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March 15, 2018

Via Electronic Delivery: ilopez@citystaug.com

Isabelle Lopez, Esq.
City of St. Augustine
75 King St.(City Hall)
St. Augustine, FL 32084

RE: City of St. Augustine
Panhandling Ordinance

Dear Ms. Lopez:

As special counsel to the City of St. Augustine tasked with rewriting the solicitation, panhandling, and begging ordinance of St. Augustine, I have forwarded certain medical studies for inclusion in the record.

The studies are: 1) Cytomegalovirus; 2) Infectious Diarrhea; 3) Leptospirosis; 4) Toxocariasis; 5) Urine Containing Sperm; 6) Zoonotic Disease transmitted by dogs, cats; 7) Principles of Epidemiology in Public Health Practice; 8) Urine Borne Diseases, The Merck Manual; 9) Open Defecation to End by 2025, Vows UN Chief, Marking World Toilet Day; 10) Stray animal and human defecation, Journal of Helminthology; 11) Human Zoonotic Infections Transmitted by Dogs and Cats, Archives of Internal Medicine; and 12) Open Defecation, Journal of Clinical and Diagnostic Research.

These studies are being submitted in correlation with and to supplement the testimony of a physician who will testify at the second reading of the ordinance to be held on March 26, 2018.

To further the understanding of both the city commission and the public, I submit the studies in light of and following numerous Supreme Court cases which have decided that such studies are pertinent, although undertaken in different geographic areas, I will further explain the legal premises for such studies at the hearing on March 26, 2018. Health-related data certainly

strengthen and amplify our record which is critical in support of the proposed ordinance. In examining the notes on the record, it is my professional opinion after 36 years of being privileged to do First Amendment work for numerous jurisdictions in the state of Florida, that our record thus far is more than sufficient to support the ordinance. I will further elaborate upon the foregoing in my closing presentation on March 26, 2018.

Respectfully submitted,

Michael H. Kahn

Michael H. Kahn
Special Counsel, City of St. Augustine

ORDINANCE NO. 2018-06

AN ORDINANCE OF THE CITY OF ST. AUGUSTINE, FLORIDA, REPEALING AND REPLACING SECTION 18-8 OF THE CODE OF THE CITY OF ST. AUGUSTINE; PROVIDING FOR FINDINGS AND INTENT; PROVIDING FOR DEFINITIONS; PROVIDING FOR PROHIBITED CONDUCT, PROXIMITY AND LOCATION RESTRICTIONS FOR SOLICITATION, PANHANDLING OR BEGGING; PROVIDING FOR PENALTIES; PROVIDING FOR INCLUSION IN THE CODE OF THE CITY OF ST. AUGUSTINE; PROVIDING FOR REPEAL OF CONFLICTING ORDINANCES; PROVIDING FOR SEVERANCE OF INVALID PROVISIONS; AND PROVIDING FOR AN EFFECTIVE DATE.

WHEREAS, § 166.041, Florida Statutes, provides for procedures for the adoption of ordinances and resolutions by municipalities; and

WHEREAS, the City of St. Augustine recognizes that solicitation, including but not limited to panhandling and begging are activities that are protected by the First Amendment to the United States Constitution; and

WHEREAS, the City of St. Augustine can adopt and enforce regulations of behavior that implicate First Amendment activity when the regulations only affect the time, place, and manner of expression, are content-neutral, are narrowly tailored to serve a significant governmental interest, and leave open ample alternative channels of communication; and

WHEREAS, the City of St. Augustine has a significant interest in providing a safe and pleasant environment and in eliminating nuisance activity, *Smith v. City of Fort Lauderdale, Florida,* 177 F. 3d 954, 956 (11th Cir. 1999); and

WHEREAS, the City of St. Augustine has experienced a significant increase in the number of complaints made to the St. Augustine Police Department regarding panhandling and problematic panhandling behaviors; and,

WHEREAS, the City Commission finds that panhandlers and beggars, sometimes use profane language when requesting money from people; that panhandlers and beggars sometimes physically touch or threaten to touch the people they solicit for money; and that panhandlers and beggars sometimes block the path of people they solicit for money, or follow the people they solicit for money in an apparent effort to intimidate people into making a donation or as retribution for refusing to make a donation; and

WHEREAS, the City Commission finds that the foregoing activities constitute "aggressive panhandling or begging," and that the increase in aggressive panhandling or begging throughout the City of St. Augustine has become extremely disturbing and disruptive to residents, visitors, and businesses, and has contributed to an enhanced sense of fear, intimidation, and disorder resulting in the loss of access to and enjoyment of public places throughout the City; and

WHEREAS, the City Commission finds that regulation of panhandling and begging, based on the time, place, or manner of the solicitation including but not limited to panhandling or begging, is a content neutral and narrowly tailored way to promote public safety, and protect residents and visitors in areas where they may be or perceive themselves to be vulnerable and/or unable to leave; and

WHEREAS, the City Commission finds that regulation of panhandling and begging, in public places where people feel particularly vulnerable and/or unable to leave

still provides ample alternative avenues of communication and are narrowly drawn to address the City's substantial interests; and

WHEREAS, the City of St. Augustine has a significant interest in preserving the safety of traffic flow and preventing traffic congestion wherever possible in the City of St. Augustine; and

WHEREAS, the City of St. Augustine has a significant interest in the safety of pedestrians and individuals traveling in vehicles throughout the City of St. Augustine; and

WHEREAS, the City of St. Augustine has a significant interest in promoting tourism, and aesthetics of historic downtown St. Augustine; and

WHEREAS, the City of St. Augustine has a significant interest in promoting the safety and convenience of its citizens on public streets. *Madsen v. Women's Health Center*, 512 U.S. 753, 768, 114 S. Ct. 2516, 129 L.Ed. 2d 593 (1994); and

WHEREAS, the City of St. Augustine has a significant interest in ensuring the public safety and order and in promoting the free flow of traffic on public streets and sidewalks; and

WHEREAS, the City of St. Augustine has a significant interest in the safety and convenience of citizens using public fora such as streets and sidewalks. *Heffron v. International Soc'y for Krishna Consciousness*, 452 U.S. 640, 650, 101 S. Ct. 2559, 69 L.Ed. 2d 298 (1981); and

WHEREAS, the City of St. Augustine has a significant interest in recognizing the safety and convenience on public roads. *Cox v. New Hampshire,* 312 U.S. 569, 574, 61 S. Ct. 762, 85 L.Ed. 1049 (1941); and

WHEREAS, the City of St. Augustine has a significant interest in controlling traffic and pedestrian congestion. *Ayres v. City of Chicago,* 125 F. 3d 1010, 1015 (7th Cir. 1997); and

WHEREAS, the City of St. Augustine has a significant interest in preventing crime, protecting the City's retail trade, maintaining property values, and generally protecting and preserving the quality of the City's neighborhoods, commercial districts and the quality of urban life. *Young v. American Mini Theaters*, 427 U.S. 50, 96 S. Ct. 440, 49 L.Ed. 2d 310 (1976); and

WHEREAS, the City of St. Augustine has a significant interest in appearance of the City and aesthetics. *Metromedia Inc. v. City of San Diego*, 453 U.S. 490, 101 S. Ct. 2882 (1981); and

WHEREAS, the City of St. Augustine has a significant interest in maintaining safe ingress and egress into and out of commercial establishments in order to, inter alia, control pedestrian congestion, facilitate pedestrian safety and order, and provide for access for emergency vehicles and personnel both fire and police to promote public health, safety, and welfare; and

WHEREAS, the City of St. Augustine has a compelling governmental interest in preserving and protecting the lives of its citizens which can be imperiled by,

inter alia, traffic and pedestrian congestion which among other things can delay deployment of life saving fire and police vehicles and personnel; and

WHEREAS, the City Commission of the City of St. Augustine has determined that the following regulations promote and protect the general health, safety, and welfare of the residents of the City of St. Augustine; and

WHEREAS, the City Commission for the City of St. Augustine finds that it is in the best interest of public health, safety, and general welfare that the following amendments be adopted consistent with the requirements of Section 166.021(4), Florida Statutes;

NOW, THEREFORE, BE IT ORDAINED BY THE CITY COMMISSION FOR THE CITY OF ST. AUGUSTINE, FLORIDA, AS FOLLOWS:

Section 1. Repeal and Replacement of Chapter 18, Article I, Section 18-8.

Chapter 18, Article I, Section 18-8 of the Code of the City of St. Augustine is hereby repealed and replaced as follows (deletions are identified using a strike-through format; additions are underlined):

~~**Sec. 18-8. - Begging, panhandling and solicitation.**~~

~~(a) *Definitions.*~~

~~(1) *After dark* means from one half hour after sunset until one-half hour before sunrise. The times of sunset and sunrise will be established by the times listed in any local publication of general distribution.~~

~~(2) *Aggressive manner* shall mean:~~

a. Approaching or speaking to a particular person or persons, or following a person before, during or after panhandling, soliciting or begging, if that conduct is intended or likely to cause a reasonable person to:

1. Fear bodily harm to oneself or to another, damage to or loss of property; or

2. Otherwise be threatened or intimidated into giving money or other thing of value; or

b. Intentionally touching or causing physical contact with another person or a vehicle operated by another person, without that person's consent, in the course of panhandling, soliciting or begging; or

c. In the course of panhandling, soliciting or begging, intentionally blocking or interfering with the safe or free passage of any pedestrian or vehicle by any means, including unreasonably causing any pedestrian or vehicle operator to take evasive action to avoid physical contact; or

d. Forcing oneself upon the company of another by continuing to solicit the individual addressed after the person to whom the panhandling, soliciting or begging is directed has made a negative response, either orally, by physical sign, by attempting to leave the presence of the person soliciting or by other negative indication.

(3) Panhandle, solicit or beg shall mean the employment of the spoken, written or printed word or other acts as are conducted in the furtherance of the purpose of immediately collecting money or any other item of value for the use of one's self or others. As used in this section, the word, "solicit," and its forms, includes begging and panhandling.

(4) Prohibited public area means those areas within historic preservation districts HP-2 and HP-3, and includes the sidewalks exterior thereto, all as more fully described below, and depicted in the map entitled "Ordinance 2006-40 No Panhandling Area" made a part hereof by reference and a copy of which is on file with the Clerk of the City of St. Augustine:

a. *Northern boundary.* Commence at a point on the northernmost sidewalk adjacent to Castillo Drive

~~located 255 feet west of the center line of South Castillo Drive; thence easterly along the northernmost sidewalk adjacent to Castillo Drive to the easternmost sidewalk adjacent to South Castillo Drive; thence southerly along the easternmost sidewalk adjacent to South Castillo Drive to the intersection of the projection of a line running along the northern boundary of the Castillo de San Marcos National Monument Reservation; thence easterly along this projection line to Matanzas Bay.~~

~~b. *Eastern boundary*. Matanzas River or Bay.~~

~~c. *Southern boundary*. Commencing at the southernmost sidewalk adjacent to King Street at the intersection with Cordova Street, thence easterly along the southernmost sidewalk adjacent to King Street along this projection line to Matanzas Bay.~~

~~d. *Western boundary*. Commencing at the westernmost sidewalk adjacent to Cordova Street at the intersection with King Street, thence northerly along the westernmost sidewalk adjacent to Cordova Street (as referenced in public records prior to the year 2000) to a point on the northernmost sidewalk adjacent to Castillo Drive located two hundred fifty five (255) feet west of the center line of South Castillo Drive.~~

~~(b) *Prohibition*. It shall be unlawful for any person within the city to:~~

~~(1) Panhandle, solicit or beg in an aggressive manner on any sidewalk, highway, street, roadway, right-of-way, parking lot, park, or other public or semi-public area or in any building lobby, entranceway, plaza or common area;~~

~~(2) Approach an operator or other occupant of a motor vehicle for the purpose of panhandling, soliciting or begging, or offering to perform a service in connection with such vehicle, or otherwise soliciting the sale of goods or services, if such panhandling, soliciting or begging is done in an aggressive manner;~~

~~(3) Panhandle, solicit or beg in an aggressive manner on private property if the owner, tenant or lawful occupant has asked the person not to solicit on the property, or has posted a sign~~

~~clearly indicating that solicitations are not welcome on the property;~~

~~(4) Panhandle, solicit or beg on any sidewalk, highway, street, roadway, right-of-way, parking lot, park, or other public or semi-public area or in any building lobby, entranceway, plaza or common area in the prohibited public area. The city manager shall post signs in such area advising the public of this prohibition;~~

~~(5) Panhandle, solicit or beg within twenty (20) feet of the entrance to any financial institution or any automatic teller machine;~~

~~(6) Panhandle, solicit or beg within twenty (20) feet of any parking meters or parking pay stations;~~

~~(7) Panhandle, solicit or beg at any lawfully permitted outdoor dining area or lawfully permitted outdoor merchandise area, provided such areas are in active use at the time;~~

~~(8) Panhandle, solicit or beg at any transit stop or taxi stand or in a public transit vehicle;~~

~~(9) Panhandle, solicit or beg while the person or persons being solicited is standing in line waiting to be admitted to a commercial establishment;~~

~~(10) Panhandle, solicit or beg by touching the person or persons being solicited without that person's consent;~~

~~(11) Panhandle, solicit or beg by blocking the path of the person or persons being solicited or blocking the entrance or exit to any building or vehicle;~~

~~(12) Panhandle, solicit or beg with the use of profane or abusive language during the solicitation or following an unsuccessful solicitation;~~

~~(13) Panhandle, solicit or beg by or with the use of any gesture or act intended to cause a reasonable person to be fearful of the solicitor or feel compelled to accede to the solicitation;~~

~~(14) Panhandle, solicit or beg while under the influence of alcohol or after having illegally used any controlled substance, as defined in the Chapter 893 of the Florida Criminal Statutes; or~~

~~(15) Panhandle, solicit or beg after dark.~~

~~(c) *Exception.* This law is not intended to limit any person from exercising his or her constitutional rights or engaging in any other constitutionally protected activity unless their conduct also violates the specific terms of this section. Rather, it is the intent of this section to protect citizens from the fear and intimidation accompanying panhandling, soliciting or begging in an aggressive manner, as prohibited herein, and to promote the health, safety and welfare of the citizens and visitors of St. Augustine.~~

~~(d) *Penalty.* Offenses under this section shall be punishable as provided by section 1-8 of this Code.~~

~~(e) *[Supplemental provisions.]* This section is not intended to replace or supersede any other ordinance or statute, but shall instead be supplemental to such ordinance or statute.~~

Section 2. Creating Chapter 18, Article I, Section 18-8. Chapter 18, Article I, Section 18-8 of the Code of the City of St. Augustine is hereby created to read as follows,

Chapter 18 - MISCELLANEOUS PROVISIONS AND OFFENSES

ARTICLE I. - IN GENERAL

Sec. 18-8. - Begging, panhandling and solicitation.

(a) *Intent.*

The purpose and intent of this article is to recognize the constitutional right of persons to solicit, including but not limited to beg and panhandle, in a peaceful and non-threatening manner; however, an increase in aggressive panhandling and begging throughout the City has become extremely disturbing and disruptive to residents and businesses, and has contributed not only to the loss of access to and enjoyment of public places but also to an enhanced sense of fear, intimidation, and disorder. Aggressive panhandling and begging usually includes approaching or following pedestrians, repetitive requests for donations of money despite refusals, the use of abusive or profane language, unwanted physical contact, and the intentional, or as incident to the aggressive panhandling and begging, blocking of pedestrian and vehicular traffic. Additionally, the

presence of panhandlers and beggars, who request money from persons in specific public areas such as outdoor cafes, automated teller machines, entrances and exits from buildings, and while standing in line to enter an event or a building, is especially troublesome because persons cannot readily escape from the undesired conduct, which often carries with it an implicit threat to both persons and property as well as incidental to the panhandling and begging activities the imperiling of the health, safety and welfare of the citizens of and visitors to St. Augustine by, among other things, blockage of ingress and egress from and into commercial businesses adjacent to public rights of way.

This article is not intended to limit any persons from exercising their constitutional right to solicit, including but not limited to beg, panhandle or solicit funds, picket, protest, or engage in any other constitutionally protected activity, when conducted in a legal manner. The goal of this article is instead to ensure the foregoing constitutional rights while through regulation acting to protect citizens from the fear and intimidation accompanying certain kinds of panhandling and begging that have become an unwelcome and overwhelming presence in the City by prohibiting aggressive panhandling and begging throughout the City and by regulating through time, place, and manner ordinance provisions regulating panhandling and begging in certain public places, based upon the foregoing significant important and substantial governmental interests set forth in the predicate clauses and an overriding compelling governmental interest to protect the health, safety and welfare of the citizens of St. Augustine and visitors from the adverse secondary effects of solicitation , including panhandling and begging, in public areas. The restrictions contained in this article are neither overbroad nor vague and are narrowly tailored to serve a substantial governmental interest, and preserve ample alternative areas for the valid exercise of constitutional rights of solicitation which they do as set forth more particularly herein.

Further, even if such regulations were to be deemed to trigger strict scrutiny, the blockage of ingress and egress into and from commercial businesses and other public areas as well as the impedance of pedestrian walkways and other public rights of way implicates the compelling governmental interest of St. Augustine in protecting the health, safety and welfare of its citizenry and visitors in preserving police and fire department access to such rights of way in order to save lives.

(b) *Definitions.*

For purposes of this article, the following words and phrases shall have the meanings ascribed to them as follows:

(1) *After dark* means from one half hour after sunset until one-half hour before sunrise. The times of sunset and sunrise will be established by the times listed in any local publication of general distribution.

(2) *Aggressive panhandling or begging* means:

a. To approach or speak to a person and demand, request or beg for money or a donation of valuable property in such a manner as would cause a reasonable person to believe that the person is being threatened with imminent bodily injury or the commission of a criminal act upon the person approached or another person in the solicited person's company, or upon property in the person's immediate possession (for example, placing oneself within 2 feet of a solicited person and/or using abusive or profane language in a loud voice while demanding or requesting money);
or

b. To maintain contact with a solicited person and continue demanding, requesting or begging for money or a donation of valuable property after the solicited person has made a negative response to an initial demand or request for money or a donation (for example, walking in front of, next to, or behind a solicited person while continuing to demand, request or beg for money from that person after that person has refused to donate or give money);

or

c. To obstruct, block or impede, either individually or as part of a group of persons, the passage or free movement of a solicited person or a person in the company of a solicited person, including persons on foot, on bicycles, in wheelchairs or operating motor vehicles or persons attempting to enter or exit motor vehicles (for example, walking, standing, sitting, laying, or placing an object in such a manner as to block passage of another person or vehicle, or to require another person or driver of a vehicle to take evasive action to avoid physical contact);

or

d. To touch or cause physical contact to a solicited person or a person in the company of a solicited person, or to touch any vehicle occupied by a solicited person or by a person in the company of the solicited person, without the person's express consent;

or

e. To engage in conduct that would reasonably be construed as intended to intimidate, compel or force a solicited person to accede to demands.

(3) *Panhandle or beg* means any demand or request made in person for an immediate donation of money or some other article of value from another person for the use of one's self or others, including but not limited for a charitable or sponsor purpose or that will benefit a charitable organization or sponsor. As used in this article, the word "solicit" and its forms is included in this definition. A solicitation is considered as having taken place regardless of whether the person making the solicitation received any contribution. Any purchase of an item for an amount far exceeding its value, under circumstances where a reasonable person would understand that the purchase is in substance a donation, constitutes a donation as contemplated in this definition.

(4) *Prohibited areas for solicitation including but not limited to panhandling and begging* means the following locations throughout the City in which it is unlawful to engage in solicitation, including but not limited to panhandling or begging, when either the panhandler or beggar or the person being panhandled is located in, on or at the following locations:

a. Within twenty (20) feet, in any direction, from any entrance or exit of commercially zoned property;
b. Within twenty (20) feet, in any direction, of any bus or trolley stop or any public transportation facility;

c. Within twenty (20) feet, in any direction, of an automated teller machine or any electronic information processing device which accepts or dispenses cash in connection with a credit, deposit or convenience account with a financial institution;

d. Within twenty (20) feet, in any direction, of any parking lot, parking garage, parking meter or parking pay station owned or operated by the City;

e. Within twenty (20) feet, in any direction, of any public restroom owned and operated by a governmental agency;

f. Within one hundred (100) feet, in any direction, of any daycare or school, including pre-kindergarten through grade 12.

(c) *Prohibited Conduct, Proximity and Location Restrictions.*

(1) It shall be unlawful for any person to engage in aggressive panhandling or begging on any sidewalk, highway, street, roadway, right-of-way, parking lot, park, or other public or semi-public area or in any public building lobby, entranceway, plaza or common area, public forum or limited public forum within the city limits of the City of St. Augustine.

(2) It shall be unlawful for any person to engage in aggressive panhandling or begging on private property if the owner, tenant or lawful occupant has asked the person not to solicit on the property, or has posted a sign clearly indicating that solicitations are not welcome on the property.

(3) It shall be unlawful for any person to engage in solicitation, including but not limited to panhandling or begging, when either the person engaged in the solicitation, including but not limited to the panhandler or beggar or the person being panhandled, is located in, on or at the following locations:

a. Within twenty (20) feet, in any direction, from any entrance or exit of commercially zoned property;

b. Within twenty (20) feet, in any direction, of any bus or trolley stop or any public transportation facility;

c. Within twenty (20) feet, in any direction, of an automated teller machine or any electronic information processing device which accepts or dispenses cash in connection with a credit, deposit or convenience account with a financial institution;

d. Within twenty (20) feet, in any direction, of any parking lot, parking garage, parking meter or parking pay station owned or operated by the City;

e. Within twenty (20) feet, in any direction, of any public restroom owned and operated by a governmental agency;

f. Within one hundred (100) feet, in any direction, of any daycare or school, including pre-kindergarten through grade 12.

(4) It shall be unlawful for any person to engage in the following prohibit conduct:

a. Approach an operator or other occupant of a motor vehicle for the purpose of panhandling, soliciting or begging, or offering to perform a service in connection with such vehicle, or otherwise soliciting the sale of goods or services, if such panhandling, soliciting or begging is done in an aggressive manner;

b. Panhandle, solicit or beg at any lawfully permitted outdoor dining area or lawfully permitted outdoor merchandise area, provided such areas are in active use at the time;

c. Panhandle, solicit or beg at any transit stop or taxi stand or in a public transit vehicle;

d. Panhandle, solicit or beg while the person or persons being solicited is standing in line waiting to be admitted to a commercial establishment

e. Panhandle, solicit or beg by touching the person or persons being solicited without that person's consent

f. Panhandle, solicit or beg with the use of profane or abusive language during the solicitation or following an unsuccessful solicitation;

g. Panhandle, solicit or beg by or with the use of any gesture or act intended to cause a reasonable person to be fearful of the solicitor or feel compelled to accede to the solicitation

h. Panhandle, solicit or beg while under the influence of alcohol or after having illegally used any controlled substance, as defined in the Chapter 893 of the Florida Criminal Statutes; or

i. Panhandle, solicit or beg after dark

(d) *Penalty.*

Any person found guilty of violating the provisions of this section shall be punished in the manner prescribed in Section 1-8 of this Code.

Section 3. Inclusion in Code. The City Commission intends that the provisions of this Ordinance shall become and shall be made part of the Code of the City of St. Augustine, that the sections of this Ordinance may be re-numbered or re-lettered and that the word ordinance may be changed to section, article or other such appropriate word or phrase in order to accomplish such intentions.

Section 4. Conflict with Other Ordinances. All ordinances or parts of ordinances in conflict herewith are hereby repealed.

Section 5. Severance of Invalid Provisions. In the event that any section, subsection, sentence, clause, phrase, word, term or provision of this Ordinance shall be held by a court of competent jurisdiction to be partially or wholly invalid, unconstitutional or unenforceable or involved for any reason whatsoever, any such invalidity, unconstitutionality, illegality, or unenforceability shall not affect any of the other or remaining terms, provisions, clauses, sentences, or sections of this Ordinance, and this Ordinance shall be read and/or applied as if the invalid, unconstitutional, illegal, or unenforceable section, subsection, sentence, clause, phrase, word, term or provision did not exist.

Section 6. Effective Date. This Ordinance shall become effective ten (10) days after passage, pursuant to § 166.041(4), Florida Statutes.

PASSED by the City Commission of the City of St. Augustine, Florida, this _____ day of _____, 2018.

_____
Nancy E. Shaver, Mayor-Commissioner

ATTEST:

_____
Darlene Galambos, City Clerk

(SEAL)

SA Health

Cytomegalovirus (CMV) infection - including symptoms, treatment and prevention

This infection is caused by cytomegalovirus (CMV). This virus occurs worldwide and humans are the only source of human CMV.

How cytomegalovirus (CMV) infection is spread

The method of spread of the infection varies. Infants usually acquire the infection while in the uterus or during passage through the birth canal, but may also be infected through breastmilk. Young children are frequently infected by contaminated saliva when sucking and sharing toys. People with weakened immune systems may have a return of a previous infection with CMV (reactivation) or may be infected with a new strain of the virus. CMV can also be transmitted during blood transfusions and organ transplants, or through sexual contact.

In developed countries more than half the population carry CMV virus by the time they reach adulthood, though this figure is much higher in developing countries.

Signs and symptoms

Infection in children and adults is usually without symptoms. Occasionally, symptoms similar to glandular fever such as fever, sore throat, swollen glands, abdomen pain and jaundice (yellowing of the skin) can occur. In certain groups, infection can result in severe disease.

These groups are:

- Infants infected before, during, or shortly after birth. Infection of a baby before birth can result in serious congenital abnormalities, with the highest risk during the first half of pregnancy and in women who have not previously been infected. CMV infection occurs in 1% or less of pregnancies, and of these cases, less than 10% are likely to have severe illness.
- People who have had a transplant, either solid organ or bone marrow/stem cell.
- People with severe immune suppression such as advanced human immunodeficiency virus (HIV) infection.

Once someone has been infected with CMV, he or she is thought to remain infected for life, even though he or she usually won't have any symptoms. People can become infected with a number of different strains of the virus. During periods of illness or stress, the virus can reactivate, and may or may not cause symptoms.

Diagnosis

Diagnosis in infants is made by growing the virus, usually from urine. Diagnosis in adults is more complicated and usually requires growing the virus, blood tests or PCR (polymerase chain reaction) tests in a pathology laboratory.

Incubation period

(time between becoming infected and developing symptoms)

3 to 12 weeks.

Infectious period

(time during which an infected person can infect others)

The virus is often shed for months in urine or saliva following infection in children and adults. Infants and immune suppressed adults can shed the virus for months to years following infection or reactivation of infection.

Treatment

Specific antiviral treatment is available for use in severe CMV infections.

Prevention

- Exclusion from childcare, preschool, school or work is not necessary
- good hand washing after handling articles contaminated with urine or saliva, particularly after changing nappies
- during pregnancy, do not share food, drinks or utensils used by young children and avoid contact with saliva when kissing a child
- there is currently no vaccine available to prevent CMV infection.

Useful links

- Hand hygiene
- Glandular fever

Last Modified: 16 Jan 2018

SA Health

Ways infectious diseases spread

Germs can spread through:

- the air as small droplets (droplet spread) or tiny aerosol particles (airborne spread)
- contact with faeces (poo) and then with the mouth (faeco-oral spread)
- contact with the skin or mucus membranes (the thin moist lining of many parts of the body such as the nose, mouth, throat and genitals) (contact spread)
- blood or other body fluids (for example, urine, saliva, breastmilk, semen and vaginal secretions).

Germs can spread:

- directly from person to person or
- indirectly from an infected person to the environment (for example toys, door handles, bench tops, bedding and toilets) and then to another person who comes in contact with the contaminated environmental source.

Germs can enter the body through the:

- mouth
- respiratory tract
- eyes
- genitals
- broken skin.

Some infections can be spread in several different ways.

There are other ways of describing how germs are spread that are commonly used. Germs can be spread through sexual contact, which is usually through semen and vaginal secretions (body fluids), but can also occur through contact with mucus membranes. Germs can spread through food or water. Many but not all the germs spread in this way are through contact with faeces and then with the mouth (faeco-oral). Germs can also spread from a mother to her unborn child, usually though blood (body fluids) but also through contact with skin or mucous membranes during delivery.

Adapted from National Health and Medical Research Council - Staying Healthy: preventing infectious disease in early childhood education and care services, 5th Edition 2012.

Spread through the air by droplets

Some infections are spread when an infected person talks, coughs or sneezes small droplets containing infectious agents into the air. Due to their size, these droplets in the air travel only a short distance (around a metre) from the infected person before falling. The droplets in the air may be breathed in by those nearby. Spread can also occur by touching the nose or mouth with droplet contaminated hands.

Examples of droplet spread diseases:

- common cold
- flu
- meningococcal disease
- rubella.

Spread through the air by aerosol

Some infections are spread when an infected person talks, breathes, coughs or sneezes tiny particles containing infectious agents into the air. These are called small particle aerosols. Due to their tiny size, small particle aerosols can travel long distances on air currents and remain suspended in the air for minutes to hours. These small particle aerosols may be breathed in by another person.

Examples of airborne spread diseases:

- chickenpox
- measles
- tuberculosis (TB)

Spread through faeces and then the mouth (faecal-oral spread)

Some infections are spread when microscopic amounts of faeces (poo) from an infected person with symptoms or an infected person without symptoms (a carrier) are taken in by another person by mouth. The faeces may be passed:

- directly from soiled hands to the mouth
- indirectly by way of objects, surfaces, food or water soiled with faeces.

Examples of diseases spread from faeces:

- *Campylobacter* infection
- *Cryptosporidium* infection
- *Giardia* infection
- hand, foot and mouth disease
- hepatitis A

- meningitis (viral)
- rotavirus infection
- *Salmonella* infection
- *Shigella* infection
- thrush
- viral gastroenteritis
- worms
- *Yersinia* infection.

Spread by skin or mucous membrane contact

Some infections are spread **directly** when skin or mucous membrane (the thin moist lining of many parts of the body such as the nose, mouth, throat and genitals) comes into contact with the skin or mucous membrane of another person. Infections are spread **indirectly** when skin or mucous membrane comes in contact with contaminated objects or surfaces.

Examples of diseases spread by skin or mucous membrane contact:

- chickenpox
- cold sores (herpes simplex infection)
- conjunctivitis
- hand, foot and mouth disease
- head lice
- molluscum contagiosum
- ringworm
- scabies
- school sores (impetigo)
- *Staphylococcus aureus* infection
- warts.

Spread through blood or other body fluids

Some infections are spread when blood or other body fluids (for example for example, urine, saliva, breastmilk, semen and vaginal secretions) from an infected person comes into contact with:

- the mucous membranes (the thin moist lining of many parts of the body such as the nose, mouth, throat and genitals), such as through kissing, breast-feeding or sexual contact or
- the bloodstream of an uninfected person, such as through a needle stick injury or a break in the skin.

Examples of diseases spread through blood or other body fluids:

- hepatitis B - blood, saliva, semen and vaginal fluids
- hepatitis C - blood
- human immunodeficiency virus (HIV) infection - blood, semen and vaginal fluids, breastmilk
- cytomegalovirus (CMV) infection - saliva, breastmilk, semen and vaginal fluids, urine
- glandular fever - saliva

Other ways of describing how infectious diseases are spread

Spread through sexual contact (sexually transmitted infections)

These infections are most commonly transmitted by sexual contact. Sexual contact means:

- genital to genital
- oral to genital
- genital to anal.

Examples of sexually transmitted infections:

- *Chlamydia* infection
- genital herpes
- genital warts
- gonorrhoea
- hepatitis B
- human immunodeficiency virus (HIV) infection
- non-specific urethritis (NSU)
- pubic lice (crabs)
- syphilis
- trichomoniasis.

Spread through food or water

These diseases result from ingestion of water or a wide variety of foods contaminated with disease-causing germs or their toxins. Often these infections are also spread by the faecal-oral route.

Examples of food or waterborne diseases:

- botulism

- *Campylobacter* infection
- cholera
- *Cryptosporidium* infection
- haemolytic uraemic syndrome
- *Listeria* infection
- *Salmonella* infection
- *Shigella* infection
- typhoid and paratyphoid
- *Yersinia* infection.

Spread from a mother to her unborn child

Some infections can be spread through the placenta from a mother to her unborn child or during delivery, or both.

Examples of diseases spread from a mother to child in this way:

- chickenpox
- hepatitis B
- rubella.

Diseases where person-to-person spread occurs rarely, if ever

Some infectious diseases are almost never spread by contact with an infected person. These diseases are usually spread by contact with an environmental source such as animals, insects, water or soil.

Examples of diseases spread by **contact with animals**:

- cat-scratch disease
- hydatid disease
- psittacosis
- Q fever
- rabies
- toxoplasmosis.

Examples of diseases **spread by insects,** and in the examples listed below, specifically by mosquitoes:

- Barmah Forest virus infection

- dengue fever
- malaria
- Ross River virus infection.

Examples of diseases spread by **contact with water or soil**:

- amoebic meningitis
- legionella infection - *Legionella pneumophila* and *Legionella longbeachae*
- tetanus.

Last Modified: 28 Feb 2017

Cytomegalovirus Survival on Common Environmental Surfaces: Opportunities for Viral Transmission

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Congenital cytomegalovirus (CMV) affects ~1 of 150 births and is a leading cause of hearing loss and intellectual disability. It has been suggested that transmission may occur via contaminated surfaces. CMV AD169 in filtered human saliva, applied to environmental surfaces, was recovered at various time points. Samples were evaluated by culture and real-time polymerase chain reaction. CMV was found viable on metal and wood to 1 hour, glass and plastic to 3 hours, and rubber, cloth, and cracker to 6 hours. CMV was cultured from 83 of 90 wet and 5 of 40 dry surfaces. CMV was more likely to be isolated from wet, highly absorbent surfaces at earlier time points.

Congenital cytomegalovirus (CMV) infection, which occurs as a result of infection during pregnancy, affects ~1 of 150 live births and is a leading cause of hearing loss and intellectual disability in the United States [1–3]. In a typical cohort of 4 million live births, this translates to ~30 000 babies born with a congenital CMV infection [1]. Of those born with CMV, ~6000 (20%) will suffer permanent sequelae (eg, sensorineural hearing loss and intellectual disability), and ~150 (0.5%) will die of complications of the infection.

CMV is an enveloped virus that establishes lifelong latency following primary infection and periodically reactivates, usually without symptoms. Reinfection with additional strains is known to occur [4]. Transmission occurs via direct contact with bodily fluids of an infected individual. In adults, asymptomatic shedding of virus in saliva and urine may persist for weeks to months after initial infection. Children, especially those congenitally infected or <3 years old, may actively shed CMV in saliva and urine for months to years [5–8].

Exposure to bodily fluids from young children poses substantial risk for CMV exposure among women of reproductive age. Consequently, recommendations set forth by various experts for reducing CMV exposure have centered on behavioral precautions, such as routinely washing hands after exposure to bodily fluids, refraining from sharing food or drink with young children, and avoiding contact with saliva when kissing young children [2, 9–12]. However, little is known about the duration of CMV viability on environmental surfaces and the extent to which bodily fluids deposited on surfaces may be sources of exposure.

Methods

Seven types of common surfaces were studied: rubber, glass, plastic, metal, sanded wood, cloth, and wheat crackers. Surfaces were categorized as either highly absorbent or poorly absorbent surfaces. Highly absorbent surfaces included cloth (100% cotton), whole wheat crackers, and sanded pine plywood. Standard window glass, steel sheet metal, plastic (Plexiglas), and rubber matting were categorized as poorly absorbent surfaces. Ten defined areas (~2 cm^2) were marked on each material for the application and recovery of virus. For poorly absorbent surfaces it was necessary to demarcate an area (using a grease pencil) on the surface to keep the virus solution within the test area.

CMV strain AD169 from a single large stock (5.96×10^9 genome equivalents/mL) was titered on human lung fibroblasts using a plaque assay and was suspended in filtered human saliva diluted 1:1 in phosphate-buffered saline (PBS), pH 7.4, at a concentration of 10^3 virons/mL. For each surface, 200 μL of viral-saliva solution (~200 viable virions) were applied to each of 10 marked areas and recovered using polyester fiber-tipped swabs (plastic shaft; BD Falcon) at time points ranging from 1 minute to 6 hours after application. When liquid was present, the remaining liquid was measured using a Pipet-Aid (Drummond Scientific Company, Broomall, PA) pipette controller and the volume adjusted to 200 μL with PBS to maximize consistency in recovery. An additional 100 μL of PBS was used to rewet the surfaces and collected onto a swab. The swab and fluids were placed into the barrel of a 3-cm^3 syringe, inserted into an open sterile 15-cm^3 centrifuge tube, and centrifuged at 200*g* for 10 minutes to maximally recover the sample. For cloth and cracker, the entire 2-cm^2 area moistened by the sample was excised, placed into a syringe

Received 2 May 2011; accepted 1 September 2011; electronically published 23 November 2011.

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The Journal of Infectious Diseases 2012;205:211–4
Published by Oxford University Press on behalf of the Infectious Diseases Society of America 2011.
DOI: 10.1093/infdis/jir722

barrel to which 100 μL of PBS was added, and centrifuged as for other specimens. Recovered liquid was measured and adjusted to 200 μL with PBS.

At each time point, observations regarding visible wetness of the material (wet or dry) were recorded. One hundred microliters of each recovered sample was immediately transferred to T25 sterile culture flasks (Corning) containing a confluent monolayer of primary human lung fibroblasts in complete minimal essential medium supplemented with 5% fetal calf serum and antibiotics (Gibco). Cultures were observed for ≥2 weeks for signs of viral growth. The remaining sample was frozen for subsequent quantitative CMV polymerase chain reaction (PCR) analysis. Viral growth in cultures was scored from 0 to 4+ based on the extent of cytopathic effect. A quantitative CMV real-time PCR assay was performed on all samples to confirm the presence of CMV. DNA copies per reaction were used as a control for sampling consistency.

Results

For each surface, the quantity of infectious virions declined with time, as measured by level of cytopathic effect produced in culture. In general, duration of CMV viability was shorter on poorly absorbent surfaces compared with highly absorbent surfaces (Figure 1*A–D*). Among poorly absorbent surfaces, CMV viability persisted longest on rubber, retaining viability for up to 6 hours. Samples applied to glass retained infectivity up to 3 hours. CMV applied to metal was rendered nonviable within 2 hours. Although viability was somewhat variable for CMV applied to plastic, viral infectivity persisted up to 3 hours. No viable virus was recovered at 1 hour for either trial of rubber, but given viability at multiple flanking time points on both sides of the timeline, this was interpreted as an experimental anomaly.

CMV survived longer on most of the highly absorbent surfaces used in the study than on poorly absorbent surfaces (Figure 1*E–G*). Viral infectivity was retained on cloth up to 6 hours. CMV applied to wheat cracker remained infectious for 6 hours in both trials, and, unlike on all other surfaces, in 1 trial retained 4+ cytopathic effect 3 hours after application. In contrast to the other highly absorbent surfaces, viral viability on sanded wood declined from a score of 4+ at 1 minute to a score of 0 at 2 hours.

The cracker surface remained visibly wet throughout the 6-hour study period. Among the other surfaces, the reduction of viability generally correlated with subjective visual observation of the surface becoming dry, although glass, cloth, and plastic retained viable virus for up to 1–2 hours after the surface appeared completely dry. This was observed in samples taken at the 5-hour sampling on cloth, 2-and 3-hour samplings on plastic, and 2-and 3-hour samplings on glass (Figure 1*A*, 1*C*, and 1*E*). No viable virus was recovered in experiments evaluating CMV survival at time points from 18 to 24 hours after application (data not shown).

As a measure of reliability and consistency of CMV sample recovery, harvested samples were separately assayed by quantitative real-time CMV PCR to estimate genome copy number. Results are displayed in the line graphs under each chart in Figure 1. These assays suggest that CMV sample recovery was highly consistent for all 10 sampling areas on all 7 surfaces. In only 1 of the trials, CMV DNA load dropped substantially at the 6-hour time point for metal (6.32 copies) and cloth (23.5 copies).

Discussion

In this systematic evaluation of CMV survival on surfaces, we found that CMV viability can sufficiently persist to enable fomite transmission. In fact, viable CMV can persist on surfaces as long as they remain wet—in some cases at least up to 6 hours. There are no additional data to conclude whether virus remains viable on surfaces that remain wet for more than 6 hours. However, the amounts of viable virus on wet surfaces decreased over a 6-hour period, suggesting that virus on wet surfaces may be less likely to survive over longer periods of time (eg, overnight). Additionally, we found that apparently dry surfaces can harbor viable virus in the 1–2-hour time period between when they are visibly dry and when, presumably, the microenvironment becomes completely dry. However, visibly wet surfaces were much more likely to harbor viable virus and to harbor higher amounts of viable virus than dry surfaces.

In most cases it appears that CMV viability is more closely related to wetness than to the particular surface where it is applied. Indeed, we found a correlation between CMV viability and the capacity of each particular material to retain moisture: with the exception of processed wood, viral viability persisted much longer in highly absorbent surfaces that would be expected to effectively retain moisture. The procedure for virus recovery from cloth and cracker, which were both centrifuged, may be most similar to a situation where cloth is placed in the mouth or a cracker is eaten. Because these highly absorbent surfaces can retain moisture internally, they may pose a higher transmission risk when they are mouthed or ingested than when only their surface is touched. CMV viability was eliminated more rapidly in wood samples than in other highly absorbent surfaces studied, suggesting a process of active neutralization. This may be attributable to substances naturally present in wood (eg, organic alcohols and aromatic liquids) or to chemicals used to treat lumber during production.

This study is the first controlled systematic research aimed at evaluating CMV viability in human fluids on surfaces over time. Few previous studies have provided evidence specifically related to opportunities for viral transmission from common surfaces. A study conducted in 1982 in a childcare center identified toddlers shedding CMV in their saliva. CMV was cultured from all 4 of the plastic toys that were swabbed immediately after removal from the mouth of a shedding child [13]. In an additional study in 1986, toys mouthed by CMV-excreting children were swabbed after removal from the child's mouth. CMV was detected on 5 of 7 toys immediately, 4 of 7 toys after 10 minutes, and 2 of 7 toys after 30 minutes [14]. Unfortunately, it is difficult

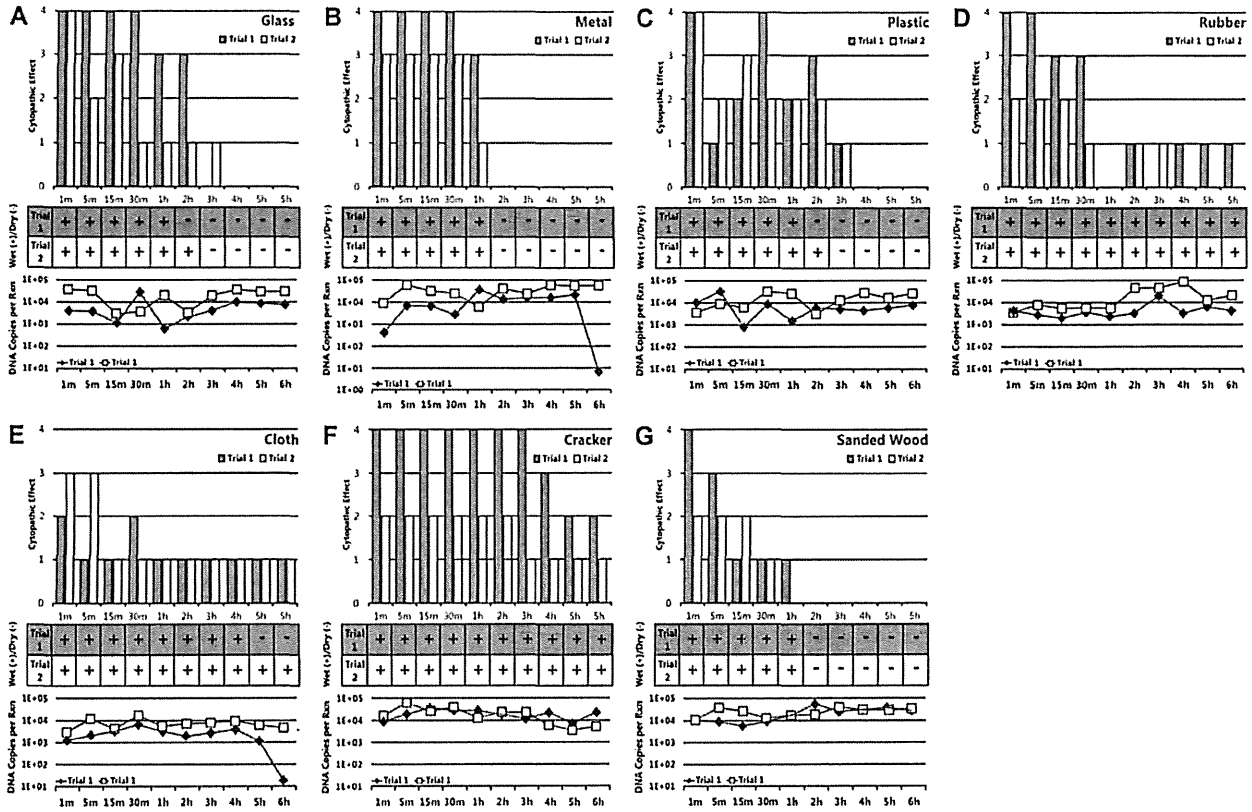

Figure 1. Cytopathic effect, dehydration, and DNA copies after inoculation of common environmental surfaces with cytomegalovirus (CMV). Cytopathic effect seen through 2-week observation of viral culture on human lung fibroblast cells and scored from 0 to 4+. Plus sign indicate wet appearance; minus signs, dry appearance. Results of quantitative real-time CMV polymerase chain reaction assay are shown as DNA copies per reaction.

to compare results between past studies and the current study, because toy surfaces were not physically described in the former. Finally, a study from 1985 investigated CMV survival on surfaces such as Plexiglas and bedding in the immediate neonatal intensive care unit environment of congenitally infected infants [15]. That study found that virus was more likely to be recovered from surfaces that came into direct contact with the infants and that higher viral titers were associated with longer persistence on surfaces.

The strengths of the current study include the evaluation of CMV survival using titered preparations of viable virus suspended in a natural fluid (saliva) and the use of objective experimental controls (CMV real-time PCR) to monitor the consistency of sample recovery from surfaces. One possible limitation was the use of laboratory strain AD169. CMV strains adapted to growth in human lung fibroblast cell culture can rapidly lose some properties found in wild-type viruses, particularly with respect to infection of other cell types (eg, epithelial cells). However, it seems unlikely that the physical properties of the virus that contribute to survival time on inanimate surfaces would be different in laboratory-adapted strains. Laboratory strains and wild-type viruses both express the receptors needed for entry into fibroblasts, and the viral envelope is acquired from the host cell and is thus identical in both types of strains. When desiccation occurs, membrane integrity is lost, and viability along with it. Because the macromolecular composition of the viral envelope is identical for laboratory strains and wild-type viruses, comparable susceptibility to desiccation is probable. Another important limitation is that recovery of specimen was not designed to reflect real-world transmission. Instead, it was designed for comparability of different surfaces and to maximize the possibility of recovering viable virus. For these reasons, we rewet dry surfaces and centrifuged the cloth and cracker. Compared with the likelihood of recovering viable virus through these methods, we would expect that a person who touches dry surfaces or touches the cloth or cracker would be less likely to transfer viable virus to their fingers. Additionally, cultures were followed for only 2 weeks. Although it is true that some foci may have appeared after 3 or 4 weeks of observation, the intent of the study was to compare viable virus on multiple surfaces and their relative survival times.

As shown, CMV in saliva can survive long enough on surfaces to pose a transmission risk. Young children commonly shed CMV for extended periods, contaminating toys, food, and other objects in

the environment. To reduce exposure to CMV, women who are pregnant should cleanse their hands after touching objects that may have been in contact with children's saliva (eg, toys, countertops), especially if the surface appears or feels wet. Additionally, surfaces that have come in contact with children's saliva should be cleansed regularly, especially when noticeably wet. Finally, women should take precautions to avoid sharing food or drink with a young child and avoid contact with saliva when kissing a young child.

Notes

Disclaimer. The views included in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

Financial support. This research was supported in part by appointments (J.D.S., D.F.P., E.D., S.L.B.) at the Centers for Disease Control and Prevention (CDC), National Center for Immunization and Respiratory Disease, Division of Viral Disease, administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the U.S. Department of Energy and the CDC. Additional funding for D.F.P. was provided by (Coordenação de Aperfeiçoamento de Pessoal de Nível) through an agreement between the Brazilian Ministry of Education and Universidade Federal de Sao Paulo, Sao Paulo, Brazil.

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Dollard SC, Grosse SD, Ross DS. New estimates of the prevalence of neurological and sensory sequelae and mortality associated with congenital cytomegalovirus infection. Rev Med Virol 2007; 17:355–63.
2. Cannon MJ, Davis KF. Washing our hands of the congenital cytomegalovirus disease epidemic. BMC Public Health 2005; 5:70.
3. Kenneson A, Cannon MJ. Review and meta-analysis of the epidemiology of congenital cytomegalovirus (CMV) infection. Rev Med Virol 2007; 17:253–76.
4. Boppana SB, Fowler KB, Pass RF, et al. Congenital cytomegalovirus infection: association between virus burden in infancy and hearing loss. J Pediatr 2005; 146:817–23.
5. Leads from the MMWR. Prevalence of cytomegalovirus excretion from children in five day-care centers. JAMA 1985; 253:1236, 1239–40.
6. Jones LA, Duke PM, Yeager AS. Cytomegalovirus infections—infant development versus day care centers. Clin Res 1984; 32:A109.
7. Murph JR, Bale JF. The natural-history of acquired cytomegalovirus infection among children in group day care. Am J Dis Child 1988; 142:843–6.
8. Rosenthal LS, Fowler KB, Boppana SB, et al. Cytomegalovirus shedding and delayed sensorineural hearing loss results from longitudinal follow-up of children with congenital infection. Pediatr Infect Dis J 2009; 28:515–20.
9. American Academy of Pediatrics. Cytomegalovirus infection. In: Pickering LK, Baker CJ, Kimberlin DW, Long SS, eds. Red Book 2009 Report of the Committee on Infectious Diseases. 28th ed. Elk Grove, IL; American Academy of Pediatrics; 2009: 275–80.
10. Adler SP, Finney JW, Manganello AM, Best AM. Prevention of child-to-mother transmission of cytomegalovirus by changing behaviors: a randomized controlled trial. Pediatr Infect Dis J 1996; 15:240–6.
11. Picone O, Vauloup-Fellous C, Cordier AG, et al. A 2-year study on cytomegalovirus infection during pregnancy in a French hospital. BJOG 2009; 116:818–23.
12. Demmler-Harrison GJ. Congenital cytomegalovirus: public health action towards awareness, prevention, and treatment. J Clin Virol 2009; 46:S1–S5.
13. Pass RF, August AM, Dworsky M, Reynolds DW. Cytomegalovirus infection in day-care center. New Engl J Med 1982; 307:477–9.
14. Hutto C, Little EA, Ricks R, Lee JD, Pass RF. Isolation of cytomegalovirus from toys and hands in a day care center. J Infect Dis 1986; 154:527–30.
15. Faix RG. Survival of cytomegalovirus on environmental surfaces. J Pediatr 1985; 106:649–52.

Clinical and Vaccine Immunology
cvi.asm.org

Accepted manuscript posted online 12 April 2017, doi: 10.1128/CVI.00024-17
Clin Vaccine Immunol June 2017 vol. 24 no. 6 e00024-17

Cytomegalovirus Virions Shed in Urine Have a Reversible Block to Epithelial Cell Entry and Are Highly Resistant to Antibody Neutralization

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ABSTRACT

Cytomegalovirus (CMV) causes sensorineural hearing loss and developmental disabilities in newborns when infections are acquired *in utero*. Pregnant women may acquire CMV from oral exposure to CMV in urine or saliva from young children. Neutralizing antibodies in maternal saliva have the potential to prevent maternal infection and, in turn, fetal infection. As CMV uses different viral glycoprotein complexes to enter different cell types, the first cells to be infected in the oral cavity could determine the type of antibodies needed to disrupt oral transmission. Antibodies targeting the pentameric complex (PC) should block CMV entry into epithelial cells but not into fibroblasts or Langerhans cells (which do not require the PC for entry), while antibodies targeting glycoprotein complexes gB or gH/gL would be needed to block entry into fibroblasts, Langerhans cells, or other cell types. To assess the potential for antibodies to disrupt oral acquisition, CMV from culture-positive urine samples (uCMV) was used to study cell tropisms and sensitivity to antibody neutralization. uCMV entered epithelial cells poorly compared with the entry into fibroblasts. CMV-hyperimmune globulin or monoclonal antibodies targeting gB, gH/gL, or the PC were incapable of blocking the entry of uCMV into either fibroblasts or epithelial cells. Both phenotypes were lost after one passage in cultured fibroblasts, suggestive of a nongenetic mechanism. These results suggest that uCMV virions have a reversible block to epithelial cell entry. Antibodies may be ineffective in preventing maternal oral CMV

acquisition but may limit viral spread in blood or tissues, thereby reducing or preventing fetal infection and disease.

KEYWORDS

cytomegalovirus neutralizing antibodies congenital infection vaccine

INTRODUCTION

Cytomegalovirus (CMV) is the leading cause of congenital abnormalities in the United States, causing serious permanent disabilities in greater than 5,500 children annually. Approximately 13% of congenitally infected infants are symptomatic at birth, and of those born infected but asymptomatic, 17 to 20% will later develop permanent sequelae, including microcephaly, brain malformations, hearing loss, vision loss, and cognitive impairment. The most common disability found in congenitally infected infants is sensorineural hearing loss, affecting about 36% of symptomatic and 12% of asymptomatic infants (1). Due to the high incidence of permanent sequelae from congenital CMV, the development of a CMV vaccine has been deemed a national priority by the Institute of Medicine (2).

Congenital CMV infections are believed to occur in many instances after the acquisition of maternal infection via an oral route during pregnancy (3). Young children with virus in their urine or saliva are the main source of maternal infections. Vaccination could potentially prevent maternal acquisition through the induction of neutralizing antibodies in maternal saliva. However, because CMV uses different viral glycoprotein complexes to enter different cell types, the types of cells that are first infected by CMV within the oral cavity could dictate which antibodies are optimal for preventing oral acquisition. For example, antibodies to the viral glycoprotein B (gB) and a trimeric complex of glycoproteins H, L, and O (gH/gL/gO) neutralize CMV entry into a variety of cell types but are less potent than antibodies recognizing epitopes of the pentameric complex (PC), a five-subunit complex of gH/gL bound to UL128, UL130, and UL131A (4–8). However, as the PC is only required for entry into certain cell types (e.g., epithelial cells, endothelial cells, monocytes, and monocyte-derived dendritic cells) and is dispensable for entry into others (e.g., fibroblasts and Langerhans-type dendritic cells) (4, 9–15), the neutralizing activity of PC-specific antibodies is limited to those cell types that require the PC for viral infection.

The cells that are first infected in the new host are not known. Due to their abundance in the oral cavity, mucosal epithelial cells are likely candidates for the initial infection. If so, a vaccine that induces PC-specific neutralizing antibodies may prevent oral acquisition. On the other hand, if initial infection occurs in cells that use a PC-independent mechanism (e.g., fibroblasts or Langerhans-type dendritic cells), PC-specific antibodies would have no efficacy and gB- or gH/gL/gO-specific antibodies may prevent acquisition. However, this rationale is based on data from cell culture-based experiments in which established cell lines such as MRC-5 fibroblasts or ARPE-19 epithelial cells were used to generate virus stocks and to study viral infection and antibody neutralization. CMV passed in cell culture may behave differently from the CMV that is transmitted via urine (uCMV)

or saliva. Indeed, it has been reported that uCMV has poor infectivity for endothelial cells (16) and is resistant to antibody neutralization (17).

These observations led us to examine both the cell type-specific tropisms of uCMV and the ability of different antibodies to neutralize it. We found that uCMV enters and replicates poorly in epithelial cells and is profoundly resistant to neutralization by CMV hyperimmune globulin (HIG) or monoclonal antibodies. Remarkably, both of these properties were rapidly lost after one passage in fibroblasts, suggesting a nongenetic rather than genetic basis. However, once uCMV had entered epithelial cells, the spread to neighboring cells was restricted by both polyclonal and monoclonal antibodies. These findings have important implications for vaccine development, as they suggest that antibodies in saliva may have little impact on oral acquisition, although antibodies in saliva and submucosal tissues may serve to limit local replication and dissemination.

RESULTS

In comparison to fibroblasts, uCMV enters epithelial cells inefficiently. Historically, fibroblasts have been used for the detection and isolation of CMV from clinical samples. The fact that CMV from clinical samples infects human umbilical vein endothelial cells far less efficiently than fibroblasts (16) led us to ask whether this phenomenon extends also to epithelial cells. Monolayer cultures of MRC-5 fibroblasts and ARPE-19 epithelial cells were simultaneously inoculated with replicate dilutions of urine sample Uxc. After incubating for 3 days, the cultures were fixed and stained for CMV immediate early (IE) antigen expression as a marker for viral entry and the initiation of replication. At higher concentrations of urine, MRC-5 cultures were heavily infected, and IE antigen-positive cells remained abundant even when the urine was diluted 320-fold (Fig. 1A). By contrast, ARPE-19 cultures contained only a few IE antigen-positive cells at the highest concentration of urine (1:20). Based on IE antigen-positive cell counts at these dilutions (Fig. 1A), the approximate ratio of fibroblast to epithelial cell infectivity is 55:1.

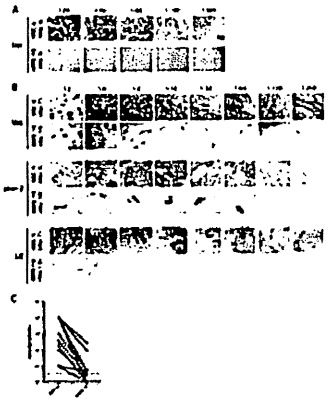

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FIG 1

uCMV enters epithelial cells inefficiently. (A) CMV-positive urine Uxc was serially diluted in medium, and replicates were added to MRC-5 or ARPE-19 monolayers. After 3 days, the cells were fixed and stained for CMV IE antigen. (B) CMV-positive urine samples Uxc, Umn-2, and U2 were serially diluted in medium, and then replicates were added to MRC-5

or ARPE-19 monolayers. Cells were fixed and stained for CMV IE antigen on the indicated dpi. Wells in which IE antigen-positive cells were not detected were not photographed. In panels A and B, the numbers within the images indicate IE-positive cell counts. (C) Eleven CMV-positive urine samples were titrated by endpoint dilution using IE antigen staining of infected MRC-5 or ARPE-19 cultures. Solid lines connect data from the same urine sample. Dashed line indicates the limit of detection (300 PFU/ml). Results below the limit of detection (no IE antigen-positive cells detected at any dilution) were arbitrarily assigned a value of 100 PFU/ml.

This experiment was repeated using urine Uxc and two additional CMV-positive urine samples, Umn-2 and U2. To ensure that infected ARPE-19 cells had adequate time to express IE proteins and to provide a more compelling readout in the form of foci of IE antigen-positive cells, MRC-5 cultures were stained 5 to 7 days postinfection (dpi) and ARPE-19 cultures were stained 13 dpi. The inoculation of MRC-5 cultures with urine samples Uxc, Umn-2, and U2 resulted in extensive infection at dilutions <1:16 (Fig. 1B). By contrast, the inoculation of ARPE-19 cultures with urine samples diluted 1:2 resulted in a few individual foci of antigen-positive cells, indicating that only a small number of infection events were successful and spread to form foci (Fig. 1B). To quantitatively compare infectivity between the two cell types, 11 CMV-positive urine samples were titrated by endpoint dilution on both cell types. Titers were 100- to 1,000-fold lower when assayed using epithelial cells versus fibroblasts (Fig. 1C). These results indicate that uCMV virions are significantly more capable of establishing productive infections in MRC-5 fibroblasts than in ARPE-19 epithelial cells.

Epithelial entry efficiency of uCMV improves within one passage in fibroblasts. Since all available laboratory strains of CMV were initially isolated from and passaged in fibroblasts, we produced a virus stock from urine Uxc that was passaged exclusively in ARPE-19 epithelial cells. After 14 passages, the culture supernatant was isolated and designated ARPE-19-adapted stock UxcAp14. An MRC-5-adapted stock, UxcMp14, was produced in parallel by passage in MRC-5 cells. The entry properties of the cell culture-passaged viruses were dramatically different from those of the parental Uxc-derived uCMV. IE antigen staining 3 dpi revealed that the ARPE-19-adapted stock UxcAp14 infected both cell types with similar efficiencies (Fig. 2A). Remarkably, the restriction to ARPE-19 entry observed for uCMV was lost following one or two passages in MRC-5 cells (Fig. 2B). ARPE-19 entry efficiency began to decline at passage four, presumably due to the increasing prevalence of a mutation disrupting *UL130* that was observed in UxcMp14. Similar results were obtained for two additional urine samples, Umn-3 and Umn-4. MRC-5 and ARPE-19 infectivity were similar for the first two MRC-5 passages but thereafter, ARPE-19 infectivity declined and infected ARPE-19 cells were not detected after Umn-3 passage 9 or Umn-4 passage 5 (Fig. 2C).

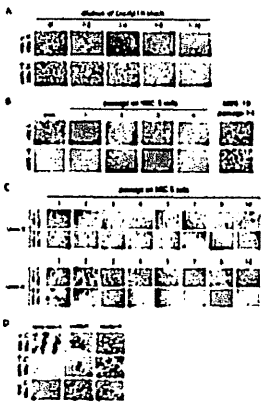

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FIG 2

Inefficient epithelial entry of uCMV is lost after one passage in cell culture. (A) ARPE-19-adapted stock UxcAp14 was serially diluted in medium and replicates were added to MRC-5 or ARPE-19 monolayers. After 3 days, the cells were fixed and stained for CMV IE antigen. (B) CMV in urine Uxc was serially passaged in MRC-5 cells. Urine Uxc or culture supernatants from each MRC-5 passage were added in replicates to MRC-5 or ARPE-19 monolayers. After 3 days, the cells were fixed and stained for CMV IE antigen. (C) CMV in urine samples Umn-3 and Umn-4 were serially passaged in MRC-5 cells, and culture supernatants from each passage were added in replicates to MRC-5 or ARPE-19 monolayers. Cells were fixed and stained for CMV IE antigen on days 4 to 7 (MRC-5) or day 13 (ARPE-19). (D) Cell monolayers were infected with matching amounts of urine Umn-4, Uxc passaged four times in MRC-5 cells (UxcMp4), or ARPE-19-adapted Uxc (UxcAp14). Cells were fixed and stained for CMV IE antigen 7 days postinfection. Arrowhead indicates an IE antigen-positive NOK cell infected with UxcMp4 virus. Numbers within images indicate IE antigen-positive cell counts.

ARPE-19 cells are derived from the retinal pigment epithelium and therefore may not accurately represent the presumed targets of uCMV during oral transmission, namely, the epithelial cells of the oral mucosa. To evaluate uCMV infectivity of mucosal epithelial cells, normal oral keratinocytes (NOKs) derived from human gingival tissue were used. Inoculation of MRC-5, ARPE-19, and NOK cultures with matching amounts of urine resulted in extensive antigen staining in MRC-5 cells, but no antigen-positive cells were detected in either the ARPE-19 or the NOK cultures (Fig. 2D). As with the ARPE-19 cells, NOK entry efficiency improved after limited MRC-5 passage, and while adaptation in ARPE-19 cells also improved virus entry efficiency in NOKs, ARPE-19-adapted virus exhibited significantly lower infectivity for NOKs than for ARPE-19 cells (Fig. 2D). Thus, to the extent that NOKs may be representative of oral mucosal epithelial cells, the restriction observed for uCMV entry into ARPE-19 cells appears to also extend to oral epithelial cells.

uCMVs are highly resistant to antibody neutralization. To confirm a previous report that uCMVs are resistant to neutralizing antibodies (17), replicate aliquots of CMV-positive urine samples were incubated in medium alone or in medium containing a high concentration (1,280 μg/ml) of HIG. The mixtures were then

added to MRC-5 or ARPE-19 monolayers and infectivity was assessed by IE antigen staining. Eleven urine samples were evaluated on MRC-5 cells but only seven had sufficient titers for evaluation on ARPE-19 cells. In all cases, 1,280 μg/ml HIG failed to neutralize CMV infectivity (Fig. 3A). However, an amniotic fluid sample was available from the same subject who, after birth, provided urine sample Ujh-1. MRC-5 infectivity of CMV in the amniotic fluid was sensitive to neutralization by HIG (Fig. 3A). Unfortunately, the viral titer of the amniotic fluid was too low to assess ARPE-19 infectivity.

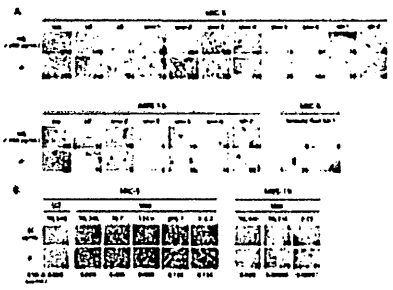

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FIG 3

Entry of uCMV into fibroblasts or epithelial cells is insensitive to antibody neutralization. (A) The indicated CMV-positive urine samples were incubated with medium (Ø) or with medium containing 1,280 μg/ml HIG for 1 h at 37°C and then were added to MRC-5 or ARPE-19 monolayers. MRC-5 cells were fixed and stained for CMV IE antigen after 5 to 7 days; ARPE-19 cells were fixed and stained after 12 to 14 days. CMV-positive amniotic fluid Ajh-1 (from the same subject as urine Ujh-1) was incubated with medium or with medium containing 1,280 μg/ml HIG for 1 h at 37°C and then added to MRC-5 monolayers. Cells were fixed and stained for CMV IE antigen after 7 days. Two foci are shown out of a total of five detected in the untreated culture; no foci were detected in the HIG-treated culture. (B) Urine samples U2 and Uxc were incubated with medium (Ø) or with medium containing 50 μg/ml of the indicated monoclonal antibodies for 1 h at 37°C and then were added to MRC-5 or ARPE-19 monolayers. Cells were fixed and stained for CMV IE antigen after three (MRC-5) or 14 (ARPE-19) days. Numbers within images indicate IE antigen-positive cell counts. IC_{50}s indicate neutralizing activities of monoclonal antibodies measured using a GFP-based assay for entry of virus ABV (a BAC-cloned virus derived from Uxc) into cells (MRC-5 or ARPE-19) matching those used in the experiments shown.

Seven monoclonal antibodies with potent neutralizing activities were used to further assess the sensitivity of uCMVs to antibody neutralization. TRL345 is a human monoclonal antibody that recognizes the AD-2 epitope of gB and neutralizes both fibroblast and epithelial entry (18). TRL310 and 2-25 are human monoclonal antibodies that recognize PC epitopes, and both selectively neutralize epithelial entry (18, 19, 20). Rabbit monoclonal antibodies 70.7, 124.4, 270.7, and

316.2 recognize a discontinuous gH/gL epitope and neutralize both fibroblast and epithelial entry (19, 20).

Only the anti-gB antibody, TRL345, had activity in these assays. At 50 μg/ml, it partially inhibited the entry of uCMV from U2 into MRC-5 cells or the entry of uCMV from Uxc into both MRC-5 cells and ARPE-19 cells (**Fig. 3B**). However, 50 μg/ml is ~2,000-fold the neutralizing 50% inhibitory concentration (IC_{50}) of TRL345 measured using cell culture-adapted viruses, and at 25 μg/ml, TRL345 had no effect (data not shown). The anti-gH/gL antibodies 70.7, 124.4, 270.7, and 316.2 had no effect on the MRC-5 infectivity of Uxc-derived virus, but given that they are 3- to 6-fold less potent than TRL345, this may be a reflection of their lower potency rather than antigen specificity.

These results confirm and extend previous findings that uCMVs are resistant to neutralizing antibodies (17). Eleven of 11 unique uCMVs were antibody resistant, and resistance extended to high concentrations of antibodies targeting a range of epitopes and viral entry mediators. The only exception was the anti-gB antibody TRL345, which, at a high concentration, partially inhibited uCMV entry into both fibroblasts and epithelial cells.

One passage in fibroblasts restores sensitivity to antibody neutralization. CMV that has been adapted for growth in cell culture is sensitive to antibody neutralization. To determine how rapidly this sensitivity to neutralization occurs upon passage, HIG neutralizing activity was assessed using Uxc-derived virus in culture supernatants after one or two passages in MRC-5 cells or after 16 passages in ARPE-19 cells. Only one passage in MRC-5 cells was sufficient to render the progeny virus sensitive to HIG neutralization using both MRC-5 cells and ARPE-19 cells as targets. The passage 16 ARPE-19-adapted virus was similarly sensitive to HIG neutralization (**Fig. 4**). One passage in MRC-5 cells was also sufficient to render CMV from urine samples U2, U3, Umn-3, and Umn-4 sensitive to neutralization by HIG (data not shown). This rapid loss of resistance implies a nongenetic mechanism of resistance, for example, the presence of novel factors associated with uCMV virions that are lost upon viral propagation in cell culture.

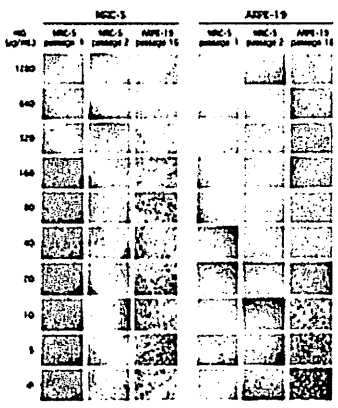

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FIG 4

Sensitivity to neutralizing antibody is established after one passage in fibroblasts or with adaptation in ARPE-19 cells. uCMV from Uxc was passaged twice in MRC-5 cells. HIG was diluted in medium, and then each dilution was combined at the indicated final concentrations with a constant amount of MRC-5 passage 1 or

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passage 2 culture supernatants or with Uxc virus adapted by 16 passages in ARPE-19 cells. The mixtures were incubated for 1 h at 37°C and then added to MRC-5 or ARPE-19 monolayers. Cells were fixed and stained for CMV IE antigen after 3 days.

HIG and monoclonal antibodies to gB and the PC can limit uCMV from spreading in epithelial cells but not in fibroblasts. HIG and monoclonal antibodies added to culture medium after infection of cells can limit the spread of CMV in ARPE-19 but not MRC-5 monolayers (19, 21). To determine if antibodies can similarly limit the spread of uCMV, MRC-5 or ARPE-19 monolayers were infected with uCMV from Uxc. After 24 h, the cultures were washed and incubated for 5 to 7 days (MRC-5) or 12 to 14 days (ARPE-19) in fresh medium alone or in medium containing HIG or monoclonal antibodies before IE antigen staining. As seen in **Fig. 5A**, neither 1,280 μg/ml HIG nor 25 μg/ml of monoclonal antibody TRL345 or 316.2 had any effect on the spread of Uxc-derived virus in MRC-5 cultures. This dose is 1,042-fold the IC_{50} of TRL345 and 187-fold the IC_{50} of 316.2 for the neutralization of MRC-5 entry. By contrast, the spread of uCMV from Uxc in ARPE-19 monolayers was inhibited by HIG, the anti-gB antibody TRL345, and the anti-PC antibodies TRL310 and 2-25 (**Fig. 5B**). Moreover, at concentrations >1.5 μg/ml, the anti-PC antibodies prevented the virus from spreading beyond the initially infected cell (**Fig. 5B**). These results suggest that uCMV is similar to cell culture-produced CMV with respect to the ability of antibodies to limit its spread in epithelial cells but not fibroblasts. Thus, in epithelial cells, the ability of uCMV to evade neutralizing antibodies appears to be lost following the initial entry event.

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FIG 5

HIG or monoclonal antibodies to gB or the PC inhibit the spread of uCMV in epithelial but not fibroblast cell monolayers. Urine Uxc was combined with medium (Ø) or medium containing HIG or monoclonal antibodies at the indicated final concentrations. After incubating for 1 h at 37°C, the mixtures were added to MRC-5 (A) or ARPE-19 (B) monolayers. Cells were fixed and stained for CMV IE antigen after 9 days (MRC-5) or 18 days (ARPE-19). IC_{50}s indicate neutralizing activities of monoclonal antibodies measured using a GFP-based assay for entry of

virus ABV into cells (MRC-5 or ARPE-19) matching those used in the experiments shown.

Treatment with saliva, trypsin, or storage at 4°C does not render uCMV sensitive to neutralizing antibodies. A monoclonal antibody capture enzyme-linked immunosorbent assay (ELISA) failed to detect uCMV in fresh urine, but the detection improved significantly after incubating at 4°C for 9 to 14 days (22). The authors suggested that uCMV may be protected by an inhibitory substance that is gradually degraded during storage. Therefore, we hypothesized that uCMV may become sensitive to antibody neutralization after incubating at 4°C or in response to the exposure to trypsin or factors in saliva that might function to counteract this protective mechanism and render uCMV sensitive to neutralization. To test this, uCMV from U2 was tested for sensitivity to neutralization by HIG after storage at 4°C for 10 or 42 days, after incubating in the presence of 54% human saliva at 37°C for 1 h, or after incubating in the presence of 170 μg/ml trypsin at 37°C for 10 min. As shown in Fig. 6, uCMV from U2 remained insensitive to HIG neutralization regardless of these treatments. These results indicate that human saliva does not contain proteases or other factors that can overcome uCMV resistance to neutralizing antibodies, and that in contrast to previously reported ELISA reactivity (22), the resistance to antibody neutralization is stable at 4°C.

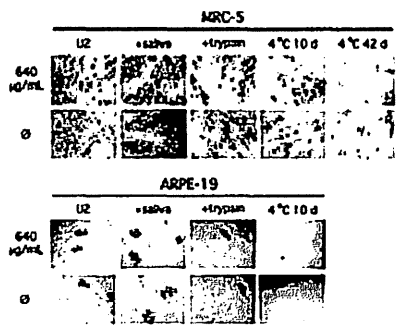

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FIG 6

uCMV resistance to antibody neutralization is not lost following storage at 4°C or exposure to saliva or trypsin. Urine U2 was pretreated as follows: incubation for 1 h at 37°C with medium (U2), incubation for 1 h at 37°C in the presence of 54% saliva from a healthy adult human subject (+saliva), incubation for 10 min at 37°C in the presence of 170 μg/ml trypsin (+trypsin), or incubation undiluted at 4°C for 10 (4°C 10 d) or 42 (4°C 42 d) days. Each sample was then combined with medium (Ø) or with HIG at a final concentration of 640 μg/ml, was incubated for 1 h at 37°C, and then was added to MRC-5 or ARPE-19 monolayers. Cells were fixed and stained for CMV IE antigen after 5 days (MRC-5) or 15 days (ARPE-19).

Urine does not contain *trans* -acting factors that render CMV insensitive to neutralizing antibodies. Urine might contain factors that inhibit the activity of neutralizing antibodies or act in *trans* to modify CMV virions and render them

resistant to neutralization. To address this, the sensitivity to HIG neutralization was measured with a virus produced in cell culture, called ABV. This virus was reconstituted from a bacterial artificial chromosome (BAC) clone that was derived from the UxcAp14 stock. A green fluorescent protein (GFP) marker cassette within the BAC origin of replication allowed assessment of ABV infectivity by the detection of GFP-positive cells. When incubated with HIG alone, ABV entry into MRC-5 cells was neutralized by HIG with an IC_{50} of approximately 10 µg/ml. When incubations also included urine U2 or urine U3 at concentrations matching those in the experiment in Fig. 3A in which uCMVs from U2 and U3 were insensitive to neutralization by HIG, the sensitivity of ABV to neutralization by HIG was unchanged (Fig. 7A). Therefore, urine samples U2 and U3 did not contain factors that, during the course of the 1 h incubation, were able to modify or render ABV virions resistant to neutralization.

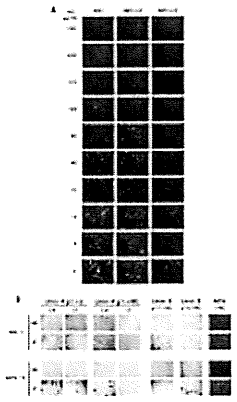

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FIG 7

uCMV resistance to antibody neutralization is not conferred by soluble factors in urine or by propagation in the presence of CMV-specific antibodies. (A) HIG was diluted in medium and then each dilution was combined at the indicated final concentrations with a constant amount of GFP-tagged virus ABV with or without a constant amount of CMV-positive urine sample U2 or U3. The mixtures were incubated for 1 h at 37°C and then added to MRC-5 monolayers. Micrographs were taken with an inverted UV microscope 5 days after infection. (B) uCMV from Umn-4 was passaged once in MRC-5 cells cultured in medium (Umn-4 p1+Ø) or in medium containing 2 mg/ml HIG (Umn-4 p1+HIG). Cell-associated (CA) or cell-free (CF) viruses were prepared 7 dpi. Additionally, cell-associated virus stocks with HIG (Umn-4p17 +HIG and Umn-3p16 +HIG) were prepared after 17 (Umn-4) or 16 (Umn-3) passages in MRC-5 cells in the presence of 2 mg/ml HIG, and cell-free ABV virus was produced by passage of virus ABV for 6 months in the presence of 2 mg/ml HIG (ABV +HIG) and then passaged once without HIG to produce a culture supernatant containing ABV virus but lacking HIG. Samples were combined with medium (Ø) or with HIG at a concentration of 1,280 µg/ml, were incubated for 1 h at 37°C, and then were added to MRC-5 or ARPE-19 monolayers. Cells infected with uCMVs derived from Umn-3 or Umn-4 were fixed and stained for CMV IE antigen after 5 days (MRC-5) or 12 days (ARPE-19); ABV-infected

cells were photographed with an inverted UV microscope 3 to 4 days after infection.

Passage in the presence of HIG does not render CMV resistant to neutralizing antibodies. CMV propagated in the presence of a gH-specific neutralizing murine monoclonal antibody becomes resistant to neutralization by a rapidly reversible (presumably nongenetic) mechanism (23). Subsequent studies found that the addition of the gH-specific human monoclonal antibody, MSL-109, to the culture medium of CMV-infected cells results in progeny virions containing MSL-109, and that somehow this provides an alternative entry pathway that is insensitive to MSL-109 neutralization (24). Given that people who shed CMV are CMV-seropositive, the resistance to antibody neutralization observed for uCMV may arise *in vivo* from CMV replication in an extracellular environment that includes high levels of CMV-specific IgG.

To test this potential mechanism, MRC-5 cells were infected with uCMV from Umn-4, and after 24 h, the medium was replaced with either medium alone or medium containing 2 mg/ml HIG. After an extensive cytopathic effect (CPE) was observed 5 to 7 dpi, cell-associated virus or virus in the culture medium was evaluated for sensitivity to neutralization by HIG. When grown in the absence of HIG, both cell-associated and cell-free Umn-4-derived virus was sensitive to HIG neutralization (Fig. 7B). Cell-associated Umn-4-derived virus produced in the presence of HIG was also sensitive, while no infectious virus was detected in the culture medium, presumably because any virus released was sensitive and neutralized by the HIG in the culture medium. To determine if long-term culture in the presence of HIG results in resistance, Umn-3- and Umn-4-derived viruses, as well as the BAC-derived virus, ABV, were passaged extensively in the presence of HIG as described above. Cell-associated Umn-3- and Umn-4-derived viruses passaged in the presence of HIG remained sensitive to neutralization by HIG (Fig. 7B), while the absence of detectable virus in the culture medium (not shown) implied the absence of resistant cell-free virus. Similarly, cell-free ABV virus passaged in the presence of HIG and then passaged once without HIG was also sensitive to neutralization by HIG (Fig. 7B). Thus, virus propagation in the presence of polyclonal antibodies does not result in antibody-resistant virus.

DISCUSSION

The discovery of the PC and elucidation of its role as a target for potent neutralizing antibodies specific for epithelial/endothelial cell entry have inspired new avenues in CMV vaccine development. In particular, vaccines that induce PC-specific salivary antibodies could block inoculum virus from infecting the epithelial cells of the oral mucosa. In support of this, saliva samples from a subset of naturally infected subjects neutralized the entry of cell culture-passaged CMV into epithelial cells (25). However, this assumes that mucosal epithelial cells are the first cells infected during oral acquisition. If this is not the case, PC-specific antibodies may have little effect. These considerations led us to evaluate the entry tropisms of CMV found in clinical specimens for fibroblasts and for epithelial cells,

and to assess the sensitivity of these infection events to neutralizing antibodies. Three key observations were made.

First, uCMV is profoundly deficient for entry into ARPE-19 epithelial cells, which in recent years have been used extensively to model CMV epithelial tropism. How accurately these cells represent CMV's interactions with epithelial cells from other tissues remains largely unknown. Using cell culture-passaged CMV, we confirmed that the PC is necessary for efficient entry into epithelial cells derived from a variety of mucosal tissues, including of the tonsil, bronchus, cervix, foreskin, and vagina (6), while in the current work, we extended these studies to include NOK cells derived from gingiva. Surprisingly, uCMV failed to infect NOK cells, but as for ARPE-19 cells, NOK infectivity was quickly acquired upon passage in fibroblasts. Gerna et al. observed very similar defects in entry of uCMV or CMV in throat washes into human umbilical vein endothelial cells (16). Taken together, these data suggest that CMV shed in urine and saliva may have a general deficiency in the ability to enter cells by the PC-mediated pathway. The molecular basis of this deficiency remains to be determined but could include low levels of functional PC in virions caused by an intrinsic property of the cells that produce CMV *in vivo* or the degradation or inactivation of the PC during or after virion release by factors in urine or saliva. That an alternative entry pathway may be utilized that is inefficient for epithelial or endothelial cell entry is discussed further below.

Second, the entry of uCMV into both fibroblasts and epithelial cells is profoundly resistant to neutralization by polyclonal antibodies in HIG and to monoclonal antibodies specific for gB, gH/gL, or the PC. However, at high concentrations, the gB-specific antibody TRL345 had a partially inhibitory effect (Fig. 3B). The fact that TRL345 is less potent than the PC-specific antibodies TRL310 and 2-25 at neutralizing cell culture-passaged virus may suggest that the mechanism of antibody resistance is less effective against antibodies that neutralize by targeting gB versus those that target the PC. Given that a recombinant gB vaccine provided partial protection against CMV acquisition (26), it is possible that, in the context of the oral cavity, vaccine-induced gB-specific antibodies can provide some efficacy by neutralizing inoculum CMV in urine or saliva.

As for the defect in epithelial cell entry, a sensitivity to antibody neutralization is acquired within one passage in fibroblasts. This implies that the two phenomena may be linked, i.e., that the mechanism that protects the virus from neutralizing antibodies also impairs its ability to enter epithelial cells. If so, the observation that CMV in throat washes is deficient in endothelial cell entry (16) may further imply that CMV in saliva may also be resistant to antibody neutralization.

The mechanism of antibody resistance is unknown. In contrast to cell culture-propagated virus, the envelope fraction of uCMV contains significant amounts of β_2 microglobulin (β_2m), and it has been proposed that this β_2m serves to prevent antibodies from binding to surface glycoproteins, thereby protecting uCMV virions from antibody neutralization and preventing their detection by an antibody capture ELISA (17). This hypothesis was supported by two experimental observations. First, the ELISA signal from uCMV in fresh culture-positive urine samples became

positive after storage at 4°C for 9 to 14 days, suggesting that uCMV glycoprotein epitopes became accessible to the monoclonal antibody as the inhibitory factors (possibly β_2m) gradually degraded (22). Second, the ELISA signal from cell culture-propagated virus could be inhibited by the addition of soluble β_2m (27). It remains uncertain whether β_2m simply occludes antibody access to the established entry mediators (gB, gH/gL/gO, or PC) without impairing their ability to engage receptors and mediate entry or, alternatively, if it inhibits the established entry pathways while perhaps facilitating a novel entry pathway that does not require gB, gH/gL/gO, or the PC. Such an alternative entry pathway could explain both resistance to antibody neutralization and the deficient infectivity of uCMV for epithelial cells if, for example, epithelial cells have low levels of the required receptor(s).

The idea that β_2m associated with uCMV might function to mediate an alternative entry pathway has been proposed and was supported by evidence that free β_2m can compete with CMV virions for binding to fibroblasts and that β_2m-coated virions bind to HLA class I-positive cells at significantly higher levels than to cells devoid of HLA class I expression (28). Evidence that antibodies to β_2m can neutralize uCMV would greatly support this model, but McKeating et al. found that uCMV was not neutralized by four monoclonal antibodies or a polyclonal rabbit antiserum raised against β_2m (17). Moreover, while the addition of β_2m to cell culture-propagated virus increases its infectivity (28), this has not been demonstrated to confer resistance to antibody neutralization, and in our hands, prolonged incubation at 4°C, shown previously to permit access of monoclonal antibodies to uCMV epitopes (22), did not render uCMV sensitive to antibody neutralization (**Fig. 6**). Thus, the proposed role for β_2m in uCMV antibody resistance and/or an alternative entry pathway requires further study.

The ability of CMV to evade antibody neutralization by the incorporation of antibodies into progeny virions, as described for MSL-109 (24), suggests an intriguing mechanism for antibody resistance of uCMV, given that uCMV is likely derived from an environment rich in CMV-specific IgG. Moreover, similar to uCMV antibody resistance, MSL-109 resistance results in impaired PC-mediated entry and is lost upon one passage in medium lacking MSL-109 (24). Unlike uCMV, MSL-109-resistant virus is specifically resistant to MSL-109 and is not cross-resistant to antibodies recognizing gB or other epitopes in gH (24). Even so, if the mechanism of MSL-109 resistance extends to other glycoprotein targets and epitopes, it is possible that the incorporation of a polyclonal mixture of antibodies into CMV virions could result in resistance to antibodies targeting a broad spectrum of epitopes. However, our failure to maintain or reconstitute resistance to antibody neutralization by propagating uCMV or cell culture-adapted CMV in the presence of polyclonal CMV-specific antibodies suggests that *in vivo* replication of CMV in the presence of CMV-specific antibodies is probably not the mechanism by which uCMV attains resistance.

Third, while neutralizing antibodies had little effect on entry, they were effective at blocking the spread of virus in ARPE-19 epithelial cell monolayers. We previously reported that HIG can inhibit the spread of cell culture-passaged and uCMV in

ARPE-19 cells (21) and have more recently extended these studies to quantitate the inhibition of cell culture-passaged CMV spreading by these and other monoclonal antibodies. We observed a close correlation between spread inhibition and neutralizing potencies, suggesting that in cultured ARPE-19 cells, CMV spread likely requires the transient release of infectious virions into the extracellular compartment (19). By contrast, cell-to-cell spread between fibroblasts uses a distinct mechanism that is highly resistant to antibody inhibition.

Our findings have two major implications. First, CMV in the form that is responsible for oral transmission appears to be optimized for infecting fibroblasts, while entry into epithelial cells (this study) and endothelial cells (16) is profoundly impaired. The fact that this impairment is rapidly lost upon propagation *in vitro* suggests a nongenetic rather than genetic cause, for example, a deficiency of the PC in the uCMV virion envelope, posttranslational modifications that impair PC function, or the interaction of the PC with inhibitory factors. These observations further suggest that CMV may have evolved mechanisms to actively block uCMV from infecting many of the cells that it initially encounters upon first entering the oral cavity, perhaps to prevent unproductive infections at sites from which dissemination may be difficult and to promote infections at sites where access to the lymphatics may be less constricted (e.g., the tonsil). If so, this may in part explain why CMV has evolved multiple entry mechanisms, as having multiple pathways allows one to be temporarily blocked while others remain fully functional.

While the cells initially targeted by uCMV remain uncertain, the data thus far suggest that they may be fibroblasts or other cells that can be infected by a PC-independent (fibroblast-like) mechanism. CMV could potentially gain access to fibroblasts through microbreaks that permit viral access to submucosal tissues, as is thought to occur during the oral transmission of human papillomaviruses, which initially infect the undifferentiated basal cell layers of mucosae (29). Alternatively, CMV infection of Langerhans-type dendritic cells does not require the PC (15), and while these cells reside in the submucosa, their dendrites can extend to the mucosal surface. Thus, CMV could initially infect Langerhans-type dendritic cells and subsequently spread to adjacent epithelial cells and/or fibroblasts. This pathway is similar to that of the measles virus, which does not initially infect mucosal epithelial cells but first infects and replicates in dendritic cells before spreading to infect adjacent pulmonary epithelial cells (30). It is also possible that uCMV selectively targets certain epithelial cells that are unique to a specific site within the oral cavity, or that uCMV entry into oral mucosal cells *in vivo* involves cellular factors that are not expressed by cells grown in culture. The development of assays that more accurately model the mucosal environment (e.g., organotypic keratinocyte cultures representing stratified/differentiated epithelium) may be useful for elucidating the initial interactions of CMV with the oral mucosa.

Second, regardless of which cell types are initially infected and which entry pathway is involved, the profound resistance of CMV in the inoculum to antibody neutralization suggests that salivary antibodies may not be highly effective at interrupting CMV acquisition. While we have shown that saliva samples from some

seropositive subjects can neutralize CMV propagated in cell culture (25), additional studies are needed to determine if these saliva samples also have the capability of neutralizing CMV in urine. In the event that saliva fails to fully neutralize CMV in the inoculum, our *in vitro* findings further suggest that CMV released from the initially infected cells or spreading to adjacent epithelial cells should be sensitive to the effects of antibody inhibition. Intervention at this stage may be crucial for limiting replication in local tissues and thereby reducing or preventing dissemination that prefaces fetal infection.

MATERIALS AND METHODS

Study populations and sample collection. CMV culture-positive urine samples were obtained from 10 congenitally infected newborns seen at Virginia Commonwealth University Medical Center, University of Minnesota Medical Center, Johns Hopkins Hospital, Baylor College of Medicine, or Texas Children's Hospital. Amniotic fluid AFjh-1 was obtained at 21.5 weeks gestation from the same subject that provided urine Ujh-1. Urine U3 was obtained from a normal healthy child attending day care in Richmond, Virginia. Urine and amniotic fluid samples were clarified of cellular debris by centrifugation at 2,600 × *g* for 5 min, and then were adjusted to 100 mM sucrose, aliquoted, and stored under liquid nitrogen. Saliva and sera were obtained from normal healthy adults. Informed consent was obtained from all subjects or their guardians, and protocols were approved by the committees for the conduct of human research at Virginia Commonwealth University, University of Minnesota, Johns Hopkins Hospital, and Baylor College of Medicine.

Antibodies. HIG (CytoGam; CSL Behring, King of Prussia, PA) was purchased from the manufacturer. TRL345 (Trellis Bioscience) is a human monoclonal antibody specific for the AD-2 (site I) epitope of gB (31). TRL310 (Trellis Bioscience) (18) is a human monoclonal antibody reconstructed from published sequences of antibody 1F11 specific for a discontinuous epitope formed by UL130 and UL131A (5). Human monoclonal 2-25 was isolated and cloned from cultured memory B cells of a healthy CMV-seropositive donor (20). Rabbit monoclonal antibodies 70.7, 124.4, 270.7, and 316.2 are described in reference 4 and recognize a discontinuous epitope formed by gH and gL (19, 20).

Cells. Human MRC-5 fetal lung fibroblasts (ATCC CCL-171) and ARPE-19 retinal pigment epithelium cells (ATCC CRL-2302) were obtained from ATCC and propagated in high glucose Dulbecco's modified Eagle medium (Gibco-BRL) supplemented with 10% fetal calf serum (HyClone Laboratories), 10,000 IU/liter penicillin, and 10 mg/liter streptomycin (Gibco-BRL). NOKs were a gift from Karl Munger (32) and were propagated using Keratinocyte-SFM supplemented with human epidermal growth factor and bovine pituitary extract (Invitrogen).

Virus adaptation for growth in ARPE-19 or MRC-5 cells. Urine Uxc was inoculated onto ARPE-19 or MRC-5 cells and serially passaged until extensive cytopathic effects were observed. The adaptation for growth in ARPE-19 cells required 10 to 12 passages over a period of 12 months. Passage 14 and passage

16 ARPE-19-adapted stocks were designated UxcAp14 and UxcAp16. Adaptation on MRC-5 cells took 10 passages over 3 months. A passage 14 fibroblast-adapted stock was designated UxcMp14. Umn-3 and Umn-4 uCMVs were serially passaged in MRC-5 cells as described above with or without 2 mg/ml HIG in the culture medium. A viral genome designated ABV was cloned as a BAC from the UxcAp14 stock using methods described previously (33). The ABV BAC contains a BAC origin of replication with GFP marker cassette inserted between *US28* and *US29* and a spontaneous deletion in the *UL146* to *UL150* region. ABV virus was reconstituted by the transfection of ABV BAC DNA into ARPE-19 cells and was amplified in ARPE-19 cultures as described previously (33). Frozen stocks were prepared from culture supernatants and the titers were determined as described previously (33).

Detection and determination of viral titers by IE antigen staining. CMV-infected cells were detected by immunohistochemical staining of IE antigen 3 to 18 dpi as described previously (34). IE antigen-positive cells in photographic images were counted manually from enlarged prints. Urine samples and virus stocks derived from urine samples were titrated by serial dilution in medium, were inoculated onto ARPE-19 or MRC-5 cultures in 96-well plates, and were stained for IE antigen on days 4 (MRC-5) or 13 (ARPE-19) postinfection.

Neutralization assays and photomicroscopy. Neutralization and spread inhibition assays were conducted as described previously (6, 21, 34). For neutralizing assays, antibodies were serially diluted in medium, were mixed with an equal volume of medium containing virus and incubated for 1 h at 37°C, and then were transferred to 96-well plates containing confluent MRC-5 or ARPE-19 cells. For spread inhibition assays, monolayers in 96-well plates were infected at a low multiplicity (50 to 150 PFU/well) and incubated for 24 h. The culture medium was then removed and replaced with medium containing serial dilutions of antibodies. Representative bright field and fluorescence images were taken using a Nikon Diaphot 300 UV microscope and 10× objective. GFP-based determination of antibody neutralizing IC_{50}s using virus ABV is described elsewhere (19).

ACKNOWLEDGMENTS

We thank Cindy Gandaria for help with processing and shipping urine samples, Karl Munger for NOK cells, Tong-Ming Fu and Merck and Co., Inc., for rabbit monoclonal antibodies and human monoclonal antibody 2-25, and Lawrence Kauvar and Trellis Bioscience for monoclonal antibodies TRL310 and TRL345.

This work was supported by grants R01AI088750 and R21AI073615 (to M.A.M.) from the National Institutes of Health.

FUNDING INFORMATION

NIAID R01AI088750 R21AI073615
Michael A. McVoy

FOOTNOTES

Received 24 January 2017.

Returned for modification 20 February 2017.

Accepted 4 April 2017.

Accepted manuscript posted online 12 April 2017.

Address correspondence to Michael A. McVoy, michael.mcvoy@vcuhealth.org.

Citation Cui X, Adler SP, Schleiss MR, Arav-Boger R, Demmler Harrison GJ, McVoy MA. 2017. Cytomegalovirus virions shed in urine have a reversible block to epithelial cell entry and are highly resistant to antibody neutralization. Clin Vaccine Immunol 24:e00024-17. https://doi.org/10.1128/CVI.00024-17.

REFERENCES

1. **Dollard SC, Grosse SD, Ross DS.** 2007. New estimates of the prevalence of neurological and sensory sequelae and mortality associated with congenital cytomegalovirus infection. Rev Med Virol 17:355-363. 10.1002/rmv.544.
CrossRef Medline Google Scholar

2. Stratton KR, Durch SJ, Lawrence RS (ed). 2000. Vaccines for the 21st century: a tool for decisionmaking. National Academies Press, Washington, DC.
Google Scholar

3. **Adler SP, Marshall B.** 2007. Cytomegalovirus infections. Pediatr Rev 28:92-100. 10.1542/pir.28-3-92. FREE Full Text

4. **Freed DC, Tang Q, Tang A, Li F, He X, Huang Z, Meng W, Xia L, Finnefrock AC, Durr E, Espeseth AS, Casimiro DR, Zhang N, Shiver JW, Wang D, An Z, Fu TM.** 2013. Pentameric complex of viral glycoprotein H is the primary target for potent neutralization by a human cytomegalovirus vaccine. Proc Natl Acad Sci U S A 110:E4997-E5005. 10.1073/pnas.1316517110.
Abstract/FREE Full Text

5. **Macagno A, Bernasconi NL, Vanzetta F, Dander E, Sarasini A, Revello MG, Gerna G, Sallusto F, Lanzavecchia A.** 2010. Isolation of human monoclonal antibodies that potently neutralize human cytomegalovirus infection by targeting different epitopes on the gH/gL/UL128-131A complex. J Virol 84:1005-1013. 10.1128/JVI.01809-09. Abstract/FREE Full Text

6. **Saccoccio FM, Sauer AL, Cui X, Armstrong AE, Habib ELSE, Johnson DC, Ryckman BJ, Klingelhutz AJ, Adler SP, McVoy MA.** 2011. Peptides from cytomegalovirus UL130 and UL131 proteins induce high titer antibodies that block viral entry into mucosal epithelial cells. Vaccine 29:2705-2711. 10.1016/j.vaccine.2011.01.079. CrossRef Medline Google Scholar

7. **Fouts AE, Chan P, Stephan JP, Vandlen R, Feierbach B.** 2012. Antibodies against the gH/gL/UL128/UL130/UL131 complex comprise the majority of the anti-cytomegalovirus (anti-CMV) neutralizing antibody response in CMV hyperimmune globulin. J Virol 86:7444-7447. 10.1128/JVI.00467-12.
Abstract/FREE Full Text

8. **Kabanova A, Marcandalli J, Zhou T, Bianchi S, Baxa U, Tsybovsky Y, Lilleri D, Silacci-Fregni C, Foglierini M, Fernandez-Rodriguez BM, Druz A, Zhang B,**

Geiger R, Pagani M, Sallusto F, Kwong PD, Corti D, Lanzavecchia A, Perez L. 2016. Platelet-derived growth factor-alpha receptor is the cellular receptor for human cytomegalovirus gHgLgO trimer. Nat Microbiol 1:16082. 10.1038/nmicrobiol.2016.82. CrossRef Google Scholar

9. Hahn G, Revello MG, Patrone M, Percivalle E, Campanini G, Sarasini A, Wagner M, Gallina A, Milanesi G, Koszinowski U, Baldanti F, Gerna G. 2004. Human cytomegalovirus UL131-128 genes are indispensable for virus growth in endothelial cells and virus transfer to leukocytes. J Virol 78:10023-10033. 10.1128/JVI.78.18.10023-10033.2004. Abstract/FREE Full Text

10. Gerna G, Percivalle E, Lilleri D, Lozza L, Fornara C, Hahn G, Baldanti F, Revello MG. 2005. Dendritic-cell infection by human cytomegalovirus is restricted to strains carrying functional UL131-128 genes and mediates efficient viral antigen presentation to $CD8^+$ T cells. J Gen Virol 86:275-284. 10.1099/vir.0.80474-0. CrossRef Medline Google Scholar

11. Wang D, Shenk T. 2005. Human cytomegalovirus virion protein complex required for epithelial and endothelial cell tropism. Proc Natl Acad Sci U S A 102:18153-18158. 10.1073/pnas.0509201102. Abstract/FREE Full Text

12. Adler B, Scrivano L, Ruzcics Z, Rupp B, Sinzger C, Koszinowski U. 2006. Role of human cytomegalovirus UL131A in cell type-specific virus entry and release. J Gen Virol 87:2451-2460. 10.1099/vir.0.81921-0. CrossRef Medline Google Scholar

13. Ryckman BJ, Rainish BL, Chase MC, Borton JA, Nelson JA, Jarvis MA, Johnson DC. 2007. Characterization of the human cytomegalovirus gH/gL/UL128-131 complex that mediates entry into epithelial and endothelial cells. J Virol 82:60-70. 10.1128/JVI.01910-07. CrossRef Google Scholar

14. Straschewski S, Patrone M, Walther P, Gallina A, Mertens T, Frascaroli G. 2011. Protein pUL128 of human cytomegalovirus is necessary for monocyte infection and blocking of migration. J Virol 85:5150-5158. 10.1128/JVI.02100-10. Abstract/FREE Full Text

15. Lauron EJ, Yu D, Fehr AR, Hertel L. 2014. Human cytomegalovirus infection of Langerhans-type dendritic cells does not require the presence of the gH/gL/UL128-131A complex and is blocked after nuclear deposition of viral genomes in immature cells. J Virol 88:403-416. 10.1128/JVI.03062-13. Abstract/FREE Full Text

16. Gerna G, Percivalle E, Sarasini A, Revello MG. 2002. Human cytomegalovirus and human umbilical vein endothelial cells: restriction of primary isolation to blood samples and susceptibilities of clinical isolates from other sources to adaptation. J Clin Microbiol 40:233-238. 10.1128/JCM.40.1.233-238.2002. Abstract/FREE Full Text

17. McKeating JA, Griffiths PD, Grundy JE. 1987. Cytomegalovirus in urine specimens has host beta 2 microglobulin bound to the viral envelope: a mechanism of evading the host immune response? J Gen Virol 68(Pt 3):785-792. 10.1099/0022-1317-68-3-785. CrossRef Medline Google Scholar

18. Kauvar LM, Liu K, Park M, DeChene N, Stephenson R, Tenorio E, Ellsworth SL, Tabata T, Petitt M, Tsuge M, Fang-Hoover J, Adler SP, Cui X, McVoy MA, Pereira L. 2015. A high-affinity native human antibody neutralizes human cytomegalovirus infection of diverse cell types. Antimicrob Agents Chemother 59:1558-1568. 10.1128/AAC.04295-14. Abstract/FREE Full Text

19. **Cui X, Freed DC, Wang D, Qiu P, Li F, Fu TM, Kauvar L, McVoy MA.** 5 April 2017. Impact of antibodies and strain polymorphisms on cytomegalovirus entry and spread in fibroblasts and epithelial cells. J Virol 10.1128/JVI.01650-16. Abstract/FREE Full Text

20. **Ha S, Li F, Troutman MC, Freed DC, Tang A, Loughney JW, Wang D, Wang IM, Vlasak J, Nickle DC, Rustandi RR, Hamm M, DePhillips PA, Zhang N, McLellan JS, Zhu H, Adler SP, McVoy MA, An Z, Fu TM.** 2017. Neutralization of diverse human cytomegalovirus strains conferred by antibodies targets viral gH/gL/pUL128-131 pentameric complex. J Virol 91:e02033-16. 10.1128/JVI.02033-16. Abstract/FREE Full Text

21. **Cui X, Lee R, Adler SP, McVoy MA.** 2013. Antibody inhibition of human cytomegalovirus spread in epithelial cell cultures. J Virol Methods 192:44-50. 10.1016/j.jviromet.2013.04.015. CrossRef Medline Google Scholar

22. **McKeating JA, Stagno S, Stirk PR, Griffiths PD.** 1985. Detection of cytomegalovirus in urine samples by enzyme-linked immunosorbent assay. J Med Virol 16:367-373. 10.1002/jmv.1890160410. CrossRef Medline Google Scholar

23. **Li L, Coelingh KL, Britt WJ.** 1995. Human cytomegalovirus neutralizing antibody-resistant phenotype is associated with reduced expression of glycoprotein H. J Virol 69:6047-6053. Abstract/FREE Full Text

24. **Manley K, Anderson J, Yang F, Szustakowski J, Oakeley EJ, Compton T, Feire AL.** 2011. Human cytomegalovirus escapes a naturally occurring neutralizing antibody by incorporating it into assembling virions. Cell Host Microbe 10:197-209. 10.1016/j.chom.2011.07.010. CrossRef Medline Google Scholar

25. **Saccoccio FM, Gallagher MK, Adler SP, McVoy MA.** 2011. Neutralizing activity of saliva against cytomegalovirus. Clin Vaccine Immunol 18:1536-1542. 10.1128/CVI.05128-11. Abstract/FREE Full Text

26. **Pass RF, Zhang C, Evans A, Simpson T, Andrews W, Huang ML, Corey L, Hill J, Davis E, Flanigan C, Cloud G.** 2009. Vaccine prevention of maternal cytomegalovirus infection. N Engl J Med 360:1191-1199. 10.1056/NEJMoa0804749. CrossRef Medline Google Scholar

27. **McKeating JA, Grundy JE, Varghese Z, Griffiths PD.** 1986. Detection of cytomegalovirus by ELISA in urine samples is inhibited by beta 2 microglobulin. J Med Virol 18:341-348. 10.1002/jmv.1890180407. CrossRef Medline Google Scholar

28. **Grundy JE, McKeating JA, Ward PJ, Sanderson AR, Griffiths PD.** 1987. Beta 2 microglobulin enhances the infectivity of cytomegalovirus and when bound to the virus enables class I HLA molecules to be used as a virus receptor. J Gen Virol 68 (Pt 3):793-803. Medline Google Scholar

29. **Moody CA, Laimins LA.** 2010. Human papillomavirus oncoproteins: pathways to transformation. Nat Rev Cancer 10:550-560. 10.1038/nrc2886. CrossRef Medline Google Scholar

30. **Mesman AW, de Vries RD, McQuaid S, Duprex WP, de Swart RL, Geijtenbeek TB.** 2012. A prominent role for DC-SIGN^{+} dendritic cells in initiation and dissemination of measles virus infection in non-human primates. PLoS One 7:e49573. 10.1371/journal.pone.0049573. CrossRef Medline Google Scholar

31. **Zydek M, Petitt M, Fang-Hoover J, Adler B, Kauvar LM, Pereira L, Tabata T.** 2014. HCMV infection of human trophoblast progenitor cells of the placenta is neutralized by a human monoclonal antibody to glycoprotein B and not by

antibodies to the pentamer complex. Viruses 6:1346-1364. 10.3390/v6031346. CrossRef Medline Google Scholar

32. **Piboonniyom SO, Duensing S, Swilling NW, Hasskarl J, Hinds PW, Munger K.** 2003. Abrogation of the retinoblastoma tumor suppressor checkpoint during keratinocyte immortalization is not sufficient for induction of centrosome-mediated genomic instability. Cancer Res 63:476-483. Abstract/FREE Full Text

33. **Cui X, Adler SP, Davison AJ, Smith L, Habib ELSE, McVoy MA.** 2012. Bacterial artificial chromosome clones of viruses comprising the Towne cytomegalovirus vaccine. J Biomed Biotechnol 2012:428498. 10.1155/2012/428498. CrossRef Medline Google Scholar

34. **Cui X, Meza BP, Adler SP, McVoy MA.** 2008. Cytomegalovirus vaccines fail to induce epithelial entry neutralizing antibodies comparable to natural infection. Vaccine 26:5760-5766. 10.1016/j.vaccine.2008.07.092. CrossRef Medline Google Scholar

BMC Infectious Diseases

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Incidence of cytomegalovirus infection among the general population and pregnant women in the United States

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Published: 2 July 2007

BMC Infectious Diseases 2007, **7**:71 doi:10.1186/1471-2334-7-71

Received: 17 January 2007
Accepted: 2 July 2007

This article is available from: http://www.biomedcentral.com/1471-2334/7/71

Abstract

Background: Cytomegalovirus (CMV) is a common opportunistic infection among HIV-infected individuals, a major source of serious complications among organ-transplant recipients, and a leading cause of hearing loss, vision loss, and mental retardation among congenitally infected children. Women infected for the first time during pregnancy are especially likely to transmit CMV to their fetuses. More children suffer serious disabilities caused by congenital CMV than by several better-known childhood maladies such as Down syndrome or fetal alcohol syndrome

Methods: Using CMV seroprevalence data from the nationally representative Third National Health and Nutrition Examination Survey, we estimated CMV incidence among the general United States population and among pregnant women. We employed catalytic models that used age-specific CMV seroprevalences as cumulative markers of past infections in order to derive estimates of three basic parameters: the force of infection, the basic reproductive rate, and the average age of infection. Our main focus was the force of infection, an instantaneous per capita rate of acquisition of infection that approximates the incidence of infection in the seronegative population.

Results: Among the United States population ages 12–49 the force of infection was 1.6 infections per 100 susceptible persons per year (95% confidence interval: 1.2, 2.4). The associated basic reproductive rate of 1.7 indicates that, on average, an infected person transmits CMV to nearly two susceptible people. The average age of CMV infection was 28.6 years. Force of infection was significantly higher among non-Hispanic Blacks (5.7) and Mexican Americans (5.1) than among non-Hispanic Whites (1.4). Force of infection was significantly higher in the low household income group (3.5) than in the middle (2.1) and upper (1.5) household income groups. Based on these CMV incidence estimates, approximately 27,000 new CMV infections occur among seronegative pregnant women in the United States each year.

Conclusion: These thousands of CMV infections in pregnant women, along with the sharp racial/ethnic disparities in CMV incidence, are compelling reasons for accelerating research on vaccines and other interventions for preventing congenital CMV disease. Nevertheless, the relatively low force of infection provides encouraging evidence that modestly effective vaccines and rates of vaccination could significantly reduce CMV transmission.

Background

Cytomegalovirus (CMV) is a common opportunistic infection among human immunodeficiency virus (HIV)-infected individuals, a major source of serious viral complications among organ-transplant recipients, and a leading cause of hearing loss, vision loss, and mental retardation among congenitally infected children. In fact, more children suffer serious disabilities caused by congenital CMV than by several better-known childhood maladies such as Down syndrome or fetal alcohol syndrome [1].

Like other herpesviruses, primary CMV infection is followed by the establishment of lifelong latent infection from which periodic reactivation is common [2,3]. Symptoms are usually absent during primary infection and reactivation, but CMV can be shed in various bodily secretions, particularly urine and saliva [4]. CMV is transmitted person-to-person via close non-sexual contact, sexual activity, breastfeeding, blood transfusions, and organ transplantation [4]. For pregnant women, important sources of infection include sexual activity and contact with the urine or saliva of young children, especially their own children [5-7].

Congenital CMV infection is most likely to occur following a primary infection in the mother during pregnancy [8]. However, maternal CMV reactivation or reinfection with a different CMV strain can also lead to fetal infection [8]. Approximately 10 percent of congenitally infected infants are symptomatic at birth, and of the 90 percent who are asymptomatic, 10–15 percent will develop symptoms over months or even years [9].

Incidence of primary CMV infections has been estimated only in small or specialized populations, such as pregnant women or day care providers. The most comprehensive study of CMV incidence was carried out by Griffiths and colleagues in the United Kingdom [10], in which they estimated that more than three seronegative women per 100 seroconvert each year. However, their study was limited to pregnant women and was hospital-based rather than population-based. Robust, nationally representative estimates of CMV incidence are essential for 1) assessing the burden of primary CMV infection in the United States population, especially among pregnant women; 2) examining whether there are racial/ethnic disparities in primary maternal infection rates, which might be responsible for racial/ethnic disparities in congenital infection rates; and 3) evaluating how effective a vaccine or other intervention must be in order to reduce the incidence of congenital CMV disease. To obtain estimates of CMV incidence in the United States, we employed mathematical models that used age-specific CMV seroprevalences from the Third National Health and Nutrition Examination Survey (NHANES III).

Methods

Study population and design

NHANES III was conducted from 1988 to 1994 and provides nationally representative estimates of the health and nutritional status of the civilian, noninstitutionalized population of the United States. In order to produce population-representative estimates, NHANES III used a multistage, stratified, clustered sample design and generated sample weights proportional to the probability of participant selection. All our analyses used the NHANES III sample weights and sample design variables to correct the CMV seroprevalence point estimates for population representativeness and the interval estimates for the multistage complex sample design. The study protocol was approved by the authors' institutional review board. More details about NHANES III can be found in the official documentation [11]. Serologic testing for CMV immunoglobulin G (IgG) was conducted as described previously [12].

The main focus of our models of CMV incidence was the age range 12–49 years. Over 90 percent of participants in this age range had sera available for CMV testing (N = 11,859) so that seroprevalence estimates were representative of the United States population. More importantly, this age range included women of childbearing age and so has key relevance for congenital CMV disease. Although surplus sera was only available for approximately 70 percent of 6–11 year-olds (N = 2,679), we also ran models in this age group to assess whether incidence rates differed by race/ethnicity. Nationally-representative CMV seroprevalence estimates were not available for children less than six years old.

Description of models

Here we give an overview of the models of CMV incidence. A more detailed description is provided in the Appendix. We employed catalytic models [13,14] that used age-specific CMV seroprevalences as cumulative markers of past infections in order to derive estimates of three basic parameters: the force of infection, the basic reproductive rate, and the average age of infection. The force of infection is the instantaneous per capita rate of acquisition of infection [13] and will be expressed in this article as the number of primary CMV infections per 100 seronegative persons per year. The basic reproductive rate is a function of the force of infection and is the average number of secondary infections produced when one infected individual is introduced into a host population where everyone is susceptible. The average age of infection is also a function of the force of infection and is the age at which an individual in a given population typically

acquires a specific infection. We considered parameter differences to be statistically significant when corresponding confidence intervals did not overlap.

Force of infection can be estimated as time-dependent, age-dependent, or both. Since our data were taken from a single, cross-sectional survey, we could not model time dependence. To evaluate age-dependence, we visually inspected the slope of the age-specific seroprevalence graph (see Appendix). We observed no extreme departures from linearity for the overall population, with the slope appearing fairly constant as a function of age. However, because we saw age-dependent changes in slope within some subpopulations (e.g., Figure 1), we used piece-wise log-linear models that allowed the slope to vary between the age groups 6–11, 12–19, and 20–49 years. With the exception of this modification for the subgroup analysis, our final models were the time- and age-independent ones proposed by Griffiths et al. [10] for modeling CMV incidence, where the force of infection is estimated as the slope of the log-linear regression line having the seronegative prevalence as the response variable and age as the explanatory variable. For all models age was treated as a continuous variable.

The models made the following assumptions: CMV infection does not affect the mortality rate; seroprevalence in newborns equals zero; the death rate is type I, meaning everyone survives until a specific age, after which the survival probability is zero; and every person in the population is equally susceptible (*i.e.*, homogeneous mixing).

Variables

We estimated the model parameters for the entire United States population and for specific population groups stratified by sex, race/ethnicity, and/or household income. Race/ethnicity was a self-reported variable that consisted of non-Hispanic Whites, non-Hispanic Blacks, Mexican

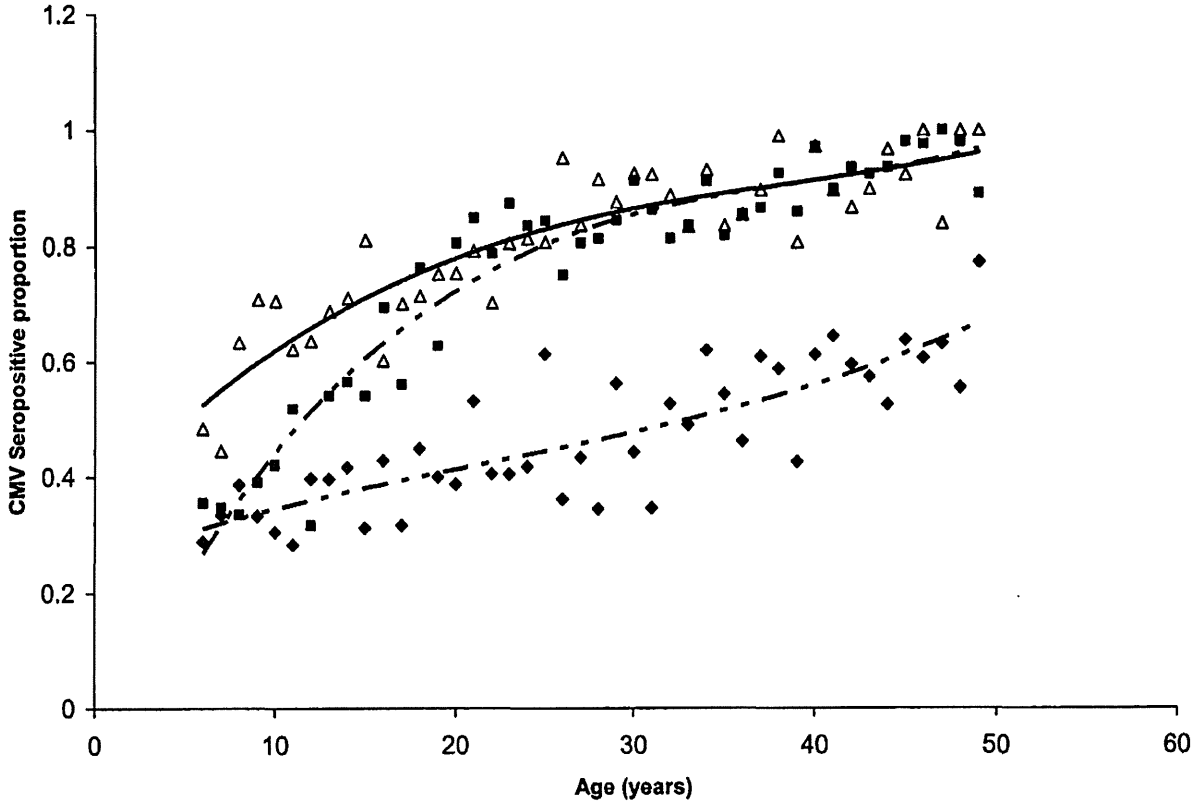

Figure 1
Age-distribution of cytomegalovirus (CMV)-seropositive proportion among U.S. women. Age-distribution of cytomegalovirus (CMV)-seropositive proportion among women in the National Health and Nutrition Examination Survey (NHANES) III, stratified by race/ethnicity. Observed seroprevalences: Δ-Mexican American, ◆-Non-Hispanic (NH) Whites, ■- NH Blacks. Adjusted third degree polynomials: ------- Mexican Americans, -------- NH Whites, --------- NH Blacks.

Americans and Others. As recommended in NHANES III documentation [15], Others was excluded from the analyses because the sample size was too small and encompassed a diverse mix of race/ethnicity. Household income was divided into low, medium, and high, as defined previously [11,12].

Estimating risk of CMV infection during pregnancy

We estimated risk of CMV infection for seronegative women during pregnancy as $risk = 100 \times [1 - e^{(-rate \times time)}]$, where rate was the force of infection per 100 women per year and time was the duration of pregnancy [16]. We multiplied this risk by the proportion of women who are CMV seronegative to obtain risk of CMV infection during pregnancy for the entire population (i.e., seronegative and seropositive) of women. We then multiplied the risk of infection in the entire population of women by the average number of live-birth pregnancies per year for the years 1988–1994 [17]. This product represented the estimated annual number of women with a primary CMV infection during pregnancy.

Results

The overall force of CMV infection in 12–49 year-olds in the United States was 1.6 per 100 persons per year (Table 1). The associated basic reproductive rate of 1.7 indicates that, on average, an infected person transmits CMV to nearly two susceptible people. The average age of CMV infection was 28.6 years. Among 12–49 year-olds, CMV force of infection was significantly higher among non-Hispanic Blacks (5.7) and Mexican Americans (5.1) than among non-Hispanic Whites (1.4) (Table 1). These differences were reflected in the average age (in years) of infection, which was 16.3 for non-Hispanic Blacks, 17.5 for Mexican Americans, and 29.3 for non-Hispanic Whites. Force of infection was significantly higher in the low household income group (3.5) than in the middle (2.1) and upper (1.5) household income groups.

We observed considerable variation in force of infection when we stratified by age and sex (Figures 1 and 2). Among adolescent girls (ages 12–19 years), non-Hispanic blacks had a substantially higher force of infection (9.9) than the other groups. In contrast, among pre-adolescent girls (ages 6–11 years), Mexican Americans had the highest force of infection (11.0). Among adolescent boys (ages 12–19 years), force of infection was highest in non-Hispanic blacks (6.4) and Mexican Americans (8.7).

Among seronegative women ages 20–49 years, risk of primary CMV infection during a full-term pregnancy was estimated to be 1.38 percent among non-Hispanic Whites, 3.40 percent among non-Hispanic Blacks, and 3.85 percent among Mexican Americans (Table 2). However, among 12–19 year-old seronegative women, risk was much higher for non-Hispanic blacks (7.33 percent) than for Mexican Americans (2.21 percent) and for non-Hispanic whites (0.15 percent). The estimated annual number of women ages 12–49 experiencing primary CMV infection during pregnancy was approximately 27,000. Most of these infections occur in non-Hispanic Whites because they are the largest racial/ethnic group in the U.S. However, non-Hispanic Blacks and Mexican Americans, especially those under age 30, are disproportionately likely to have pregnancies in which they experience primary CMV infections.

Discussion

Robust estimates of the frequency of new CMV infections are essential for understanding and preventing viral transmission. This study provides the first estimates of CMV incidence that are based on population-representative data. We found that among CMV-seronegative individuals aged 12–49 in the United States, nearly one in 60 seroconverts each year.

This relatively low force of infection indicates that CMV is less easily transmitted than some other infections, such as measles or rubella. For these infections, high vaccine efficacy and coverage are required in order to interrupt transmission [18]. In contrast, a CMV vaccine would not need to have such high efficacy and coverage to substantially prevent CMV transmission. Griffiths et al. [10], who estimated forces of CMV infection of 3.1–3.5/100 persons/year in the United Kingdom, showed that modest rates of vaccination (~60 percent) would be able to eradicate CMV infection from the human population. Our estimates, which are similar but even lower overall (force of infection = 1.6/100 persons/year), provide further evidence that modestly effective vaccines and rates of vaccination could significantly reduce CMV transmission.

Our models identified large racial/ethnic disparities in the frequencies of new CMV infections. The force of infection for CMV was considerably higher in non-Hispanic Blacks and Mexican Americans than in non-Hispanic Whites. The nearly three-fold differences in risk of primary CMV infection among seronegative women could be responsible for much of the racial/ethnic disparities in rates of infants born with congenital CMV [19]. Racial/ethnic differences were especially pronounced among adolescent girls (ages 12–19 years), among whom primary infection was 50 times more likely in seronegative non-Hispanic blacks and 15 times more likely in seronegative Mexican Americans than in non-Hispanic whites. These higher forces of infection (i.e., incidence in seronegative individuals) suggest that CMV is circulating more frequently in these racial/ethnic groups. Thus, seropositive, pregnant non-Hispanic blacks and Mexican Americans may be at a higher risk of suffering re-infection with a different strain

Table 1: CMV force of infection, basic reproductive rates, and average age of infection among persons 12–49 years old in the United States.

	Force of Infection (95% CI)*		Basic reproductive rate (95% CI)		Average age of infection in years (95% CI)	
Entire U.S. population	1.6	1.3–1.9	1.7	1.5–1.8	28.6	27.3–29.4
Sex						
Female	1.8	1.3–2.2	1.7	1.5–1.9	28.0	26.2–29.9
Male	1.5	1.1–1.8	1.6	1.4–1.8	29.1	27.7–30.6
Race/Ethnicity						
Non-Hispanic Black	5.7	5.1–6.2	4.1	3.7–4.4	16.3	15.1–17.5
Mexican American	5.1	4.3–5.6	3.7	3.2–4.2	17.5	15.8–19.4
Non-Hispanic White	1.4	1.1–1.8	1.6	1.4–1.7	29.3	27.9–30.6
Income per family size						
Low	3.5	2.8–4.5	2.7	2.1–3.2	21.9	19.0–24.9
Middle	2.1	1.6–2.6	1.9	1.7–2.2	26.7	24.9–28.6
High	1.5	1.1–1.9	1.6	1.4–1.8	28.9	27.5–30.4

*Number of infections per 100 susceptible persons per year. We considered parameter differences to be statistically significant when corresponding confidence intervals did not overlap. CI, confidence interval.

of CMV, which also places their infants at risk of symptomatic congenital CMV [8]. These disparities indicate that interventions, such as vaccines or education campaigns, may need to be tailored to meet the needs of different racial/ethnic groups and different age groups.

In addition to race/ethnicity, low household income was a risk factor for CMV infection. People with low household income may be more likely to have a larger family and experience crowding, thus facilitating CMV transmission via close contact. However, because force of infection

Table 2: Risk and frequency of CMV primary infection during pregnancy in the United States.

Ages (years)	% Seronegative	Risk among seronegative women/100 pregnancies*	Risk for all women/100 pregnancies	No. live-birth pregnancies (100's)†	No. women with primary infection during live-birth pregnancies
Non-Hispanic White					
12–19	61.0	0.15	0.09	2320	209
20–29	56.7	1.38	0.78	12140	9469
30–39	49.4	1.38	0.68	9120	6201
40–49	38.9	1.38	0.54	510	275
Subtotal				24090	16154
Non-Hispanic Black					
12–19	42.6	7.33	3.12	1330	4150
20–29	17.8	3.40	0.61	3060	1867
30–39	13.4	3.40	0.46	1350	621
40–49	5.3	3.40	0.18	80	14
Subtotal				5820	6652
Mexican American					
12–19	30.1	2.21	0.67	1220	817
20–29	17.5	3.85	0.67	3990	2673
30–39	10.5	3.85	0.40	1700	680
40–49	6.8	3.85	0.26	100	26
Subtotal				7010	4196
Total				36940	27002

Risk is computed as 100 [1-exp(-rate × time)], where rate is force of infection per 100 women per year (0.2/100 and 1.8/100 for NH-White, 9.9/100 and 4.5/100 for NH-Black, and 2.9/100 and 5.1/100 for Mexican American) and time is duration of pregnancy in years, i.e., 40/52 = 0.77 years.
†From National Vital Statistics Report, Vol. 49, No. 4, June 6, 2001 [17].

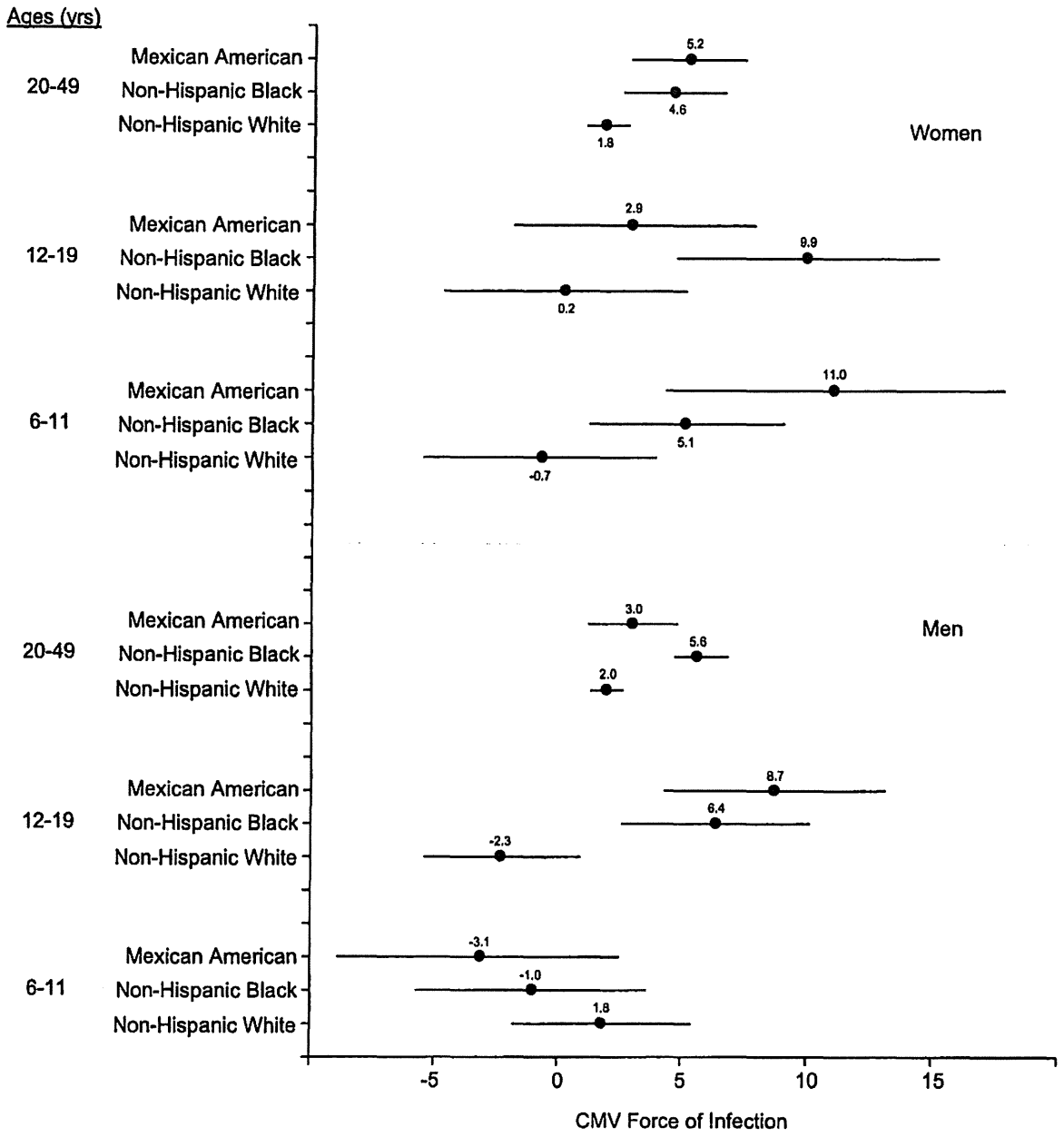

Figure 2

Cytomegalovirus (CMV) force of infection. Cytomegalovirus (CMV) force of infection stratified by sex, age group (6–11, 12–19, and 20–49 years), and race/ethnicity. Circles represent point estimates and lines represent 95% confidence intervals. Negative values for force of infection can occur because the models treat the CMV seroprevalences as if they come from a single cohort followed over time, when in fact they are age-specific seroprevalences of a population at a single point in time. Thus, in the younger ages where the sample sizes are smaller, it is possible for an older age group to have a somewhat lower seroprevalence than a younger age group, which can lead to a negative value for force of infection. We considered force of infection differences to be statistically significant when corresponding confidence intervals did not overlap.

was more strongly associated with race/ethnicity than with household income, high-risk racial/ethnic groups may have a higher prevalence of additional factors related to CMV transmission, such as increased exposure to CMV while caring for young children. A more detailed analysis of risk factors for CMV infection in NHANES III can be found in Staras et al. [12].

Among women ages 20–49 years, force of infection appeared to be independent of age, suggesting that risk of infection during pregnancy is fairly constant during these ages, and that interventions to prevent congenital CMV must target all women of childbearing age. CMV had a higher force of infection than infections transmitted primarily via sex or injection drug use, such as herpes simplex virus type 2 (HSV-2) or hepatitis B virus (HBV). This suggests either that CMV is more easily transmissible through such behaviors [20] or, more likely, that CMV is transmitted via other, additional routes. Given that CMV has been shown to be transmitted via urine or saliva during close, non-sexual contact, it is likely that this sort of transmission plays a major role in the dynamics of CMV infection [7].

We estimated that each year in the United States more than 27,000 pregnant women experience primary CMV infection and are thus at high risk of giving birth to a child with congenital CMV infection. This estimate does not include any fetal losses that may have been caused by primary CMV infection, nor does it include the many pregnancies affected by CMV reactivation or reinfection among seropositive women. The burden of primary CMV infections during pregnancy falls disproportionately on disadvantaged women--those of low income and racial/ethnic minorities. Furthermore, teenaged minority women are at especially high risk of primary CMV infections during pregnancy, due to their high prevalence of susceptibility, high force of infection, and high pregnancy rates.

The risk of primary CMV infection during pregnancy among seronegative women is similar to previous estimates [4]. For seronegative women, CMV infection represents one of the highest risks for fetal damage that they experience during pregnancy [21]. Because CMV transmission is potentially preventable [1], CMV antibody screening prior to or near the beginning of pregnancy should be evaluated as a means of identifying women at high risk for having congenitally infected infants. Studies should pursue whether knowledge of high risk status is a useful motivational tool for modifying behaviors, such as hand hygiene, for reducing risk of infection [22]. Such screening may also lead to the administration of CMV hyperimmuneglobulins or antiviral drugs for prevention or therapy of fetal infection and disease [23,24].

In this study the modeling assumptions appeared to have been reasonably satisfied. On a population level, CMV infection does not contribute significantly to mortality among infected individuals. Nearly all members (≈99 percent) of the population are susceptible at birth, and infection is believed to induce life-long immunity. The type I death-rate cut-off was chosen as 70 years to approximate the U.S. life expectancy during the years that NHANES III was conducted, but modifying the cut-off had little effect on the model results. The assumption of homogeneous mixing is unlikely to be completely true, but because CMV infection is common and has multiple transmission modes, susceptible individuals are likely to have similar risks of exposure to CMV.

An important limitation of our models was that the data were from a single, cross-sectional study so that time trends were not able to be addressed. Thus, high CMV seroprevalence in cohorts of older people might not reflect current incidence and could cause the models to overestimate the force of infection [12]. We sought to minimize this potential bias by focusing most of our analyses on a limited age range (12–49 years). It is also important to note that our younger, age-specific force of infection estimates (i.e., for ages 6–11 and 12–19 years) were imprecise, with wide confidence intervals. Furthermore, the models implicitly assumed that seroprevalence was monotonically increasing with age, as if this cross-sectional study were a cohort study in which seroprevalence was measured at various ages of follow-up. However, this assumption was violated for some of the younger subpopulations. As a result, we occasionally obtained negative estimates for the force of infection (Figure 2), although these estimates were not statistically different from zero.

The calculations of risk of primary infection during pregnancy required several assumptions, one of which was that the force of infection was the same for pregnant and non-pregnant women. Women who are pregnant may have fewer sex partners (and thus lower risk of exposure to CMV) during pregnancy; on the other hand, pregnant women may be more likely than non-pregnant women to be exposed to young children (a group that frequently sheds CMV). Pregnant women may also have a higher risk of acquiring infections because of pregnancy-induced immune depression [25].

Based on our models, we would estimate that more than one million United States women have experienced primary CMV infections during pregnancy since CMV was first isolated 50 years ago [26,27]. A substantial proportion of these infections would have led to congenital infections, leaving thousands of children with lifelong disabilities. Children from disadvantaged racial/ethnic groups are likely to have been disproportionately

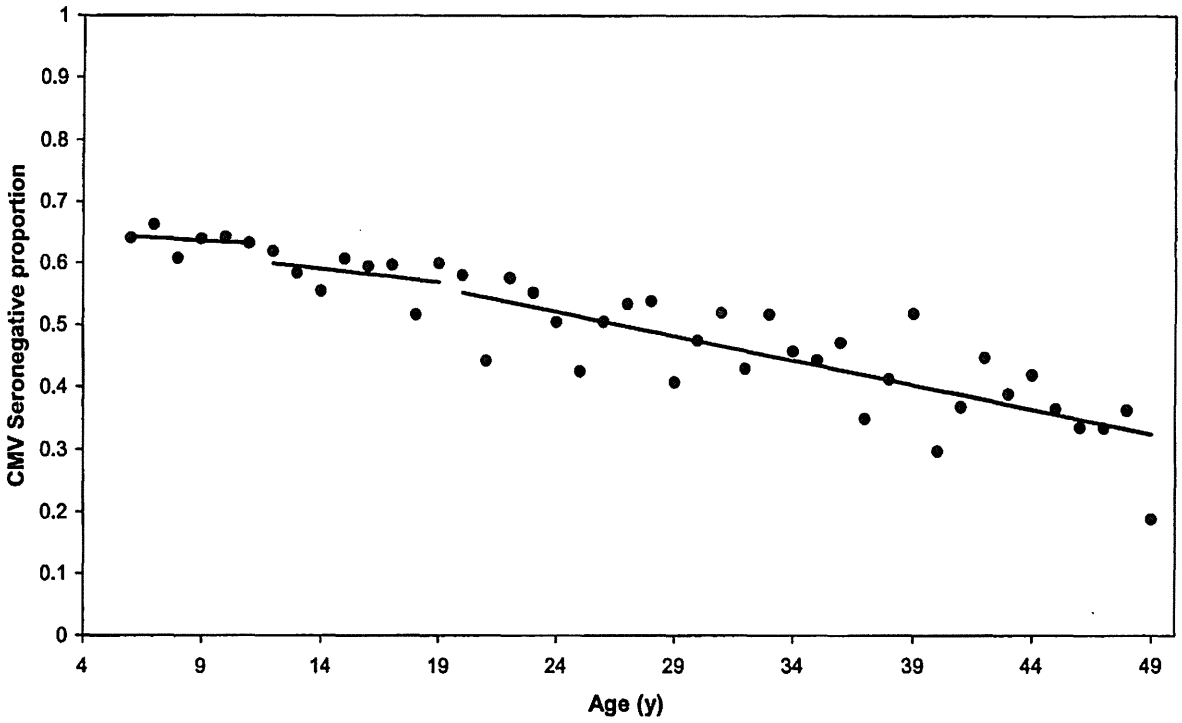

Figure 3
Example of piece-wise log-linear model among non-Hispanic black women.

impacted. These many affected children are a compelling argument for accelerating research on vaccines and other interventions for the prevention of congenital CMV [28].

Conclusion

Each year, thousands of CMV infections occur in pregnant women in the United States, putting numerous unborn babies at risk for serious disabilities. Incidence of CMV infection in pregnant women is not distributed evenly, but exhibits sharp racial/ethnic disparities, especially affecting non-Hispanic blacks and Mexican Americans. Because of the magnitude of the problem and its associated health disparities, there is an urgent need to accelerate research on vaccines and other interventions for preventing congenital CMV disease. Nevertheless, the low incidence of CMV infection relative to other vaccine-preventable infections provides encouraging evidence that

Table 3: Comparison of force of infection for different viruses for selected* age ranges.

Virus	Force of infection (per 100 persons per year)	Ages modeled	Study sample	Citation
Measles	20	11–17	Lit. review – misc. sources	[18]
Mumps	12	11–17	Lit. review – misc. sources	[18]
Rubella	10	11–17	Lit. review – misc. sources	[18]
Varicella	6	≥ 10	Convenience sample	[30]
CMV†	3.1 and 3.5	16–40	Hospital-based	[10]
CMV	1.8	12–49	Population-based	Current study
HSV-2	0.84	≥ 12	Population-based	[31]
Hepatitis B	0.15	6–39	Population-based	[32]

*Ages were selected to be roughly comparable with the ages we modeled; in general, young children were not selected for comparison because they often had much higher forces of infection. †Patients were recruited from 2 different hospitals.

modestly effective vaccines and rates of vaccination could significantly reduce CMV transmission.

Competing interests

The author(s) declare that they have no competing interests.

Authors' contributions

FABC designed and carried out the mathematical modeling and statistical analyses and drafted the manuscript. SASS participated in the design and implementation of the CMV testing of the NHANES III specimens. SCD coordinated and supervised the CMV testing of the NHANES III specimens. MJC conceived of the study, participated in its design and coordination, and helped to draft the manuscript. All authors read and approved the final manuscript and revised it critically for important intellectual content.

Appendix

To estimate CMV incidence by using the force of infection, we used the catalytic model approach described in Farrington [14] and Anderson [13]. We began by assuming that the force of infection was age-dependent, so that

$$S^{-}(a) = e^{-\int_0^a \lambda(x)dx},$$

where a is age and $S^{-}(a)$ is the age distribution for the seronegatives. To assess the shape of the integral above we proceeded as Farrington, by visual inspections. $\lambda(x)$was evaluated as an exponential decay function and as a polynomial of third or lesser degree. Despite permitting $\lambda(x)$ to be a complicated function, force of infection was approximately constant as a function of age (i.e., force of infection was age-independent). Therefore, we used the log-linear approach where the force of infection is the slope of the regression model (i.e., $\lambda(x)$ equals the constant λ) given by $ln(S^{-}(a)) = -(\beta_0 + \lambda a)$. This model, used by Griffiths [10] to estimate CMV force of infection, also seemed to fit the NHANES III data in most cases, where β_0 plays the role of the natural logarithm of the age-adjusted seronegative proportion. We made one modification to this model when we estimated force of infection within subgroups: λ was treated as constant within pieces of the age range, namely, 6–11 years, 12–19 years, and 20–49 years (Figure 3).

With the age-independent assumption, the average age of infection, A, and the basic reproductive rate, R_0, were estimated by:

$$A = \frac{1}{\lambda}\left(\frac{1-(1+\lambda L)\exp(-\lambda L)}{1-\exp(-\lambda L)}\right), \quad R_0 = \frac{\lambda L}{1-\exp(-\lambda L)},$$

where $L = 70$ is the threshold age for the type I death rate.

When estimating force of infection for different subgroup categories, one category was chosen to be the referent category and the others were represented by indicator variables and were included in the models with interaction for age. For example, in the case of race/ethnicity, which had 3 categories and White as the referent category, the model was:

$$\ln(S^{-}(a)) = -(\beta_0 + \lambda_0 a + \beta_1 \delta[White - Black] + \beta_2 \delta[Mexican] + \lambda_1 \delta[White - Black]a + \lambda_2 \delta[Mexican]a),$$

where $\delta[X] = 1$ if X and 0 otherwise.

The final models were estimated using the STATA 8.0 (College Station, TX) svypoisson command (log-linear model), which is appropriate for complex survey estimation. The sample weight, cluster, and strata variables suggested by the NHANES III analytical guidelines were used to adjust the estimates for the sample design. The variance was estimated by the linearization method [29]. The R_0 and A and their confidence intervals were estimated using the nlcom command for non-linear transformations of the regression parameters.

Acknowledgements

We thank Kay Radford, Ashley Schoenfisch and Minal Amin for serologic testing and Geraldine McQuillan for technical assistance. This study was supported in part by funding from the National Vaccine Program Office. Fernando Colugnati received support from the Brazilian Centro Nacional de Pesquisa while he was a guest researcher at the CDC. This research was supported in part by an appointment to the Research Participation Program at the Centers for Disease Control and Prevention administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the U.S. Department of Energy and CDC (stipend support for Stephanie A.S. Staras).

References

1. Cannon MJ, Davis KF: **Washing our hands of the congenital cytomegalovirus disease epidemic.** *BMC Public Health* 2005, **5**:70.
2. Alford CA, Stagno S, Pass RF, Britt WJ: **Congenital and perinatal cytomegalovirus infections.** *Rev Infect Dis* 1990, **12 Suppl 7**:S745-S753.
3. Brown HL, Abernathy MP: **Cytomegalovirus infection.** *Semin Perinatol* 1998, **22**:260-266.
4. Stagno S: **Cytomegalovirus.** In *Infectious diseases of the fetus and newborn infant* Edited by: Remington JS and Klein JO. Philadelphia, W.B. Saunders Company; 2001:389-424.
5. Pass RF, Hutto C, Ricks R, Cloud GA: **Increased rate of cytomegalovirus infection among parents of children attending day-care centers.** *N Engl J Med* 1986, **314**:1414-1418.
6. Adler SP: **Cytomegalovirus and child day care: risk factors for maternal infection.** *Pediatr Infect Dis J* 1991, **10**:590-594.
7. Fowler KB, Pass RF: **Risk factors for congenital cytomegalovirus infection in the offspring of young women: exposure to**

young children and recent onset of sexual activity. *Pediatrics* 2006, 118:e286-e292.
8. Boppana SB, Rivera LB, Fowler KB, Mach M, Britt WJ: **Intrauterine transmission of cytomegalovirus to infants of women with preconceptional immunity.** *N Engl J Med* 2001, 344:1366-1371.
9. Stagno S, Whitley RJ: **Herpesvirus infection of pregnancy.** *N Engl J Med* 1985, 313:1270-1274.
10. Griffiths PD, McLean A, Emery VC: **Encouraging prospects for immunisation against primary cytomegalovirus infection.** *Vaccine* 2001, 19:1356-1362.
11. **Plan and operation of the Third National Health and Nutrition Examination Survey, 1988-94. Series 1: programs and collection procedures.** *Vital Health Stat 1* 1994, (32):1-407.
12. Staras SAS, Dollard SC, Radford KW, Flanders WD, Pass RF, Cannon MJ: **Seroprevalence of cytomegalovirus infection in the United States, 1988-1994.** *Clin Infect Dis* 2006, 43:1143-1151.
13. Anderson RM, May RM: *Infectious diseases of humans–dynamics and control* Oxford, United Kingdom, Oxford Scientific Publications; 1991.
14. Farrington CP: **Modelling forces of infection for measles, mumps and rubella.** *Stat Med* 1990, 9:953-967.
15. Centers for Disease Control and Prevention: **Analytic and Reporting Guidelines: The Third National Health and Nutrition Examination Survey, NHANES III (1988-1994).** Hyattsville, MD, National Center for Health Statistics; 1996.
16. Ades AE: **Methods for estimating the incidence of primary infection in pregnancy: a reappraisal of toxoplasmosis and cytomegalovirus data.** *Epidemiol Infect* 1992, 108:367-375.
17. Ventura SJ, Mosher WD, Curtin SC, Abma JC, Henshaw S: **Trends in pregnancy rates for the United States, 1976-97: an update.** *Natl Vital Stat Rep* 2001, 49(4):1-9.
18. Edmunds WJ, Gay NJ, Kretzschmar M, Pebody RG, Wachmann H: **The pre-vaccination epidemiology of measles, mumps and rubella in Europe: implications for modelling studies.** *Epidemiol Infect* 2000, 125:635-650.
19. Fowler KB, Stagno S, Pass RF: **Maternal age and congenital cytomegalovirus infection: screening of two diverse newborn populations, 1980-1990.** *J Infect Dis* 1993, 168:552-556.
20. Stover CT, Smith DK, Schmid DS, Pellett PE, Stewart JA, Klein RS, Mayer K, Vlahov D, Schuman P, Cannon MJ: **Prevalence of and risk factors for viral infections among human immunodeficiency virus (HIV)-infected and high-risk HIV-uninfected women.** *J Infect Dis* 2003, 187:1388-1396.
21. Cannon MJ, Pellett PE: **Risk of congenital cytomegalovirus infection.** *Clin Infect Dis* 2005, 40:1701-1702.
22. Adler SP, Finney JW, Manganello AM, Best AM: **Prevention of child-to-mother transmission of cytomegalovirus among pregnant women.** *J Pediatr* 2004, 145:485-491.
23. Nigro G, Adler SP, La Torre R, Best AM: **Passive immunization during pregnancy for congenital cytomegalovirus infection.** *N Engl J Med* 2005, 353:1350-1362.
24. Adler SP, Nigro G, Pereira L: **Recent advances in the prevention and treatment of congenital cytomegalovirus infections.** *Semin Perinatol* 2007, 31:10-18.
25. Yip L, McCluskey J, Sinclair R: **Immunological aspects of pregnancy.** *Clin Dermatol* 2006, 24:84-87.
26. SMITH MG: **Propagation in tissue cultures of a cytopathogenic virus from human salivary gland virus (SGV) disease.** *Proc Soc Exp Biol Med* 1956, 92:424-430.
27. ROWE WP, HARTLEY JW, WATERMAN S, TURNER HC, HUEBNER RJ: **Cytopathogenic agent resembling human salivary gland virus recovered from tissue cultures of human adenoids.** *Proc Soc Exp Biol Med* 1956, 92:418-424.
28. Ross DS, Dollard SC, Victor M, Sumartojo E, Cannon MJ: **The epidemiology and prevention of congenital cytomegalovirus infection and disease: activities of the Centers for Disease Control and Prevention Workgroup.** *J Womens Health (Larchmt)* 2006, 15:224-229.
29. *Analysis of Complex Surveys* Edited by: Skinner CJ, Holt D and Smith TMF. Chichester, John Wiley & Sons; 1989.
30. Gidding HF, MacIntyre CR, Burgess MA, Gilbert GL: **The seroepidemiology and transmission dynamics of varicella in Australia.** *Epidemiol Infect* 2003, 131:1085-1089.
31. Armstrong GL, Schillinger J, Markowitz L, Nahmias AJ, Johnson RE, McQuillan GM, St Louis ME: **Incidence of herpes simplex virus type 2 infection in the United States.** *Am J Epidemiol* 2001, 153:912-920.
32. Coleman PJ, McQuillan GM, Moyer LA, Lambert SB, Margolis HS: **Incidence of hepatitis B virus infection in the United States, 1976-1994: estimates from the National Health and Nutrition Examination Surveys.** *J Infect Dis* 1998, 178:954-959.

Pre-publication history

The pre-publication history for this paper can be accessed here:

http://www.biomedcentral.com/1471-2334/7/71/prepub

BMC Public Health

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Washing our hands of the congenital cytomegalovirus disease epidemic

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Published: 20 June 2005

BMC Public Health 2005, 5:70 doi:10.1186/1471-2458-5-70

Received: 09 December 2004

Accepted: 20 June 2005

This article is available from: http://www.biomedcentral.com/1471-2458/5/70

Abstract

Background: Each year in the United States, an estimated 40,000 children are born with congenital cytomegalovirus (CMV) infection, causing an estimated 400 deaths and leaving approximately 8000 children with permanent disabilities such as hearing or vision loss, or mental retardation. More children are affected by serious CMV-related disabilities than by several better-known childhood maladies, including Down syndrome, fetal alcohol syndrome, and spina bifida.

Discussion: Congenital CMV is a prime target for prevention not only because of its substantial disease burden but also because the biology and epidemiology of CMV suggest that there are ways to reduce viral transmission. Because exposure to the saliva or urine of young children is a major cause of CMV infection among pregnant women, it is likely that good personal hygiene, especially hand-washing, can reduce the risk of CMV acquisition. Experts agree that such measures are likely to be efficacious (*i.e.*, they will work if consistently followed) and the American College of Obstetricians and Gynecologists recommends that physicians counsel pregnant women about preventing CMV acquisition through careful attention to hygiene. However, because of concerns about effectiveness (*i.e.*, Will women consistently follow hygienic practices as the result of interventions?), the medical and public health communities appear reluctant to embrace primary CMV prevention via improved hygienic practices, and educational interventions are rare. Current data on the effectiveness of such measures in preventing CMV infection are promising, but limited. There is strong evidence, however, that educational interventions can prevent other infectious diseases with similar transmission modes, suggesting that effective interventions can also be found for CMV. Until a CMV vaccine becomes available, effective educational interventions are needed to inform women about congenital CMV prevention.

Summary: Perhaps no single cause of birth defects and developmental disabilities in the United States currently provides greater opportunity for improved outcomes in more children than congenital CMV. Given the present state of knowledge, women deserve to be informed about how they can reduce their risk of CMV infection during pregnancy, and trials are needed to identify effective educational interventions.

Background

The history of public health in twentieth century America is replete with successes in the prevention of birth defects and childhood disabilities. Vaccines have virtually eliminated polio, congenital rubella syndrome, and *Haemophilus influenzae* meningitis [1-3]. Educational efforts aimed at preventing fetal alcohol syndrome have reduced maternal alcohol consumption during pregnancy [4]. Prenatal vitamins and folic acid fortification of cereals have lowered rates of neural tube defects [5], while antiretroviral treatments have caused the occurrence of mother-to-child human immunodeficiency virus (HIV) transmission to plummet [6]. Notably absent from the list of successes, however, is the prevention of congenital cytomegalovirus (CMV) disease [7].

Perhaps no single cause of birth defects and developmental disabilities in the United States currently provides greater opportunity for improved outcomes in more children than congenital CMV. Each year in the United States, an estimated 40,000 children are born with congenital CMV infection, causing an estimated 400 deaths and leaving approximately 8000 children with permanent disabilities such as hearing or vision loss, or mental retardation [8]. The direct annual economic costs of caring for these children are estimated at $1-$2 billion [8,9]. More children are adversely affected by congenital CMV disease than by several better-known childhood diseases or syndromes (Figure 1). Congenital CMV is a prime target for prevention not only because of its substantial disease burden but also because the biology and epidemiology of CMV suggest that there are ways to reduce viral transmission. Unfortunately, by missing prevention opportunities, we in the medical and public health communities are washing our hands of the congenital CMV disease epidemic.

CMV and congenital CMV disease

As with other human herpesviruses, initial infection by CMV (also known as primary infection) is followed by the establishment of lifelong latent infection, from which periodic reactivation is common [10,11]. Symptoms are usually absent during primary infection and reactivation, but CMV is shed in various bodily secretions, particularly

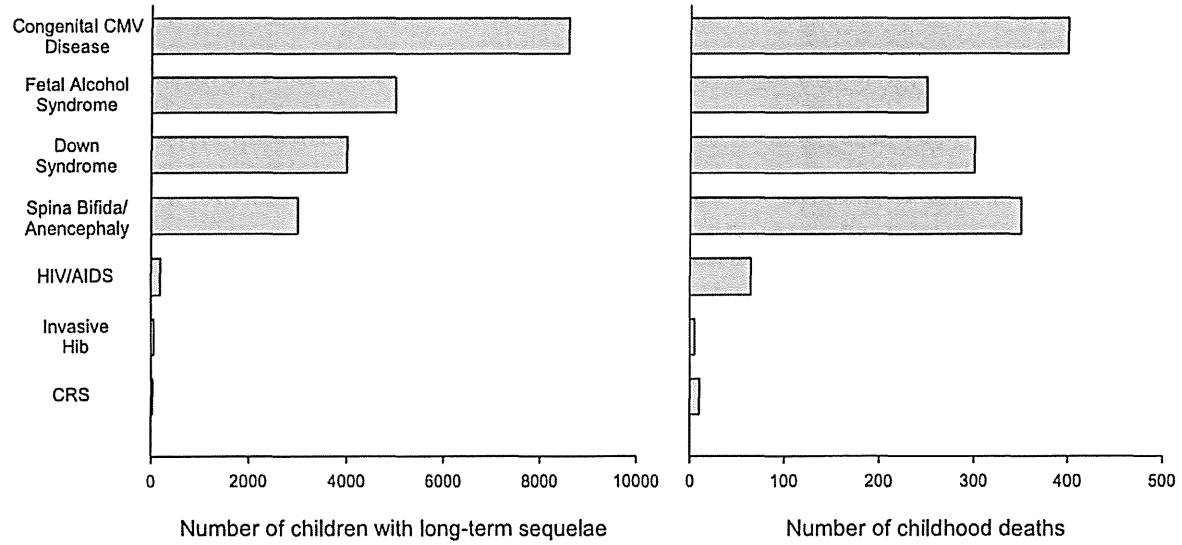

Figure 1

Estimates of the annual burden of prominent childhood diseases and syndromes in the US [3, 5, 6, 8, 51–57]. Assumes 4 million live births per year and 20 million children <5 years of age. Childhood deaths were defined as those occurring <1 year after birth except for Haemophilus influenzae type B (Hib) (<5 years) and HIV/AIDS (<13 years). Where applicable, numbers represent means of published estimates. All estimates should be considered useful for rough comparisons only since surveillance methodology and diagnostic accuracy varied over different studies. CRS, congenital rubella syndrome.

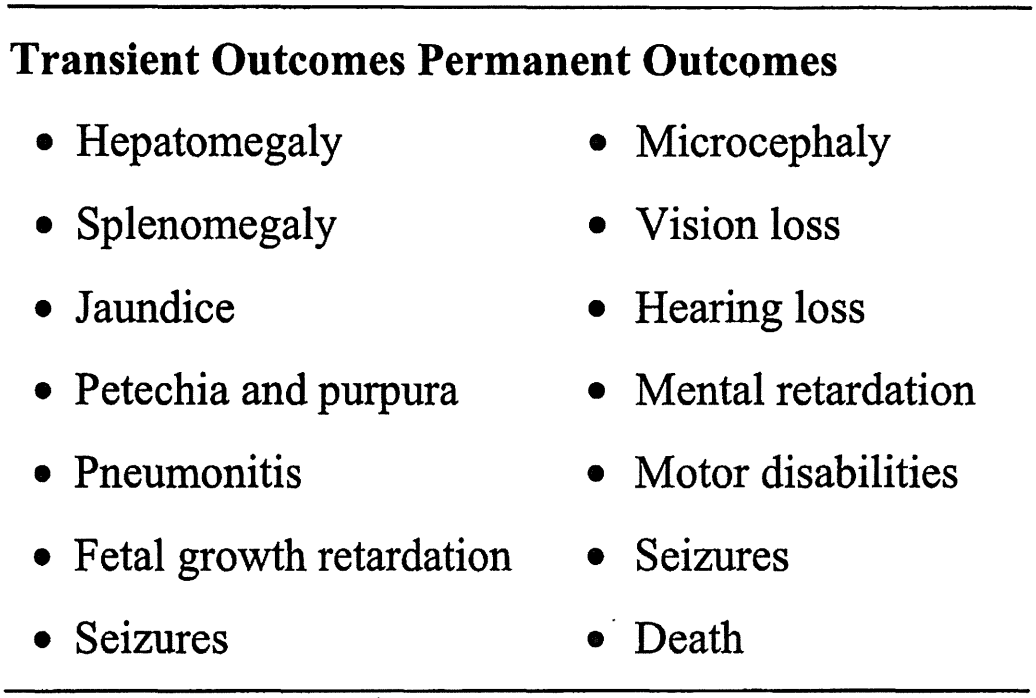

Figure 2
Transient and permanent outcomes among children with congenital CMV disease.

urine and saliva [12]. CMV excretion can be continuous or intermittent, generally lasting several weeks in adults but often continuing for months or years in young children [13-15]. CMV infection is widespread, with estimates of CMV seroprevalence in the United States ranging from 40% to 80% [16-18].

CMV is transmitted person-to-person via close non-sexual contact, sexual activity, breastfeeding, blood transfusions, and organ transplantation [12]. CMV has not been shown to be transmitted via respiratory secretions or aerosolized virus. For the pregnant woman, the most likely source of infection may be contact with the urine or saliva of young children, especially her own children [19,20].

Congenital CMV disease is most likely to occur following a primary infection in the mother. Primary infections occur in 1%-4% of seronegative, pregnant women and lead to fetal infection in 40%-50% of these pregnancies. Maternal CMV reactivation or reinfection with a different CMV strain leads to fetal infection in about 1% of seropositive, pregnant women. Approximately 10% of congenitally infected infants are symptomatic at birth, and of the 90% who are asymptomatic, 10%-15% will develop symptoms over months or even years (Figure 2) [21]. Permanent sequelae can result from CMV infection of the fetus during any trimester, but infection during early fetal development is likely to be especially damaging [22,23]. Since few newborns are screened for CMV, the true impact of congenital CMV infection is underappreciated.

Discussion

Reducing the burden of congenital CMV disease

Of all the ways to fight congenital CMV disease, the development of a vaccine is viewed as the most promising. Considerable progress has been made over the last 30 years, but insufficient interest by vaccine manufacturers (Stanley Plotkin, personal communication) and technical challenges make it is uncertain when a vaccine will become available [24]. Avenues for improving outcomes in congenitally infected children have also been explored, including anti-CMV therapies (e.g., ganciclovir) for seri-

- Thoroughly wash hands with soap and warm water after activities such as:
  - Diaper changes
  - Feeding or bathing child
  - Wiping child's runny nose or drool
  - Handling child's toys
- Do not share cups, plates, utensils, toothbrushes, or food
- Do not kiss on or near the mouth
- Do not share towels or washcloths
- Clean toys, countertops and other surfaces that come in contact with urine or saliva.

Figure 3
Hygienic practices to reduce risk of CMV infection for women who are pregnant or planning to become pregnant. When interacting with young children, women should assume the children are secreting CMV in their urine and saliva.

ously infected infants [25,26] and supportive care, such as hearing screening, language therapy, and special education [27,28]. In contrast, insufficient emphasis has been given to preventing CMV infection in pregnant women. While women may be infected via several routes, the remainder of this article focuses on preventing transmission via the important child-to-mother route, by encouraging hygienic practices such as frequent hand washing.

A number of experts have suggested that women be educated about hygienic practices for preventing CMV transmission from young children, and there is little dispute over what the prevention guidelines should entail (Figure 3) [7,11,29-32]. This consensus is reflected in current American College of Obstetricians and Gynecologists guidelines, which recommend that physicians counsel pregnant women about preventing CMV acquisition through careful attention to hygiene [33]. Nevertheless, hygienic practices do not appear to be widely discussed by healthcare providers and prospective mothers are often unaware of both CMV disease and the potential benefits of hygienic practices. The virtual absence of a prevention message has been due, in part, to the low profile of congenital CMV. Infection is usually asymptomatic in both mother and infant, and when symptoms do occur, they are non-specific, so most CMV infections go undiagnosed. The prevention message has also been hindered by a sense that infection is unavoidable. For example, a number of authors have urged prevention education for women on the one hand but on the other hand, they have noted that "CMV is neither preventable nor treatable..."[34],"...it is not certain that infections in pregnant women can be prevented by avoiding exposure" [35], "...it is doubtful whether parents will comply with these [behavioral measures in nonstudy settings..." [36], "...there is very little evidence for the efficacy of these strategies and even less for their practical implementation...", and "The only effective prevention strategy relies upon the development of a vaccine." [37] Given the relative invisibility of CMV disease and these mixed messages about prevention education, it is not surprising that healthcare providers do not discuss CMV with their patients and that women are unaware of the risks of CMV infection.

Preventing CMV infection through hygienic practices

Why the ambivalence toward hygienic practices? Studies have shown that transmission of CMV via the urine and saliva of children is a major cause of infection among

pregnant women [19,20]. In addition, more than 100 years of evidence conclusively demonstrates that hand washing reduces risk of infection for a wide range of pathogens [38]. Thus, nearly everyone would agree that, in theory, hand washing can prevent CMV infection because hands are an important vehicle for transmission. The concern, then, is not the *efficacy* of hygienic practices (i.e., Will they work if consistently followed?) but, instead, the *effectiveness* of interventions to promote them (i.e., Will women consistently follow hygienic practices as the result of interventions?).

It is important to recognize the implications of the consensus that hygienic practices are efficacious for preventing CMV transmission. Individual women have the right to know that, under ideal conditions, risk of child-to-mother CMV transmission can be reduced by proper hygienic practices. This is equivalent to the ethical obligation to inform individuals that, under ideal conditions, safer sexual practices will reduce the risk of acquiring HIV. This obligation is independent of whether any particular educational program or intervention is effective. All women of childbearing age, whether they are CMV seropositive or seronegative, carry some risk of new CMV infection during pregnancy and thus should be informed of hygienic practices that reduce that risk. As Revello and Gerna aptly remind us, "...withholding information on possible medical interventions is unethical (and legally risky)" [39]. The terrible burden of congenital CMV disease (Figure 1) should make the provision of such information a priority.

As there is consensus on the efficacy of hygienic practices in preventing CMV transmission, the next step is to evaluate the effectiveness of educational interventions in preventing CMV transmission. Current evidence of effectiveness is promising, but limited. In one study, after non-pregnant women were educated about CMV prevention, hygienic practices improved [40]. In a small study of Houston families, Demmler and colleagues found that behavioral changes prevented transmission of CMV (unpublished report described in Yow and Demmler [7]). Adler and colleagues studied the effectiveness of hygienic practices in a randomized, controlled trial of 39 seronegative, non-pregnant women with young children who were shedding CMV [31]. Although the study was underpowered to detect significant differences in infection rates between the intervention and control groups (and thus the intervention was deemed unsuccessful by some), seroconversion rates decreased as CMV education and support increased. Furthermore, in the same study, 14 pregnant women were educated regarding hygienic practices and then followed for comparison with the randomized groups; none of the women seroconverted – a significant difference compared with the randomized groups. A more recent study also reported that pregnant women who received an intervention involving hygienic practices were significantly less likely to acquire CMV infection than were non-pregnant women [41].

More conclusive evidence of effectiveness can be found in the literature on community-based interventions for the prevention of other infectious diseases with similar transmission modes. For example, a meta-analysis found that community intervention trials that encouraged washing hands with soap reduced the risk of diarrheal diseases by 47% [42]. Hand-washing programs reduced respiratory illness among military recruits [43] and children in day-care [44], and interventions involving hand sanitizers reduced absenteeism among elementary school teachers and children [45]. An in-depth review of the literature would be useful for determining the key factors associated with the success of these and other community interventions.

Although all women of childbearing age deserve to be informed about CMV, interventions for preventing CMV transmission are most likely to be effective for pregnant women, who tend to be highly motivated, often changing behavior to protect the health of their developing fetuses. As a case in point, 25% of low-income smokers spontaneously quit smoking during pregnancy [46]; this percentage is higher than that achieved by most smoking cessation programs [47]. Studies by Adler and colleagues suggest that motivation for avoiding CMV infection is considerably higher among pregnant than non-pregnant women [31,41].

In sum, the evidence to date gives every indication that effective interventions can be found for preventing CMV infection among pregnant women. Thus, the paradigm must shift from wondering whether such interventions will be effective to developing and evaluating interventions until effective ones are identified.

Next steps

Given the consequences of allowing the congenital CMV disease epidemic to continue unabated, it is imperative that women receive the educational message about congenital CMV disease prevention (see http://www.cdc.gov/cmv) [48]. Promotion of this message could translate years of careful CMV research into an immediate public health benefit. Encouraging hygienic practices would be relatively inexpensive (requires no laboratory testing or additional doctor visits), ethically responsible (allows women to make informed decisions), and likely to prevent disabilities and save lives. Based on studies of the economic impact of future CMV vaccines, which estimate savings of $50,000 per quality adjusted life-year saved [8], it is likely that CMV education efforts would provide a highly favorable cost-benefit ratio as well. CMV educa-

tional messages should emphasize hygienic practices as a precaution for all women who are pregnant or planning to become pregnant, and reasonable but not extreme measures for minimizing risk during interactions with young children (Figure 3). In many instances, a CMV education message could build upon and strengthen other public health messages about infection prevention through improved hand hygiene. Once effective hand-hygiene messages are identified, more ambitious goals might also be considered, such as prevention of sexual transmission or transmission between children in daycare.

Enlisting the support of healthcare providers to convey the gravity of CMV infection and the importance of good hygienic practices is crucial. Healthcare providers have many opportunities to provide women with such information, such as during annual gynecological exams or well-baby visits as women accompany their children to the pediatrician's office. Other steps, such as community education by healthcare providers (including information sessions with daycare directors, daycare providers, and parents) and provision of information by the Public Health Service and other professional organizations, can supplement the healthcare provider's information [49]. Success in delivering the message will depend on the active involvement of all relevant healthcare professionals.

In addition, trials are needed to identify effective community-based interventions for preventing CMV transmission to pregnant women. These trials will be important for quantifying the effectiveness of the proposed hygienic practices and for assessing the proportion of CMV infections that result from child-to-mother transmission as opposed to other routes, such as sexual transmission. However, such trials should not delay nor hinder the educational effort, just as we would not wait to inform skydivers about the prudence of parachute use because the results of controlled trials are not yet in. "As with many interventions intended to prevent ill health, the effectiveness of parachutes has not been subjected to rigorous evaluation by using randomised controlled trials...*[One option] is that we accept that, under exceptional circumstances, common sense might be applied when considering the potential risks and benefits of interventions* [our emphasis]."[50] Common sense tells us that avoiding CMV-laden secretions will prevent transmission and that the potential benefits of educational interventions far outweigh the potential risks.

Although an effective CMV vaccine would be ideal and vaccine development deserves increased support, hope for a vaccine tomorrow should not stand in the way of a vigorous educational message today. Prevention through improved hygienic practices will not be easy, but washing our hands of the problem by staying the present course guarantees that each year in the United States, hundreds of children will die and thousands of others will swell the ranks of CMV-affected children growing up with serious disabilities. Just as the medical and public health communities are successfully meeting the challenges posed by polio, rubella, HIV, and neural tube defects, now is the time to meet the congenital CMV challenge head on by making awareness and prevention high priorities.

Summary

• Each year in the United States, congenital CMV

- causes an estimated 400 deaths

- leaves more than 8000 children with permanent disabilities such as hearing or vision loss, or mental retardation

• Exposure to the saliva or urine of young children is a major cause of CMV infection among pregnant women.

• Risk of CMV infection is likely to be reduced by careful attention to good personal hygiene, especially handwashing.

• Women should be informed about how to reduce their risk of CMV infection during pregnancy.

• Trials are needed to identify educational interventions that are effective in preventing CMV infection.

Competing interests

The author(s) declare that they have no competing interests.

Authors' contributions

MJC and KFD contributed equally to the drafting and critical revision of the manuscript. Both authors read and approved the final manuscript.

Acknowledgements

We thank Larry Anderson, Pamela Buchalter, Gail Demmler, Sheila Dollard, Philip Pellett, Stanley Plotkin, Scott Schmid, Stephanie Staras, and Laura Wagner for helpful discussions and comments.

This research was supported in part by an appointment (KFD) to the Research Participation Program at the Centers for Disease Control and Prevention, National Center for Infectious Diseases, Division of Viral and Rickettsial Diseases administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the U.S. Department of Energy and the CDC.

References

1. Centers for Disease Control and Prevention: **Progress toward global eradication of poliomyelitis, 2002.** *MMWR Morb Mortal Wkly Rep* 2003, **52:366-369.**
2. Watson JC, Hadler SC, Dykewicz CA, Reef S, Phillips L: **Measles, mumps, and rubella--vaccine use and strategies for elimina-**

tion of measles, rubella, and congenital rubella syndrome and control of mumps: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 1998, 47:1-57.
3. Centers for Disease Control and Prevention: **Progress toward elimination of Haemophilus influenzae type b invasive disease among infants and children--United States, 1998-2000.** *MMWR Morb Mortal Wkly Rep* 2002, 51:234-237.
4. Hankin JR: **Fetal alcohol syndrome prevention research.** *Alcohol Res Health* 2002, 26:58-65.
5. Centers for Disease Control and Prevention: **Spina bifida and anencephaly before and after folic acid mandate--United States, 1995-1996 and 1999-2000.** *MMWR Morb Mortal Wkly Rep* 2004, 53:362-365.
6. Sullivan JL, Luzuriaga K: **The changing face of pediatric HIV-1 infection.** *N Engl J Med* 2001, 345:1568-1569.
7. Yow MD, Demmler GJ: **Congenital cytomegalovirus disease--20 years is long enough.** *N Engl J Med* 1992, 326:702-703.
8. Institute of Medicine(U.S.) Committee to Study Priorities for Vaccine Development: *Vaccines for the 21st Century: A Tool for Decision making* Washington D.C., National Academy Press; 2000:460.
9. Dobbins JG, Stewart JA, Demmler GJ: **Surveillance of congenital cytomegalovirus disease, 1990-1991. Collaborating Registry Group.** *Mor Mortal Wkly Rep CDC Surveill Summ* 1992, 41:35-39.
10. Alford CA, Stagno S, Pass RF, Britt WJ: **Congenital and perinatal cytomegalovirus infections.** *Rev Infect Dis* 1990, 12 Suppl 7:S745-S753.
11. Brown HL, Abernathy MP: **Cytomegalovirus infection.** *Semin Perinatol* 1998, 22:260-266.
12. Stagno S: **Cytomegalovirus.** In *Infectious diseases of the fetus and newborn infant* Edited by: Remington JS and Klein JO. Philadelphia, W.B. Saunders Company; 2001:389-424.
13. Zanghellini F, Boppana SB, Emery VC, Griffiths PD, Pass RF: **Asymptomatic primary cytomegalovirus infection: virologic and immunologic features.** *J Infect Dis* 1999, 180:702-707.
14. Pass RF, Stagno S, Dworsky ME, Smith RJ, Alford CA: **Excretion of cytomegalovirus in mothers: observations after delivery of congenitally infected and normal infants.** *J Infect Dis* 1982, 146:1-6.
15. Noyola DE, Demmler GJ, Williamson WD, Griesser C, Sellers S, Llorente A, Littman T, Williams S, Jarrett L, Yow MD: **Cytomegalovirus urinary excretion and long term outcome in children with congenital cytomegalovirus infection. Congenital CMV Longitudinal Study Group.** *Pediatr Infect Dis J* 2000, 19:505-510.
16. Montgomery JR, Mason EOJ, Williamson AP, Desmond MM, South MA: **Prospective study of congenital cytomegalovirus infection.** *South Med J* 1980, 73:590-3, 595.
17. Murph JR, Souza IE, Dawson JD, Benson P, Petheram SJ, Pfab D, Gregg A, O'Neill ME, Zimmerman B, Bale JF]: **Epidemiology of congenital cytomegalovirus infection: maternal risk factors and molecular analysis of cytomegalovirus strains.** *Am J Epidemiol* 1998, 147:940-947.
18. Stagno S, Dworsky ME, Torres J, Mesa T, Hirsh T: **Prevalence and importance of congenital cytomegalovirus infection in three different populations.** *J Pediatr* 1982, 101:897-900.
19. Pass RF, Hutto C, Ricks R, Cloud GA: **Increased rate of cytomegalovirus infection among parents of children attending day-care centers.** *N Engl J Med* 1986, 314:1414-1418.
20. Adler SP: **Cytomegalovirus and child day care: risk factors for maternal infection.** *Pediatr Infect Dis J* 1991, 10:590-594.
21. Stagno S, Whitley RJ: **Herpesvirus infection of pregnancy.** *N Engl J Med* 1985, 313:1270-1274.
22. Preece PM, Blount JM, Glover J, Fletcher GM, Peckham CS, Griffiths PD: **The consequences of primary cytomegalovirus infection in pregnancy.** *Arch Dis Child* 1983, 58:970-975.
23. Stagno S, Pass RF, Cloud G, Britt WJ, Henderson RE, Walton PD, Veren DA, Page F, Alford CA: **Primary cytomegalovirus infection in pregnancy. Incidence, transmission to fetus, and clinical outcome.** *JAMA* 1986, 256:1904-1908.
24. Plotkin SA: **Is there a formula for an effective CMV vaccine?** *J Clin Virol* 2002, 25:S13 - S21.
25. Kimberlin DW, Lin CY, Sanchez PJ, Demmler GJ, Dankner W, Shelton M, Jacobs RF, Vaudry W, Pass RF, Kiell JM, Soong SJ, Whitley RJ: **Effect of ganciclovir therapy on hearing in symptomatic congenital cytomegalovirus disease involving the central nervous system: a randomized, controlled trial.** *J Pediatr* 2003, 143:16-25.
26. Michaels MG, Greenberg DP, Sabo DL, Wald ER: **Treatment of children with congenital cytomegalovirus infection with ganciclovir.** *Pediatr Infect Dis J* 2003, 22:504-509.
27. Fowler KB, McCollister FP, Dahle AJ, Boppana S, Britt WJ, Pass RF: **Progressive and fluctuating sensorineural hearing loss in children with asymptomatic congenital cytomegalovirus infection.** *J Pediatr* 1997, 130:624-630.
28. Guralnick MJ: **Effectiveness of early intervention for vulnerable children: a developmental perspective.** *Am J Ment Retard* 1998, 102:319-345.
29. Kinney JS, Onorato IM, Stewart JA, Pass RF, Stagno S, Cheeseman SH, Chin J, Kumar ML, Yaeger AS, Herrmann KL, .: **Cytomegaloviral infection and disease.** *J Infect Dis* 1985, 151:772-774.
30. Onorato IM, Morens DM, Martone WJ, Stansfield SK: **Epidemiology of cytomegaloviral infections: recommendations for prevention and control.** *Rev Infect Dis* 1985, 7:479-497.
31. Adler SP, Finney JW, Manganello AM, Best AM: **Prevention of child-to-mother transmission of cytomegalovirus by changing behaviors: a randomized controlled trial.** *Pediatr Infect Dis J* 1996, 15:240-246.
32. Demmler GJ: **Cytomegalovirus.** In *Textbook of pediatric infectious disease* 4th edition. Edited by: Feigin RG and Cherry JD. Philadelphia, W.B. Saunders Company; 1998:1732-1751.
33. American College of Obstetricians and Gynecologists: *Perinatal viral and parasitic infections. ACOG Practice Bulletin 20* 20th edition. Washington, DC, ACOG; 2000.
34. Vielfaure MKM, Milhalchuk D: **Cytomegalovirus: no prevention, no cure.** *Can Nurse* 1995, 91:25-28.
35. Pass RF: **Cytomegalovirus infection.** *Pediatr Rev* 2002, 23:163-170.
36. Gaytant MA, Steegers EA, Semmekrot BA, Merkus HM, Galama JM: **Congenital cytomegalovirus infection: review of the epidemiology and outcome.** *Obstet Gynecol Surv* 2002, 57:245-256.
37. Nigro G, Anceschi MM, Cosmi EV: **Clinical manifestations and abnormal laboratory findings in pregnant women with primary cytomegalovirus infection.** *BJOG* 2003, 110:572-577.
38. Larson E: **A causal link between handwashing and risk of infection? Examination of the evidence.** *Infect Control* 1988, 9:28-36.
39. Revello MG, Gerna G: **Diagnosis and management of human cytomegalovirus infection in the mother, fetus, and newborn infant.** *Clin Microbiol Rev* 2002, 15:680-715.
40. Finney JW, Miller KM, Adler SP: **Changing protective and risky behaviors to prevent child-to-parent transmission of cytomegalovirus.** *J Appl Behav Anal* 1993, 26:471-472.
41. Adler SP, Finney JW, Manganello AM, Best AM: **Prevention of child-to-mother transmission of cytomegalovirus among pregnant women.** *J Pediatr* 2004, 145:485-491.
42. Curtis V, Cairncross S: **Effect of washing hands with soap on diarrhoea risk in the community: a systematic review.** *Lancet Infect Dis* 2003, 3:275-281.
43. Ryan MA, Christian RS, Wohlrabe J: **Handwashing and respiratory illness among young adults in military training.** *Am J Prev Med* 2001, 21:79-83.
44. Roberts L, Smith W, Jorm L, Patel M, Douglas RM, McGilchrist C: **Effect of infection control measures on the frequency of upper respiratory infection in child care: a randomized, controlled trial.** *Pediatrics* 2000, 105:738-742.
45. Hammond B, Ali Y, Fendler E, Dolan M, Donovan S: **Effect of hand sanitizer use on elementary school absenteeism.** *Am J Infect Control* 2000, 28:340-346.
46. Ockene J, Ma Y, Zapka J, Pbert L, Valentine GK, Stoddard A: **Spontaneous cessation of smoking and alcohol use among low-income pregnant women.** *Am J Prev Med* 2002, 23:150-159.
47. Lawrence D, Graber JE, Mills SL, Meissner HI, Warnecke R: **Smoking cessation interventions in U.S. racial/ethnic minority populations: an assessment of the literature.** *Prev Med* 2003, 36:204-216.
48. Centers for Disease Control and Prevention: **CMV.** 2004 [http://www.cdc.gov/cmv].
49. Osterholm MT, Reves RR, Murph JR, Pickering LK: **Infectious diseases and child day care.** *Pediatr Infect Dis J* 1992, 11:S31 - S41.
50. Smith GC, Pell JP: **Parachute use to prevent death and major trauma related to gravitational challenge: systematic review of randomised controlled trials.** *BMJ* 2003, 327:1459-1461.

51. Centers for Disease Control and Prevention: **HIV/AIDS Surveillance Report. 2001, 13(No.2):39.**
52. Groseclose SL, Brathwaite WS, Hall PA, Connor FJ, Sharp P, Anderson WJ, Fagan RF, Aponte JJ, Jones GF, Nitschke DA, Chang MH, Doyle T, Dhara R, Jajosky RA, Hatmaker JD: **Summary of notifiable diseases--United States, 2002.** *MMWR Morb Mortal Wkly Rep* 2004, **51**:1-84.
53. May PA, Gossage JP: **Estimating the prevalence of fetal alcohol syndrome. A summary.** *Alcohol Res Health* 2001, **25**:159-167.
54. Habbick BF, Nanson JL, Snyder RE, Casey RE: **Mortality in foetal alcohol syndrome.** *Can J Public Health* 1997, **88**:181-183.
55. Nembhard WN, Waller DK, Sever LE, Canfield MA: **Patterns of first-year survival among infants with selected congenital anomalies in Texas, 1995-1997.** *Teratology* 2001, **64**:267-275.
56. Vintzileos AM, Ananth CV, Smulian JC, Day-Salvatore DL, Beazoglou T, Knuppel RA: **Cost-benefit analysis of prenatal diagnosis for Down syndrome using the British or the American approach.** *Obstet Gynecol* 2000, **95**:577-583.
57. Davidoff MJ, Petrini J, Damus K, Russell RB, Mattison D: **Neural tube defect-specific infant mortality in the United States.** *Teratology* 2002, **66 Suppl 1**:S17-S22.

Pre-publication history

The pre-publication history for this paper can be accessed here:

http://www.biomedcentral.com/1471-2458/5/70/prepub

Infectious Diarrhea

So what happens when there's no place to go? The result is an unhealthy environment contaminated by human waste. This in turn, can contaminate a community's land and water which increases the risk of disease.

People living in communities lacking sanitary facilities are more likely to come into contact with human waste via their water or food which puts them at increased risk for infection.

Inadequate waste disposal accelerates the spread of disease that lives in soil, food, and water.

Water-, sanitation-, and hygiene-related diseases, including diarrhea, cholera (http://www.cdc.gov/cholera/index.html), hepatitis (http://www.cdc.gov/hepatitis/) and dysentery (bloody diarrhea), are some of the primary causes of illness and death in these settings, especially among young children. In fact, every 20 seconds (http://www.un.org/waterforlifedecade/sanitation.shtml) , a child dies as a result of poor sanitation. In addition, poor sanitation is associated with other diseases such as intestinal worms, trachoma and schistosomiasis and contributes to malnutrition in children.

Solutions

So what's being done to change the state of global sanitation? In November of 2013, UN Member States passed a resolution to encourage behavioral change and implement policies to increase access to proper sanitation. The resolution also calls for an end to open-air defecation, as it is harmful to public health.

- Sanitation initiatives such as Community-Led Total Sanitation have focused on ending open defecation and have increased access to latrines and toilets in many countries.
- Interventions to improve lighting such as a Solar Light Program in Haiti may lead to safer conditions for nighttime use of latrines for women and children.
- CDC has been supporting several sanitation interventions in Haiti as well as alternative sanitation options (http://www.elrha.org/map-location/cdc-alternative-sanitation-call1/) with *Sanivation* and with the UN High Commissioner for Refugees (http://www.unhcr.org/pages/49c3646cef.html) .
- Initiatives supported by UNICEF and other partners are increasing access to water and sanitation in schools

Finally, there's World Toilet Day (http://www.un.org/en/events/toiletday/) , designated by the UN to raise awareness for the billion people who do not have access to clean, safe sanitation.

Media centre

Sanitation

Fact sheet
Updated July 2017

Key facts

- In 2015, 39% of the global population (2.9 billion people) used a safely managed sanitation service – defined as use of a toilet or improved latrine, not shared with other households, with a system in place to ensure that excreta are treated or disposed of safely.
- 27% of the global population (1.9 billion people) used private sanitation facilities connected to sewers from which wastewater was treated.
- 13% of the global population (0.9 billion people) used toilets or latrines where excreta were disposed of in situ.
- 68% of the world's population (5.0 billion people) used at least a basic sanitation service.
- 2.3 billion people still do not have basic sanitation facilities such as toilets or latrines.
- Of these, 892 million still defecate in the open, for example in street gutters, behind bushes or into open bodies of water.
- At least 10% of the world's population is thought to consume food irrigated by wastewater.
- Poor sanitation is linked to transmission of diseases such as cholera, diarrhoea, dysentery, hepatitis A, typhoid and polio.
- Inadequate sanitation is estimated to cause 280 000 diarrhoeal deaths annually and is a major factor in several neglected tropical diseases, including intestinal worms, schistosomiasis, and trachoma. Poor sanitation also contributes to malnutrition.

Introduction

Hygienic sanitation facilities are crucial for public health. Since 1990, the number of people gaining access to improved sanitation has risen from 54% to 68% but some 2.3 billion people still do not have toilets or improved latrines.

In 2010, the UN General Assembly recognized access to safe and clean drinking water and sanitation as a human right, and called for international efforts to help countries to provide safe, clean, accessible and affordable drinking water and sanitation.

Despite progress, the 2015 Millennium Development Goal target to halve the proportion of the population without access to improved sanitation facilities was missed by almost 700 million people.

Sanitation and health

Some 842 000 people in low- and middle-income countries die as a result of inadequate water, sanitation, and hygiene each year, representing 58% of total diarrhoeal deaths. Poor sanitation is believed to be the main cause in some 280 000 of these deaths.

Diarrhoea remains a major killer but is largely preventable. Better water, sanitation, and hygiene could prevent the deaths of 361 000 children aged under 5 years each year.

Open defecation perpetuates a vicious cycle of disease and poverty. The countries where open defection is most widespread have the highest number of deaths of children aged under 5 years as well as the highest levels of malnutrition and poverty, and big disparities of wealth.

Benefits of improving sanitation

Benefits of improved sanitation extend well beyond reducing the risk of diarrhoea. These include:

- reducing the spread of intestinal worms, schistosomiasis and trachoma, which are neglected tropical diseases that cause suffering for millions;
- reducing the severity and impact of malnutrition;
- promoting dignity and boosting safety, particularly among women and girls;
- promoting school attendance: girls' school attendance is particularly boosted by the provision of separate sanitary facilities; and
- potential recovery of water, renewable energy and nutrients from faecal waste.

A WHO study in 2012 calculated that for every US$ 1.00 invested in sanitation, there was a return of US$ 5.50 in lower health costs, more productivity, and fewer premature deaths.

Challenges

In 2013, the UN Deputy Secretary General issued a call to action on sanitation that included the elimination of open defecation by 2025. Achieving universal access to a basic drinking water source appears within reach, but universal access to basic sanitation will require additional efforts.

The situation of the urban poor poses a growing challenge as they live increasingly in mega cities where sewerage is precarious or non-existent and space for toilets and removal of waste is at a premium. Inequalities in access are compounded when sewage removed from wealthier households is discharged into storm drains, waterways or landfills, polluting poor residential areas.

Limited data available on this topic suggests that a large proportion of wastewater in developing countries is discharged partially treated or untreated directly into rivers, lakes or the ocean.

Wastewater is increasingly seen as a resource providing reliable water and nutrients for food production to feed growing urban populations. Yet this requires:

- management practices that ensure wastewater is sufficiently treated and safely reused;
- institutional oversight and regulation; and
- public education campaigns to inform people about wastewater use.

WHO's response

As the international authority on public health, WHO leads global efforts to prevent transmission of diseases, advising governments on health-based regulations.

On sanitation, WHO monitors global burden of disease and the level of sanitation access and analyses what helps and hinders progress. Such monitoring gives Member States and donors global data to help decide how to invest in providing toilets and ensuring safe management of wastewater and excreta.

WHO works with partners on promoting effective risk assessment and management practices for sanitation through *Sanitation safety planning, Guidelines on safe use of wastewater, excreta and greywater*, and forthcoming Sanitation and Health Guidelines and *Global strategy on water, sanitation and hygiene and neglected tropical diseases*.

Sanitation safety planning
Guidelines for the safe use of wastewater, excreta and greywater

WHO, along with UNICEF and other partners, are implementing a global action plan for ending preventable child deaths from pneumonia and diarrhoea by 2025. This aims to meet several prevention and treatment targets, including promoting universal access to drinking water, sanitation, and hygiene in health care facilities and homes by 2030.

Increasing people's access to improved sanitation, combined with delivering preventive chemotherapy, is also part of the five global public health strategies for the control and elimination of neglected tropical diseases.

For more information contact:

WHO Media centre
E-mail: mediainquiries@who.int

Related links

- Water factsheet
- Sanitation safety planning
- WHO/UNICEF Joint Monitoring Programme
- Urgent call to action on sanitation
- WHO guidelines for the safe use of wastewater, excreta and greywater
- Ending preventable child deaths from pneumonia and diarrhoea by 2025
- GLAAS
- GEMI
- Report: Progress on drinking water, sanitation and hygiene

Infographics

Progress on drinking-water, sanitation and hygiene, 2017

Published in final edited form as:

Epidemiol Infect. 2016 October ; 144(13): 2790–2801. doi:10.1017/S0950268815003040.

Giardiasis outbreaks in the United States, 1971–2011

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Summary

Giardia intestinalis is the leading parasitic aetiology of human enteric infections in the United States, with an estimated 1.2 million cases occurring annually. To better understand transmission, we analysed data on all giardiasis outbreaks reported to the Centers for Disease Control and Prevention for 1971–2011. The 242 outbreaks, affecting ~41 000 persons, resulted from waterborne (74.8%), foodborne (15.7%), person-to-person (2.5%), and animal contact (1.2%) transmission. Most (74.6%) waterborne outbreaks were associated with drinking water, followed by recreational water (18.2%). Problems with water treatment, untreated groundwater, and distribution systems were identified most often during drinking water-associated outbreak investigations; problems with water treatment declined after the 1980s. Most recreational water-associated outbreaks were linked to treated swimming venues, with pools and wading pools implicated most often. Produce was implicated most often in foodborne outbreaks. Additionally, foods were most commonly prepared in a restaurant and contaminated by a food handler. Lessons learned from examining patterns in outbreaks over time can help prevent future disease. Groundwater and distribution system vulnerabilities, inadequate pool disinfection, fruit and vegetable contamination, and poor food handler hygiene are promising targets for giardiasis prevention measures.

Keywords

Community outbreaks; foodborne infections; *Giardia lamblia*; waterborne infections; zoonoses

Introduction

Giardia intestinalis is the leading parasitic aetiology of human enteric infections in the United States [1]. An estimated 1.2 million cases of giardiasis and 3581 hospitalizations occur annually [2], resulting in US$34 million in direct hospitalization costs [3]. Giardiasis is typically characterized by gastrointestinal symptoms including diarrhoea, bloating, and abdominal cramps; asymptomatic infections also occur frequently [4, 5]. Ingestion of as few as ten *Giardia* cysts has been shown to cause infection in a dosing study [6]. Cysts are excreted in the faeces of an infected person or animal [7]. Infected individuals might excrete

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Declaration Of Interest: None.

up to a billion cysts in their stool each day for several months, and cysts are immediately infectious upon excretion [6, 8, 9]. Cysts are environmentally stable and moderately resistant to chlorine disinfection, and thus can survive in water, food, or on surfaces for prolonged periods of time [4, 7].

US national data on reported cases of giardiasis show a gradual decrease in disease rates from 1996 to 2001; subsequently, rates from 2002 to 2010 remained relatively stable, coinciding with the disease becoming nationally notifiable in 2002 [10]. A recent decline in reported cases during 2011 to 2012 has been noted [11]. For most cases of illness, the source of the infection cannot be identified, and thus data from national case reports cannot be used to describe pathogen transmission [10]. Insight into the major drivers of *Giardia* transmission could inform effective prevention messages and measures.

The transmission pathways of giardiasis in the United States have not previously been well-described. Outbreak investigations provide an opportunity to gain insight into pathogen transmission. In the United States, local or state public health officials detect and investigate clusters of illnesses to determine whether there is a common source, as is the case in an outbreak; once an outbreak is detected, the investigation is used to find related cases of illnesses, to control the outbreak, and to prevent similar occurrences from happening in the future. Investigation methods and jurisdictions vary by state and locality, depending on local priorities and resources. States occasionally request epidemiological or laboratory assistance from the federal Centers for Disease Control and Prevention (CDC); CDC does not direct or participate in outbreak investigations without an invitation. States voluntarily report outbreak investigation summary information to CDC, and CDC has periodically published reports on disease outbreaks by mode of transmission since the early 1970s [12–15]. To date, there has been no overarching evaluation over several decades of the impact of changes to policies and surveillance activities, pathogen-specific risk factors, or patterns in outbreak occurrence. To gain a better understanding of *Giardia* transmission across all modes of transmission, we reviewed and analysed national data on all giardiasis outbreaks reported to CDC for 1971–2011.

Methods

An outbreak was defined as two or more cases of giardiasis that were epidemiologically linked by time or location of exposure to a common source. Outbreaks were investigated by and at the discretion of local or state public health agencies, and states voluntarily reported outbreaks to CDC surveillance systems. Since 2009, enteric disease outbreaks have been reported to CDC via the electronic National Outbreak Reporting System (NORS) [12]. Before 2009, waterborne outbreaks were reported to CDC using standard paper-based reporting forms, starting in 1971 for drinking water-associated outbreaks and in 1978 for recreational water-associated outbreaks. Foodborne outbreaks were reported to CDC using standard paper-based reporting forms starting in 1973, and using standard electronic forms starting in 1998. Systematic reporting of enteric disease outbreaks resulting from person-to-person, animal contact, environmental, and unknown transmission modes started with NORS implementation, although some outbreaks from these transmission modes were reported to CDC's foodborne outbreak surveillance system before 2009.

For this review and analysis, we included data from all outbreak reports finalized as of 25 February 2013 in CDC outbreak surveillance systems for which at least one listed aetiology was *Giardia* and the date of illness onset of the earliest case occurred during 1971–2011. To facilitate characterization of outbreaks, we analysed data elements that were consistently collected across the different outbreak surveillance systems and over time, including: state, year and month of illness onset of the earliest case, the number of outbreak-associated cases, the implicated pathogen(s), the setting of the outbreak, and the modes and vehicles of transmission. To describe contributing factors, we summarized data specific to each transmission mode, such as the type of drinking water system or the food preparation setting.

Descriptors for foodborne and other enteric disease outbreaks were maintained as reported to CDC. For waterborne outbreaks, we used the implicated water vehicle to classify the outbreaks as being associated with drinking water, recreational water, or other water. The drinking water category included outbreaks linked to contaminated drinking water or commercially bottled water. Implicated drinking water systems were categorized according to the U.S. Environmental Protection Agency (EPA) water system definitions for community, non-community, individual, or commercially bottled water [16]. Drinking water source was defined as groundwater, surface water, mixed, or unknown. Outbreak deficiencies, which characterize water system problems implicated during outbreak investigations, were previously assigned by a panel of CDC and EPA scientists that routinely reviews the outbreak reports. Outbreaks resulting from problems with the source water were classified as having untreated surface water or untreated groundwater deficiency. Outbreaks caused by circumstances at the treatment facility that allowed contamination, such as disruption in disinfection, were assigned treatment deficiency. Outbreaks involving problems in the distribution system under the jurisdiction of the water utility (e.g. cross-connection and backflow) were assigned distribution system deficiency. Outbreaks resulting from problems outside the jurisdiction of the water utility were assigned plumbing deficiency (e.g. cross-connection and back-flow within a building) or deficiency at point-of-use (e.g. contamination of a container). The recreational water category included outbreaks linked to contaminated untreated water (e.g. in lakes) or treated water (e.g. chlorinated or filtered) in at least partially man-made venues (e.g. in pools). The remaining waterborne outbreak category included outbreaks linked to consumption of non-potable or non-recreational water (e.g. drinking directly from a stream) and outbreaks in which the particular contaminated water source was undetermined. Data were summarized descriptively using SAS v. 9.3 (SAS Institute Inc., USA). To analyse the potential impact of drinking water regulations, the time period was divided into two periods: before implementation of the Surface Water Treatment Rule (SWTR) (1971–1989) and post-SWTR (1990–2011), a period in which several new microbial rules were implemented. Frequencies were compared using χ^2 tests or Fisher's exact tests when expected cell counts were <5, and differences in mean number of annual outbreaks were evaluated using *t* tests.

Results

Giardia was implicated in 242 reported outbreaks during 1971–2011, which resulted from waterborne ($n = 181$, 74.8%), foodborne ($n = 38$, 15.7%), person-to-person ($n = 6$, 2.5%),

animal contact ($n = 3$, 1.2%), and unknown ($n = 14$, 5.8%) transmission (Table 1). The 242 outbreaks were associated with 40 939 cases of illness. The median number of cases per outbreak was 18 (range 2–5449). Waterborne outbreaks resulted in the largest total number of cases ($n = 40027$, 97.8%) and the highest median number of cases per outbreak (23 cases).

Waterborne giardiasis outbreaks peaked in the early 1980s, which correlated with the implementation of an EPA-funded project to support intensive water-borne disease surveillance in Colorado, Vermont, and Washington for 2-year periods during 1980–1983 (Fig. 1) [17]. Following the implementation of NORS in 2009, reporting of giardiasis outbreaks resulting from person-to-person, animal contact, and unknown modes of transmission increased.

Giardiasis outbreaks occurred year-round but displayed a summer peak (Fig. 2). About half of all reported outbreaks started during June–September (51.6%), with July the most frequent month (17.8%). The summer peak was most pronounced for outbreaks associated with recreational water; only three of 33 outbreaks occurred outside the months of June–September.

Three-quarters of waterborne giardiasis outbreaks ($n = 135$, 74.6%) were associated with drinking water, followed by recreational water ($n = 33$, 18.2%) and other water ($n = 13$, 7.2%). More than two-thirds of all reported drinking water-associated outbreaks (96 outbreaks) occurred during the 19-year period 1971–1989; the remaining 39 outbreaks occurred during the 22-year period 1990–2011 (Fig. 3a). The mean annual number of reported drinking water-associated outbreaks decreased during the second time period (5.3 in 1971–1989 vs. 1.8 in 1990–2011, $P < 0.001$); this difference was less pronounced but still evident after excluding outbreaks that occurred during 1980–1983 from the three states with intensive waterborne disease surveillance activities (3.9 *vs.* 1.8, $P < 0.001$). The first reported recreational water-associated giardiasis outbreak occurred in 1985; since that time, the annual number of reported recreational water-associated outbreaks ranged from zero to four.

For drinking water-associated outbreaks, plotting the deficiencies identified over the 41-year period revealed changes in the causes of outbreaks over time (Fig. 3b). Overall, the most common deficiencies were treatment (62.2%), untreated groundwater source (13.3%), and distribution system deficiencies (11.9%). Deficiencies in water treatment were identified for almost three-quarters (74.0%) of the pre-1990 drinking water-associated outbreaks and one-third (33.3%) of outbreaks that occurred during 1990–2011 ($\chi^2 = 19.5$, $P < 0.001$). The mean annual number of outbreaks assigned treatment deficiencies also decreased after the 1980s (4.2 *vs.* 0.7, $P < 0.001$). The proportions of outbreaks assigned untreated groundwater source deficiencies (6.3% in 1971–1989 *vs.* 33.3% in 1990–2011, $\chi^2 = 16.8$, $P < 0.001$) and distribution system deficiencies (8.3% for 1971–1989 *vs.* 20.5% for 1990–2011, Fisher's exact $P = 0.074$) increased after 1989, but the mean annual numbers of outbreaks assigned these deficiencies did not differ by time period. The last outbreak associated with an untreated surface water deficiency occurred in 1995.

The majority of reported drinking water-associated outbreaks and outbreak cases involved community water systems (68.1% of outbreaks, 83.8% of outbreak cases) and systems with a surface water source (65.2% of outbreaks, 79.2% of outbreak cases) (Table 2). Drinking water-associated outbreaks most frequently involved systems serving a community/ municipality (43.9%), resort (9.5%), camp/cabin/recreational area (8.8%), or private residence (7.4%).

Most recreational water-associated outbreaks ($n = 22$, 66.7%) and outbreak cases ($n = 9295$, 96.5%) were linked to treated recreational water venues (Table 3). *Giardia* was the only pathogen identified for most of these outbreaks (72.7% for both treated and untreated water), but most cases of illness resulted from outbreaks of multiple aetiologies (93.2% in treated water and 67.9% in untreated water). Of treated recreational water-associated outbreaks, the most common swimming venues were pools (50.0%) and wading pools (36.4%). Most (91.0%) outbreak cases resulted from three waterpark-associated outbreaks; two of these outbreaks, accounting for 8449 (90.9%) of all treated recreational water-associated outbreak cases, were caused by aetiological agents *Giardia* and *Cryptosporidium*. Among the untreated recreational water-associated outbreaks, most outbreaks (72.7%) and cases (94.7%) were associated with lakes. Outdoor areas/parks were the most frequent settings of untreated recreational water-associated outbreaks (36.4%), while beaches were the setting of the most resulting cases (67.9%).

For waterborne outbreaks associated with water other than drinking water or recreational water, the most commonly implicated water type was a river/stream, which was linked to six outbreaks (46.2%) and 48.4% of outbreak cases. Two outbreaks (15.4%) were associated with a puddle, canal, or swamp; two outbreaks (15.4%) were associated with wastewater or partially treated wastewater; one outbreak (7.7%) was associated with a spring, and two outbreaks had an unreported water type.

Most of the 38 foodborne outbreaks (60.5%) had no food vehicle reported; these outbreaks resulted in half of the foodborne giardiasis outbreak cases. Of the 15 with a food implicated, produce (20.0%) was the most commonly implicated food category, followed by ice (13.3%) and fruit (13.3%). Raw vegetables, a salad bar, unspecified vegetables, and fresh fruit were implicated in the produce and fruit-associated outbreaks. Chicken salad, home-canned salmon, ice cream, oysters, and sandwiches were each linked to one outbreak. There were three outbreaks for which either multiple foods or a meal were implicated but the particular contaminated ingredient was undetermined. These included an outbreak linked to lettuce, onions, coffee, and tea; an outbreak linked to chicken parmesan and lettuce salad; and an outbreak for which the food items were not specified. Of the 36 outbreaks with a food preparation setting identified, the most common settings were restaurant or delicatessen (44.4%), followed by private home (19.4%). Seven of nine foodborne outbreaks with information on contributing factors had involvement of a food worker or other food handler; one outbreak involving produce had contaminated raw product and process failures that permitted pathogen survival, and two outbreaks had factors related to inadequate cleaning or storage of food in a contaminated environment.

Six person-to-person outbreaks were linked to 28 cases of illness. Settings of person-to-person outbreaks included households ($n = 3$), childcare ($n = 1$), and unknown ($n = 2$). Three outbreaks and 20 outbreak cases resulted from animal contact transmission; these were associated with rabbits at a petting zoo, cattle at a farm, and a pet reptile at a long-term care facility.

Discussion

This review and analysis of national giardiasis outbreak surveillance data highlights the historical and ongoing ability of *Giardia* to cause outbreaks through multiple transmission routes. To our knowledge, this analysis is the first of its kind to examine giardiasis outbreaks associated with all modes of transmission over several decades and to summarize the outbreaks in the context of changes to policies and disease surveillance activities. The results provide insight into the impact of general and pathogen-specific prevention measures already undertaken, including drinking water regulations, pool codes, and food codes. The findings also help clarify which transmission modes and preventive measures have been important drivers of giardiasis outbreaks in the United States and suggest additional areas to target with future prevention efforts.

Most reported giardiasis outbreaks and outbreak cases were associated with waterborne transmission. *Giardia* is moderately chlorine-tolerant and is able to survive in untreated or inadequately chlorinated drinking or recreational water. *Giardia* has a low infectious dose, infected persons can excrete large numbers of infectious cysts for prolonged periods of time, and cysts can survive in the environment. Thus, if faecal waste from one infected person is diluted in a water body or supply, many others can become infected with *Giardia* by ingesting contaminated recreational or drinking water. The Clean Water Act (CWA) of 1972, which regulates pollutant discharges and quality standards for surface waters, was one of the first regulations to control waste discharge into surface waters that could potentially be used for drinking, agriculture, or recreation [18].

While most of the waterborne giardiasis outbreaks and outbreak cases were drinking water-associated, the number of reported drinking water-associated outbreaks decreased after the 1980s, possibly resulting from a reduction in outbreaks associated with treatment deficiencies (i.e. circumstances at the treatment facility allowed contamination). The Safe Drinking Water Act (SDWA) of 1974 authorizes the U.S. EPA to regulate the public drinking water supply for health-based contaminants, largely by focusing on water-treatment standards. The 1996 amendments added additional measures to increase source water protection, operator training, and funding for water system improvements [16]. No outbreaks with untreated surface water deficiencies were reported after 1995. These decreases in the proportion of giardiasis outbreaks associated with treatment and surface water deficiencies followed the implementation of several key drinking water regulations, including the 1989 SWTR, which requires 99.9% removal or inactivation of *Giardia* in public water systems with surface water sources or groundwater sources influenced by surface water [19]. Additional protections from the 1998 Interim Enhanced Surface Water Treatment Rule, the 2002 Long Term 1 Enhanced Surface Water Treatment Rule, and the 2006 Long Term 2

Enhanced Surface Water Treatment Rule likely also help to reduce *Giardia* in public drinking water systems with surface water sources [20–22].

In contrast, untreated groundwater source and distribution system deficiencies were identified throughout the study period. The 2006 Ground Water Rule specifies monitoring criteria and corrective action requirements for public groundwater systems with documented deficiencies demonstrating a high risk for faecal contamination [23]. However, this rule does not address individual water systems (e.g. private wells), which the federal government does not have the authority to regulate. These systems were associated with about 8% of the drinking water-associated outbreaks in this analysis; however, these are difficult to detect by public health agencies and through outbreak surveillance, since they might only impact a single household or neighbourhood. Continued efforts to educate and motivate private well owners to inspect, maintain, and test the water from their private wells could improve water quality and reduce the spread of disease [24]. The continued occurrence of giardiasis outbreaks with distribution system deficiencies suggests that drinking water distribution system infrastructure vulnerabilities are an ongoing public health concern. In a separate analysis summarizing all drinking water-associated outbreaks in the United States from 1971–2006, distribution system deficiencies were linked to 10% of outbreaks, and untreated groundwater source deficiencies were linked to 30% of outbreaks, indicating that efforts to remediate and prevent these types of deficiencies could have widespread public health benefits that extend beyond *Giardia* prevention [25].

Giardiasis outbreaks occur in both treated and untreated recreational water venues. Outbreaks associated with treated recreational water venues are preventable and indicate inadequate operation of the implicated venues, including failure to maintain appropriate chlorine and pH levels. Violations of disinfectant level and pH code standards have been well-documented in routine inspections of public treated recreational water venues, including wading pools [26]. These violations in wading pools are especially risky for pathogen transmission, as young children have the highest rates of giardiasis and ingest water during swimming activities [11, 27]. Requiring operators of public treated recreational water venues to successfully complete training to operate these venues has been associated with improved water quality [28, 29].

In contrast to treated recreational water venues, untreated recreational waters, by their nature, have a lack of available chemical remediation options. The Beaches and Environmental Assessment and Coastal Health (BEACH) Act of 2000 sets national water quality monitoring and reporting standards for the Great Lakes and marine coastal recreational waters [30, 31]. Still, even on days where the water quality is considered acceptable for bathing by bacterial indicator standards, crowded beach waters might contain human pathogens, including *Giardia* and *Cryptosporidium*, likely because of direct microbial input from bathers and re-suspension of sediment [32, 33]. Ultimately, the occurrence of inadequately maintained disinfectant and pH levels in treated recreational water venues and the lack of chemical remediation options for untreated recreational water venues underscore the need for healthy swimming behaviours (e.g. not swimming while ill with diarrhoea) to minimize water contamination and thus help prevent pathogen

transmission. Health communication campaigns can increase awareness of and possibly the practice of healthy swimming behaviours [34].

Poor hygiene practices by food handlers might have contributed to the occurrence of many foodborne giardiasis outbreaks: several of the outbreak reports cited a lack of hand washing and infectious, sometimes asymptomatic, food workers as the cause [35, 36]. Our finding that produce items were the most frequently identified foods in giardiasis outbreaks is consistent with studies of risk factors of sporadic giardiasis, which have found links between giardiasis and eating lettuce and green salads [37, 38]. There are numerous places in the production chain where produce can become contaminated, and many of the mechanisms of contamination have a water quality, sanitation, or hygiene component [39, 40]. These include contamination by irrigation water or runoff in the field, by water used in processing and packing, or by unwashed hands during harvesting, processing, packing, transport, or at point-of-use [40–42]. Prevention of produce contamination is especially important, since fruits and vegetables are often eaten raw without a step to inactivate pathogens [43]. Implementation of the Food Safety Modernization Act (FSMA) Proposed Rule for Produce Safety, when finalized, is expected to prevent future produce-associated outbreaks by setting safety requirements for produce on farms during growing, harvesting, packing, and holding. Requirements include setting standards for personnel hygienic practices, establishing a water quality profile for agricultural water, treating and applying soil amendments, controlling potential hazards introduced by animals, and keeping equipment, buildings, and tools cleaned and maintained [44].

Outbreaks with person-to-person, animal contact, environmental, or unknown transmission modes were the least commonly reported; however, there were only 2 years of systematic data collection available for inclusion in this analysis. Published studies have implicated person-to-person transmission of giardiasis in institutional settings such as child care and nursing homes that resulted in disease spread to caregivers, other contacts, or into the community [45–48]. The case counts from these person-to-person outbreaks were higher than those for the outbreaks included in this analysis; however, these occurred before the implementation of NORS and were not reported to CDC. Animal contact was implicated in only three reported giardiasis outbreaks, consistent with current molecular epidemiological data suggesting that animal contact transmission of giardiasis is relatively uncommon because animals are more often infected with species-specific assemblages that do not cause disease in humans [7].

There were several limitations to this review and analysis. National surveillance systems do not capture all outbreaks that occur, and epidemiological, laboratory, and environmental health information are not uniformly collected during outbreak investigations. This limits inferences that can be made using outbreak surveillance data and underscores the usefulness of uniform data collection during outbreak investigations [49], in addition to systematic reporting. The peak in reported waterborne outbreaks seen here during a period of intensive waterborne disease surveillance in three states indicates that the sensitivity of passive outbreak surveillance could be low and that detection and reporting can improve if resources are allocated to increase surveillance capacity at the state level. Differences in public health capacity or reporting practices across states and over time should be considered when

interpreting increases and decreases in reported outbreaks. Additionally, there were limited years of outbreak data for the non-water and non-food modes of transmission, thus caution should be exercised in interpreting the relative importance of the different modes of transmission in giardiasis outbreaks. The implementation of comprehensive enteric disease outbreak reporting through NORS is an important step to help standardize data collection across years and modes of transmission [12]. Additionally, the inclusion of multiple-pathogen outbreaks in our analysis means that the number of outbreak-associated cases reported here includes an unknown number of cases of other illnesses. In particular, the magnitude of some recreational water-associated giardiasis outbreaks might have been driven by transmission of the extremely chlorine-tolerant parasite *Cryptosporidium* [50]; omitting the nine muti-pathogen outbreaks would have excluded 90% of outbreak cases from the recreational water outbreaks.

In summary, this analysis of giardiasis outbreaks reported to CDC over 41 years suggests that these outbreaks can be prevented when resources are allocated for preventive measures. Regulations, facilities, and services for drinking water treatment and management appear to have prevented waterborne transmission of giardiasis in public surface water systems. Groundwater and distribution system vulnerabilities might be promising areas to target with future preventive measures. Treated recreational venue maintenance and operation, in combination with health promotion efforts to improve swimmer hygiene in both treated and untreated recreational waters could prevent outbreaks associated with these settings. For foodborne outbreaks, focusing on food handler and consumer hygiene and the prevention of produce contamination could prevent outbreaks.

Molecular epidemiology has the potential to expand our understanding of *Giardia* transmission. The United States does not systematically perform molecular typing on *Giardia* isolates nationally, and molecular studies are seldom undertaken in outbreak investigations. Implementation of a surveillance system that incorporates molecular-based laboratory testing of *Giardia* specimens could enable the detection of additional outbreaks, including those associated with distributed food products or animal contact. Our findings demonstrate the need and value in investing resources in public health surveillance activities as a way to help evaluate preventive measures already in place and suggest areas where future efforts might decrease illness incidence. Each outbreak provides valuable information about pathogen transmission, and the lessons learned from examining patterns in outbreaks over time can be used to prevent future disease.

Acknowledgments

The authors gratefully acknowledge the work of local, state, and territorial health departments in investigating and reporting outbreaks and thank Virginia Roberts for assistance with extracting and interpreting outbreak data, and Sarah Collier for assistance with analyses. This research was supported in part by an appointment to the Research Participation Program at the Centers for Disease Control and Prevention administered by the Oak Ridge Institute for Science and Education through and interagency agreement between the U.S. Department of Energy and CDC.

The findings and conclusions in this article are those of the authors, and do not necessarily represent the official position of the U.S. CDC.

References

1. Kappus KD, et al. Intestinal parasitism in the United States: update on a continuing problem. American Journal of Tropical Medicine and Hygiene. 1994; 50:705–713. [PubMed: 8024063]
2. Scallan E, et al. Foodborne illness acquired in the United States – major pathogens. Emerging Infectious Diseases. 2011; 17:7–15. [PubMed: 21192848]
3. Collier SA, et al. Direct healthcare costs of selected diseases primarily or partially transmitted by water. Epidemiology and Infection. 2012; 140:2003–2013. [PubMed: 22233584]
4. Huang DB, White AC. An updated review on *Cryptosporidium* and *Giardia*. Gastroenterology Clinics of North America. 2006; 35:291–314. [PubMed: 16880067]
5. Nash TE, et al. Experimental human infections with *Giardia lamblia*. Journal of Infectious Diseases. 1987; 156:974–984. [PubMed: 3680997]
6. Rendtorff RC. The experimental transmission of human intestinal protozoan parasites. II. Giardia lamblia cysts given in capsules. American Journal of Hygiene. 1954; 59:209–220. [PubMed: 13138586]
7. Xiao L, Fayer R. Molecular characterisation of species and genotypes of cryptosporidium and giardia and assessment of zoonotic transmission. International Journal for Parasitology. 2008; 38:1239–1255. [PubMed: 18479685]
8. Danciger M, Lopez M. Numbers of giardia in the feces of infected children. American Journal of Tropical Medicine and Hygiene. 1975; 24:237–242. [PubMed: 1119665]
9. Pickering LK, et al. Occurrence of *Giardia lamblia* in children in day care centers. Journal of Pediatrics. 1984; 104:522–526. [PubMed: 6707812]
10. Yoder JS, et al. Giardiasis surveillance – United States, 2009–2010. Morbidity and Mortality Weekly Report Surveillance Summaries. 2012; 61:13–23. [PubMed: 22951494]
11. Painter JE, et al. Giardiasis Surveillance – United States, 2011–2012. Morbidity and Mortality Weekly Report Surveillance Summaries. 2015; 64:15–25.
12. Hall AJ, et al. Acute gastroenteritis surveillance through the National Outbreak Reporting System, United States. Emerging Infectious Disease. 2013; 19:1305–1309.
13. Gargano JW, et al. Surveillance for waterborne disease outbreaks associated with drinking water and other nonrecreational water – United States, 2009–2010. Morbidity and Mortality Weekly Report. 2013; 62:714–720. [PubMed: 24005226]
14. Gould LH, et al. Surveillance for foodborne disease outbreaks – United States, 1998–2008. Morbidity and Mortality Weekly Report Surveillance Summaries. 2013; 62:1–34.
15. Hlavsa MC, et al. Recreational water-associated disease outbreaks – United States, 2009–2010. Morbidity and Mortality Weekly Report. 2014; 63:6–10. [PubMed: 24402466]
16. Environmental Protection Agency. [Accessed 26 May 2015] Understanding the Safe Drinking Water Act. http://water.epa.gov/law.sregs/guidance/sdwa/upload/2009_08_28_sdwa_fs_30ann_sdwa_web.pdf
17. Harter L, et al. A three-state study of waterborne disease surveillance techniques. American Journal of Public Health. 1985; 75:1327–1328. [PubMed: 4051072]
18. Envrionmental Protection Agency. Clean Water Act. 1972 33 U.S.C. §1251 et seq.
19. Environmental Protection Agency. Drinking water, national primary drinking water regulations, filtration, disinfection, turbidity, *Giardia lamblia*, viruses, *Legionella*, and heterotrophic bacteria; Final Rule. Federal Register. 1989; 54:27486–27541.
20. Environmental Protection Agency. National primary drinking water regulations: interim enhanced surface water treatment; Final Rule. Federal Register. 1998; 63:69478–69521.
21. Environmental Protection Agency. National primary drinking water regulations: long term 1 enhanced surface water treatment rule; Final Rule. Federal Register. 2002; 67:1812–1844.
22. Environmental Protection Agency. National primary drinking water regulations: long term 2 enhanced surface water treatment rule; Final Rule. Federal Register. 2006; 71:654–786.
23. Environmental Protection Agency. National *Primary Drinking Water Regulations: Ground Water Rule*; Final Rule. Federal Register. 2006; 71:65573–65660.

24. Knobeloch L, et al. Private drinking water quality in rural Wisconsin. Journal of Environmental Health. 2013; 75:16–20.
25. Craun GF, et al. Causes of outbreaks associated with drinking water in the United States from 1971 to 2006. Clinical Microbiology Reviews. 2010; 23:507–528. [PubMed: 20610821]
26. Centers for Disease Control and Prevention. Violations identified from routine swimming pool inspections – selected states and counties, United States, 2008. Morbidity and Mortality Weekly Report. 2010; 59:582–587. [PubMed: 20489681]
27. Dufour AP, et al. Water ingestion during swimming activities in a pool: a pilot study. Journal of Water and Health. 2006; 4:425–430. [PubMed: 17176813]
28. Buss BF, et al. Association between swimming pool operator certification and reduced pool chemistry violations – Nebraska, 2005–2006. Journal of Environmental Health. 2009; 71:36–40.
29. Johnston K, Kinziger M. Certified operators: does certification provide significant results in real-world pool and spa chemistry? International Journal of Aquatic Research and Education. 2007; 1:18–33.
30. Environmental Protection Agency. [Accessed 26 May 2015] Beaches Environmental Assessment and Coastal Health Act of 2000. Public Law 106-284. http://water.epa.gov/lawsregs/lawsguidance/beachrules/upload/2009_04_13_beaches_files_beachbill.pdf
31. Wade TJ, et al. Rapidly measured indicators of recreational water quality are predictive of swimming-associated gastrointestinal illness. Environmental Health Perspectives. 2006; 114:24–28. [PubMed: 16393653]
32. Graczyk TK, et al. Relationships among bather density, levels of human waterborne pathogens, and fecal coliform counts in marine recreational beach water. Parasitology Research. 2010; 106:1103–1108. [PubMed: 20145953]
33. Graczyk TK, et al. Bather density and levels of cryptosporidium, giardia, and pathogenic microsporidian spores in recreational bathing water. Parasitology Research. 2007; 101:1729–1731. [PubMed: 17823816]
34. Hlavsa MC, et al. Promotion of healthy swimming after a statewide outbreak of cryptosporidiosis associated with recreational water venues – Utah, 2008– 2009. Morbidity and Mortality Weekly Report. 2012; 61:348. [PubMed: 22592274]
35. Mintz ED, et al. Foodborne giardiasis in a corporate office setting. Journal of Infectious Diseases. 1993; 167:250–253. [PubMed: 8418177]
36. Quick R, et al. Restaurant-associated outbreak of giardiasis. Journal of Infectious Diseases. 1992; 166:673–676. [PubMed: 1500757]
37. Espelage W, et al. Characteristics and risk factors for symptomatic *Giardia lamblia* infections in Germany. BMC Public Health. 2010:10–41. [PubMed: 20064237]
38. Stuart JM, et al. Risk factors for sporadic giardiasis: a case-control study in southwestern England. Emerging Infectious Diseases. 2003; 9:229–233. [PubMed: 12603995]
39. Ailes EC, et al. Microbial concentrations on fresh produce are affected by postharvest processing, importation, and season. Journal of Food Protection. 2008; 71:2389–2397. [PubMed: 19244889]
40. Lynch MF, Tauxe RV, Hedberg CW. The growing burden of foodborne outbreaks due to contaminated fresh produce: risks and opportunities. Epidemiology and Infection. 2009; 137:307–315. [PubMed: 19200406]
41. Lewis HC, et al. Outbreaks of Shigella sonnei infections in Denmark and Australia linked to consumption of imported raw baby corn. Epidemiology and Infection. 2009; 137:326–334. [PubMed: 19134229]
42. Sivapalasingam S, et al. A multistate outbreak of *Salmonella enterica* serotype Newport infection linked to mango consumption: impact of water-dip disinfestation technology. Clinical Infectious Diseases. 2003; 37:1585–1590. [PubMed: 14689335]
43. Burnett SL, Beuchat LR. Human pathogens associated with raw produce and unpasteurized juices, and difficulties in decontamination. Journal of Industrial Microbiology & Biotechnology. 2001; 27:104–110. [PubMed: 11641768]
44. Food and Drug Administration. Standards for the growing, harvesting, packing, and holding of produce for human consumption; Proposed Rule. Federal Register. 2014; 79:58433–58473.

45. Black RE, et al. Giardiasis in day-care centers: evidence of person-to-person transmission. Pediatrics. 1977; 60:486–491. [PubMed: 905014]

46. Polis MA, et al. Transmission of *Giardia lamblia* from a day care center to the community. American Journal of Public Health. 1986; 76:1142–1144. [PubMed: 3740341]

47. White KE, et al. An outbreak of giardiasis in a nursing home with evidence for multiple modes of transmission. Journal of Infectious Diseases. 1989; 160:298–304. [PubMed: 2760485]

48. Katz DE, et al. Prolonged outbreak of giardiasis with two modes of transmission. Epidemiology and Infection. 2006; 134:935–941. [PubMed: 16569269]

49. Council to Improve Foodborne Outbreak Response (CIFOR). Guidelines for foodborne disease outbreak response. 2nd. Atlanta: Council of State and Territorial Epidemiologists; 2014. http://www.cifor.us/documents/CIFOR%20Industry%20Guidelines/CIFOR-Industry-Guideline.pdf [Accessed May 26, 2015]

50. Centers for Disease Control and Prevention. Community-wide cryptosporidiosis outbreak – Utah, 2007. Morbidity and Mortality Weekly Report. 2008; 57:989–993. [PubMed: 18784640]

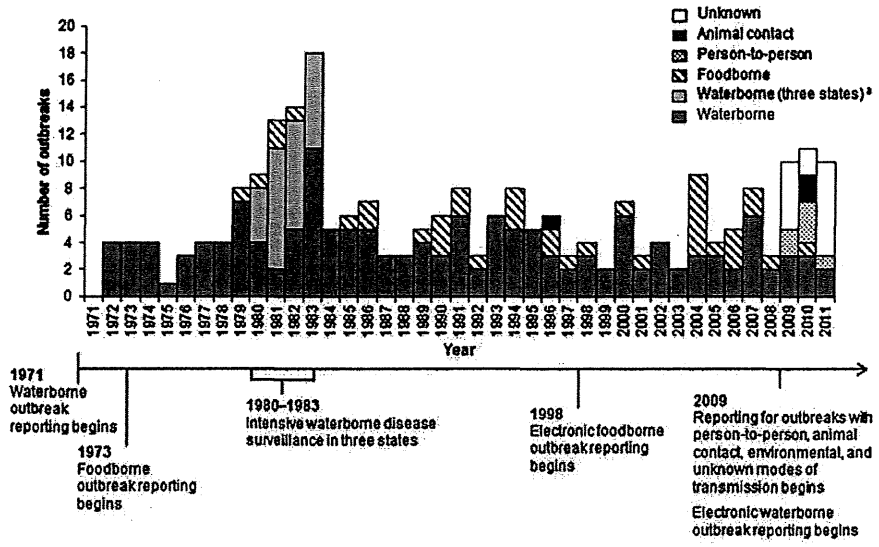

Fig. 1.

Giardiasis outbreaks, by year and mode of transmission, United States, 1971–2011 (n = 242). The timeline below the figure outlines key changes in disease surveillance activities and systems. [a] Waterborne outbreaks reported from Colorado, Vermont, and Washington; these states received 2-year funding from the U.S. Environmental Protection Agency to support intensive waterborne disease surveillance.

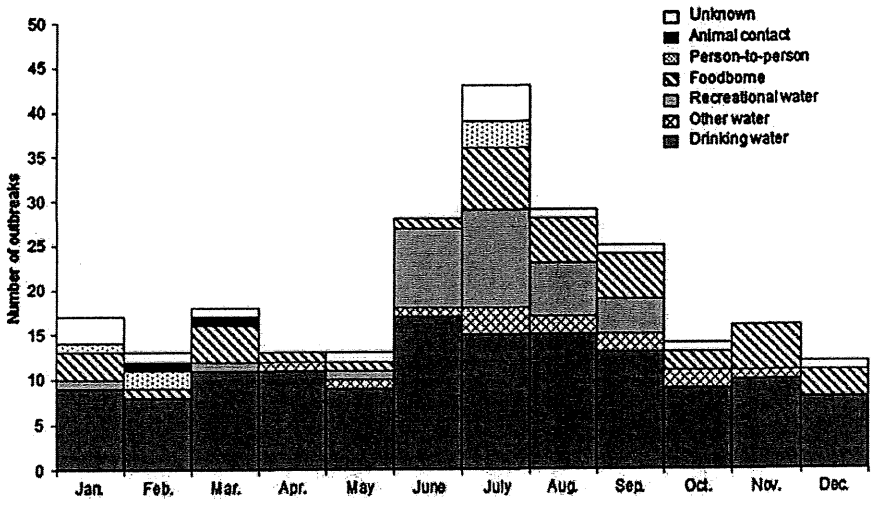

Fig. 2.

Giardiasis outbreaks, by mode of transmission and month of first reported illness, United States, 1971–2011 ($n = 241$). Information on the month of first illness was missing for one outbreak associated with animal contact transmission.

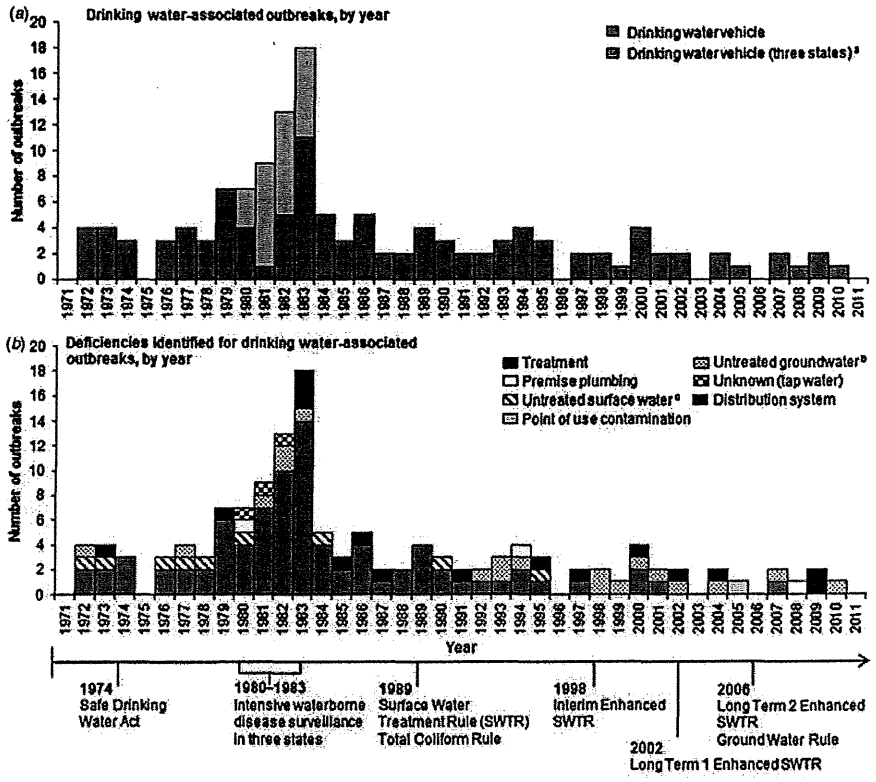

Fig. 3.

Waterborne giardiasis outbreaks, by year, water vehicle, and outbreak deficiency, United States, 1971–2011. (*a*) Number of waterborne outbreaks, by year and implicated water vehicle ($n = 181$). [a] Waterborne outbreaks reported from Colorado, Vermont, and Washington; these states received 2-year funding from the U.S. Environmental Protection Agency to support intensive waterborne disease surveillance. (*b*) Deficiencies identified during drinking water outbreak investigations, by year, and timeline of key drinking water regulations and surveillance activities; each deficiency represents one outbreak ($n = 135$). [b] One outbreak was assigned an additional distribution system deficiency. [c] One outbreak was assigned an additional plumbing deficiency.

Table 1

Giardiasis outbreaks reported in the United States (1971–2011), by mode of transmission (n = 242)

	Outbreaks		Cases		No. of cases per outbreak		
Transmission mode	***n***	**(%)**	***n***	**(%)**	**Median**	**Minimum**	**Maximum**
All modes	242	(100)	40 939	(100)	18	2	5449
Waterborne*	181	(74.8)	40 027	(97.8)	23	2	5449
Foodborne†	38	(15.7)	825	(2.0)	16	2	82
Person-to-person‡	6	(2.5)	28	(0.1)	4	3	9
Animal contact‡	3	(1.2)	20	(0.0)	6	2	12
Unknown‡	14	(5.8)	39	(0.1)	2	2	6

*Systematic waterborne disease outbreak reporting began in 1971.

†Systematic foodborne disease outbreak reporting began in 1973.

‡Systematic enteric disease outbreak reporting from person-to-person, animal contact, environmental, and unknown transmission modes began in 2009. There were no *Giardia* outbreaks reported with environmental mode of transmission.

Table 2

Aetiology, water system, water source, and deficiencies of drinking water-associated giardiasis outbreaks (n = 135) and outbreak cases (n = 30 237), United States, 1971-2011

	Outbreaks		Cases	
Characteristic/category	***n***	**(%)**	***n***	**(%)**
Aetiology				
Giardia only	127	(94.1)	28 222	(93.3)
Giardia and other pathogens *	8	(5.9)	2015	(6.7)
Water system †				
Community	92	(68.1)	25 329	(83.8)
Non-community	30	(22.2)	3367	(11.1)
Individual	11	(8.1)	50	(0.2)
Non-community and individual	1	(0.7)	1450	(4.8)
Commercially bottled water	1	(0.7)	41	(0.1)
Water source ‡				
Surface water	88	(65.2)	23 948	(79.2)
Groundwater	42	(31.1)	5804	(19.2)
Mixed	2	(1.5)	312	(1.0)
Unknown	3	(2.2)	173	(0.6)
Deficiency §				
Treatment	84	(62.2)	23 697	(78.4)
Untreated groundwater	18	(13.3)	368	(1.2)
Distribution system	16	(11.9)	3818	(12.6)
Untreated surface water	8	(5.9)	197	(0.7)
Plumbing	3	(2.2)	365	(1.2)
Untreated groundwater and distribution system	1	(0.7)	1450	(4.8)
Untreated surface water and plumbing	1	(0.7)	189	(0.6)
Point-of-use contamination	1	(0.7)	41	(0.1)
Unknown (tap water)	3	(2.2)	112	(0.4)

* Multiple aetiological agents were identified during investigations of eight outbreaks. *Giardia and Cryptosporidium* were identified in two outbreaks, *Giardia and Campylobacter* were identified in one outbreak, *Giardia, Campylobacter*, and *Entamoeba* were identified in one outbreak, *Giardia, Campylobacter*, and norovirus were identified in one outbreak, *Giardia* and *Entamoeba* were identified in one outbreak, *Giardia* and *Salmonella* were identified in one outbreak, and *Giardia, Shigella, Cryptoprodium*, and *Clostridium* were identified in one outbreak.

† Community and non-community systems are public water systems that are operated by a water utility and regulated for safety by the U.S. Environmental Protection Agency (EPA). Individual water systems are small systems that are not operated by a water utility and are not regulated for safety by the EPA.

‡ Surface water sources include lakes, rivers, reservoirs, and ponds. Groundwater sources include wells, springs, and other sources that extract water from an aquifer. Mixed sources are made up of both groundwater and surface water sources.

§ Deficiencies are identified by CDC and EPA scientists that review the outbreak reports; assigned deficiencies provide information about how the water became contaminated and help explain why the outbreak occurred.

Table 3

Aetiology, swimming venue, and setting of recreational water-associated giardiasis outbreaks (n = 33) and outbreak cases (n = 9635), by treatment type, United States, 1971–2011

	Treated recreational water					Untreated recreational water				
		Outbreaks		Cases			Outbreaks		Cases	
Characteristic	Category	*n*	(%)	*n*	(%)	Category	*n*	(%)	*n*	(%)
All outbreaks		22	(66.7)	9295	(96.5)		11	(33.3)	340	(3.5)
Aetiology	*Giardia* only	16	(72.7)	632	(6.8)	*Giardia* only	8	(72.7)	109	(32.1)
	Giardia and other pathogens *	6	(27.3)	8663	(93.2)	*Giardia* and other pathogens *	3	(27.3)	231	(67.9)
Venue	Pool	11	(50.0)	513	(5.5)	Lake	8	(72.7)	322	(94.7)
	Wading pool	8	(36.4)	3273	(35.2)	River	2	(18.2)	8	(2.4)
	Interactive outdoor fountain	1	(4.5)	55	(0.6)	Unknown	1	(9.1)	10	(2.9)
	Wading pool, wave pool	1	(4.5)	5449	(58.6)					
	Unknown	1	(4.5)	5	(0.1)					
Setting	Community	5	(22.7)	227	(2.4)	Outdoor area/park	4	(36.4)	38	(11.2)
	Club	4	(18.2)	189	(2.0)	Beach	3	(27.3)	231	(67.9)
	Water park	3	(13.6)	8456	(91.0)	Club	1	(9.1)	48	(14.1)
	Outdoor area/park	3	(13.6)	295	(3.2)	Camp	1	(9.1)	4	(1.2)
	Childcare	2	(9.1)	16	(0.2)	Unknown	2	(18.2)	19	(5.6)
	Childcare and community	1	(4.5)	63	(0.7)					
	Hotel	1	(4.5)	7	(0.1)					
	Private residence	1	(4.5)	3	(0.0)					
	Unknown	2	(9.1)	39	(0.4)					

* Multiple aetiological agents were identified during investigations of nine outbreaks. *Giardia* and *Cryptosporidium* were identified in the six treated recreational water-associated outbreaks and in two untreated recreational water-associated outbreaks. In one untreated recreational water-associated outbreak, *Giardia* and *E. coli* O157:H7 were identified ($n = 8$ cases).

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Informed Health Online [Internet]. Cologne, Germany: Institute for Quality and Efficiency in Health Care (IQWiG); 2006-.

Acute infectious diarrhea: Common germs and routes of infection

Created: May 4, 2016; Next update: 2019.

In Germany, infectious diarrhea is most commonly caused by the norovirus. Infants and young children often have rotavirus infections as well. Viral infections are usually quite intense but short. Bacterial infectious diarrhea is also widespread in adults.

Infections with the highly contagious norovirus or rotavirus typically start with sudden and severe symptoms such as diarrhea or vomiting. These symptoms usually go away after a few days. Since 2013, the German Standing Committee on Vaccination (STIKO) has recommended that infants be vaccinated against the rotavirus.

Bacterial infectious diarrhea is most often caused by eating contaminated or spoiled food. Like viruses, bacteria such as Campylobacter or Salmonella can also be transmitted through direct contact with infected people or by touching contaminated objects.

Rotaviruses

Rotaviruses most commonly infect infants and young children between the ages of 6 and 24 months. A child's first rotavirus infection usually causes severe symptoms, whereas later infections are milder. If children have several rotavirus infections, they become immune to the virus, but the immunity does not last for the rest of their lives. Adults under the age of 60 have fewer infections than children, and usually have milder symptoms as well. Common sources of infection include long-distance travel and their own children.

Rotavirus infections are more common in older people and those with weakened immune systems. Their symptoms may be so severe that hospital treatment is needed.

It usually takes about one to three days for rotavirus symptoms to start. They then last about two to six days. Common symptoms include:

- Sudden watery diarrhea (often with mucus in it)
- Vomiting
- Stomach ache
- Fever
- Feeling weak and dazed from loss of fluids
- Breathing difficulties in half of all affected people

Noroviruses

In Germany, norovirus infections are especially common in children under the age of five years and adults over the age of 70. Infection is possible at any time of the year, but most often occurs between October and March.

It can take anywhere from six hours to two days for a norovirus infection to start causing symptoms. The symptoms often start quite suddenly, and include:

- Severe diarrhea
- Severe projectile vomiting
- Stomach ache
- Sometimes nausea, headaches, muscle ache, exhaustion and a mild fever

The symptoms usually only last about 12 to 48 hours. If too much fluid is lost due to severe diarrhea and vomiting, people may feel dizzy, drowsy and faint too.

How do the viruses spread?

Noroviruses and rotaviruses are most often transmitted through direct contact with infected people, touching contaminated objects and, less often, eating contaminated food. The viruses, which are found in stool and vomit, can be transferred through direct contact. Noroviruses can also be spread to others through tiny droplets in the air when someone vomits.

Viruses may survive on toilets, door handles or clothing, where they remain infectious for several days. Indirect infection is possible if you touch a contaminated object with your hand, and then touch your mouth with your hand. So if you want to protect yourself and others from infection, it's especially important to wash your hands regularly and thoroughly.

If viruses enter food through droplets, contaminated objects or poor hand hygiene, eating that food can lead to an infection. But, unlike bacteria, the viruses don't multiply when on food.

Rotaviruses are still found in stool about eight days after the symptoms have died down. Noroviruses can still be detected up to 14 days later. These viruses are only rarely found in stool for longer than that, apart from in people with weakened immune systems: Noroviruses may still be found in their stool several months or even years after initial infection.

What vaccines are available?

Since August 2013, the German Standing Committee on Vaccination (STIKO) has recommended rotavirus vaccinations for infants under the age of six months. Two vaccines are available: Both are used as an oral vaccine that is swallowed, instead of an injection.

Depending on the vaccine used, two or three doses of the vaccine are given, with an interval of at least four weeks between the doses. The series of vaccinations should be started at the age of six to twelve weeks and then finished by the time the infant is 24 or 32 weeks old at the latest. The exact ages will depend on which vaccine is used. Experts estimate that a rotavirus vaccination will offer protection for about two years.

Current studies show that, within a period of up to two years,

- about 40 out of 1,000 children who are not vaccinated have a severe rotavirus infection, compared to only
- about 6 out of 1,000 children who are vaccinated.

Vaccination is not recommended for older children or adults. Side effects of rotavirus vaccinations include mild diarrhea, vomiting or a fever. But they usually pass quickly.

There is no norovirus vaccine.

What kinds of bacteria cause diarrhea?

Many different types of bacteria can cause infectious diarrhea. Most of them aren't a problem in Germany due to good general hygiene. In Germany, bacterial infectious diarrhea is most commonly caused by Campylobacter or Salmonella. In rare cases, diarrhea is caused by certain strains of Escherichia coli, such as EHEC (enterohemorrhagic Escherichia coli) or Yersinia.

Bacteria often multiply in food, and can't usually be detected based on a certain smell or taste. All types of bacteria can be spread from one person to another through direct contact or by touching contaminated objects as well.

Campylobacter

The most common form of bacterial diarrhea is caused by Campylobacter. These bacteria can live in raw meat - usually poultry, but also ground beef, for example. They are also sometimes found in raw milk (unpasteurized) or dairy products. It is also possible to pick up the bacteria from the stool of infected pets or contaminated waters.

In order to prevent Campylobacter infections, it is important to keep a clean and hygienic kitchen. Excess water from defrosting poultry or other types of meat should be disposed of immediately. It is important to cook meat well in order to make sure that any bacteria have been killed. Cutting boards used to prepare raw meat should be carefully cleaned each time they are used, and kitchen towels should be washed at a temperature of at least 60 degrees Centigrade.

Symptoms typically start two to five days after infection. They include

- fever, headache and muscle ache, followed by
- severe stomach ache and abdominal cramps,
- nausea and diarrhea.

In very rare cases, Campylobacter infections can cause complications like rheumatic joint inflammations or paralysis.

These problems usually last up to one week. But many infections run their course undetected. It can take between two to four weeks until the stool no longer contains bacteria. This may take even longer in people who have weakened immune systems.

Salmonella

Salmonella bacteria are most often transmitted through raw foods. Eggs, egg-based foods like mayonnaise or dessert mousse, raw meat, and undercooked meat products are the most common sources of Salmonella infections. These bacteria can also get into food as a result of poor hygiene in the kitchen, for example through the use of contaminated cutting boards. Salmonella symptoms start within six hours to three days after infection, and last several days. They include the following:

- Sudden diarrhea
- Headache and stomach ache
- Sometimes vomiting and a slight fever too

Very rarely, and mostly in people who have weakened immune systems, Salmonella infections can also cause blood poisoning (septicemia).

People who have been infected with Salmonella are often still contagious up to one month after the symptoms have disappeared. Young children and older people may still have bacteria in their stool for several weeks or months.

EHEC

EHEC (enterohemorrhagic Escherichia coli) can be spread through raw foods such as meat products that have not been cooked enough. The bacteria can also live on unwashed vegetables and in unpasteurized fruit juices or unpasteurized milk. The stool of infected cattle and other animals that chew the cud (e.g. goats, sheep) is also contagious. EHEC bacteria are sometimes found in bodies of water too.

EHEC infections are most common in children and infants. They can sometimes pass without causing any symptoms, but in most cases the following symptoms arise three to ten days after infection:

- Watery diarrhea
- Nausea, vomiting and stomach ache
- Sometimes fever as well

Very rarely, hemolytic uremic syndrome (HUS) may develop too. HUS is a blood clotting disorder that can damage the kidneys and become life-threatening.

The bacteria may still be present in stool for several weeks after the symptoms have cleared up. In very rare cases, EHEC can cause bloody diarrhea accompanied by stomach cramps.

Yersinia

Although rare, Yersinia can be spread through contaminated meat, milk or water. Pork that has not been thoroughly cooked is one main source of infection. Signs of yersiniosis include stomach ache, diarrhea and a moderate fever. The symptoms usually last 5 to 14 days.

Sources

Dalby-Payne JR, Elliott EJ. Gastroenteritis in children. BMJ Clin Evid 2011; 2011.

Gottlieb T, Heather CS. Diarrhoea in adults (acute). BMJ Clin Evid 2011; 02: 901.

Robert Koch-Institut (RKI). Campylobacter-Enteritis. RKI-Ratgeber für Ärzte. March 26, 2015.

Robert Koch-Institut (RKI). EHEC-Erkrankung. RKI-Ratgeber für Ärzte. March 26, 2015.

Robert Koch-Institut (RKI). Infektionsepidemiologisches Jahrbuch meldepflichtiger Krankheiten für 2014. March 01, 2015.

Robert Koch-Institut (RKI). Norovirus-Gastroenteritis. RKI-Ratgeber für Ärzte. July 26, 2008.

Robert Koch-Institut (RKI). Rotaviren-Gastroenteritis. RKI-Ratgeber für Ärzte. July 31, 2013.

Robert Koch-Institut (RKI). Salmonellose. RKI-Ratgeber für Ärzte. April 20, 2016.

Soares-Weiser K, Maclehose H, Bergman H, Ben-Aharon I, Nagpal S, Goldberg E et al. Vaccines for preventing rotavirus diarrhoea: vaccines in use. Cochrane Database Syst Rev 2012; (11): CD008521. [PubMed: 22336845]

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IQWiG Institute for Quality and Efficiency in Health Care

Media centre

Diarrhoeal disease

Fact sheet
Updated May 2017

Key facts

- Diarrhoeal disease is the second leading cause of death in children under five years old. It is both preventable and treatable.
- Each year diarrhoea kills around 525 000 children under five.
- A significant proportion of diarrhoeal disease can be prevented through safe drinking-water and adequate sanitation and hygiene.
- Globally, there are nearly 1.7 billion cases of childhood diarrhoeal disease every year.
- Diarrhoea is a leading cause of malnutrition in children under five years old.

Diarrhoeal disease is the second leading cause of death in children under five years old, and is responsible for killing around 525 000 children every year. Diarrhoea can last several days, and can leave the body without the water and salts that are necessary for survival. In the past, for most people, severe dehydration and fluid loss were the main causes of diarrhoea deaths. Now, other causes such as septic bacterial infections are likely to account for an increasing proportion of all diarrhoea-associated deaths. Children who are malnourished or have impaired immunity as well as people living with HIV are most at risk of life-threatening diarrhoea.

Diarrhoea is defined as the passage of three or more loose or liquid stools per day (or more frequent passage than is normal for the individual). Frequent passing of formed stools is not diarrhoea, nor is the passing of loose, "pasty" stools by breastfed babies.

Diarrhoea is usually a symptom of an infection in the intestinal tract, which can be caused by a variety of bacterial, viral and parasitic organisms. Infection is spread through contaminated food or drinking-water, or from person-to-person as a result of poor hygiene.

Interventions to prevent diarrhoea, including safe drinking-water, use of improved sanitation and hand washing with soap can reduce disease risk. Diarrhoea should be treated with oral rehydration solution (ORS), a solution of clean water, sugar and salt. In addition, a 10-14 day

supplemental treatment course of dispersible 20 mg zinc tablets shortens diarrheoa duration and improves outcomes.

There are three clinical types of diarrhoea:

- acute watery diarrhoea – lasts several hours or days, and includes cholera;
- acute bloody diarrhoea – also called dysentery; and
- persistent diarrhoea – lasts 14 days or longer.

Scope of diarrhoeal disease

Diarrhoeal disease is a leading cause of child mortality and morbidity in the world, and mostly results from contaminated food and water sources. Worldwide, 780 million individuals lack access to improved drinking-water and 2.5 billion lack improved sanitation. Diarrhoea due to infection is widespread throughout developing countries.

In low-income countries, children under three years old experience on average three episodes of diarrhoea every year. Each episode deprives the child of the nutrition necessary for growth. As a result, diarrhoea is a major cause of malnutrition, and malnourished children are more likely to fall ill from diarrhoea.

Dehydration

The most severe threat posed by diarrhoea is dehydration. During a diarrhoeal episode, water and electrolytes (sodium, chloride, potassium and bicarbonate) are lost through liquid stools, vomit, sweat, urine and breathing. Dehydration occurs when these losses are not replaced.

The degree of dehydration is rated on a scale of three.

1. Severe dehydration (at least two of the following signs):
    - lethargy/unconsciousness
    - sunken eyes
    - unable to drink or drink poorly
    - skin pinch goes back very slowly (≥2 seconds)
2. Some dehydration (two or more of the following signs):
    - restlessness, irritability
    - sunken eyes
    - drinks eagerly, thirsty
3. No dehydration (not enough signs to classify as some or severe dehydration).

Causes

Infection: Diarrhoea is a symptom of infections caused by a host of bacterial, viral and parasitic organisms, most of which are spread by faeces-contaminated water. Infection is more common when there is a shortage of adequate sanitation and hygiene and safe water for drinking,

cooking and cleaning. Rotavirus and *Escherichia coli*, are the two most common etiological agents of moderate-to-severe diarrhoea in low-income countries. Other pathogens such as *cryptosporidium* and *shigella* species may also be important. Location-specific etiologic patterns also need to be considered.

Malnutrition: Children who die from diarrhoea often suffer from underlying malnutrition, which makes them more vulnerable to diarrhoea. Each diarrhoeal episode, in turn, makes their malnutrition even worse. Diarrhoea is a leading cause of malnutrition in children under five years old.

Source: Water contaminated with human faeces, for example, from sewage, septic tanks and latrines, is of particular concern. Animal faeces also contain microorganisms that can cause diarrhoea.

Other causes: Diarrhoeal disease can also spread from person-to-person, aggravated by poor personal hygiene. Food is another major cause of diarrhoea when it is prepared or stored in unhygienic conditions.Unsafe domestic water storage and handling is also an important risk factor. Fish and seafood from polluted water may also contribute to the disease.

Prevention and treatment

Key measures to prevent diarrhoea include:

- access to safe drinking-water;
- use of improved sanitation;
- hand washing with soap;
- exclusive breastfeeding for the first six months of life;
- good personal and food hygiene;
- health education about how infections spread; and
- rotavirus vaccination.

Key measures to treat diarrhoea include the following:

- Rehydration: with oral rehydration salts (ORS) solution. ORS is a mixture of clean water, salt and sugar. It costs a few cents per treatment. ORS is absorbed in the small intestine and replaces the water and electrolytes lost in the faeces.
- Zinc supplements: zinc supplements reduce the duration of a diarrhoea episode by 25% and are associated with a 30% reduction in stool volume.
- Rehydration: with intravenous fluids in case of severe dehydration or shock.
- Nutrient-rich foods: the vicious circle of malnutrition and diarrhoea can be broken by continuing to give nutrient-rich foods – including breast milk – during an episode, and by giving a nutritious diet – including exclusive breastfeeding for the first six months of life – to children when they are well.

- Consulting a health professional , in particular for management of persistent diarrhoea or when there is blood in stool or if there are signs of dehydration.

WHO response

WHO works with Member States and other partners to:

- promote national policies and investments that support case management of diarrhoea and its complications as well as increasing access to safe drinking-water and sanitation in developing countries;
- conduct research to develop and test new diarrhoea prevention and control strategies in this area;
- build capacity in implementing preventive interventions, including sanitation, source water improvements, and household water treatment and safe storage;
- develop new health interventions, such as the rotavirus immunization; and
- help to train health workers, especially at community level.

For more information contact:
WHO Media centre
Telephone: +41 22 791 2222
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Jamison DT, Breman JG, Measham AR, et al., editors. Disease Control Priorities in Developing Countries. 2nd edition. Washington (DC): The International Bank for Reconstruction and Development / The World Bank; 2006. Co-published by Oxford University Press, New York.

Chapter 19 Diarrheal Diseases

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Diarrheal diseases remain a leading cause of preventable death, especially among children under five in developing countries. This chapter reviews and prioritizes a number of available interventions.

The normal intestinal tract regulates the absorption and secretion of electrolytes and water to meet the body's physiological needs. More than 98 percent of the 10 liters per day of fluid entering the adult intestines are reabsorbed (Keusch 2001). The remaining stool water, related primarily to the indigestible fiber content, determines the consistency of normal feces from dry, hard pellets to mushy, bulky stools, varying from person to person, day to day, and stool to stool. This variation complicates the definition of *diarrhea,* which by convention is present when three or more stools are passed in 24 hours that are sufficiently liquid to take the shape of the container in which they are placed. The frequent passage of formed stool is not diarrhea (Black and Lanata 2002). Although young nursing infants tend to have five or more motions per day, mothers know when the stooling pattern changes and their children have diarrhea (Ronsmans, Bennish, and Wierzba 1988). The interval between two episodes is also arbitrarily defined as at least 48 hours of normal stools. These definitions enable epidemiologists to count incidence, relapses, and new infections.

Transmission

Diarrhea is caused by infectious organisms, including viruses, bacteria, protozoa, and helminths, that are transmitted from the stool of one individual to the mouth of another, termed *fecaloral transmission.* Some are well known, others are recently discovered or emerging new agents, and presumably many remain to be identified. They differ in the route from the stool to the mouth and in the number of organisms needed to cause infection and illness. Among bacteria, the ability to

survive stomach acid is an important determinant of the inoculum size required to cause illness. For example, *Shigella* bacteria are resistant to low pH, and a few thousand organisms suffice, which are readily transferred by direct person-to-person contact or through contamination of inanimate objects, such as a cup. In contrast, bacteria readily killed by acid, such as *Vibrio cholerae,* require millions of organisms to cause illness, and therefore must first multiply in food or water to an infectious dose. Some pathogens, such as rotavirus, display a sharp host species preference, and others have a broad host range. Among *Salmonella* bacteria, certain bio-serotypes are adapted to infect animals and pose no threat to humans, and others are adapted to humans and do not infect animals. The majority, however, are not adapted to a specific host and can infect either humans or domestic animals, thus facilitating transmission of these organisms to humans. Less than a dozen of the more than 2,500 individual *Salmonella* cause the majority of human infections, reflecting the requirement for genes that encode essential virulence factors.

The ability to identify virulence genes and their products has led to new molecular approaches to epidemiology and diagnosis, and undoubtedly will lead to new measures to prevent and treat diarrhea. Molecular methods also allow the separation of organisms that otherwise appear to be identical. Nonpathogenic *Escherichia coli* in normal stool cannot be separated from diarrhea-causing *E. coli* by standard methods; however, identification of virulence genes or factors distinguishes five groups of *E. coli* that cause illnesses ranging from cholera-like watery diarrhea to neonatal diarrhea, persistent diarrhea, and bloody diarrhea (Nataro and Kaper 1998).

Laboratory Diagnosis

Etiologic diagnosis of diarrhea is valuable for public health interventions and case management. Microbiological culture and microscopy remain the standard, despite their limited sensitivity. Their effectiveness is further reduced by antibiotic use, and patients with severe illness are more likely both to be cultured and to have taken antibiotics. Even when cultures are positive, the delay in laboratory identification limits their cost-effectiveness for managing individual patients. The information is always epidemiologically and clinically important; however, during epidemics, culturing every patient is unnecessary when the causative organism is known. Antimicrobial resistance data are essential to guide initial antibiotic choices.

New rapid tests to detect inflammatory mediators or white or red blood cells in stool offer the promise of distinguishing between secretory and inflammatory disease and optimizing case management (Huicho and others 1996). High background levels, probably from frequent infections, limits the use of such tests in developing countries, where they would be most useful (Gill and others 2003).

Simple microscopy for protozoa or helminths can be quick and effective when the proper sample is obtained and a well-trained technician is available to examine a fresh specimen, but these prerequisites are often not available in developing countries. Newer immunological and nucleic acid–based tests to detect pathogen-specific factors hold great promise for all diarrhea agents, but

they are too expensive or require specialized instrumentation and trained technicians. For the foreseeable future, then, syndromic diagnosis will be the norm.

Syndromic Diagnosis

Three major diarrhea syndromes exist. They are acute watery diarrhea, which results in varying degrees of dehydration; persistent diarrhea, which lasts 14 days or longer, manifested by malabsorption, nutrient losses, and wasting; and bloody diarrhea, which is a sign of the intestinal damage caused by inflammation. The three are physiologically different and require specific management. Syndromic diagnosis provides important clues to optimal management and is both programmatically and epidemiologically relevant.

Acute watery diarrhea can be rapidly dehydrating, with stool losses of 250 milliliters per kilogram per day or more, a quantity that quickly exceeds total plasma and interstitial fluid volumes and is incompatible with life unless fluid therapy can keep up with losses. Such dramatic dehydration is usually due to rotavirus, enterotoxigenic *E. coli*, or *V. cholerae* (the cause of cholera), and it is most dangerous in the very young.

Persistent diarrhea is typically associated with malnutrition, either preceding or resulting from the illness itself (Ochoa, Salazar-Lindo, and Cleary 2004). Even though persistent diarrhea accounts for a small percentage of the total number of diarrhea episodes, it is associated with a disproportionately increased risk of death. In India, persistent diarrhea accounted for 5 percent of episodes but 14 percent of deaths, and a mortality rate three times higher than briefer episodes (Bhan and others 1989). In Pakistan, persistent diarrhea accounted for 8 to 18 percent of episodes but 54 percent of deaths (Khan and others 1993). In Bangladesh, persistent diarrhea associated with malnutrition was responsible for nearly half of diarrhea deaths, and the relative risk for death among infants with persistent diarrhea and severe malnutrition was 17 times greater than for those with mild malnutrition (Fauveau and others 1992). Persistent diarrhea occurs more often during an episode of bloody diarrhea than an episode of watery diarrhea, and the mortality rate when bloody diarrhea progresses to persistent diarrhea is 10 times greater than for bloody diarrhea without persistent diarrhea. HIV infection is another risk factor for persistent diarrhea in both adults and children (Keusch and others 1992). Management focuses on overcoming the nutritional alterations initiated by persistent diarrhea.

Bloody diarrhea, defined as diarrhea with visible or microscopic blood in the stool, is associated with intestinal damage and nutritional deterioration,often with secondary sepsis.Some dehydration—rarely severe—is common, as is fever. Clinicians often use the term *bloody diarrhea* interchangeably with dysentery; however,dysentery is a syndrome consisting of the frequent passage of characteristic, small-volume, bloody mucoid stools; abdominal cramps; and tenesmus, a severe pain that accompanies straining to pass stool. Those features show the severity of the inflammation. Agents that cause bloody diarrhea or dysentery can also provoke a form of diarrhea that clinically is not bloody diarrhea, although mucosal damage and inflammation are present, and fecal blood and white blood cells are usually detectable by microscopy. The release

of host-derived cytokines causes fever, altering host metabolism and leading to the breakdown of body stores of protein, carbohydrate, and fat and the loss of nitrogen and other nutrients. Those losses must be replenished during convalescence, which takes much longer than the illness does to develop. For these reasons, bloody diarrhea calls for management strategies that are markedly different than those for watery or persistent diarrhea. New bouts of infection that occur before complete restoration of nutrient stores can initiate a downward spiral of nutritional status terminating in fatal protein-energy malnutrition (Keusch 2003).

Diarrhea, Environment, and Poverty

Diarrheal disease affects rich and poor, old and young, and those in developed and developing countries alike, yet a strong relationship exists between poverty, an unhygienic environment, and the number and severity of diarrheal episodes—especially for children under five.

Poverty is associated with poor housing, crowding, dirt floors, lack of access to sufficient clean water or to sanitary disposal of fecal waste, cohabitation with domestic animals that may carry human pathogens, and a lack of refrigerated storage for food—all of which increase the frequency of diarrhea. Poverty also restricts the ability to provide age-appropriate, nutritionally balanced diets or to modify diets when diarrhea develops so as to mitigate and repair nutrient losses. The impact is exacerbated by the lack of adequate, available, and affordable medical care. Thus, the young suffer from an apparently never-ending sequence of infections, rarely receive appropriate preventive care, and too often encounter the health care system when they are already severely ill.

Although the presence of blood in the stool is a recognized danger signal, prompting more urgent care seeking, even these patients either are not treated early or receive poor medical care. Ironically, the poor spend considerable amounts on inappropriate care and useless drugs purchased from local shops and untrained practitioners. If antibiotics are properly prescribed, poverty often limits the purchase of a full course of treatment or leads to cessation of treatment as soon as symptoms improve, even though the infection has not been cured.

Public Health Significance of Diarrheal Illnesses

Continuing surveillance and longitudinal studies allow tracking of current levels and trends in diarrhea incidence and mortality and provide the basis for future projections and for evaluations of different control strategies.

Morbidity

Comparisons over time of the global burden of diarrheal diseases have revealed secular trends and demonstrated the impact of public health interventions (Bern and others 1992; Kosek, Bern, and Guerrant 2003; Snyder and Merson 1982). The long-term consequences of diarrhea are only now being systematically assessed and are not reflected in earlier studies.

Reviews in 1992 (Bern and others) and 2003 (Kosek, Bern, and Guerrant) are similar in many ways—for example, assessing morbidity at least twice weekly—but differ significantly in the use of different sources for data on children under five and in the inclusion of studies differing in design and data collection protocols (and only the later study includes data from China). Remarkably, the estimated median incidence of diarrheal disease in children under five in developing countries has not changed much since the early 1990s (figure 19.1): 3.2 episodes per child per year in 2003 (Parashar and others 2003) compared with 3.5 episodes per child per year in 1993 (Jamison and others 1993). However, many fewer surveys were available for the most recent review (31 in 20 countries) compared with the 1993 consensus (276 in 60 countries), reflecting diminished support for the systematic collection of incidence data. Incidence rates in Sub-Saharan Africa and Latin America are clearly greater than in Asia or the Western Pacific, while subject to greater data limitations from individual countries. Incidence continues to show a peak in infants age 6 to 11 months, dropping steadily thereafter.

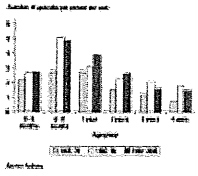

Figure 19.1

Median Age-specific Incidences for Diarrheal Episodes per Child per Year from Three Reviews of Prospective Studies in Developing Areas, 1955–2000

The seemingly lower estimates of diarrheal incidence before 1980 (Snyder and Merson 1982) are likely due to methodological differences. These estimates are not precise or directly comparable; the trends are most relevant. The persistently high rates of diarrhea throughout the 1990s despite intensive efforts at control, particularly among children age 6 to 24 months, is of particular concern. Early childhood diarrhea during periods of critical postnatal development may have long-term effects on linear growth and on physical and cognitive functions.

Data on the incidence of shigellosis, the principal cause of bloody diarrhea in developing countries, are even more limited. Kotloff and others' (1999) review of studies on *Shigella* infection estimates that more than 113 million episodes occur every year in children under five in developing countries, or 0.2 episodes of bloody diarrhea per year caused by *Shigella* species.

Mortality

Bern and others (1992); Kosek, Bern, and Guerrant (2003); and Snyder and Merson (1982) also estimate diarrheal mortality using data from longitudinal studies with active surveillance in place (figure 19.2). The estimate before 1980 was 4.6 million deaths per year. This estimate dropped to 3.3 million per year between 1980 and 1990 and to 2.6 million per year between 1990 and 2000. Two other studies (Parashar and others 2003; Boschi-Pinto and Tomaskovic forthcoming) report even lower figures for 1990–2000: 2.1 million and 1.6 million deaths per year, respectively. Methodological variations (inclusion of studies with different designs and data collection methods and inclusion of data from China, different sources for estimating the number of children under five, and different strategies for calculating mortality for this age group) may

account for some of the striking differences. However, the end of the 20th century witnessed significant reductions in diarrheal deaths in children under five.

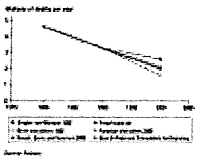

Figure 19.2

Estimates of Diarrhea Mortality, 1975–2000

This steady decline in diarrheal mortality, despite the lack of significant changes in incidence, is most likely due to modern case management (introduced since the 1980s) and to the improved nutrition of infants and children. Major recommendations include the following:

- counseling mothers to begin suitable home-prepared rehydration fluids immediately on the onset of diarrhea
- treating mild to moderate dehydration early with oral rehydration solution (ORS), reserving intravenous electrolytes for severe dehydration
- continuing breastfeeding and complementary foods during diarrhea and increasing intake afterward
- limiting antibiotic use to cases of bloody diarrhea or dysentery and avoiding antidiarrheal and antimotility drugs
- advising mothers to increase fluids and continue feeding during future episodes.

Victora and others' (2000) review provides evidence that this strategy, and especially oral rehydration therapy (ORT), has influenced the outcome of dehydrating diarrhea. Data from 99 national surveys carried out in the mid 1990s and compiled by the United Nations Children's Fund (UNICEF) increasingly show that diarrhea patients are appropriately managed in most parts of the world,with overall use rates of ORS or recommended home fluids reaching 49 percent. Country case studies in Brazil, the Arab Republic of Egypt, Mexico, and the Philippines showed a dramatic reduction of diarrhea mortality as ORT use rates increased from close to zero in the early 1980s to 35 percent in Brazil, 50 percent in Egypt, 81 percent in Mexico, and 33 percent in the Philippines in the early 1990s. Hospital admissions for diarrhea also plummeted (Victora and others 2000). As mortality attributable to acute dehydration decreased, the proportionate mortality associated with persistent diarrhea increased. Data from Brazil and Egypt suggest that even relatively low ORT use rates can positively affect mortality,because ORT use tends to be much higher for severe illness (Victora and others 2000).

Worldwide mortality caused by *Shigella* infection is estimated to be 600,000 deaths per year among children under five, or a quarter to a third of all diarrhea-related mortality in this age group (Kotloff and others 1999). Because mortality caused by bloody diarrhea is not tracked separately, it is difficult to assess the impact of standard case management recommendations, and disease-specific trends cannot be tracked. In the past few years, however, data from the

International Centre for Diarrheal Disease Research, in Bangladesh, have shown a marked decrease in the rate of hospitalization caused by *Shigella,* especially *S. dysenteriae* type 1, the most severe form of shigellosis. Some investigators have suggested that this decrease may be because *Shigella* infections are now in the low part of a 10-year cycle (Legros 2004). The observed change could also be explained by better case management with more efficacious antimicrobials. More comprehensive, syndrome-specific surveillance data will be required if rational control priorities are to be set, because the options for dehydrating and bloody diarrheal diseases differ substantially.

Despite national data that indicate a significant decline in mortality (Baltazar, Nadera, and Victora 2002; Miller and Hirschhorn 1995), diarrheal diseases remain among the five top preventable killers of children under five in developing countries and among the top two in many.

Long-Term Consequences

The long-term consequences of diarrheal diseases remain poorly studied, and analyses of global trends have not considered them. Niehaus and others (2002) recently evaluated the long-term consequences of acute diarrheal disease on psychomotor and cognitive development in young children. Following a cohort of 47 children in a poor urban community in northeastern Brazil, they correlated the number of diarrheal episodes in the first two years of life with measures of cognitive function obtained four to seven years later. They found a significant inverse correlation (average decrease of 5.6 percent) between episodes of early diarrheal disease and overall intellectual capacity and concentration, even when controlling for maternal education or helminth infection, which are known to be independent predictors of malnutrition and cognitive defects. Test scores were also 25 to 65 percent lower in children with an earlier history of persistent diarrhea.

Recent evidence suggests that genetic factors may also be involved in the developmental response to repeated diarrhea (Oria and others 2005). Better and more sensitive assessment tools are needed to define the relationships between diarrheal diseases and developmental disorders and to calculate individual and societal costs and the cost-effectiveness of interventions. In addition, early childhood malnutrition resulting from any cause reduces physical fitness and work productivity in adults (Dobbing 1990).

Preventive Strategies

Strategies for controlling diarrheal diseases have remained substantially unchanged since the 1993 edition of this volume (Martinez, Phillips, and Feachem 1993). The World Health Organization (WHO 2004) recently reevaluated these interventions to determine the extent to which they have been effectively implemented and their effect.

Promotion of Exclusive Breastfeeding

Exclusive breastfeeding means no other food or drink, not even water, is permitted, except for supplements of vitamins and minerals or necessary medicines. The optimal duration of exclusive breastfeeding is six months (WHO 2001). A meta-analysis of three observational studies in developing countries shows that breastfed children under age 6 months are 6.1 times less likely to die of diarrhea than infants who are not breastfed (WHO Collaborative Study Team 2000). Exclusive breastfeeding protects very young infants from diarrheal disease in two ways: first, breast milk contains both immune (specific) and nonimmune (nonspecific) antimicrobial factors; second, exclusive breastfeeding eliminates the intake of potentially contaminated food and water. Breast milk also provides all the nutrients most infants need up to age 6 months. When exclusive breastfeeding is continued during diarrhea, it also diminishes the adverse impact on nutritional status.

Those data underpin the global campaign to promote exclusive breastfeeding for the first six months of life by increasing both the initiation and the duration of exclusive breastfeeding. The strategies include the following:

- hospital policies and actions to encourage breastfeeding and discourage bottle feeding
- counseling and education provided by peers or health workers
- mass media and community education
- mothers' support groups.

Interventions focused on hospital practices apply where most women deliver in such facilities. Such interventions have shown up to a 43 percent increase in exclusive breastfeeding with good institutional policies and retraining of health staff (Westphal and others 1995). Interventions focused on education and counseling increase exclusive breastfeeding by 4 to 64 percent (Sikorski and others 2002). Peer-counseled women are less likely to stop exclusive breastfeeding than are those who receive either professional support or no support, and their infants are 1.9 to 2.9 times less likely to have diarrhea (Barros and others 1995; Haider and others 1996). No large-scale peer counseling programs exist; therefore, feasibility is unknown. Community-based mother's support groups are sustainable, but they have low coverage and are biased toward women who are already motivated to breastfeed (Bhandari and others 2003). Mass media can be effective where media coverage is high, where production skills are good, and where it addresses barriers to breastfeeding instead of just proclaiming its benefits. We found no studies that examined the relationship between breastfeeding promotion and diarrheal disease mortality; however, estimates suggest such promotion could decrease all-cause mortality in children under five by 13 percent (Jones and others 2003).

Maternal HIV infection has put a new wrinkle in the "breast is best" dogma because of the risk of transmission of infection to the infant (De Cock and others 2000). There is a trade-off, however, between the risk of mortality associated with replacement feeding and the risk of HIV infection, especially where safe replacement feeding is difficult. For women who are HIV-negative or

whose status is unknown, WHO currently recommends exclusive breastfeeding for at least six months (WHO 2000). The best option for HIV-positive women is acceptable, affordable, sustainable, and safe replacement feeding. If this option is not possible, there are four alternatives: (a) heat-treated breast milk, (b) HIV-negative wet nurses, (c) uncontaminated donor milk, or (d) exclusive breastfeeding for six months and rapid discontinuation thereafter (WHO 2003).

A danger of promoting replacement feeding is that uninfected women or women with unknown HIV status will adopt the practice. Even in high-prevalence communities, the best option for women with unknown status for the overall health of their children appears to be exclusive breastfeeding for six months. In Coutsoudis and others' (1999) cohort study in South Africa, the risk of mother-to-infant transmission of HIV after three months of exclusive breastfeeding was similar to that with no breastfeeding and significantly lower than that with mixed feeding. Providing antiretroviral therapy to the mother should significantly extend the period of safe breastfeeding for the initially HIV-negative infants of HIV-positive mothers.

Improved Complementary Feeding Practices

Ideally, complementary foods should be introduced at age 6 months, and breastfeeding should continue for up to two years or even longer to increase birth intervals (WHO 2003). There is a strong inverse association between appropriate, safe complementary feeding and mortality in children age 6 to 11 months. Malnutrition is an independent risk predictor for the frequency and severity of diarrheal illness. There is a vicious cycle in which sequential diarrheal disease leads to increasing nutritional deterioration, impaired immune function, and greater susceptibility to infection. The cycle may be broken by interventions to decrease infection incidence to reduce malnutrition (Keusch and Scrimshaw 1986) or improving nutritional status to reduce the burden of infection (Victora and others 1999).

Improved feeding practices to prevent or treat malnutrition could save as many as 800,000 lives per year (Jones and others 2003). Pediatricians have long been aware of an increase in diarrhea incidence during weaning from exclusive breast milk feeding. Microbial contamination of complementary foods (Mondal and others 1996) and nutritionally inadequate diets during and after diarrhea episodes (Badruddin and others 1991) increase the risk. Contamination of complementary foods can potentially be reduced by educating caregivers on hygienic practices (Guptill and others 1993), improving home food storage (English and others 1997), fermenting foods to reduce pathogen multiplication (Kimmons and others 1999), or ingesting nonpathogenic probiotic microorganisms that colonize the gut and help resist pathogens (Allen and others 2004). These interventions have not been evaluated at scale in communities, and effectiveness trials are lacking.

We could not find any reports on the effects of complementary feeding interventions on mortality. Five efficacy trials to improve the intake of complementary foods noted a net increase in energy intake of between 65 and 300 kilocalories a day and improvements of 0.25 to 0.46

standard deviations in weight-for-age and 0.04 to 0.35 standard deviations in height-for-age (Caulfield, Huffman, and Piwoz 1999). By extrapolation, this increment in growth should translate into a 2 to 13 percent reduction in deaths associated with malnutrition (Black and others 1995).

Brown, Dewey, and Allen (1998) reviewed experiences with large-scale complementary feeding interventions in 14 countries. They demonstrate that it is possible to provide nutritionally improved complementary foods in diverse cultural settings and that poor mothers are willing to prepare new foods their children will eat. However, caregivers face considerable time and resource constraints in providing such foods, especially during episodes of illness. A pilot study in Brazil that implemented nutritional counseling through the Integrated Management of Childhood Illness Program reported significant weight gain in children age one year or more, but not in younger children (Santos and others 2001).

Unfortified complementary foods do not meet all essential micronutrient requirements. Although improvements in vitamin A status do not significantly reduce the incidence of diarrhea and other common childhood illnesses, vitamin A supplementation can reduce the frequency of severe diarrhea (Barreto and others 1994) and mortality (Ross and others 1995). Chapter 28 describes interventions to promote vitamin A intake. Zinc supplementation also reduces the incidence of diarrhea.

Rotavirus Immunization

Almost all infants acquire rotavirus diarrhea early in life, and rotavirus accounts for at least one-third of severe and potentially fatal watery diarrhea episodes—primarily in developing countries, where an estimated 440,000 vaccine-preventable rotavirus deaths per year occur (Parashar and others 2003), compared with about a dozen in a developed country such as France (Fourquet and others 2003). An effective rotavirus vaccine would have a major effect on diarrhea mortality in developing countries.

In 1998, a quadrivalent Rhesus rotavirus–derived vaccine that reduced the frequency of severely dehydrating rotavirus—but not the overall incidence of rotavirus infections—was licensed in the United States (Glass and others 1999). It was cost-effective, even at US$100 for a full course of immunization, when direct economic losses resulting from health care expenses and indirect costs of lost productivity and wages for the caretakers were considered (Tucker and others 1998). The strategy was clear: use the high-priced vaccine routinely in industrial countries to subsidize its use in developing countries. However, postmarketing surveillance detected an apparent increase in a relatively rare event, intussusception, a condition in which the intestine telescopes on itself, causing a potentially serious obstruction (CDC 1999a). The relationship was strongest with the first dose of vaccine given with the first or second dose of diphtheria-pertussis-tetanus vaccine (Peter and others 2002), although this was counterbalanced by a decrease in the incidence of intussusception in older children (Murphy and others 2003).

The overall reduced incidence in immunized infants compared with nonimmunized infants in these studies suggested that the vaccine may actually protect against later adverse events. Nonetheless, the ensuing controversy led to a reversal of the recommendation for universal immunization in the United States and withdrawal of the vaccine from the market, precluding the possibility of its deployment in developing countries (CDC 1999b). Because very young infants are less prone to develop intussusception, initial immunization at birth might have been entirely safe.

Despite this setback, efforts to produce an effective and safe rotavirus vaccine continue. The Rhesus vaccine has been relicensed to another manufacturer, and new vaccines derived from human or bovine rotavirus are undergoing field trials in developing countries (Dennehy 2005). A monovalent human rotavirus vaccine was introduced in Mexico in 2005. The entry of both China and India into rotavirus vaccine development and their potential for manufacturing quality vaccines at low cost will make it easier to deploy an effective vaccine where it is really needed.

Cholera Immunization

Endemic cholera is primarily a pediatric disease, although adult morbidity and mortality are significant, especially during epidemics. The lethality of cholera is due to the physiological consequences of rapid and profound dehydration. Oral rehydration therapy has dramatically improved survival and reduced the cost of treatment. Wherever parenteral and oral rehydration is readily available, even in epidemic situations, a cholera mortality rate above 1 percent indicates failure of the public health system to provide appropriate case management.

A vaccine would further reduce the morbidity and mortality associated with cholera in endemic areas; however, developing an effective, safe vaccine has proven difficult. The most immunogenic and protective vaccines tested thus far are administered orally. Two such vaccines have been licensed: an attenuated live vaccine and a heat-killed vaccine combined with recombinant cholera toxin B subunit, which functions as an immunoadjuvant (Graves and others 2000; Ryan and Calderwood 2000). Many developing countries can produce the killed vaccine, especially without cholera toxin B. Current oral cholera vaccines appear to be safe and offer reasonable protection for a limited period; however, the main users have been individual travelers from industrial countries who may be exposed to the risk of cholera while traveling in endemic areas.

The use of oral cholera vaccine in mass vaccination campaigns as an adjunct to good case management, disposal of fecal waste, and access to safe water during humanitarian disasters has recently been reviewed (WHO 1999). Analysis of an outbreak in Micronesia suggested that a single dose was useful in limiting the spread of cholera (Calain and others 2004). But because ORT is so inexpensive and useful in preventing death, immunization is not a high priority. Only Vietnam routinely deploys cholera vaccine.

Operational information on the costs, logistics, and availability of vaccines for use by global programs and on the vulnerable populations in high-risk settings who would benefit from cholera

vaccine remains limited. Although scientific interest in a cholera vaccine remains high, its public health priority is less than that of a vaccine for rotavirus or *Shigella*.

Measles Immunization

Measles is known to predispose to diarrheal disease secondary to measles-induced immunodeficiency. Feachem and Koblinsky (1983) estimate that measles vaccine given to 45 to 90 percent of infants would prevent 44 to 64 percent of measles cases, 0.6 to 3.8 percent of diarrheal episodes, and 6 to 26 percent of diarrheal deaths among children under five. Global measles immunization coverage is now approaching 80 percent, and the disease has been eliminated from the Americas, raising hopes for global elimination in the near future (GAVI 2005), with a predictable reduction in diarrhea as well.

Improved Water and Sanitary Facilities and Promotion of Personal and Domestic Hygiene

Human feces are the primary source of diarrheal pathogens. Poor sanitation, lack of access to clean water, and inadequate personal hygiene are responsible for an estimated 90 percent of childhood diarrhea (WHO 1997). Promotion of hand washing reduces diarrhea incidence by an average of 33 percent (Huttly, Morris, and Pisani 1997); it works best when it is part of a package of behavior change interventions. Effects on mortality have not been demonstrated. However, the required behavior change is complex, and significant resources are needed. Antiseptic soaps are more costly than plain hand soap and confer little advantage. Washing hands after defecating or handling children's feces and before handling food is recommended, but it entails an average of 32 hand washes a day and consumes 20 liters of water (Graef, Elder, and Booth 1993). If soap is too costly, ash or mud can be used, but access to water remains essential (Esrey 1996).

Six rigorous observational studies demonstrated a median reduction of 55 percent in all-cause child mortality associated with improved access to sanitation facilities (Esrey, Feachem, and Hughes 1985). The greatest effect of improving sanitation systems will be in areas of high population density and wherever the entire community, rather than single households, adopts the intervention. Current technology can be costly and difficult to maintain, and in some settings it is simply not feasible.

Case Management

Two recent advances in managing diarrheal disease—(a) newly formulated ORS containing lower concentrations of glucose and salts and (b) zinc supplementation—used in combination with promotion of exclusive breastfeeding, general nutritional support, and selective and appropriate use of antibiotics, can further reduce the number of diarrheal deaths among children. Families and communities are key to achieving case management goals by making these recommendations routine practice in homes and health facilities.

New Oral Rehydration Solutions

For more than 25 years, UNICEF and WHO have recommended a single formulation of glucose-based ORS considered optimal for cholera, irrespective of cause or age group affected. This formulation has proven effective and without significant adverse effects (Ruxin 1994), but because watery stools persist and duration of diarrhea is not reduced, mothers' and health workers' acceptance of current ORSs has been suboptimal.

During the past 20 years, efforts to improve ORS to treat dehydration from all types of diarrhea and reduce stool output or duration have continued—for example, by reducing the sodium content in line with sodium losses for noncholera diarrhea. Compared with standard ORS, lower sodium and glucose ORS reduces stool output, vomiting, and the need for intravenous fluids (Hanh, Kim, and Garner 2001). If household use increases, new ORS can reduce childhood deaths from non-cholera diarrhea (Duggan and others 2004), and it appears to be as effective as standard ORS for children or adults with cholera. A WHO expert group now recommends that ORS containing 75 milliequivalents of sodium and 75 millimoles of glucose per liter (total osmolarity, 245 milliosmoles per liter) be used everywhere (WHO and UNICEF 2004).

Zinc Supplementation

A review of all relevant clinical trials indicates that zinc supplements given during an episode of acute diarrhea reduce both duration and severity and could prevent 300,000 deaths in children each year (Black 2003). WHO and UNICEF now recommend that all children with acute diarrhea be given zinc in some form for 10 to 14 days during and after diarrhea (10 milligrams per day for infants younger than 6 months and 20 milligrams per day for those older than 6 months) (WHO and UNICEF 2004).

Pilot studies in Brazil, Egypt, Ethiopia, India, Mali, Pakistan, and the Philippines that include zinc routinely in the management of acute diarrhea not only show an improvement over ORS alone but also suggest two important new effects: (a) use rates of ORS increase, and (b) use rates of antidiarrheals and antimicrobials decrease significantly (Baqui and others 2004). Large community-based studies are being implemented to corroborate these potentially important findings.

Management of Bloody Diarrhea

The primary treatment for shigellosis, the most common and severe cause of bloody diarrhea, is antimicrobials. The choice of effective, safe, and inexpensive oral drugs for use in developing countries has, however, become problematic because of the increasing prevalence of antimicrobial drug resistance (Salam 1998). Tetracycline, ampicillin, and the fixed-ratio combination of trimethoprim and sulfmethoxazole, once used as first-line treatment, are no longer reliably effective. When epidemic dysentery caused by multidrug-resistant *S. dysenteriae* type 1 appeared in Africa and Asia in the 1980s and 1990s, nalidixic acid was pressed into use (Salam and Bennish 1988). Nalidixic acid is a drug used primarily for urinary tract infections, but

it is also effective against *Shigella*. Clinical responses were initially excellent, but with continued use, resistance to nalidixic acid has been increasing in many parts of the world (Dutta and others 2003).

A number of other drugs have been tested and shown effective, including ceftriaxone, azithromycin, pivmecillinam, and some new generation 5-fluoroquinolones, such as ciprofloxacin (Salam 1998). Because of its effectiveness, safety, ease of administration by the oral route, short course, and low cost (US$0.10 for a three-day course for a 15-kilogram child), ciprofloxacin is the current drug of choice for shigellosis (Zimbasa Dysentery Study Group 2002). However, ciprofloxacin-resistant strains are already appearing (Pazhani and others 2004), and it is only a matter of time before resistance becomes widespread, especially if the drug is readily available and indiscriminately used. Because of these concerns, development of a vaccine for *Shigella* is critical. The Diseases of the Most Impoverished initiative, supported by the Bill & Melinda Gates Foundation (Nossal 2003), which promotes vaccine development for *Shigella*, cholera, and typhoid, is a significant advance since the previous edition of this volume.

Cost-Effectiveness of Interventions

Cost-effectiveness ratios of diarrheal disease interventions were calculated by World Bank region in terms of disability-adjusted life years (DALYs) averted for a model population of 1 million, following the standardized guidelines of the Disease Control Priorities Project for economic analyses (see chapter 15). Europe and Central Asia were excluded because data were lacking owing to the low prevalence of disease. Input variables included (a) region-specific diarrhea morbidity rates adapted from Kosek, Bern, and Guerrant (2003); (b) region-specific underlying mortality rates and age structures provided by the Disease Control Priorities Project; (c) median intervention effectiveness rates (that is, percentage of diarrheal morbidity reduction and percentage of diarrheal mortality reduction); and (d) median per capita intervention costs gathered from the literature and from personal communications (table 19.1).

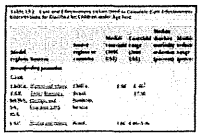

Table 19.1

Cost and Effectiveness Values Used to Calculate Cost-Effectiveness Ratios for Select Interventions for Diarrhea for Children under Age Five.

Because approximately 90 percent of all cases in the developing world occur in children under five, the analysis focused on this age group alone. Uniform intervention effectiveness rates were assumed for all regions because region-specific information was not available. Regional variations in cost-effectiveness were due to regional variations in the prevalence of diarrheal disease, in the diarrhea-attributable morbidity and mortality, and in the intervention cost, where region-specific information was available.

Disability-adjusted life years are averted through the avoidance of cotemporaneous disability and mortality attributable to diarrhea. We did not consider long-term developmental and cognitive effects of childhood diarrhea or the external benefits of interventions unrelated to diarrhea (for instance, benefits of measles immunization unrelated to diarrhea or other health benefits of improved public water and sanitation). Therefore, our estimates err on the conservative side.

We explored two general categories of interventions: early interventions that take place within the first year of life—breastfeeding promotion and immunizations for rotavirus (with the prototype Rhesus reassortant tetravalent vaccine), cholera (with live oral vaccine), and measles—and other interventions that treat an entire cohort of children under five simultaneously (improved water and sanitation). For early interventions, cost-effectiveness ratios were calculated by considering the cost of treating all newborns in a single year and the benefits (DALYs averted) from those treatments that occur over the first five years of life. These benefits include avoided mortality that allows individuals to live to the expected life expectancy for the region. Other interventions included ORT and improved water and sanitation infrastructure. Because a single year of these interventions yields only cotemporaneous benefits—because effectively treated individuals do not necessarily live to life expectancy given that they are likely to be reinfected the next year—we calculated cost-effectiveness of a five-year intervention. Analysis of a five-year intervention enabled us to consider the case in which an entire cohort of children age zero to four avoids early childhood diarrheal mortality because of the intervention and receives the benefit of living to life expectancy.

Disability and deaths averted for those benefiting from improved water and sanitation were calculated from only the fraction of the model populations currently without access. For each region, the proportion of rural and urban children age zero to four currently without access to improved water and sanitation was calculated using region-specific information from *World Bank Development Indicators* (World Bank 2002) for 2000. Infrastructure improvements for rural and urban populations were considered separately because of differences in infrastructure type and cost, although the same effectiveness rates were used for both.

The per child treatment costs and effectiveness rates used are presented in table 19.1. Cost per treatment of ORT varied widely depending on the type and method of ORT implemented. Oral rehydration therapy can be as inexpensive as US$0.02 per child treated—the cost of a home remedy with sugar and salt. However, treatment can become substantially more expensive if commercially manufactured ORS is used or if there are substantial personnel or infrastructure costs (Martinez, Phillips, and Feachem 1993). Finally, our analysis considered only long-run marginal costs (which vary with the number of individuals treated) and did not include fixed costs of initiating a program where none currently exists.

Figure 19.3 shows the cost-effectiveness of all interventions over the first five years of life. Two interventions administered during the first year of life—breastfeeding promotion (US$930 per DALY) and measles immunization (US$981 per DALY)—were the most cost-effective. ORT (US$1,062 per DALY) and water and sanitation in rural areas (US$1,974 per DALY) were the

next most cost-effective, but only if they were implemented continuously for five years, thereby allowing an entire cohort of effectively treated children age zero to four to survive past the age at which they are most at risk for diarrheal infection, disability, and mortality. Rotavirus immunization (US$2,478 per DALY), cholera immunization (US$2,945 per DALY), and water and sanitation in urban areas (US$6,396 per DALY) were the least cost-effective.

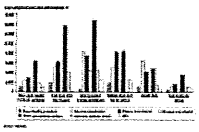

Figure 19.3

Cost-Effectiveness: Intervention at Birth through Age 5 with Benefits That Occur over Five Years (age 0–4)

Among the early interventions, breastfeeding promotion was less effective than other interventions but also less expensive than rotavirus and measles vaccination (table 19.1). Cholera vaccination was less expensive than breastfeeding promotion, but it was also many times less effective because of the significantly higher prevalence of diarrhea that is not related to cholera—making cholera vaccination the least cost-effective of the early interventions considered. Oral rehydration therapy and water and sanitation interventions were more effective than breastfeeding and vaccination interventions in reducing morbidity and mortality caused by diarrhea, but they were also more expensive. However, our analysis for water and sanitation did not consider the benefits of this intervention other than those related to health, and the high cost-effectiveness ratio is more a limitation of our methodology than of the intervention itself.

The high cost-effectiveness ratio for ORT is attributable to the high variation in reported treatment costs, which may inflate the median cost used in this analysis (table 19.2). Given the range of reported treatment costs (table 19.1), the cost-effectiveness ratio of ORT could be as low as US$4 per DALY or as high as US$2,124 per DALY in low- and middle-income countries. High variation in reported treatment costs results in high variation in cost-effectiveness for the other regions as well. There remains little doubt, however, about the effect of widespread use of ORT on diarrhea morbidity and mortality and about the associated direct and indirect cost savings for treatment and hospitalization.

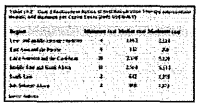

Table 19.2

Cost-Effectiveness Ratios of Oral Rehydration Therapy Interventions Based on Minimum, Median, and Maximum per Capita Costs (2001 US$/DALY).

Research Agenda

Good evidence now supports the view that promoting ORT in conjunction with other key interventions, preventive as well as curative, has had a large role in the marked reduction in deaths of children caused by diarrhea (Victora and others 2000). Preventive strategies—such as breastfeeding, improving complementary feeding and using micronutrient supplementation or

fortification, and increasing coverage with the full set of Expanded Programme on Immunization vaccines (especially measles vaccine)—are all useful and effective (GAVI 2005). Failure to separately track the full impact of bloody diarrhea—especially *Shigella* infection—on morbidity and mortality or to effectively implement good clinical management (including guidelines for and control over the use of antibiotics) has contributed to the continuing burden of bloody diarrhea and dysentery worldwide and the alarming increase in antibiotic resistance. The challenges for the next decade will be to increase or ensure universal appropriate implementation of these interventions in developing countries and to avoid a situation in which they compete for funding and staff time. Delivery of good-quality services is essential, and much remains to be learned through research before this requirement can be met.

Other interventions, such as vaccines against rotavirus, *Shigella,* or cholera, are either not yet available or not ready for universal administration. Progress toward the development of these vaccines, with the highest priority for the first two, is encouraging, but further investments in research and development will be required before large-scale implementation of these interventions can be considered. The cost of these vaccines will remain a major constraint for poor people, who cannot pay for the costs of development and ensure reasonable profits for industry. However, increased public investment in fundamental and applied research, vaccine purchase schemes, and development of low-cost, high-quality manufacturing capacity in developing countries may change the prevailing dynamics. By creating public-private partnerships for vaccine development, organized as targeted product development programs, the public sector, private foundations, and industry are taking steps toward these goals.

Because of the fecal-oral transmission of enteric pathogens, improving the supply of safe water and the ability to safely dispose of fecal waste are the best ways to reduce the burden of morbidity and mortality. However, major investments and critical improvements in water and sanitary waste disposal on the necessary scale are unlikely to occur in the next decade or two. Local low-tech solutions can be useful, and enhanced efforts to find ways to improve water cleanliness at the point of use and to build simple latrines that will be used consistently are needed (chapter 41). However, in the face of HIV and the attention being given to tuberculosis and malaria, coordinated efforts to build safe water and sanitation capacity at the local level, one village at a time, that are sufficient to significantly influence the burden of illness are unlikely—even though many more infants and children die each year of preventable and treatable diarrhea than of HIV/AIDS.

The cycle of research, followed by implementation, followed by research has enabled the development of improved tools to manage diarrheal diseases—tools that have the potential to further drive down diarrhea mortality. The challenge is to achieve high coverage and good practice with ORT and correct diarrhea case management, including antimicrobial and nutrition interventions. Interventions to integrate health care through programmatic initiatives such as the Integrated Management of Childhood Illness program, critically evaluated elsewhere in this book (chapter 63), could be essential to ensure this high coverage. Some concern remains that in low-

resource settings such targeted vertical programs may be abandoned, to the detriment of the goals for disease burden reduction that they were established to achieve.

The challenge posed by the case management of bloody diarrhea is a different matter. Until a vaccine is available, the keystone for managing bloody diarrhea will continue to be the early use of effective antimicrobial agents. That is made difficult by increasing drug resistance, aided by the widespread indiscriminate and inappropriate use of antimicrobials, and the increasingly difficult task of finding a safe, inexpensive, and effective oral agent and then ensuring that the drug is given in a clinically optimal manner. From a technical perspective, the development of a vaccine against *Shigella* infections is still in its infancy and in need of greater investment. For both watery and bloody diarrhea, the challenge of developing drugs to normalize the pathophysiology caused by the infection remains a scientific challenge and a distant hope.

Conclusions

Existing interventions to prevent or treat diarrheal diseases have proven their efficacy in reducing mortality, but a major challenge for the next 10 years will be to scale up these interventions to achieve universal utilization coverage. The United Nations Millennium Development Goal to reduce the mortality rate among children under five by two-thirds by 2015 will be easier to attain if the scale-up goals are reached. New products and tools could significantly improve the efficacy of these interventions—for example, rapid specific diagnostics, new treatment strategies based on reversing the pathophysiology of the infection, simple and effective ways to produce clean water and control human waste, and vaccines to prevent illness. However, these products and tools will not become widely available in time to influence the achievement of the Millennium Development Goals. Continued investment in diarrheal disease research across the spectrum of basic, social and behavioral, and applied investigations is, therefore, essential, including expanded behavioral research to understand how parents assess risk and how actionable health messages can be presented in different cultures and settings.

References

1. Allen S. J., Okoko B., Martinez E., Gregorio G., Dans L. F. Probiotics for Treating Infectious Diarrhea. Cochrane Database Systematic Reviews. 2004;(2):CD003048. [PubMed: 15106189]
2. Badruddin S., Islam A., Hendricks K. H., Bhutta Z. A., Shaikh S. A., Snyder J. D., Molla A. M. Dietary Risk Factors Associated with Acute and Persistent Diarrhea in Karachi, Pakistan. American Journal of Clinical Nutrition. 1991;51:745–49. [PubMed: 1897481]
3. Baltazar J. C., Nadera D. P., Victora C. G. Evaluation of the National Control of Diarrhoeal Diseases Programme in the Philippines, 1980–93. Bulletin of the World Health Organization. 2002;80:637–43. [PMC free article: PMC2567577] [PubMed: 12219155]
4. Baqui A. H., Black R. E., El Arifeen S., Yunus M., Zaman K., Begum N. et al. Zinc Therapy for Diarrhoea Increased the Use of Oral Rehydration Therapy and Reduced the

Use of Antibiotics in Bangladeshi Children. Journal of Health, Population, and Nutrition. 2004;22(4):440–42. [PubMed: 15663177]

5. Barreto M. L., Santos L. M. P., Assis A. M. O., Araujo M. P. N., Farenzena G. G., Santos P. A. B., Fiaccone R. L. Effect of Vitamin A Supplementation on Diarrhoea and Acute Lower Respiratory-Tract Infections in Young Children in Brazil. Lancet. 1994;344:228–31. [PubMed: 7913157]
6. Barros F. C., Semer T. C., Tonioli Filho S., Tomasi E., Victora C. G. The Impact of Lactation Centers on Breastfeeding Patterns, Morbidity, and Growth: A Birth Cohort Study. Acta Paediatrica. 1995;84:1221–26. [PubMed: 8580615]
7. Bern C., Martines J., de Zoysa I., Glass R. I. The Magnitude of the Problem of Diarrhoeal Disease: A Ten-Year Update. Bulletin of the World Health Organization. 1992;70:705–14. [PMC free article: PMC2393403] [PubMed: 1486666]
8. Bhan M. K., Bhandari N., Sazawal S., Clemens J., Raj P. Descriptive Epidemiology of Persistent Diarrhoea among Young Children in Rural North India. Bulletin of the World Health Organization. 1989;67:281–88. [PMC free article: PMC2491248] [PubMed: 2670297]
9. Bhandari N., Bahl R., Mazumdar S., Martines J., Black R. E., Bhan M. K. Infant Feeding Study Group: Effect of Community-Based Promotion of Exclusive Breastfeeding on Diarrhoeal Illness and Growth: A Cluster Randomised Controlled Trial. Lancet. 2003;361:1418–23. [PubMed: 12727395]
10. Black M. M., Dubowitz H., Hutcheson J., Berenson-Howard J., Starr R. H. Jr. A Randomized Clinical Trial of Home Intervention for Children with Failure to Thrive. Pediatrics. 1995;95:807–14. [PubMed: 7539121]
11. Black R. E. Zinc Deficiency, Infectious Disease, and Mortality in the Developing World. Journal of Nutrition. 2003;133(Suppl. 1):1485S–89S. [PubMed: 12730449]
12. Black, R. E., and C. F. Lanata. 2002. "Epidemiology of Diarrheal Diseases in Developing Countries." In *Infections of the Gastrointestinal Tract,* 2nd ed., ed. M. J. Blaser, P. D. Smith, J. I. Ravdin, H. B. Greenberg, and R. L. Guerrant, 11–29. Philadelphia: Lippincott, Williams, and Wilkins.
13. Boschi-Pinto, C., and L. Tomaskovic. Forthcoming. "Deaths from Diarrhoeal Diseases among Children under Five Years of Age in the Developing World: A Review." *Bulletin of the World Health Organization.*
14. Brown, K., K. Dewey, and L. Allen. 1998. *Complementary Feeding of Young Children in Developing Countries: A Review of Current Scientific Knowledge.* WHO/NUT/98.1. Geneva: World Health Organization.
15. Calain P., Chaine J. P., Johnson E., Hawley M. L., O'Leary M. J., Oshitani H., Chaignat C. L. Can Oral Cholera Vaccination Play a Role in Controlling a Cholera Outbreak? Vaccine. 2004;22:2444–51. [PubMed: 15193408]
16. Caulfield L. E., Huffman S. L., Piwoz E. G. Interventions to Improve Intake of Complementary Foods by Infants 6 to 12 Months of Age in Developing Countries: Impact

on Growth and on the Prevalence of Malnutrition and Potential Contribution to Child Survival. Food and Nutrition Bulletin. 1999;20:183–200.

17. CDC (U.S. Centers for Disease Control and Prevention). Intussusception among Recipients of Rotavirus Vaccine: United States, 1998–1999. Morbidity and Mortality Weekly Reports. 1999a;48:577–81. [PubMed: 10428095]
18. ———1999bSuspension of Rotavirus Vaccine after Reports of Intussusceptions: United States, 1999 Morbidity and Mortality Weekly Reports 53(34):786–89. [PubMed: 15343145]
19. Cookson S. T., Stamboulian D., Demonte J., Quero L., De Arquiza C. M., Aleman A. et al. A Cost-Benefit Analysis of Programmatic Use of CVD 103-Hgr Live Oral Cholera Vaccine in a High-Risk Population. International Journal of Epidemiology. 1997;26:212–19. [PubMed: 9126522]
20. Coutsoudis A., Pillay K., Spooner E., Kuhn L., Coovadia H. M. Influence of Infant-Feeding Patterns on Early Mother-to-Child Transmission of HIV-1 in Durban, South Africa: A Prospective Cohort Study. Lancet. 1999;354:471–76. [PubMed: 10465172]
21. De Cock K. M., Fowler M. G., Mercier E., de Vincenzi I., Saba J., Hoff E. et al. Prevention of Mother-to-Child HIV Transmission in Resource-Poor Countries: Translating Research into Policy and Practice. Journal of the American Medical Association. 2000;283:1175–82. [PubMed: 10703780]
22. Dennehy P. H. Rotavirus Vaccines: An Update. Current Opinion in Pediatrics. 2005;17:88–92. [PubMed: 15659970]
23. de Zoysa I., Feachem R. G. Interventions for the Control of Diarrhoeal Diseases among Young Children: Rotavirus and Cholera Immunization. Bulletin of the World Health Organization. 1985;63:569–83. [PMC free article: PMC2536413] [PubMed: 3876173]
24. Dobbing J. Early Nutrition and Later Achievement. Proceedings of the Nutrition Society. 1990;49:103–18. [PubMed: 2236081]
25. Duggan C., Fontaine O., Pierce N. F., Glass R. I., Mahalanabis D., Alam N. H. et al. Scientific Rationale for a Change in the Composition of Oral Rehydration Solution. Journal of the American Medical Association. 2004;291:2628–31. [PubMed: 15173155]
26. Duke T. Haemophilus influenzae Type B Vaccine in Papua New Guinea: What Can We Expect, and How Should We Determine Priority for Child Health Interventions? Papua and New Guinea Medical Journal. 1999;42:1–4. [PubMed: 11061000]
27. Dutta S., Dutta D., Dutta P., Matsushita S., Bhattacharya S. K., Yoshida S. *Shigella dysenteriae* Serotype 1, Kolkata, India. Emerging Infectious Diseases. 2003;9:1471–74. [PMC free article: PMC3035535] [PubMed: 14718096]
28. English R. M., Badcock J. C., Giay T., Ngu T., Waters A. M., Bennett S. A. Effect of Nutrition Improvement Project on Morbidity from Infectious Diseases in Preschool Children in Vietnam: Comparison with Control Commune. British Medical Journal. 1997;315:1122–25. [PMC free article: PMC2127738] [PubMed: 9374884]
29. Esrey S. A. Water, Waste, and Well-Being: A Multicountry Study. American Journal of Epidemiology. 1996;143:608–23. [PubMed: 8610678]

30. Esrey S. A., Feachem R., Hughes J. M. Interventions for the Control of Diarrhoeal Diseases among Young Children: Improving Water Supplies and Excreta Disposal Facilities. Bulletin of the World Health Organization. 1985;63:757–72. [PMC free article: PMC2536385] [PubMed: 3878742]
31. Esrey S. A., Potash J. B., Roberts L., Shiff C. Effects of Improved Water Supply and Sanitation on Ascariasis, Diarrhoea, Dracunculiasis, Hookworm Infection, Schistosomiasis, and Trachoma. Bulletin of the World Health Organization. 1991;69:609–21. [PMC free article: PMC2393264] [PubMed: 1835675]
32. Fauveau V., Henry F. J., Briend A., Yunus M., Chakraborty J. Persistent Diarrhea as a Cause of Childhood Mortality in Rural Bangladesh. Acta Paediatrica Supplement. 1992;381:12–14. [PubMed: 1421926]
33. Feachem R. G. A., Koblinsky M. A. Interventions for the Control of Diarrhoeal Diseases among Young Children: Measles Immunization. Bulletin of the World Health Organization. 1983;61:641–52. [PMC free article: PMC2536152] [PubMed: 6414730]
34. ———1984Interventions for the Control of Diarrhoeal Diseases among Young Children: Promotion of Breast-Feeding Bulletin of the World Health Organization 62271–91. [PMC free article: PMC2536296] [PubMed: 6610496]
35. Fourquet F., Desenclos J. C., Maurage C., Baron S. Acute Gastroenteritis in Children in France: Estimates of Disease Burden through National Hospital Discharge Data. Archives of Pediatrics. 2003;10:861–68. [PubMed: 14550973]
36. GAVI (Global Alliance for Vaccines and Immunization). 2005."Outcomes: Most Recent Data on the Impact of Support from GAVI/The Vaccine Fund and the Work of GAVI Partners." http://www.vaccinealliance.org/General_Information /About_alliance/progupdate.php.
37. Gill C., Lau J., Gorbach S. L., Hamer D. H. Diagnostic Accuracy of Stool Assays for Inflammatory Bacterial Gastroenteritis in Developed and Resource-Poor Countries. Clinical Infectious Diseases. 2003;37:365–75. [PubMed: 12884161]
38. Glass R. I., Bresee J. S., Parashar U. D., Holman R. C., Gentsch J. R. First Rotavirus Vaccine License: Is There Really a Need? Acta Paediatrica Supplement. 1999;88:2–8. [PubMed: 10088904]
39. Graeff, J. A., J. P. Elder, and E. M. Booth. 1993. *Communication for Health and Behavior Change: A Developing Country Perspective*. San Francisco, CA: Jossey Bass.
40. Graves P., Deeks J., Demicheli V., Pratt M., Jefferson T. Vaccines for Preventing Cholera. Cochrane Database Systematic Reviews. 2000;(4):CD000974. [PubMed: 11034693]
41. Guptill K. S., Esrey S. A., Oni G. A., Brown K. H. Evaluation of a Face-to-Face Weaning Food Intervention in Kwara State, Nigeria: Knowledge, Trial, and Adoption of a Home-Prepared Weaning Food. Social Science and Medicine. 1993;36:665–72. [PubMed: 8456336]
42. Haider R., Islam A., Hamadani J., Amin N. J., Kabir I., Malek M. A. et al. Breastfeeding Counselling in a Diarrhoeal Hospital. Bulletin of the World Health Organization. 1996;74:173–79. [PMC free article: PMC2486910] [PubMed: 8706233]

43. Hanh S. K., Kim Y. J., Garner P. Reduced Osmolarity Oral Rehydrations Solution for Treating Dehydration Due to Diarrhoea in Children: A Systematic Review. British Medical Journal. 2001;323:81–85. [PMC free article: PMC34542] [PubMed: 11451782]
44. Horton S., Claquin P. Cost-Effectiveness and User Characteristics of Clinic-Based Services for the Treatment of Diarrhea: A Case Study in Bangladesh. Social Science and Medicine. 1983;17:721–29. [PubMed: 6410515]
45. Horton S., Sanghvi T., Phillips M., Fielder J., Perez-Escamilla R., Lutter C. et al. Breastfeeding Promotion and Priority Setting in Health. Health Policy and Planning. 1996;11:156–68. [PubMed: 10158457]
46. Huicho L., Campos M., Rivera J., Guerrant R. L. Fecal Screening Tests in the Approach to Acute Infectious Diarrhea: A Scientific Overview. Pediatric Infectious Disease. 1996;15:486–94. [PubMed: 8783344]
47. Huttly S. R., Morris S. S., Pisani V. Prevention of Diarrhoea in Young Children in Developing Countries. Bulletin of the World Health Organization. 1997;75:163–74. [PMC free article: PMC2486931] [PubMed: 9185369]
48. Islam M. A., Mahalanabis D., Majid N. Use of Rice-Based Oral Rehydration Solution in a Large Diarrhea Treatment Centre in Bangladesh: In-House Production, Use, and Relative Cost. Journal of Tropical Medicine and Hygiene. 1994;97:341–46. [PubMed: 7966535]
49. Jamison, D. T., H. W. Mosley, A. R. Measham, and J. L. Bobadilla. 1993. *Disease Control Priorities in Developing Countries*. Oxford, U.K.: Oxford University Press.
50. Jones G., Steketee R. W., Black R. E., Bhutta Z. A., Morris S. S. the Bellagio Child Survival Study Group. . How Many Child Deaths Can We Prevent This Year? Lancet. 2003;362:65–71. [PubMed: 12853204]
51. Keusch, G. T. 2001. "Toxin-Associated Gastrointestinal Disease: A Clinical Overview." In *Molecular Medical Microbiology*, ed. M. Sussman, 1083–88. New York: Academic Press.
52. ———2003The History of Nutrition: Malnutrition, Infection, and Immunity Journal of Nutrition 133336S–40S. [PubMed: 12514322]
53. Keusch G. T., Scrimshaw N. S. Selective Primary Health Care: Strategies for Control of Disease in the Developing World—XXIII. The Control of Infection to Reduce the Prevalence of Infantile and Childhood Malnutrition. Reviews of Infectious Diseases. 1986;8:273–87. [PubMed: 3085193]
54. Keusch G. T., Thea D. M., Kamenga M., Kakanda K., Mbala M., Davachi F. Persistent Diarrhea Associated with AIDS. Acta Paediatrica Scandinavica. 1992;381(Suppl.):45–48. [PubMed: 1421940]
55. Khan S. R., Jalil F., Zaman S., Lindblad B. S., Karlberg J. Early Child Health in Lahore, Pakistan: X—Mortality. Acta Paediatrica Supplement. 1993;390:109–17. [PubMed: 8219459]
56. Kimmons J. E., Brown K. H., Lartey A., Collison E., Mensah P. P., Dewey K. G. The Effects of Fermentation and/or Vacuum Flask Storage on the Presence of Coliforms in Complementary Foods Prepared for Ghanaian Children. International Journal of Food Science and Nutrition. 1999;50:195–201. [PubMed: 10627835]

57. Kosek M., Bern C., Guerrant R. L. The Global Burden of Diarrhoeal Disease, as Estimated from Studies Published between 1992 and 2000. Bulletin of the World Health Organization. 2003;81:197–204. [PMC free article: PMC2572419] [PubMed: 12764516]
58. Kotloff K. L., Winickoff J. P., Ivanoff B., Clemens J. D., Swerdlow D. L., Sansonetti P. J. et al. Global Burden of Shigella Infections: Implications for Vaccine Development and Implementation of Control Strategies. Bulletin of the World Health Organizaton. 1999;77:651–66. [PMC free article: PMC2557719] [PubMed: 10516787]
59. Legros D. Shigellosis: Report of a Workshop. Journal of Health, Population and Nutrition. 2004;22:445–49. [PubMed: 15663179]
60. Martinez, J., M. Phillips, and R. G. A. Feachem. 1993. "Diarrheal Diseases." In *Disease Control Priorities in Developing Countries*, ed. D. Jamison, W. H. Moseley, A. R. Measham, and J. S. Bobadilla, 91–115. Oxford, U.K.: Oxford University Press.
61. Miller P., Hirschhorn N. The Effect of a National Control of Diarrheal Diseases Program on Mortality: The Case of Egypt. Social Science and Medicine. 1995;40:S1–30. [PubMed: 7638641]
62. Mondal S. K., Gupta P. G., Gupta D. N., Ghosh S., Sikder S. N., Rajendran K. et al. Occurrence of Diarrheal Diseases in Relation to Infant Feeding Practices in a Rural Community in West Bengal, India. Acta Paediatrica. 1996;85:1159–62. [PubMed: 8922075]
63. Murphy B. R., Morens D. M., Simonsen L., Chanock R. M., La Montagne J. R., Kapikian A. Z. Reappraisal of the Association of Intussusception with the Licensed Live Rotavirus Vaccine Challenges Initial Conclusions. Journal of Infectious Diseases. 2003;187(8):1301 –8. [PubMed: 12696010]
64. Narula D., Tiwari L., Puliyeh J. M. Rotavirus Vaccines. Lancet. 2004;364:245–46. [PubMed: 15262096]
65. Nataro J., Kaper J. B. Diarrheagenic Escherichia coli. Clinical Microbiological Reviews. 1998;11:142–201. [PMC free article: PMC121379] [PubMed: 9457432]
66. Niehaus M. D., Moore S. R., Patrick P. D., Derr L. L., Lorntz B., Lima A. A., Guerrant R. L. Early Childhood Diarrhea Is Associated with Diminished Cognitive Function 4 to 7 Years Later in Children in a Northeast Brazilian Shantytown. American Journal of Tropical Medicine and Hygiene. 2002;66:590–93. [PubMed: 12201596]
67. Nossal G. J. Gates, GAVI, the Glorious Global Funds, and More: All You Ever Wanted to Know. Immunology and Cell Biology. 2003;81:20–22. [PubMed: 12534942]
68. Ochoa T. J., Salazar-Lindo E., Cleary T. G. Management of Children with Infection-Associated Persistent Diarrhea. Seminars in Pediatric Infectious Diseases. 2004;15:229–36. [PubMed: 15494946]
69. Oria R. B., Patrick P. D., Zhang H., Lorntz B., de Castro Costa C. M., Brito G. A. et al. APOE4 Protects Cognitive Development in Children with Heavy Diarrhea Burdens in Northeast Brazil. Pediatric Research. 2005;57:310–16. [PubMed: 15611352]
70. Parashar U. D., Bresee J. S., Gentsch J. R., Glass R. I. Rotavirus. Emerging Infectious Diseases. 1998;4:561–70. [PMC free article: PMC2640254] [PubMed: 9866732]

71. Parashar U. D., Hummelman E. G., Bresee J. S., Miller M. A., Glass R. I. Global Illness and Deaths Caused by Rotavirus Disease in Children. Emerging Infectious Diseases. 2003;9:565–72. [PMC free article: PMC2972763] [PubMed: 12737740]
72. Pazhani G. P., Sarkar B., Ramamurthy T., Bhattacharya S. K., Takeda Y., Niyogi S. K. Clonal Multidrug-Resistant Shigella Dysenteriae Type 1 Strains Associated with Epidemic and Sporadic Dysenteries in Eastern India. Antimicrobial Agents and Chemotherapy. 2004;48:681–84. [PMC free article: PMC321556] [PubMed: 14742238]
73. Peter G., Myers M. G. the National Vaccine Advisory Committee, and the National Vaccine Program Office. . Intussusception, Rotavirus, and Oral Vaccines: Summary of a Workshop. Pediatrics. 2002;110:e67. [PubMed: 12456934]
74. Phillips, M. A., R. G. A. Feachem, and A. Mills. 1987. *Options for Diarrhoeal Disease Control: The Cost and Cost-Effectiveness of Selected Interventions for the Prevention of Diarrhea.* London: Evaluation and Planning Centre for Health Care.
75. Qualls N., Robertson R. Potential Uses of Cost Analyses in Child Survival Programs: Evidence from Africa. Health Policy and Planning. 1989;4:50–61.
76. Ronsmans C., Bennish M. L., Wierzba T. Diagnosis and Management of Dysentery by Community Health Workers. Lancet. 1988;8610:552–55. [PubMed: 2900931]
77. Ross D. A., Kirkwood B. R., Binka F. N., Arthur P., Dollimore N., Morris S. S. et al. Child Morbidity and Mortality Following Vitamin A Supplementation in Ghana: Time since Dosing, Number of Doses, and Time of Year. American Journal of Public Health. 1995;85:1246–51. [PMC free article: PMC1615567] [PubMed: 7661232]
78. Ruxin J. N. Magic Bullet: The History of Oral Rehydration Therapy. Medical History. 1994;38:363–97. [PMC free article: PMC1036912] [PubMed: 7808099]
79. Ryan E. T., Calderwood S. B. Cholera Vaccines. Clinical Infectious Diseases. 2000;31:561 –65. [PubMed: 10987721]
80. Salam M. A. Antimicrobial Therapy for Shigellosis: Issues on Antimicrobial Resistance. Japanese Journal of Medical Science and Biology. 1998;51(Suppl.):S43–62. [PubMed: 10211436]
81. Salam M. A., Bennish M. L. Therapy for Shigellosis: I. Randomized, Double-Blind Trial of Nalidixic Acid in Childhood Shigellosis. Journal of Pediatrics. 1988;113:901–7. [PubMed: 3054035]
82. Santos I., Victora C. G., Martines J., Goncalves H., Gigante D. P., Valle N. J., Pelto G. Nutrition Counseling Increases Weight Gain among Brazilian Children. Journal of Nutrition. 2001;131:2866–73. [PubMed: 11694610]
83. Shann F. Immunization: Dramatic New Evidence. Papua and New Guinea Medical Journal. 2000;43:24–29. [PubMed: 11407613]
84. Shepard, D. S., L. E. Brenzel, and K. T. Nemeth. 1986. "Cost-Effectiveness of Oral Rehydration Therapy for Diarrheal Diseases." Technical Note 86–26, Population, Health and Nutrition Department, World Bank, Washington, DC.
85. Sikorski J., Renfrew M. J., Pindoria S., Wade A. Support for Breastfeeding Mothers. Cochrane Database of Systematic Reviews. 2002;(1):CD001141. [PubMed: 11869593]

86. Snyder J. D., Merson M. H. The Magnitude of the Global Problem of Acute Diarrhoeal Disease: A Review of Active Surveillance Data. Bulletin of the World Health Organization. 1982;60:604–13. [PMC free article: PMC2536091] [PubMed: 6982783]
87. Tucker A. W., Haddix A. C., Bresee J. S., Holman R. C., Parashar U. D., Glass R. I. Cost-Effectiveness Analysis of a Rotavirus Immunization Program for the United States. Journal of the American Medical Association. 1998;279:1371–76. [PubMed: 9582045]
88. Victora C. G., Bryce J., Fontaine O., Monasch R. Reducing Deaths from Diarrhoea through Oral Rehydration Therapy. Bulletin of the World Health Organization. 2000;78:1246–55. [PMC free article: PMC2560623] [PubMed: 11100619]
89. Victora C. G., Kirkwood B. R., Ashworth A., Black R. E., Rogers S., Sazawal S., Campbell H. Potential Interventions for the Prevention of Childhood Pneumonia in Developing Countries: Improving Nutrition. American Journal of Clinical Nutrition. 1999;70:309–20. [PubMed: 10479192]
90. Westphal M. F., Taddei J. A., Venancio S. I., Bogus C. M. Breastfeeding Training for Health Professionals and Resultant Institutional Changes. Bulletin of the World Health Organization. 1995;73:461–68. [PMC free article: PMC2486792] [PubMed: 7554017]
91. WHO (World Health Organization). 1997. *Health and Environment in Sustainable Development Five Years after the Health Summit.* WHO/EHG/97.8. Geneva: WHO.
92. ———. 1999. "Potential Use of Oral Cholera Vaccines in Emergency Situations." WHO/CDS/CSR/EDC/99.4. Report of a WHO meeting, Geneva, May 12–13.
93. ———. 2000. "New Data on the Prevention of Mother-to-Child Transmission of HIV and Their Policy Implications." Report of a WHO technical consultation on behalf of a United Nations Population Fund, United Nations Children's Fund, and Joint United Nations Programme on HIV/AIDS interagency task team on mother-to-child transmission of HIV, Geneva, October 11–13.
94. ———. 2001. "The Optimal Duration of Exclusive Breastfeeding: Results of a WHO Systematic Review." http://www.who.int/inf-pr-2001/en/note2001-07.html. [PubMed: 11803895]
95. ———. 2003. *HIV and Infant Feeding—Framework for Priority Action.* Geneva: WHO.
96. ———. 2004. *Family and Community Practices That Promote Child Survival, Growth, and Development—A Review of Evidence*. Geneva: WHO.
97. WHO Collaborative Study Team. Effect of Breastfeeding on Infant and Child Mortality Due to Infectious Diseases in Less Developed Countries: A Pooled Analysis. Lancet. 2000;355:1104. [PubMed: 10841125]
98. WHO and UNICEF (United Nations Children's Fund). 2001. "Reduced Osmolarity Oral Rehydration Salts (ORS) Formulation." WHO/FCH/CAH/01.22. Report from a meeting of experts jointly organized by the United Nations Children's Fund and the World Health Organization, Geneva.
99. ———. 2004. *Joint Statement: Clinical Management of Acute Diarrhoea.* WHO/FCH/CAH/04.7. Geneva: WHO; New York: UNICEF.

100. World Bank. 2002. *World Development Indicators*. CD-ROM. Washington, DC: World Bank.
101. Zimbasa (Zimbabwe, Bangladesh, South Africa) Dysentery Study Group. Multicenter, Randomized, Double Blind Clinical Trial of Short Course versus Standard Course Oral Ciprofloxacin for Shigella Dysenteriae Type 1 Dysentery in Children. Pediatric Infectious Disease Journal. 2002;21:1136–41. [PubMed: 12488664]

Bookshelf ID: NBK11764 PMID: 21250340

Leptospirosis Information

Human-to-human transmission

An infected human will shed leptospires in their urine for a period both during and after the illness, and so can present a risk of infection to others but only in a specific way. General social interations are prefectly safe, as the bacteria are not airborne. Saliva is not considered a high risk, as the bacteria cannot tolerate the acidity of the human mouth for very long, so although we advise against it the risks from sharing food, cutlery or cups is very small. Items that can dry between uses, such as towels, are also of extremely low risk once they are dry – but handling very wet bedding, blood-soaked clothing or similar can present a risk.

The presence of bacteria in the urine means that leptospirosis is a sexually-transmitted

infection, since during intercourse there will be the opportunity for small volumes of urine to exchange between partners. There are several documented cases of human infection via sex, but insufficient data to show if a particular sexual practice is more or less risky (though any event where urine can come into contact with damaged skin is hazardous).

In humans, viable bacteria will be present in the urine after about 2 days from the exposure (day E2), and they will usually remain present for a few weeks after illness, but there are recorded cases of humans shedding the bacteria for up to 11 months. Treatment with antibiotics can reduce the symptoms and also reduce this shedding time, but in most patients their urine contains detectable bacteria for smany weeks after clinical recovery. Our advice is to assume some risk from urine for up to 12 months after the acute illness has faded.

The risks from direct blood transfer are actually less of an issue, as the bacteria are present in the blood for only a short time (typically they appear in noticeable levels at day E1 (first day after exposure), and reduce on or before day S10 (10th day of symptoms). It does remain a concern for medical staff handling samples and treating injuries, but due to the rapid death of leptospira when dried the usual blood-related hazards such as

needlestick injuries are far less important than when dealing with viruses.

Pregnant mothers may pass the infection on to their fetus, see below for information.

- Human testing methods
- Preventative medication (prophylatic antibiotics)
- Issues with human pregnancy
- Issues with children and infants
- Human vaccines
- Survival of leptospires in the environment
- Risks from contaminated food and drink
- Protecting employees in the workplace
- Where can I get information cards to carry?
- Overview of human leptospirosis – guide for the public
- Education and advice for teachers and schools
- Human-to-human transmission
- Getting infected
- Culturing leptospires
- News

Leptospirosis Information

Overview of leptospirosis

Any infection with the leptospira bacterium is called 'leptospirosis'. In a small number of rare cases, the infection causes serious damage to the body organs and jaundice. This severe form of the infection is called 'Weils Disease' after the doctor who first identified it. Tens of thousands of people contract the infection every year, and most recover completely with treatment. If untreated, the patient MAY recover, though if the infection is serious the patient may not survive the organ damage caused by the bacteria. The onset of symptoms is rapid, and in severe cases decline is equally rapid therefore **all patients should be identified and treated as soon as physically possible.**

An infection of leptospirosis resembles a cold or influenza infection in the initial stages. The incubation period is from 4 to 10 days,

LEPTOSPIROSIS INFORMATION:

depending on the method of infection and your susceptibility. Not all exposed persons catch the disease.

- Early symptoms are fever, chills, muscular aches and pains, loss of appetite, and nausea when lying down. These can easily be mistaken for influenza, menengitis or the classic physician's excuse, 'FUO' or Fever of Unknown Origin.
- Later symptoms include bruising of the skin, anaemia, sore eyes, nose bleeds and jaundice. The fever lasts for approximately five days, then a significant deterioration follows.

Symptoms vary from case to case, and with underlying medical condition. Often, exposure to leptospira is combined with exposure to other waterborne organisms and symptoms of other infections may also exist. The initial appearance of a fever is reliable and anyone experiencing a fever after exposure to high risk water should assume they have contracted the infection until proved otherwise.

If you show any of the above symptoms after exposure to high risk areas, it is extremely important to contact your doctor as soon as possible. You should always tell your doctor that you suspect Leptospira infection, as many general practitioners do not associate fever-

related symptoms to the infection without a helpful hint. If you miss or ignore the early symptoms and start seeing the later and more serious indications of the disease then it is advised that you present yourself directly to a hospital accident and emergency department, again stating that you suspect you have leptospirosis. The later symptoms are the result of serious damage to the organs of the body and require urgent treatment with antibiotics not usually available outside a hospital.

There are over 200 'serovars' of Leptospira bacterium that can cause the disease, and an infection from one strain will provide immunity but only to that strain. Exposure to other strains will still cause infection. It is usual for more than one strain to exist within a specific population of infected animals, so immunity to one type is no great advantage to reducing your risks

Treatment with antibiotics is only effective if started rapidly after symptoms develop.
The antibiotics of choice are only available via hospital doctors.
Kidney dialysis may be necessary in some cases.

- Human testing methods
- Preventative medication (prophylatic antibiotics)
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Recognition of leptospiral LPS by human TLR4. Patients with severe leptospirosis experience a cytokine storm characterized by high levels of IL-6, TNF-alpha, and IL-10. Patients with the HLA DQ6 allele are at higher risk of disease, suggesting a role for lymphocyte stimulation by a leptospiral superantigen. Leptospirosis typically presents as a nonspecific, acute febrile illness characterized by fever, myalgia, and headache and may be confused with other entities such as influenza and dengue fever. Newer diagnostic methods facilitate early diagnosis and antibiotic treatment. Patients progressing to multisystem organ failure have widespread hematogenous dissemination of pathogens. Nonoliguric (high output) renal dysfunction should be supported with fluids and electrolytes. When oliguric renal failure occurs, prompt initiation of dialysis can be life saving. Elevated bilirubin levels are due to hepatocellular damage and disruption of intercellular junctions between hepatocytes, resulting in leaking of bilirubin out of bile caniliculi. Hemorrhagic complications are common and are associated with coagulation abnormalities. Severe pulmonary hemorrhage syndrome due to extensive alveolar

Curr Top Microbiol Immunol. Author manuscript; available in PMC 2015 May 25.
Published in final edited form as:
Curr Top Microbiol Immunol. 2015; 387: 65–97.
doi: 10.1007/978-3-662-45059-8_5
PMCID: PMC4442676
NIHMSID: NIHMS690013

Leptospirosis in Humans

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Abstract

Go to:

Leptospirosis is a widespread and potentially fatal zoonosis that is endemic in many tropical regions and causes large epidemics after heavy rainfall and flooding. Infection results from direct or indirect exposure to infected reservoir host animals that carry the pathogen in their renal tubules and shed pathogenic leptospires in their urine. Although many wild and domestic animals can serve as reservoir hosts, the brown rat (*Rattus norvegicus*) is the most important source of human infections. Individuals living in urban slum environments characterized by inadequate sanitation and poor housing are at high risk of rat exposure and leptospirosis. The global burden of leptospirosis is expected to rise with demographic shifts that favor increases in the number of urban poor in tropical regions subject to worsening storms and urban flooding due to climate change. Data emerging from prospective surveillance studies suggest that most human leptospiral infections in endemic areas are mild or asymptomatic. Development of more severe outcomes likely depends on three factors: epidemiological conditions, host susceptibility, and pathogen virulence (Fig. 1). Mortality increases with age, particularly in patients older than 60 years of age. High levels of bacteremia are associated with poor clinical outcomes and, based on animal model and in vitro studies, are related in part to poor recognition of leptospiral LPS by human TLR4. Patients with severe leptospirosis experience a cytokine storm characterized by high levels of IL-6, TNF-alpha, and IL-10. Patients with the HLA DQ6 allele are at higher risk of disease, suggesting a role for lymphocyte stimulation by a leptospiral superantigen. Leptospirosis typically presents as a nonspecific, acute febrile illness characterized by fever, myalgia, and headache and may be confused with other entities such as influenza and dengue fever. Newer diagnostic methods facilitate early diagnosis and antibiotic treatment. Patients progressing to multisystem organ failure have widespread hematogenous dissemination of pathogens. Nonoliguric (high output) renal dysfunction should be supported with fluids and electrolytes. When oliguric renal failure occurs, prompt initiation of dialysis can be life saving. Elevated bilirubin levels are due to hepatocellular damage and disruption of intercellular junctions between hepatocytes, resulting in leaking of bilirubin out of bile caniliculi. Hemorrhagic complications are common and are associated with coagulation abnormalities. Severe pulmonary hemorrhage syndrome due to extensive alveolar

hemorrhage has a fatality rate of >50 %. Readers are referred to earlier, excellent summaries related to this subject (Adler and de la Peña-Moctezuma 2010; Bharti et al. 2003; Hartskeerl et al. 2011; Ko et al. 2009; Levett 2001; McBride et al. 2005).

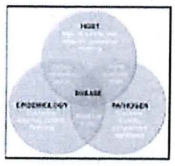


Fig. 1

Factors contributing to leptospirosis. Development of leptospirosis depends on three types of factors (*epidemiology*, *host*, and *pathogen*) and their interactions. Epidemiologic factors include sanitation, housing, rainfall, and whether flooding occurs. ...

1 Epidemiology and Surveillance

Go to:

1.1 Sources of Infection

Pathogenic leptospires are widespread in nature, reflecting maintenance in the kidneys of many wild and domestic reservoir hosts. The leptospiral life cycle involves shedding in the urine, persistence in the ambient environment, acquisition of a new host, and hematogenous dissemination to the kidneys through the glomerulus or peritubular capillaries. Once leptospires gain access to the renal tubular lumen of the kidney, they colonize the brush border of the proximal renal tubular epithelium, from which urinary shedding can persist for long periods of time without significant ill effects on the reservoir host. For this reason, leptospiral infection of the reservoir host can be considered a commensal relationship (Fig. 1).

Small mammals are the most important reservoirs, with large herbivores as additional significant sources of infection. Pathogenic *Leptospira* species have been isolated from hundreds of mammalian species, including bats and pinnipeds (see the chapter by W.A. Ellis, this volume). In addition, leptospires have been recovered from poikilothermic animals such as frogs and toads, and it is possible that these animals play a role in the circulation of leptospirosis in the environment, although they may not be significant reservoirs of human infection. Only a few studies have reported isolation of leptospires from amphibians (Babudieri et al. 1973; Everard et al. 1988; Gravekamp et al. 1991). However, the results justify further attempts to understand the role of amphibians in maintaining leptospires in nature (Adler and de la Peña-Moctezuma 2010; Bharti et al. 2003; Hartskeerl et al. 2011; Ko et al. 2009; Levett 2001; McBride et al. 2005; Felzemburgh et al. 2014).

Leptospirosis is primarily a zoonosis, with humans serving as accidental hosts. However, it is worth noting that transient leptospiral shedding does occur during human infection and human-to-human infection, although extremely rare, has occurred through sexual intercourse (Doeleman 1932; Harrison and Fitzgerald 1988) and during lactation (Bolin and Koellner 1988). Transplacental transmission may occur if infection occurs during pregnancy, resulting in abortion (Chung et al. 1963) or still birth (Coghlan and Bain 1969; Faine et al. 1984).

1.2 Transmission

Portals of entry include cuts and abrasions or mucous membranes such as the conjunctival, oral, or genital surfaces. Exposure may occur through either direct contact with an infected animal or through indirect contact via soil or water contaminated with urine from an infected animal. Individuals with occupations at risk for direct contact with potentially infected animals include veterinarians, abattoir workers, farm workers (particularly in dairy milking situations), hunters and trappers, animal shelter workers, scientists, and technologists handling animals in laboratories or during fieldwork. The magnitude of the risk depends on the local prevalence of leptospiral carriage and the degree and frequency of exposure. Most of these infections are preventable by the use of appropriate personal

protective equipment such as rubber boots, gloves, and protective eyewear. Since many of these infections are covered by occupational health and safety regulations, local risk assessments and training are essential (Steneroden et al. 2011).

Indirect contact with water or soil contaminated with leptospires is much more common, and can be associated with occupational, recreational, or avocational activities. In addition to the risks associated with outdoor work listed above, sewer work, military exercises, and farming in high rainfall tropical regions are recognized; the latter is by far the most important numerically. Agricultural workers at risk for leptospirosis include rice field workers, taro farmers, banana farmers, and sugar cane and pineapple field harvesters (Levett 2001). These occupations involve activities likely to result in exposure of cuts and abrasions to soil and water contaminated with the urine of rodents and other animals attracted to food sources. For example, banana workers accounted for two-thirds of the reported leptospirosis cases in a tropical region of Queensland, Australia (Smythe et al. 1997).

Recreational exposures include all freshwater water sports including caving (Self et al. 1987), canoeing (Waitkins 1986), kayaking (Jevon et al. 1986; Shaw 1992), rafting (Wilkins et al. 1988), and triathlons (Morgan et al. 2002; Sejvar et al. 2003). The importance of this type of exposure has increased over the past 20 years as the popularity of adventure sports and races has increased, and also because the relative cost of travel to exotic destinations has decreased (Lau et al. 2010a). Competitive events create the potential for large outbreaks; 80 and 98 leptospirosis cases occurred as part of the 2000 Eco-challenge competition (Fig. 2a) (Sejvar et al. 2003) and 1998 Springfield triathlon (Morgan et al. 2002), respectively. Participants in international events may become ill after having returned home, often to multiple destination countries, which complicates recognition and investigation of outbreaks. In many series, the incidence of leptospirosis is much higher in males than females (Everard et al. 1992; Guerra-Silveira and Abad-Franch 2013; Katz et al. 2011). However, it seems likely that gender differences in leptospirosis incidence are due entirely to exposure-related bias, as reports of leptospirosis outbreaks related to athletic events where males and females have similar levels of exposure have found no significant effects of gender on development of illness (Morgan et al. 2002; Sejvar et al. 2003).

Fig. 2
Epidemiologic settings for leptospirosis. **a** A high proportion of contestants in the 2000 Eco-Challenge multisport race held in Malaysian Borneo developed leptospirosis. Of 189 participants contacted by the Centers for Disease Control, 80 (42 %) met the ...

Avocational exposures are by far the most important exposures, affecting millions of people living in tropical regions. As illustrated in Fig. 2b, lack of adequate sanitation and poor housing combine to exacerbate the risk of exposure to leptospires in both rural and urban slum communities (Bharti et al. 2003; Felzemburgh et al. 2014; Hotez et al. 2008; Reis et al. 2008). The role of poor housing is also suggested by the study of Maciel et al. (2008), which showed a greatly increased risk (odds ratio 5.29) of leptospirosis exposure among individuals who live in the same household as a leptospirosis patient. These factors are most likely surrogates for rat exposure, as proximity to uncollected trash and sighting of rats increased the risk of leptospirosis among residents of urban slums (Reis et al. 2008). The recognition of large outbreaks following excess rainfall events (Ahern et al. 2005; Dechet et al. 2012; Ko et al. 1999; Zaki and Shieh 1996) led to the labeling of leptospirosis as an emerging infectious disease two decades ago (Levett 1999). More recently, the interaction of urbanization and climate change has been identified as a significant risk for both increased incidence and increasing frequency of outbreaks of leptospirosis (Lau et al. 2010b). The need for interdisciplinary research to understand the

effects of anthropogenic change and its effect on the epidemiology of leptospirosis has been proposed (Vinetz et al. 2005).

1.3 Global Burden of Disease

Early studies of leptospirosis incidence concentrated on occupational disease, primarily in developed countries related to leptospirosis in livestock animals (Alston and Broom 1958; Faine et al. 1999). As the importance of the disease in tropical countries became better recognized, guidelines were developed for the diagnosis and control of leptospirosis (Faine 1982). As diagnostic methods became more widely available, numerous epidemiologic studies were reported from many countries. An initial attempt to gather global data on the incidence of leptospirosis was published over 15 years ago (WHO 1999). Based on global data collected by International Leptospirosis Society surveys, the incidence was estimated to be 350,000–500,000 severe leptospirosis cases annually (Ahmed et al. 2012). Despite these efforts, the global burden of leptospirosis was felt to be largely underestimated for a number of reasons, including the fact that the vast majority of countries either lack a notification system or notification is not mandatory (Ahmed et al. 2012). To address these shortcomings, the WHO established the Leptospirosis Burden Epidemiology Reference Group (LERG) (Abela-Ridder et al. 2010). The LERG met for the first time in 2009 and for a second time in 2010. The specific objectives of the second LERG meeting were: (1) To review and appraise the revised systematic review of mortality, morbidity, and disability from human leptospirosis; (2) To review a draft disease transmission model for leptospirosis and provide technical input for the further development and refinement of the model; (3) To assemble preliminary estimates of the disease burden; (4) To identify gaps in knowledge and research; and (5) To advise WHO on the next steps for estimation of the burden of human leptospirosis and the implications for policy. The resulting LERG report included a systematic literature review that estimated the overall global annual incidence of endemic and epidemic human leptospirosis at 5 and 14 cases per 100,000 population, respectively (WHO 2011). Endemic human leptospirosis rates varied by region from 0.5/100,000 population in Europe to 95/100,000 population in Africa.

2 Pathology

Go to:

The first step in the pathogenesis of leptospirosis is penetration of tissue barriers to gain entrance to the body. Potential portals of entry include the skin via a cut or abrasion and the mucous membranes of the conjunctivae or oral cavity. The importance of the oral mucosa as a portal of entry is indicated by a number of studies that found that swallowing while swimming in contaminated water is a risk factor for infection (Corwin et al. 1990; Lingappa et al. 2004; Stern et al. 2010).

The second step in pathogenesis is hematogenous dissemination. Unlike other pathogenic spirochetes such as *B. burgdorferi* and *T. pallidum*, which cause skin lesions indicating establishment of infection in the skin, pathogenic leptospires make their way into the bloodstream and persist there during the leptospiremic phase of the illness. Results from inoculation of blood into leptospiral medium and detection of leptospiremia by quantitative PCR are more likely to be positive during the first 8 days of fever (Agampodi et al. 2012) prior to antibody formation and clearance of organisms from the bloodstream. Quantitative PCR has documented leptospiremia levels as high as 10^6/ml of blood (Agampodi et al. 2012), which is similar to the burden of spirochetes seen in the blood of patients with relapsing fever (Stoenner et al. 1982). Levels of $>10^4$ leptospires/ml in the bloodstream have been associated with severe outcomes (Segura et al. 2005; Truccolo et al. 2001), although a more recent larger study suggests that leptospires with lower virulence may be able to achieve even higher leptospiral bloodstream burdens without causing severe complications (Agampodi et al. 2012).

The levels of bacteremia that occur during leptospirosis are similar to those found in infections caused by the relapsing fever *Borreliae* (Stoenner et al. 1982), and very different from those found in

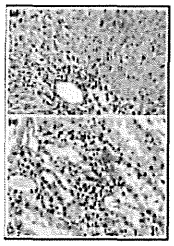
bacteremia caused by *E. coli* and other *Enterobacteriaceae*, in which concentrations are typically <1 cfu/ml (Yagupsky and Nolte 1990). Part of the explanation for these differences is the human innate immune response. Human TLR4 is able to detect *E. coli* lipopolysaccharide (LPS) at extremely low concentrations, but is unable to recognize leptospiral LPS (Werts et al. 2001). A likely explanation for this difference in reactivity of human TLR4 is structural differences between the lipid A component of *E. coli* and leptospiral LPS; leptospiral LPS has a unique methylated phosphate residue not found in any other form of lipid A (Que-Gewirth et al. 2004). In contrast to human TLR4, mouse TLR4 is able to recognize leptospiral LPS (Nahori et al. 2005), suggesting that the murine innate immune response is adapted to leptospiral infection. This notion is consistent with differences in the pathogenesis of leptospirosis between humans and mice; humans are accidental hosts that experience potentially fatal outcomes and rarely transmit infection, while mice are resistant to fatal infection and serve as natural reservoirs.

The importance of TLR4 in determining the outcome of infection was demonstrated in studies showing that young (but not adult) C3H/HeJ mice lacking TLR4 are susceptible to lethal infection with *L. interrogans* (Viriyakosol et al. 2006). However, TLR4 is only one component of the innate immune response to leptospirosis. Both human and mouse TLR2 are able to recognize the polysaccharide or 2-keto-3-deoxyoctonoic acid (KDO) component of leptospiral LPS (Nahori et al. 2005; Werts 2010). Only when both TLR4 and TLR2 are mutated do adult C57BL/6 J mice experience lethal leptospirosis infections (Nahori et al. 2005). Presumably, TLR2 and other innate immune response mechanisms are responsible for the host response to leptospiral infection that leads to symptoms of disease. TLR2, TLR4, and TLR5 have been shown to be required for virulent leptospires to induce expression of the cytokines IL-6 and TNF-alpha in whole blood (Goris et al. 2011a).

When high levels of leptospiremia occur during infection, innate immune mechanisms eventually trigger tissue-based and systemic responses to infection that lead to severe outcomes such as a sepsis-like syndrome or organ failure. Patients with severe leptospirosis have evidence of a "cytokine storm" with higher levels of IL-6, TNF-alpha, and a number of other cytokines than patients with mild disease (Reis et al. 2013). In particular, IL-6 and IL-10 levels were independent predictors of death, suggesting that overproduction of IL-10 may inhibit a protective Th1 immune response. Superantigen stimulation of nonspecific T cell activation may also play a role in human leptospirosis. A study of athletes participating in the Springfield triathalon found that the human leukocyte antigen (HLA) DQ6 was an independent risk factor for development of leptospirosis after exposure to lake water contaminated with virulent leptospires (Lingappa et al. 2004). The structural location of HLA-DQ6 polymorphisms associated with disease suggested a superantigen mechanism for this HLA-dependent susceptibility (Lingappa et al. 2004), although the identity of any such antigen(s) remains unknown.

The liver is a major target organ in leptospirosis. Pathology reports from autopsy specimens from fatal cases of leptospirosis have reported congested sinusoids and distention of the space of Disse, located between the sinusoids and hepatocytes (Arean 1962). Immunohistochemistry studies have documented large numbers of leptospires between hepatocytes in animal models. A recent, elegant study has documented leptospiral infiltration of Disse's space and preferential leptospiral attachment to and invasion of the perijunctional region between hepatocytes (Miyahara et al. 2014). Additionally, hepatocyte apoptosis has been documented in leptospirosis (Merien et al. 1998). Together, hepatocellular damage and disruption of hepatocyte intercellular junctions (Fig. 3a) leads to leakage of bile from bile canaliculi into sinusoidal blood vessels, which accounts for the elevated levels of direct bilirubin seen in icteric forms of leptospirosis. Occasionally, elevation of indirect bilirubin levels may also occur in the setting of leptospirosis-induced hemolysis (Avdeeva et al. 2002).

Fig. 3



Histopathology of leptospirosis. a Histology of the liver typically shows lack of the normal adhesion between hepatocytes, a hallmark of the disease (*photograph credit* Thales De Brito). **b** Typical renal histopathology showing acute tubular necrosis and ...

Pathological changes in the lung are extremely common in leptospirosis. In the 1962 study of fatal leptospirosis cases by Arean, all 33 cases were found to have pulmonary petechiae on the pleural surfaces and 60 % of patients had gross hemorrhage on the cut surfaces of the lungs (Arean 1962). Histologically, hemorrhage was found to occur in both the alveolar septa and intra-alveolar spaces (Arean 1962). A recent Brazilian study of patients with severe pulmonary hemorrhage syndrome (SPHS) performed immunohistochemistry on pulmonary tissue and found finely granular material representing leptospiral antigen within macrophages in septa and alveoli (Silva et al. 2002b). The guinea pig model of leptospirosis replicates the pulmonary hemorrhage seen in humans and studies of this animal model also revealed extensive deposition of immunoglobulin and complement along the alveolar basement membrane (Nally et al. 2004). Petechiae and hemorrhage are noted in a number of different organs beside the lungs and may be related, at least in part, to the coagulation abnormalities associated that frequently occur in severe leptospirosis (Wagenaar et al. 2010). The concept that SPHS is a manifestation of severe systemic disease rather than a strictly pulmonary problem is consistent with the finding that risk factors for SPHS include not only the respiratory rate but also hypokalemia, elevated serum creatinine, shock, and the Glasgow Coma Scale Score (Marotto et al. 2010).

Renal involvement varies in severity from mild nonoliguric renal dysfunction to complete renal failure, a hallmark of Weil's syndrome. The polyuria observed in mild leptospirosis appears to be due to reduced expression of the sodium–hydrogen exchanger 3, resulting in decreased reabsorption of sodium and fluid by the proximal tubule (Araujo et al. 2010). The histologic changes vary in intensity and typically involve tubular changes and interstitial nephritis (Fig. 3b). Tubular damage includes thinning and/or necrosis of the tubular epithelium and distention of the tubular lumen with hyaline casts and cellular debris (Arean 1962). In the reservoir host, the tubular lumen is a key site of colonization in the leptospiral life cycle and immunohistochemistry can show large numbers of organisms attached to the brush border of proximal tubular epithelium. In humans, an inflammatory response is triggered by recognition of leptospiral lipoproteins such as LipL32 by TLR2 on renal tubular epithelial cells, resulting in induction of nitric oxide synthase (iNOS) and monocyte chemoattractant protein-1 (Yang et al. 2006). Tubular inflammation results in interstitial nephritis characterized by edema and infiltration with lymphocytes, monocytes, and plasma cells, and occasionally neutrophils (Arean 1962; Sitprija and Evans 1970). This interstitial nephritis increases in extent and intensity during the first 2 weeks of illness. Most patients with acute renal failure due to leptospirosis who survive regain normal renal function. However, some patients have persistent renal dysfunction associated with tubular atrophy and interstitial fibrosis on kidney biopsy (Herath et al. 2014).

3 Clinical Features

Go to:

Leptospirosis ranges in severity from a mild, self-limited febrile illness to a fulminant life-threatening illness. When illness occurs, a broad array of organ systems may be involved, reflecting the systemic nature of the infection. As a result, the signs and symptoms of leptospirosis are protean and frequently mistaken for other causes of acute febrile syndrome.

3.1 Incubation Phase

The incubation phase from exposure to onset of symptoms averages from 7 to 12 days, though it can be as short as 3 days or as long as a month. The remarkable variability in the duration of the incubation phase is evident in the 6–29 day lag between exposure and onset of symptoms among 52 athletes who developed laboratory-confirmed leptospirosis after participating (all on the same day) in the Springfield Triathalon (Morgan et al. 2002).

3.2 Presentation

Patients typically present with sudden onset of fever, chills, and headache. These signs and symptoms are nonspecific and also occur with other causes of acute febrile syndrome that, depending on the setting, could also be caused by influenza, dengue fever, or malaria. The headache is often severe and has been described as a bitemporal, frontal throbbing headache accompanied by retro-orbital pain and photophobia.

Muscle pain and tenderness is common and characteristically involves the calves and lower back. A tip-off to identification of leptospirosis is conjunctival suffusion (dilatation of conjunctival vessels without purulent exudate), which occurs frequently in leptospirosis, but is uncommon in other infectious diseases. Additional ocular findings typically include subconjunctival hemorrhages and icterus (Fig. 4a). Rash is uncommon in leptospirosis and when it occurs in the setting of an acute febrile illness, may suggest an alternative diagnosis such as dengue or chikungunya fever (Burt et al. 2012; Zaki and Shanbag 2010). An erythematous rash limited to the pretibial areas of both legs appearing on about the fourth day of illness was a feature of an outbreak of "Fort Bragg Fever" which also included headache, malaise, and splenomegaly among soldiers in North Carolina, the etiology of which was later determined to be *L. interrogans* serovar Autumnalis (Gochenour et al. 1952).

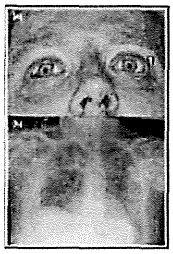


Fig. 4
Clinical presentation of leptospirosis. **a** Subconjunctival hemorrhages and icterus in a 37-year-old man who kept pet rats presented with sudden onset of fever, myalgia, and severe headache. On admission he had abnormal liver and kidney function. Serological ...

A nonproductive cough has been noted in 20–57 % of leptospirosis patients and can potentially lead clinicians to incorrectly diagnose the patient with influenza or another respiratory illness. Gastrointestinal symptoms are frequently observed, and may include nausea, vomiting, diarrhea, and abdominal pain. Nausea and other gastrointestinal symptoms may contribute to dehydration in patients with high-output nonoliguric renal failure caused by leptospirosis. The abdominal pain may be due to acalculous cholecystitis and/or pancreatitis. In patients admitted to the hospital for leptospirosis, abdominal pain associated with abnormal serum amylase and/or lipase levels is relatively common (O'Brien et al. 1998). It should be kept in mind that impaired renal function alone can elevate pancreatic enzyme levels when the creatinine clearance is less than 50 ml/min (Collen et al. 1990). While most cases of pancreatitis due to leptospirosis are self-limited, some cases are more severe and associated with fatal outcomes (Spichler et al. 2007).

Severe leptospirosis is characterized by dysfunction of multiple organs including the liver, kidneys, lungs, and brain. The combination of jaundice and renal failure, known as Weil's disease, was first described in 1886 (Weil 1886) and remains one of the most clinically recognizable forms of leptospirosis (see the chapter by B. Adler, this volume). Evidence of organ dysfunction indicates a more advanced stage of infection, yet may develop suddenly and be present in a large percentage of patients at the time of presentation.

Leptospirosis patients are typically found to have mild to moderate elevations in levels of liver transaminases and direct (conjugated) bilirubin. The frequency of jaundice varies widely among case series, perhaps due in part to the virulence of the causative organism. Katz et al. (2001) found a strong association between infection with the Icterohaemorrhagiae serogroup and jaundice and elevated bilirubin levels. Acute hemolytic anemia can contribute to jaundice which, not surprisingly, is more common in leptospirosis patients with glucose-6-phosphate dehydrogenase deficiency (Avdeeva et al. 2002). Such patients have a higher percentage of unconjugated (i.e., indirect) bilirubin. Many patients have leukocytosis and thrombocytopenia, though usually not to the extent that would cause spontaneous hemorrhage. Leukopenia in the setting of thrombocytopenia and anemia can suggest bone marrow suppression.

Clinical signs of bleeding are common and occur in the majority of patients with severe leptospirosis. Most bleeding manifestations are mild, including petechiae, ecchymoses, and epistaxis. However, some patients have severe gastrointestinal (melena or hematemesis) or pulmonary hemorrhage. Thrombocytopenia frequently occurs, although usually not to the extent that would cause spontaneous hemorrhage. In a study of severe leptospirosis performed in the Netherlands, all patients had coagulation disorders, including prolongation of the prothrombin time (PT) and the length of the PT was associated with severe bleeding manifestations (Wagenaar et al. 2010).

The kidney is a major target organ in leptospirosis, perhaps due to the intrinsic renal-tropic homing ability of leptospires in their reservoir hosts. The kidneys are commonly involved, as manifested by elevations in serum blood urea nitrogen and creatinine levels and findings on urinalysis of pyuria, hematuria, and elevated urine protein levels (Katz et al. 2001). Leptospirosis causes a unique nonoliguric potassium wasting nephropathy characterized by impaired sodium reabsorption and potassium wasting (Seguro et al. 1990). When poor oral intake due to nausea and high-output renal failure combine to cause dehydration, patients are at risk of oliguria and renal failure, a frequent cause of death in areas where peritoneal or hemodialysis is not available.

Progression to severe leptospirosis and circulatory collapse may be accompanied by acute respiratory distress syndrome (ARDS). As in other causes of ARDS, leptospirosis causes diffuse lung injury characterized by impaired gas exchange and the need for mechanical ventilation. Massive hemoptysis, representing extensive alveolar hemorrhage, is an ominous complication of leptospirosis associated with fatality rates >50 % (Gouveia et al. 2008). Pulmonary hemorrhage associated with leptospirosis was first reported in Switzerland in 1943 (Moeschlin 1943), and since then has been reported with increasing frequency from a variety of locations (Park et al. 1989). Leptospirosis-associated severe pulmonary hemorrhage syndrome (SHPS) can occur sporadically or in outbreaks that can be confused clinically with viral pneumonitis (Sehgal et al. 1995; Trevejo et al. 1998). For example, a 1995 outbreak of SPHS that occurred after heavy rainfall and flooding in Nicaragua was initially thought to be due to hantavirus pulmonary syndrome until silver staining and immunohistochemistry of postmortem lung tissue revealed leptospires (Trevejo et al. 1998). SPHS can present as hemoptysis associated with cough or may be discovered after patients undergo pulmonary intubation (Yersin et al. 2000). Chest radiographs show diffuse alveolar infiltrates (Fig. 4b). Epidemiologic evidence suggests that SPHS may be a relatively new problem, suggesting emergence of a new clone of *L. interrogans* with enhanced virulence. However, it is also possible that SPHS is an old problem that is finally being recognized and documented.

As noted above, headache is frequently severe and when accompanied by meningismus may prompt performance of lumbar puncture. Typical findings on CSF examination include a lymphocytic predominance with total cell counts of up to 500/mm^3, protein levels between 50 and 100 mg/mL, and normal glucose levels, consistent with aseptic meningitis (Berman et al. 1973). Depending on the epidemiologic setting, leptospirosis may be a predominant cause of aseptic meningitis in some areas

(Silva et al. 2002a). Patients with aseptic meningitis due to leptospirosis may be anicteric, making the diagnosis more challenging (Berman et al. 1973; Karande et al. 2005). In severe leptospirosis, altered mental status may be an indicator of meningoencephalitis. A variety of other neurologic complications may also occur including hemiplegia, transverse myelitis, and Guillain–Barré syndrome (Levett 2001).

3.3 Risk Factors for Morbidity and Mortality

In an active surveillance study of 326 cases of leptospirosis in Salvador, Brazil, the strongest independent predictor of a fatal outcome was altered mental status (odds ratio 9.12), which typically began with confusion and obtundation without focal neurologic signs (Ko et al. 1999). Other independent risk factors for death identified in the Salvador study included oliguria (odds ratio 5.28), age over 36 years (odds ratio 4.38), and respiratory insufficiency (2. 56) (Ko et al. 1999). The risk of a fatal outcome increases with increased age; compared to individuals aged 19–29, the increased risk of death rose from 3.7-fold for 40–49 year olds to 7.3-fold among those 60 or older (Lopes et al. 2004). Lung involvement, as indicated by dyspnea (odds ratio 11.7) or alveolar infiltrates on chest X-ray (odds ratio 7.3), was found to be associated with mortality in a retrospective study of 68 leptospirosis cases at Pointe-à-Pître Hospital in the French West Indies, along with oliguria (odds ratio 9), repolarization abnormalities on electrocardiogram (odds ratio 5.95), and a white blood count >12,900/mm^3 (odds ratio 2.54) (Dupont et al. 1997). A retrospective review of leptospirosis cases associated with an outbreak of leptospirosis in India identified pulmonary involvement and altered mental status as independent predictors of death (Pappachan et al. 2004). Additional poor prognostic signs identified in other studies include acute renal failure, hypotension, and arrhythmias (Daher et al. 1999; Panaphut et al. 2002).

3.4 Recovery Phase

With proper supportive care (see Management below), most leptospirosis patients recover completely (Spichler et al. 2011). Patients with acute renal failure who require dialysis typically regain most of their renal function, although there may be evidence of persistent mild renal impairment (Covic et al. 2003). In addition, there is growing recognition that many patients suffer from chronic postleptospirosis symptoms. In a recent study of laboratory-confirmed leptospirosis patients in the Netherlands, 30 % of patients experienced persistent complaints after acute leptospirosis (PCAC) characterized by fatigue, myalgia, malaise, headache, and weakness (Goris et al. 2013a). Of patients with PCAC, 21 % reported that their complaints lasted for more than 24 months.

Ocular involvement in the form of uveitis is well-known to occur during the convalescent phase of leptospirosis. Eye involvement ranges in severity from insidious onset of mild anterior uveitis to acute, severe panuveitis involving the anterior, middle, and posterior segments of the eye (Rathinam 2005). Leptospiral uveitis may occur either as a single, self-limited event or as a series of recurrent episodes, which appears to occur more frequently in patients with severe uveitis. In one study, 80 % of patients had leptospiral DNA in the aqueous humor, detected by PCR (Chu et al. 1998). However, the relative contributions of infection and autoimmunity are uncertain. There are parallels between recurrent uveitis in humans and equine recurrent uveitis (ERU) and autoimmunity to lens proteins has been suggested to play a role in ERU (Verma et al. 2010).

4 Diagnosis

Go to:

Diagnosis of leptospirosis may be accomplished by direct detection of the organism or its components in body fluid or tissues, by isolation of leptospires in cultures, or by detection of specific antibodies (Hartskeerl et al. 2011; Schreier et al. 2013). The collection of appropriate specimens and selection of tests for diagnosis depend upon the timing of collection and the duration of symptoms (Fig. 5). For detailed descriptions of historical methods see the following publications (Faine et al. 1999; Galton 1962; Levett 2001; Sulzer and Jones 1978; Turner 1968, 1970; Wolff 1954).

Fig. 5
Biphasic nature of leptospirosis and relevant investigations at different stages of disease. Specimens *1* and *2* for serology are acute-phase specimens, *3* is a convalescent-phase sample which may facilitate detection of a delayed immune response, and *4* ...

4.1 Molecular Diagnosis

Leptospiral DNA has been amplified from serum, urine, aqueous humor, CSF, and a number of organs post mortem (Levett 2004). Conventional PCR and other assays such as LAMP and NASBA were reviewed recently (Ahmed et al. 2012) and will not be discussed further. Many quantitative PCR assays have been described, which target a number of different genes (Ahmed et al. 2009; Merien et al. 2005; Palaniappan et al. 2005; Smythe et al. 2002; Stoddard et al. 2009). Assays developed for diagnostic use can be considered in two broad categories, targeting either housekeeping genes, such as *rrs*, *gyrB*, or *secY*, or pathogen-specific genes such as *lipL32*, *lig*, or *lfb1* (Ahmed et al. 2012). Examples of these two types of quantitative assay were evaluated in a large case-control study in a high-prevalence population in Thailand (Thaipadunpanit et al. 2011), that confirmed earlier reports that PCR detection in blood samples collected at admission to hospital was more sensitive than culture, but serology using the microscopic agglutination test (MAT) ultimately detected more cases (Brown et al. 1995). Real-time PCR assays have been used to quantify the bacterial load in leptospirosis (Agampodi et al. 2012; Segura et al. 2005; Tubiana et al. 2013).

A limitation of PCR-based diagnosis of leptospirosis is the current inability of PCR assays to identify the infecting serovar. While this is not significant for individual patient management, the identity of the serovar has both epidemiological and public health value. Serovar identification requires isolation of the infecting strain from patients or carrier animals. However, whole genome sequencing has recently been applied to the diagnosis of neurological leptospirosis (Wilson et al. 2014) and it is probable that direct serovar identification will be possible in the near future, limited only by the quality of sequences obtained from specimens.

4.2 Isolation and Identification of Leptospires

Culture of leptospires requires specialized media (see the chapter by C.E. Cameron, this volume). Leptospires can be recovered from humans during the acute phase of the illness and during the so-called immune phase. Leptospiremia occurs during the first stage of the disease, beginning before the onset of symptoms and has usually declined by the end of the first week of the acute illness. Timing of culture of different specimens depends upon an accurate date of onset of symptoms, so a careful history is essential. Blood cultures should be taken as soon as possible after the patient's presentation. One or two drops of blood are inoculated into 5–10 ml semisolid or liquid medium at the bedside (Turner 1970). Multiple cultures yield higher recovery rates, but this is rarely possible. Inoculation of media with dilutions of blood samples may increase recovery (Sulzer and Jones 1978). Leptospires have been shown to survive in commercially available conventional blood culture media for periods of time ranging from 48 h to 4 weeks (Palmer and Zochowski 2000). Blood cultures with no growth can be used to inoculate leptospiral culture medium (Turner 1970).

Other samples that may be cultured during the first week of illness include CSF and peritoneal dialysate. Urine should be cultured from the beginning of the second week of symptomatic illness. The duration of urinary excretion varies, but may be several weeks (Bal et al. 1994). Survival of leptospires in voided human urine is limited, so urine should be collected into sterile phosphate buffered saline (Turner 1970). Contamination of urine cultures is a major problem and the use of selective media containing 5-fluorouracil or other antimicrobial agents (see the chapter by C.E. Cameron, this volume)

is strongly recommended. Cultures are incubated at 28–30 °C and examined weekly by dark field microscopy, for up to 13 weeks.

Isolated leptospires are identified either by serological methods, or more recently, by molecular techniques. Traditional methods relied on cross-agglutinin absorption (Dikken and Kmety 1978). The number of laboratories which can perform these identification methods is small. Monoclonal antibodies are available for identification of many, but not all, serovars (Korver et al. 1988). The limitations of these approaches are discussed by Hartskeerl and Smythe (see the chapter by R.A. Hartskeerl and L.D. Smythe, this volume).

Molecular methods for identification and subtyping have been studied extensively. Increasingly, sequence-based identification of *Leptospira* is becoming the standard (Ahmed et al. 2012) and this can be performed on the products of diagnostic PCR (Ganoza et al. 2010; Perez and Goarant 2010). Pulsed-field gel electrophoresis (PFGE) has been shown to identify most serovars (Galloway and Levett 2010; Herrmann et al. 1992), but complements, rather than replaces, serological identification (Ahmed et al. 2012). Identification of serovars by whole genome sequencing will likely become standardized in the near future (Ahmed et al. 2012).

Strain subtyping for epidemiological purposes can be accomplished by simple methods using restriction enzymes or variations of PCR conditions that can generate banding patterns that allow strains to be differentiated (Ahmed et al. 2012). However, reproducibility is poor, particularly between laboratories. More recently, sequence-based methods such as MLVA (Pavan et al. 2008; Salaun et al. 2006; Slack et al. 2005) and MLST (Ahmed et al. 2006, 2011; Boonsilp et al. 2013; Leon et al. 2009; Thaipadungpanit et al. 2007) have been applied. These methods are reproducible and can yield significant information at a subserovar level (Boonsilp et al. 2013). MLST data can be analyzed online (http://leptospira.mlst.net).

4.3 Serological Diagnosis

Most cases of leptospirosis are diagnosed by serology, because capacity for culture and PCR is limited. IgM antibodies are detectable in the blood 5–7 days after the onset of symptoms. Serological methods can be divided into those which are genus-specific and those which are serogroup-specific. The use of agglutination tests was described soon after the first isolation of the organism and the microscopic agglutination test remains the definitive serological investigation in both human and animals.

4.3.1 Microscopic Agglutination Test In the microscopic agglutination test (MAT), patients' sera are reacted with live antigen suspensions of leptospiral serovars. After incubation, the serum/antigen mixtures are examined microscopically for agglutination and the titers are determined. The MAT can be a complex test to control, perform, and interpret (Turner 1968). Live cultures must be maintained of all the serovars required for use as antigens. The range of antigens used should include serovars representative of all serogroups (Faine 1982; Turner 1968) and locally common serovars (Torten 1979). A wide range of antigens is used in order to detect infections with uncommon, or previously undetected, serovars (Katz et al. 1991). The MAT is a serogroup-specific assay and cannot be relied upon to detect the infecting serovar (Levett 2003; Murray et al. 2011; Smythe et al. 2009).

The MAT is read by dark field microscopy. The endpoint is the highest dilution of serum in which 50 % agglutination occurs. Because of the difficulty in detecting when 50 % of the leptospires are agglutinated, the endpoint is determined by the presence of approximately 50 % free, unagglutinated leptospires, by comparison with the control suspension (Faine 1982). Considerable effort is required to reduce the subjective effect of observer variation, even within laboratories.

Interpretation of the MAT is complicated by the high degree of cross-reaction that occurs between different serogroups, especially in acute-phase samples. Patients often have similar titers to all serovars of an individual serogroup, but "paradoxical" reactions, in which the highest titers are detected to a

serogroup unrelated to the infecting one, may also occur (Alston and Broom 1958; Levett 2001). The broad cross-reactivity in the acute phase, followed by relative serogroup specificity in convalescent samples, results from the detection in the MAT of both IgM and IgG antibodies (Adler and Faine 1978).

Paired sera are required to confirm a diagnosis with certainty. A fourfold or greater rise in titre between paired sera confirms the diagnosis, regardless of the interval between samples. The interval between first and second samples depends very much on the delay between onset of symptoms and presentation of the patient. If symptoms typical of leptospirosis are present, then an interval of 3–5 days may be adequate to detect rising titers. However, if the patient presents earlier in the course of the disease, or if the date of onset is not known precisely, then an interval of 10–14 days between samples is more appropriate. Less often, seroconversion does not occur with such rapidity, and a longer interval between samples (or repeated sampling) is necessary. MAT serology is insensitive in early acute-phase specimens (Appassakij et al. 1995; Brandão et al. 1998; Cumberland et al. 1999). Moreover, patients with fulminant leptospirosis may die before seroconversion occurs (Brown et al. 1995; Cumberland et al. 1999; Ribeiro et al. 1994).

Acute infection is suggested by a single elevated titer detected in association with an acute febrile illness. The magnitude of such a titer is dependent upon the background level of exposure in the population, and hence the seroprevalence. The application of single titers for presumptive diagnosis has been reviewed (Levett 2001) and will not be discussed further. Titers following acute infection may be extremely high (≥25,600) and may take months, or even years, to fall to low levels (Alston and Broom 1958; Blackmore et al. 1984; Cumberland et al. 2001; Lupidi et al. 1991; Romero et al. 1998). Rarely, seroconversion may be delayed for many weeks after recovery, and longer serological follow-up will be necessary to confirm the diagnosis.

The MAT is the most appropriate test to employ in epidemiological sero-surveys, since it can be applied to sera from any animal species, and because the range of antigens utilized can be expanded or decreased as required. It is usual to use a titer ≥100 as evidence of past exposure (Faine 1982). However, conclusions about infecting serovars cannot be drawn without isolates; MAT data can give only a general impression about which serogroups are present within a population (Everard and Everard 1993).

4.3.2 Other Serological Tests Because of the complexity of the MAT, rapid screening tests for leptospiral antibodies in acute infection have been developed. IgM antibodies become detectable during the first week of illness, allowing the diagnosis to be confirmed and treatment to be initiated while it is likely to be most effective. IgM detection has repeatedly been shown to be more sensitive than MAT when the first specimen is taken early in the acute phase of the illness (Cumberland et al. 1999; Goris et al. 2011b; Ribeiro et al. 1994; Winslow et al. 1997).

Detection of IgM using ELISA has been employed widely, most often using antigen prepared from cultures of *L. biflexa*, although pathogenic species have also been used. Several products are available commercially. Recombinant antigens have also been employed, but none has been evaluated widely (Signorini et al. 2013). Specificity of IgM detection by ELISA is affected by the antigen used in the assay, by the presence of antibodies due to previous exposure (in endemic regions), and by the presence of other diseases (Bajani et al. 2003).

More recently, IgM detection assays have been developed in several rapid test formats intended for use in laboratories without extensive instrumentation, or potentially in field settings. These have included two dipstick formats (Smits et al. 2000a; Levett and Branch 2002), latex agglutination (Smits et al. 2000b, 2001a), lateral flow (Smits et al. 2001b) and dual path platform (Nabity et al. 2012).

However, there are significant limitations to early diagnosis using any serological test (Goris et al. 2011b; Signorini et al. 2013) and testing of a second sample should be considered mandatory.

Moreover, confirmation of rapid diagnostic test results by a reference test has been recommended (Goris et al. 2013b).

4.3.3 Evaluation of Serological Tests Evaluation of serological tests for leptospirosis has been problematic because there are few laboratories equipped to perform the definitive serological test (MAT), and there are fewer laboratories with the capacity to isolate and identify leptospires from patients. A large body of the literature consists of reports on studies that have been ill-designed and which use less than perfect case definitions, leading to misleading estimates of sensitivity and specificity. Ideally, new serological assays should be evaluated in clinical trials of consecutive patients investigated using a case definition which includes both MAT and culture results, and which are conducted in multiple regions, where different leptospiral serovars are prevalent and where the differential diagnoses may vary widely (Smits et al. 2000a, b). Assays may perform differently in different populations (Desakorn et al. 2012; Levett and Branch 2002). Alternatively, well-designed studies conducted in individual centers may be compared, providing the limitations of this approach are recognized (Levett 2001). Evaluations performed using collections of sera in reference laboratories may be useful for determining sensitivity of assays, but specificity is dependent upon the selection of noncase sera representative both of other diseases and the normal population. Parallel studies in clinical and reference settings may yield quite different results (Bajani et al. 2003; Hull-Jackson et al. 2006).

5 Management

Go to:

Most leptospirosis cases are mild and resolve spontaneously. Early initiation of antimicrobial therapy may prevent some patients from progressing to more severe disease. Identification of leptospirosis in its early stages is largely a clinical diagnosis and relies on a high index of suspicion based on the patient's risk factors, exposure history, and presenting signs and symptoms. Rapid diagnostic tests for leptospirosis are improving, but a negative result should not be relied on to rule out early infection. For these reasons, empirical therapy should be initiated as soon as the diagnosis of leptospirosis is suspected.

Therapy for patients with leptospirosis severe enough to merit hospitalization usually involves intravenous penicillin (1.5 million units IV every 6 h), ampicillin (0.5–1 g IV every 6 h), ceftriaxone (1 g IV every 24 h), or cefotaxime (1 g IV every 6 h). Ceftriaxone has been shown to be noninferior to penicillin for serious leptospirosis (Panaphut et al. 2003) and in addition to once daily dosing has the added benefit of intramuscular administration as an alternative to intravenous therapy in settings where hospitalization is not possible. Adult outpatients with early disease should receive either doxycycline 100 mg orally twice per day or azithromycin 500 mg orally once per day. When the dosage is adjusted for weight, either azithromycin or amoxycillin can also be given to pregnant women and children. These recommendations are based on in vitro susceptibility data (Hospenthal and Murray 2003; Ressner et al. 2008), animal studies (Alexander and Rule 1986; Truccolo et al. 2002), and clinical experience including a randomized, placebo-controlled, double-blinded study which found that doxycycline therapy shortened the duration of illness due to leptospirosis by 2 days and improved fever, malaise, headache, and myalgias (McClain et al. 1984). Doxycycline treatment also prevented shedding of organisms in the urine.

There are strong grounds for administering antibiotics as soon as possible to patients with risk factors and clinical features of severe leptospirosis. A placebo-controlled trial of intravenous penicillin for leptospirosis conducted in the Philippines found that penicillin shortened the duration of fever, abnormal renal function, and hospitalization and prevented leptospiral shedding in the urine (Watt et al. 1988). A flaw in this study was that a number of patients in both groups had received antibiotics prior to entry into the study. A second placebo-controlled study of intravenous penicillin for leptospirosis patients was conducted in Barbados, most of whom were icteric. Although the Barbados study failed to

show significant differences between the penicillin and placebo groups, patients receiving penicillin had a lower mortality rate than patients receiving placebo (2.6 vs. 7.3 %, respectively) (Edwards et al. 1988). It can be difficult to demonstrate a beneficial effect of antibiotics in patients who have already begun to experience some degree of organ dysfunction, which of course cannot be reversed with antibiotics. As imperfect as these studies are, they are likely to be the only placebo-controlled studies that will ever be conducted, given the ethical barriers to placebo-controlled studies involving life-threatening illnesses caused by antibiotic-susceptible bacteria.

Severe leptospirosis is a medical emergency requiring both antibiotics and proper supportive therapy to improve mortality rates. Patients with severe leptospirosis are frequently found to have a unique form of potassium wasting high-output renal dysfunction (Abdulkader et al. 1996; Seguro et al. 1990). For this reason, patients should receive intravenous hydration to correct dehydration and prevent oliguric renal failure. Potassium supplementation should be included for patients with hypokalemia. When oliguric renal failure occurs, early initiation of peritoneal or hemodialysis can be lifesaving and is usually needed only on a short-term basis (Andrade et al. 2008). In a comparative study, prompt initiation of daily dialysis in critically ill leptospirosis patients reduced mortality from 67 to 17 % (Andrade et al. 2007). Patients with respiratory failure who require intubation typically have poor pulmonary compliance (i.e., "stiff lungs") and have been found to benefit from ventilation with low tidal volumes (<6 mL/kg) to reduce ventilation pressures, protect patients from alveolar injury, and improve survival rates (Amato et al. 1998).

5.1 Antimicrobial Susceptibilities

Leptospires are susceptible to β-lactams, macrolides, tetracyclines, fluoroquinolones, and streptomycin (Alexander and Rule 1986; Faine et al. 1999). Problems in the determination of susceptibility include the long incubation time required, the use of media containing serum, and the difficulty in quantifying growth accurately. These constraints have limited the development of rapid, standardized methods for susceptibility testing. Most studies have used a limited range of laboratory strains and/or a small number of antimicrobial agents. However, microdilution methods have been described recently (Murray et al. 2004; Ressner et al. 2008), which will facilitate the study of large numbers of isolates against a wide range of antimicrobial agents, with the potential of identifying new agents for prophylaxis or treatment of leptospirosis.

6 Prevention

Go to:

Strategies for prevention of leptospirosis are based on awareness of leptospirosis epidemiology and transmission mechanisms, as presented earlier in this chapter. Once the local epidemiology and transmission risks have been defined, it is possible to greatly mitigate risk by taking steps to reduce exposure and implement protective measures, immunization, and pre- or postexposure chemoprophylaxis.

From a global perspective, human leptospirosis is strongly linked to poverty wherever poor housing standards and local infrastructure result in exposure to rodent reservoirs. Rodent abatement efforts may have short-term benefit but rodenticides create risks for children and wildlife and are not good long-term solutions. Housing construction that prevents rodents from invading residential living spaces greatly reduces risk. Flood control projects that prevent inundation of residential areas would greatly reduce the potential for leptospirosis outbreaks. These measures are difficult to implement, but should be recognized as an important part of an overall prevention strategy.

Occupational activities that put workers at risk through exposure to contaminated water or infected animals should be identified. Personal protective equipment such as gloves, boots, goggles, and overalls for workers in high-risk occupations are important to prevent exposure of mucous membranes and skin,

but can be difficult to implement in hot and humid environments. Abrasions, cuts, and damaged skin are particularly important as portals of entry. Walking barefoot and water sports in endemic areas are notoriously high-risk activities. The 2001 Eco-challenge multi-sport competition in Borneo involving jungle trekking and leach bites followed by prolonged emersion in the rain-swollen Segama River resulted in an astounding 42 % attack rate and illustrates how endemic factors and susceptible hosts combine to create high-risk exposures (Sejvar et al. 2003).

Source reduction through immunization of agricultural and companion animals with killed whole-cell vaccines is an extremely important strategy for reducing the risk of human leptospirosis. Humans may also become infected through exposure to acutely or chronically infected animals that are shedding leptospires in their urine. Diagnosis and treatment of infected animals, and immunization of uninfected companion and agricultural animals is another cornerstone of leptospirosis prevention and is covered in chapter by W.A. Ellis, this volume.

6.1 Human Leptospirosis Vaccines

Immunization of humans with killed, whole-cell vaccines has generally been restricted to individuals in high-risk occupations and in response to floods and epidemics. One of the first reports of human leptospirosis immunization involved the vaccination of thousands of miners in Japan using a culture-derived *L. interrogans* serovar Icterohaemorrhagiae vaccine (Wani 1933). Although local and generalized reactions were common, a significant decrease in the incidence of leptospirosis among the miners was observed. Immunization of large populations at risk of leptospirosis due to extensive flooding has been performed in China (Chen 1985). A Cuban leptospirosis vaccine trial involving >100,000 persons reported that local pain and "general discomfort" were significantly greater than in a control group given a recombinant hepatitis B vaccine (Martinez et al. 2004). The vaccine showed an efficacy of >97 % against the prevalent local serovars. Concern over reactions to host proteins led to the development of a leptospiral vaccine derived from leptospires grown in a chemically defined medium (Shenberg and Torten 1973); however, growth in protein media is generally poorer and such media have not gained widespread use.

Some of the most detailed safety and efficacy studies involved a leptospirosis vaccination program for Parisian sewer workers. In response to a request by the City of Paris, the Pasteur Institute developed a killed, whole-cell vaccine derived from *L. interrogans* serovar Icterohaemorrhagiae strain Verdun. Mailloux et al. (1983) examined the safety of this vaccine and reported three systemic (nausea) reactions and seven local reactions among 1,157 immunizations of 454 vaccines. Importantly, after the vaccine was introduced in 1979, the incidence of leptospirosis dropped from 1.3 cases per year (29 cases from 1951 to 1979) to zero (no cases reported from 1981 to 1988) during a 7 year follow-up period. The recommended vaccination protocol involves two booster doses after the initial immunization followed by reimmunization every 2 years. More recent reports of safety and efficacy have been published since the vaccine was marketed as Spirolept™ (Benbrick et al. 2001; Laurichesse et al. 2007; Pouliquen and Catilina 2000).

As described in chapter by B. Adler, this volume, the active component of killed, whole-cell vaccines is leptospiral LPS, a serovar-specific antigen (Chapman et al. 1990). LPS-based immunity is generally considered to provide protection against homologous or closely related, but not heterologous, serovars. For example, Fukumura (1984) reported that individuals immunized with a serovar Pyrogenes vaccine were protected from infection by that serovar but not from serovars Autumnalis and Hebdomadis with antigenically unrelated LPS, leading to development of a trivalent vaccine consisting of all three serovars. Research on development of leptospirosis vaccines with a low side-effect profile that induce long-lasting, cross-protective immunity is focused on an improved understanding of the leptospiral outer membrane (see the chapters by D.A. Haake and W.R. Zückert and by B. Adler, this volume).

6.2 Chemoprophylaxis

Unavoidable short-term exposure can be mitigated by chemoprophylaxis. Pre-exposure prophylaxis with doxycycline (200 mg orally once per week) was effective for military personnel undergoing high-risk jungle training exercises (Takafuji et al. 1984). Doxycycline has also been studied for postexposure prophylaxis of local populations after heavy rainfall in endemic areas (Gonsalez et al. 1998; Sehgal et al. 2000). One of these two studies found that postexposure doxycycline prophylaxis reduced the incidence of symptomatic disease (Sehgal et al. 2000). Alternatives to doxycycline, such as azithromycin or amoxicillin, have not been studied, but may be considered in pregnant women and children and individuals at risk of photosensitivity.

Acknowledgments

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Current work in Prof. Haake's laboratory is supported by NIH Grant R01 AI034431 and a VA Merit Award.

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References

Go to:

1. Abdulkader RC, Seguro AC, Malheiro PS, Burdmann EA, Marcondes M. Peculiar electrolytic and hormonal abnormalities in acute renal failure due to leptospirosis. Am J Trop Med Hyg. 1996;54:1–6. [PubMed]
2. Abela-Ridder B, Sikkema R, Hartskeerl RA. Estimating the burden of human leptospirosis. Int J Antimicrob Agents. 2010;36(Suppl 1):S5–S7. [PubMed]
3. Adler B, Faine S. The antibodies involved in the human immune response to leptospiral infection. J Med Microbiol. 1978;11:387–400. [PubMed]
4. Adler B, de la Peña-Moctezuma A. *Leptospira* and leptospirosis. Vet Microbiol. 2010;140:287–296. [PubMed]
5. Agampodi SB, Matthias MA, Moreno AC, Vinetz JM. Utility of quantitative polymerase chain reaction in leptospirosis diagnosis: association of level of leptospiremia and clinical manifestations in Sri Lanka. Clin Infect Dis. 2012;54:1249–1255. [PMC free article] [PubMed]
6. Ahern M, Kovats RS, Wilkinson P, Few R, Matthies F. Global health impacts of floods: epidemiologic evidence. Epidemiol Rev. 2005;27:36–46. [PubMed]
7. Ahmed A, Engelberts MF, Boer KR, Ahmed N, Hartskeerl RA. Development and validation of a real-time PCR for detection of pathogenic *Leptospira* species in clinical materials. PLoS ONE. 2009;4:e7093. [PMC free article] [PubMed]
8. Ahmed A, Grobusch MP, Klatser PR, Hartskeerl RA. Molecular approaches in the detection and characterization of *Leptospira*. J Bacteriol Parasitol. 2012;3:1000133.
9. Ahmed A, Thaipadungpanit J, Boonsilp S, Wuthiekanun V, Nalam K, Spratt BG, Aanensen DM, Smythe LD, Ahmed N, Feil EJ, Hartskeerl RA, Peacock SJ. Comparison of two multilocus sequence based genotyping schemes for *Leptospira* species. PLoS Negl Trop Dis. 2011;5:e1374. [PMC free article] [PubMed]
10. Ahmed N, Devi SM, de Valverde ML, Vijayachari P, Machang'u RS, Ellis WA, Hartskeerl RA. Multilocus sequence typing method for identification and genotypic classification of pathogenic *Leptospira* species. Ann Clin Microbiol Antimicrob. 2006;5:28. [PMC free article] [PubMed]

11. Alexander AD, Rule PL. Penicillins, cephalosporins, and tetracyclines in treatment of hamsters with fatal leptospirosis. Antimicrob Agents Chemother. 1986;30:835–839. [PMC free article] [PubMed]
12. Alston JM, Broom JC. Leptospirosis in man and animals. E. & S. Livingstone; Edinburgh: 1958.
13. Amato MB, Barbas CS, Medeiros DM, Magaldi RB, Schettino GP, Lorenzi-Filho G, Kairalla RA, Deheinzelin D, Munoz C, Oliveira R, Takagaki TY, Carvalho CR. Effect of a protective-ventilation strategy on mortality in the acute respiratory distress syndrome. N Engl J Med. 1998;338:347–354. [PubMed]
14. Andrade L, Cleto S, Seguro AC. Door-to-dialysis time and daily hemodialysis in patients with leptospirosis: impact on mortality. Clin J Am Soc Nephrol. 2007;2:739–744. [PubMed]
15. Andrade L, de Francesco Daher E, Seguro AC. *Leptospiral* nephropathy. Semin Nephrol. 2008;28:383–394. [PubMed]
16. Appassakij H, Silpapojakul K, Wansit R, Woodtayakorn J. Evaluation of the immunofluorescent antibody test for the diagnosis of human leptospirosis. Am J Trop Med Hyg. 1995;52:340–343. [PubMed]
17. Araujo ER, Seguro AC, Spichler A, Magaldi AJ, Volpini RA, De Brito T. Acute kidney injury in human leptospirosis: an immunohistochemical study with pathophysiological correlation. Virchows Arch. 2010;456:367–375. [PubMed]
18. Arean VM. The pathologic anatomy and pathogenesis of fatal human leptospirosis (Weil's disease) Am J Pathol. 1962;40:393–423. [PMC free article] [PubMed]
19. Avdeeva MG, Moisova DL, Gorodin VN, Kostomarov AM, Zotov SV, Cherniavskaia OV. The role glucose-6-phosphate dehydrogenase in pathogenesis of anemia in leptospirosis. Klin Med (Mosk) 2002;80:42–44. [PubMed]
20. Babudieri B, Carlos ER, Carlos ET., Jr Pathogenic *Leptospira* isolated from toad kidneys. Trop Geogr Med. 1973;25:297–299. [PubMed]
21. Bajani MD, Ashford DA, Bragg SL, Woods CW, Aye T, Spiegel RA, Plikaytis BD, Perkins BA, Phelan M, Levett PN, Weyant RS. Evaluation of four commercially available rapid serologic tests for diagnosis of leptospirosis. J Clin Microbiol. 2003;41:803–809. [PMC free article] [PubMed]
22. Bal AE, Gravekamp C, Hartskeerl RA, de Meza-Brewster J, Korver H, Terpstra WJ. Detection of leptospires in urine by PCR for early diagnosis of leptospirosis. J Clin Microbiol. 1994;32:1894–1898. [PMC free article] [PubMed]
23. Benbrick E, Pouliquen P, Domont A. Evaluation de la tolerance de la vaccination contre *Leptospira icterohaemorrhagiae* chez 50 employe's de canaux. Arch Mal Prof. 2001;62:35–40.
24. Berman SJ, Tsai C, Holms K, Fresh JW, Walten RH. Sporadic anicteric leptospirosis in South Vietnam. A study of 150 patients. Ann Intern Med. 1973;79:167–173. [PubMed]
25. Bharti AR, Nally JE, Ricaldi JN, Matthias MA, Diaz MM, Lovett MA, Levett PN, Gilman RH, Willig MR, Gotuzzo E, Vinetz JM. Leptospirosis: a zoonotic disease of global importance. Lancet Infect Dis. 2003;3:757–771. [PubMed]
26. Blackmore DK, Schollum LM, Moriarty KM. The magnitude and duration of titres of leptospiral agglutinins in human sera. NZ Med J. 1984;97:83–86. [PubMed]
27. Bolin CA, Koellner P. Human-to-human transmission of *Leptospira interrogans* by milk. J Infect Dis. 1988;158:246–247. [PubMed]
28. Boonsilp S, Thaipadungpanit J, Amornchai P, Wuthiekanun V, Bailey MS, Holden MT, Zhang C, Jiang X, Koizumi N, Taylor K, Galloway R, Hoffmaster AR, Craig S, Smythe LD, Hartskeerl RA, Day NP, Chantratita N, Feil EJ, Aanensen DM, Spratt BG, Peacock SJ. A single multilocus sequence typing (MLST) scheme for seven pathogenic *Leptospira* species. PLoS Negl Trop Dis. 2013;7:e1954. [PMC free article] [PubMed]

29. Brandão AP, Camargo ED, da Silva ED, Silva MV, Abrão RV. Macroscopic agglutination test for rapid diagnosis of human leptospirosis. J Clin Microbiol. 1998;36:3138–3142. [PMC free article] [PubMed]
30. Brown PD, Gravekamp C, Carrington DG, Van de Kemp H, Hartskeerl RA, Edwards CN, Everard COR, Terpstra WJ, Levett PN. Evaluation of the polymerase chain reaction for early diagnosis of leptospirosis. J Med Microbiol. 1995;43:110–114. [PubMed]
31. Burt FJ, Rolph MS, Rulli NE, Mahalingam S, Heise MT. Chikungunya: a re-emerging virus. Lancet. 2012;379:662–671. [PubMed]
32. Chapman AJ, Faine S, Adler B. Antigens recognized by the human immune response to vaccination with a bivalent hardjo/pomona leptospiral vaccine. FEMS Microbiol Immunol. 1990;2:111–118. [PubMed]
33. Chen T. Development and present status of leptospiral vaccine and technology of production of the vaccine in China. Nihon Saikingaku Zasshi. 1985;40:755–762. [PubMed]
34. Chu KM, Rathinam R, Namperumalsamy P, Dean D. Identification of *Leptospira* species in the pathogenesis of uveitis and determination of clinical ocular characteristics in South India. J Infect Dis. 1998;177:1314–1321. [PubMed]
35. Chung HL, Ts'ao WC, Mo PS, Yen C. Transplacental or congenital infection of leptospirosis. Clinical and experimental observations. Chin Med J. 1963;82:777–782. [PubMed]
36. Coghlan JD, Bain AD. Leptospirosis in human pregnancy followed by death of the foetus. Brit Med J. 1969;1:228–230. [PMC free article] [PubMed]
37. Collen MJ, Ansher AF, Chapman AB, Mackow RC, Lewis JH. Serum amylase in patients with renal insufficiency and renal failure. Am J Gastroenterol. 1990;85:1377–1380. [PubMed]
38. Corwin A, Ryan A, Bloys W, Thomas R, Deniega B, Watts D. A waterborne outbreak of leptospirosis among United States military personnel in Okinawa, Japan. Int J Epidemiol. 1990;19:743–748. [PubMed]
39. Covic A, Goldsmith DJ, Gusbeth-Tatomir P, Seica A, Covic M. A retrospective 5-year study in Moldova of acute renal failure due to leptospirosis: 58 cases and a review of the literature. Nephrol Dial Transplant. 2003;18:1128–1134. [PubMed]
40. Cumberland PC, Everard COR, Levett PN. Assessment of the efficacy of the IgM enzyme-linked immunosorbent assay (ELISA) and microscopic agglutination test (MAT) in the diagnosis of acute leptospirosis. Am J Trop Med Hyg. 1999;61:731–734. [PubMed]
41. Cumberland PC, Everard COR, Wheeler JG, Levett PN. Persistence of anti-leptospiral IgM, IgG and agglutinating antibodies in patients presenting with acute febrile illness in Barbados 1979 –1989. Eur J Epidemiol. 2001;17:601–608. [PubMed]
42. Daher E, Zanetta DM, Cavalcante MB, Abdulkader RC. Risk factors for death and changing patterns in leptospirosis acute renal failure. Am J Trop Med Hyg. 1999;61:630–634. [PubMed]
43. Dechet AM, Parsons M, Rambaran M, Mohamed-Rambaran P, Florendo-Cumbermack A, Persaud S, Baboolal S, Ari MD, Shadomy SV, Zaki SR, Paddock CD, Clark TA, Harris L, Lyon D, Mintz ED. Leptospirosis outbreak following severe flooding: a rapid assessment and mass prophylaxis campaign; Guyana, January–February 2005. PLoS ONE. 2012;7:e39672. [PMC free article] [PubMed]
44. Desakorn V, Wuthiekanun V, Thanachartwet V, Sahassananda D, Chierakul W, Apiwattanaporn A, Day NP, Limmathurotsakul D, Peacock SJ. Accuracy of a commercial IgM ELISA for the diagnosis of human leptospirosis in Thailand. Am J Trop Med Hyg. 2012;86:524–527. [PMC free article] [PubMed]
45. Dikken H, Kmety E. Serological typing methods of leptospires. In: Bergan T, Norris JR, editors. Methods in microbiology. Academic Press; London: 1978. pp. 259–307.
46. Doeleman FPJ. Ziekte van Weil, rechstreeks overgebracht van mensch op mensch. Ned Tijdschr Geneeskd. 1932;76:5057.

47. Dupont H, Dupont-Perdrizet D, Perie JL, Zehner-Hansen S, Jarrige B, Daijardin JB. Leptospirosis: prognostic factors associated with mortality. Clin Infect Dis. 1997;25:720–724. [PubMed]
48. Edwards CN, Nicholson GD, Hassell TA, Everard CO, Callender J. Penicillin therapy in icteric leptospirosis. Am J Trop Med Hyg. 1988;39:388–390. [PubMed]
49. Everard CO, Carrington D, Korver H, Everard JD. Leptospires in the marine toad (*Bufo marinus*) on Barbados. J Wildl Dis. 1988;24:334–338. [PubMed]
50. Everard COR, Bennett S, Edwards CN, Nicholson GD, Hassell TA, Carrington DG, Everard JD. An investigation of some risk factors for severe leptospirosis on Barbados. J Trop Med Hyg. 1992;95:13–32. [PubMed]
51. Everard JD, Everard COR. Leptospirosis in the Caribbean. Rev Med Microbiol. 1993;4:114–122.
52. Faine S. Guidelines for the control of leptospirosis. World Health Organization; Geneva: 1982.
53. Faine S, Adler B, Christopher W, Valentine R. Fatal congenital human leptospirosis. Zentralbl Bakteriol Mikrobiol Hyg [A] 1984;257:548. [PubMed]
54. Faine S, Adler B, Bolin C, Perolat P. *Leptospira* and leptospirosis. 2. MediSci; Melbourne: 1999.
55. Felzemburgh RD, Ribeiro GS, Costa F, Reis RB, Hagan JE, Melendez AX, Fraga D, Santana FS, Mohr S, Dos Santos BL, Silva AQ, Santos AC, Ravines RR, Tassinari WS, Carvalho MS, Reis MG, Ko AI. Prospective study of leptospirosis transmission in an urban slum community: role of poor environment in repeated exposures to the *Leptospira* agent. PLoS Negl Trop Dis. 2014;8:e2927. [PMC free article] [PubMed]
56. Fukumura K. Epidemiological studies on leptospirosis in Okinawa. Part 1. Prevalence of leptospirosis in Izena Island and the prevention by vaccination. Yamaguchi Med J. 1984;33:257–268.
57. Galloway RL, Levett PN. Application and validation of PFGE for serovar identification of *Leptospira* clinical isolates. PLoS Negl Trop Dis. 2010;4:e824. [PMC free article] [PubMed]
58. Galton MM. Methods in the laboratory diagnosis of leptospirosis. Ann NY Acad Sci. 1962;98:675–684.
59. Ganoza CA, Matthias MA, Saito M, Cespedes M, Gotuzzo E, Vinetz JM. Asymptomatic renal colonization of humans in the peruvian Amazon by *Leptospira*. PLoS Negl Trop Dis. 2010;4:e612. [PMC free article] [PubMed]
60. Gochenour WS, Smadel JE, Jackson EB, Evans LB, Yager RH. Leptospiral etiology of Fort Bragg fever. Publ Hlth Rep. 1952;67:811–813. [PMC free article] [PubMed]
61. Gonsalez CR, Casseb J, Monteiro FG, Paula-Neto JB, Fernandez RB, Silva MV, Camargo ED, Mairinque JM, Tavares LC. Use of doxycycline for leptospirosis after high-risk exposure in Sao Paulo, Brazil. Rev Inst Med Trop Sao Paulo. 1998;40:59–61. [PubMed]
62. Goris MG, Wagenaar JF, Hartskeerl RA, van Gorp EC, Schuller S, Monahan AM, Nally JE, van der Poll T, van't Veer C. Potent innate immune response to pathogenic *Leptospira* in human whole blood. PLoS ONE. 2011a;6:e18279. [PMC free article] [PubMed]
63. Goris MGA, Leeflang MMG, Boer KR, Goeijenbier M, van Gorp ECM, Wagenaar JFP, Hartskeerl RA. Establishment of valid laboratory case definition for human leptospirosis. J Bacteriol Parasitol. 2011b;S5-001:1–8.
64. Goris MG, Kikken V, Straetemans M, Alba S, Goeijenbier M, van Gorp EC, Boer KR, Wagenaar JF, Hartskeerl RA. Towards the burden of human leptospirosis: duration of acute illness and occurrence of post-leptospirosis symptoms of patients in the Netherlands. PLoS ONE. 2013a;8:e76549. [PMC free article] [PubMed]
65. Goris MGA, Leeflang MMG, Loden M, Wagenaar JFP, Klatser PR, Hartskeerl RA, Boer KR. Prospective evaluation of three rapid diagnostic tests for diagnosis of human leptospirosis. PLoS Negl Trop Dis. 2013b;7:e2290. [PMC free article] [PubMed]

66. Gouveia EL, Metcalfe J, de Carvalho ALF, Aires TSF, Villalobos-Bisneto JC, Queiroz A, Santos AC, Salgado K, Reis MG, Ko AI. Leptospirosis-associated severe pulmonary hemorrhage syndrome, Salvador, Brazil. Emerg Infect Dis. 2008;14:505–508. [PMC free article] [PubMed]
67. Gravekamp C, Korver H, Montgomery J, Everard COR, Carrington D, Ellis WA, Terpstra WJ. Leptospires isolated from toads and frogs on the island of Barbados. Zentralbl Bakteriol. 1991;275:403–411. [PubMed]
68. Guerra-Silveira F, Abad-Franch F. Sex bias in infectious disease epidemiology: patterns and processes. PLoS ONE. 2013;8:e62390. [PMC free article] [PubMed]
69. Harrison NA, Fitzgerald WR. Leptospirosis—can it be a sexually transmitted disease? Postgrad Med J. 1988;64:163–164. [PMC free article] [PubMed]
70. Hartskeerl RA, Collares-Pereira M, Ellis WA. Emergence, control and re-emerging leptospirosis: dynamics of infection in the changing world. Clin Microbiol Infect. 2011;17:494–501. [PubMed]
71. Herath NJ, Kularatne SA, Weerakoon KG, Wazil A, Subasinghe N, Ratnatunga NV. Long term outcome of acute kidney injury due to leptospirosis? A longitudinal study in Sri Lanka. BMC Res Notes. 2014;7:398. [PMC free article] [PubMed]
72. Herrmann JL, Bellenger E, Perolat P, Baranton G, Saint Girons I. Pulsed-field gel electrophoresis of *Not*I digests of leptospiral DNA: a new rapid method of serovar identification. J Clin Microbiol. 1992;30:1696–1702. [PMC free article] [PubMed]
73. Hospenthal DR, Murray CK. In vitro susceptibilities of seven *Leptospira* species to traditional and newer antibiotics. Antimicrob Agents Chemother. 2003;47:2646–2648. [PMC free article] [PubMed]
74. Hotez PJ, Bottazzi ME, Franco-Paredes C, Ault SK, Periago MR. The neglected tropical diseases of Latin America and the Caribbean: a review of disease burden and distribution and a roadmap for control and elimination. PLoS Negl Trop Dis. 2008;2:e300. [PMC free article] [PubMed]
75. Hull-Jackson C, Glass MB, Ari MD, Bragg SL, Branch SL, Whittington CU, Edwards CN, Levett PN. Evaluation of a commercial latex agglutination assay for serological diagnosis of leptospirosis. J Clin Microbiol. 2006;44:1853–1855. [PMC free article] [PubMed]
76. Jevon TR, Knudson MP, Smith PA, Whitecar PS, Blake RL. A point-source epidemic of leptospirosis. Postgrad Med. 1986;80:121–129. [PubMed]
77. Karande S, Patil S, Kulkarni M, Joshi A, Bharadwaj R. Acute aseptic meningitis as the only presenting feature of leptospirosis. Pediatr Infect Dis J. 2005;24:390–391. [PubMed]
78. Katz AR, Manea SJ, Sasaki DM. Leptospirosis on Kauai: investigation of a common source waterborne outbreak. Am J Public Health. 1991;81:1310–1312. [PMC free article] [PubMed]
79. Katz AR, Ansdell VE, Effler PV, Middleton CR, Sasaki DM. Assessment of the clinical presentation and treatment of 353 cases of laboratory-confirmed leptospirosis in hawaii, 1974 –1998. Clin Infect Dis. 2001;33:1834–1841. [PubMed]
80. Katz AR, Buchholz AE, Hinson K, Park SY, Effler PV. Leptospirosis in Hawaii, USA, 1999 –2008. Emerg Infect Dis. 2011;17:221–226. [PMC free article] [PubMed]
81. Ko AI, Galvao Reis M, Ribeiro Dourado CM, Johnson WD, Jr, Riley LW. The Salvador Leptospirosis Study Group (1999) Urban epidemic of severe leptospirosis in Braz. Lancet. 354:820–825. [PubMed]
82. Ko AI, Goarant C, Picardeau M. *Leptospira*: the dawn of the molecular genetics era for an emerging zoonotic pathogen. Nat Rev Microbiol. 2009;7:736–747. [PMC free article] [PubMed]
83. Korver H, Kolk AHJ, Vingerhoed J, van Leeuwen J, Terpstra WJ. Classification of serovars of the Icterohaemorrhagiae serogroup by monoclonal antibodies. Israel J Vet Med. 1988;44:15–18.
84. Lau C, Smythe L, Weinstein P. Leptospirosis: an emerging disease in travellers. Travel Med Infect Dis. 2010a;8:33–39. [PubMed]
85. Lau CL, Smythe LD, Craig SB, Weinstein P. Climate change, flooding, urbanisation and leptospirosis: fuelling the fire? Trans R Soc Trop Med Hyg. 2010b;104:631–638. [PubMed]

86. Laurichesse H, Gourdon F, Smits HL, Abdoe TH, Estavoyer JM, Rebika H, Pouliquen P, Catalina P, Dubray C, Beytout J. Safety and immunogenicity of subcutaneous or intramuscular administration of a monovalent inactivated vaccine against *Leptospira interrogans* serogroup Icterohaemorrhagiae in healthy volunteers. Clin Microbiol Infect. 2007;13:395–403. [PubMed]
87. Leon A, Pronost S, Fortier G, Andre-Fontaine G, Leclercq R. Multilocus sequence analysis for typing *Leptospira interrogans* and *Leptospira kirschneri*. J Clin Microbiol. 2009;48:581–585. [PMC free article] [PubMed]
88. Levett PN. Leptospirosis: re-emerging or re-discovered disease? J Med Microbiol. 1999;48:417–418. [PubMed]
89. Levett PN. Leptospirosis. Clin Microbiol Rev. 2001;14:296–326. [PMC free article] [PubMed]
90. Levett PN, Branch SL. Evaluation of two enzyme-linked immunosorbent assay methods for detection of immunoglobulin M antibodies in acute leptospirosis. Am J Trop Med Hyg. 2002;66:745–748. [PubMed]
91. Levett PN. Usefulness of serologic analysis as a predictor of the infecting serovar in patients with severe leptospirosis. Clin Infect Dis. 2003;36:447–452. [PubMed]
92. Levett PN. Leptospirosis: a forgotten zoonosis? Clin Appl Immunol Rev. 2004;4:435–448.
93. Lingappa J, Kuffner T, Tappero J, Whitworth W, Mize A, Kaiser R, McNicholl J. HLA-DQ6 and ingestion of contaminated water: possible gene-environment interaction in an outbreak of leptospirosis. Genes Immun. 2004;5:197–202. [PubMed]
94. Lopes AA, Costa E, Costa YA, Sacramento E, De Oliveira AR, Junior, Lopes MB, Lopes GB. Comparative study of the in-hospital case-fatality rate of leptospirosis between pediatric and adult patients of different age groups. Rev Inst Med Trop Sao Paulo. 2004;46:19–24. [PubMed]
95. Lupidi R, Cinco M, Balanzin D, Delprete E, Varaldo PE. Serological follow-up of patients in a localized outbreak of leptospirosis. J Clin Microbiol. 1991;29:805–809. [PMC free article] [PubMed]
96. Maciel EA, de Carvalho AL, Nascimento SF, de Matos RB, Gouveia EL, Reis MG, Ko AI. Household transmission of *Leptospira* infection in urban slum communities. PLoS Negl Trop Dis. 2008;2:e154. [PMC free article] [PubMed]
97. Mailloux M, Lambert R, Chenu M. Human vaccination against leptospirosis icterohaemorrhagiae. Med Hyg (Geneve) 1983;41:1025–1030.
98. Marotto PC, Ko AI, Murta-Nascimento C, Seguro AC, Prado RR, Barbosa MC, Cleto SA, Eluf-Neto J. Early identification of leptospirosis-associated pulmonary hemorrhage syndrome by use of a validated prediction model. J Infect. 2010;60:218–223. [PMC free article] [PubMed]
99. Martinez R, Perez A, del Quinones MC, Cruz R, Alvarez A, Armesto M, Fernandez C, Menendez J, Rodriguez I, Baro M, Diaz M, Rodriguez J, Sierra G, Obregon AM, Toledo ME, Fernandez N. Efficacy and safety of a vaccine against human leptospirosis in Cuba. Rev Panam Salud Publica. 2004;15:249–255. [PubMed]
100. McBride AJ, Athanazio DA, Reis MG, Ko AI. Leptospirosis. Curr Opin Infect Dis. 2005;18:376–386. [PubMed]
101. McClain JBL, Ballou WR, Harrison SM, Steinweg DL. Doxycycline therapy for leptospirosis. Ann Intern Med. 1984;100:696–698. [PubMed]
102. Merien F, Truccolo J, Rougier Y, Baranton G, Perolat P. In vivo apoptosis of hepatocytes in guinea pigs infected with *Leptospira interrogans* serovar icterohaemorrhagiae. FEMS Microbiol Lett. 1998;169:95–102. [PubMed]
103. Merien F, Portnoi D, Bourhy P, Charavay F, Berlioz-Arthaud A, Baranton G. A rapid and quantitative method for the detection of *Leptospira* species in human leptospirosis. FEMS Microbiol Lett. 2005;249:139–147. [PubMed]

104. Miyahara S, Saito M, Kanemaru T, Villanueva SY, Gloriani NG, Yoshida SI. Destruction of the hepatocyte junction by intercellular invasion of *Leptospira* causes jaundice in a hamster model of Weil's disease. Int J Exp Pathol. 2014;95:271–281. [PMC free article] [PubMed]
105. Moeschlin S. Lungeninfiltrate beim Ikterus infeciosus Weil. Schweitz Med Wochenschr. 1943;73:1227–1230.
106. Morgan J, Bornstein SL, Karpati AM, Bruce M, Bolin CA, Austin CC, Woods CW, Lingappa J, Langkop C, Davis B, Graham DR, Proctor M, Ashford DA, Bajani M, Bragg SL, Shutt K, Perkins BA, Tappero JW. Outbreak of leptospirosis among triathlon participants and community residents in Springfield, Illinois, 1998. Clin Infect Dis. 2002;34:1593–1599. [PubMed]
107. Murray CK, Ellis MW, Hospenthal DR. Susceptibility of *Leptospira* serovars to antimalarial agents. Am J Trop Med Hyg. 2004;71:685–686. [PubMed]
108. Murray CK, Gray MR, Mende K, Parker TM, Samir A, Rahman BA, Habashy EE, Hospenthal DR, Pimentel G. Use of patient-specific *Leptospira* isolates in the diagnosis of leptospirosis employing microscopic agglutination testing (MAT) Trans R Soc Trop Med Hyg. 2011;105:209–213. [PubMed]
109. Nabity SA, Ribeiro GS, Lessa Aquino C, Takahashi D, Damião AO, Gonçalves AH, Miranda-Filho DB, Greenwald R, Esfandiari J, Lyashchenko KP, Reis MG, Medeiros MA, Ko AI. Accuracy of a dual path platform (DPP) assay for the rapid point-of-care diagnosis of human leptospirosis. PLoS Negl Trop Dis. 2012;6:e1878. [PMC free article] [PubMed]
110. Nahori MA, Fournie-Amazouz E, Que-Gewirth NS, Balloy V, Chignard M, Raetz CR, Saint Girons I, Werts C. Differential TLR recognition of leptospiral lipid A and lipopolysaccharide in murine and human cells. J Immunol. 2005;175:6022–6031. [PubMed]
111. Nally JE, Chantranuwat C, Wu XY, Fishbein MC, Pereira MM, Da Silva JJ, Blanco DR, Lovett MA. Alveolar septal deposition of immunoglobulin and complement parallels pulmonary hemorrhage in a guinea pig model of severe pulmonary leptospirosis. Am J Pathol. 2004;164:1115–1127. [PMC free article] [PubMed]
112. O'Brien MM, Vincent JM, Person DA, Cook BA. Leptospirosis and pancreatitis: a report of ten cases. Pediatr Infect Dis J. 1998;17:436–438. [PubMed]
113. Palaniappan RU, Chang YF, Chang CF, Pan MJ, Yang CW, Harpending P, McDonough SP, Dubovi E, Divers T, Qu J, Roe B. Evaluation of *lig*-based conventional and real time PCR for the detection of pathogenic leptospires. Mol Cell Probes. 2005;19:111–117. [PubMed]
114. Palmer MF, Zochowski WJ. Survival of leptospires in commercial blood culture systems revisited. J Clin Pathol. 2000;53:713–714. [PMC free article] [PubMed]
115. Panaphut T, Domrongkitchaiporn S, Thinkamrop B. Prognostic factors of death in leptospirosis: a prospective cohort study in Khon Kaen, Thailand. Int J Infect Dis. 2002;6:52–59. [PubMed]
116. Panaphut T, Domrongkitchaiporn S, Vibhagool A, Thinkamrop B, Susaengrat W. Ceftriaxone compared with sodium penicillin G for treatment of severe leptospirosis. Clin Infect Dis. 2003;36:1507–1513. [PubMed]
117. Pappachan MJ, Mathew S, Aravindan KP, Khader A, Bharghavan PV, Kareem MM, Tuteja U, Shukla J, Batra HV. Risk factors for mortality in patients with leptospirosis during an epidemic in northern Kerala. Natl Med J India. 2004;17:240–242. [PubMed]
118. Park SK, Lee SH, Rhee YK, Kang SK, Kim KJ, Kim MC, Kim KW, Chang WH. Leptospirosis in Chonbuk Province of Korea in 1987: a study of 93 patients. Am J Trop Med Hyg. 1989;41:345–351. [PubMed]
119. Pavan ME, Cairó F, Brihuega B, Samartino L. Multiple-locus variable-number tandem repeat analysis (MLVA) of *Leptospira interrogans* serovar Pomona from Argentina reveals four new genotypes. Comp Immunol Microbiol Infect Dis. 2008;31:37–45. [PubMed]
120. Perez J, Goarant C. Rapid *Leptospira* identification by direct sequencing of the diagnostic PCR products in New Caledonia. BMC Microbiol. 2010;10:325. [PMC free article] [PubMed]

121. Pouliquen P, Catilina P. Enquete de pharmacosurveillance aupres des medecins vaccinateurs. Rev Med Trav. 2000;27:83–88.
122. Que-Gewirth NS, Riberio AA, Kalb SR, Cotter RJ, Bulach DM, Adler B, Saint Girons I, Werts C, Raetz CRH. A methylated phosphate group and four amide-linked acyl chains in *Leptospira interrogans* lipid A. J Biol Chem. 2004;279:25420–25429. [PMC free article] [PubMed]
123. Rathinam SR. Ocular manifestations of leptospirosis. J Postgrad Med. 2005;51:189–194. [PubMed]
124. Reis EA, Hagan JE, Ribeiro GS, Teixeira-Carvalho A, Martins-Filho OA, Montgomery RR, Shaw AC, Ko AI, Reis MG. Cytokine response signatures in disease progression and development of severe clinical outcomes for leptospirosis. PLoS Negl Trop Dis. 2013;7:e2457. [PMC free article] [PubMed]
125. Reis RB, Ribeiro GS, Felzemburgh RD, Santana FS, Mohr S, Melendez AX, Queiroz A, Santos AC, Ravines RR, Tassinari WS, Carvalho MS, Reis MG, Ko AI. Impact of environment and social gradient on *Leptospira* infection in urban slums. PLoS Negl Trop Dis. 2008;2:e228. [PMC free article] [PubMed]
126. Ressner RA, Griffith ME, Beckius ML, Pimentel G, Miller RS, Mende K, Fraser SL, Galloway RL, Hospenthal DR, Murray CK. Antimicrobial susceptibilities of geographically diverse clinical human isolates of *Leptospira*. Antimicrob Agents Chemother. 2008;52:2750–2754. [PMC free article] [PubMed]
127. Ribeiro MA, Assis CSN, Romero EC. Serodiagnosis of human leptospirosis employing immunodominant antigen. Serodiagn Immunother Infect Dis. 1994;6:140–144.
128. Romero EC, Caly CR, Yasuda PH. The persistence of leptospiral agglutinins titers in human sera diagnosed by the microscopic agglutination test. Rev Inst Med Trop São Paulo. 1998;40:183–184. [PubMed]
129. Salaun L, Merien F, Gurianova S, Baranton G, Picardeau M. Application of multilocus variable-number tandem-repeat analysis for molecular typing of the agent of leptospirosis. J Clin Microbiol. 2006;44:3954–3962. [PMC free article] [PubMed]
130. Schreier S, Doungchawee G, Chadsuthi S, Triampo D, Triampo W. Leptospirosis: current situation and trends of specific laboratory tests. Expert Rev Clin Immunol. 2013;9:263–280. [PubMed]
131. Segura ER, Ganoza CA, Campos K, Ricaldi JN, Torres S, Silva H, Cespedes MJ, Matthias MA, Swancutt MA, Lopez Linan R, Gotuzzo E, Guerra H, Gilman RH, Vinetz JM. Clinical spectrum of pulmonary involvement in leptospirosis in a region of endemicity, with quantification of leptospiral burden. Clin Infect Dis. 2005;40:343–351. [PMC free article] [PubMed]
132. Seguro AC, Lomar AV, Rocha AS. Acute renal failure of leptospirosis: nonoliguric and hypokalemic forms. Nephron. 1990;55:146–151. [PubMed]
133. Sehgal SC, Murhekar MV, Sugunan AP. Outbreak of leptospirosis with pulmonary involvement in North Andaman. Indian J Med Res. 1995;102:9–12. [PubMed]
134. Sehgal SC, Sugunan AP, Murhekar MV, Sharma S, Vijayachari P. Randomized controlled trial of doxycycline prophylaxis against leptospirosis in an endemic area. Int J Antimicrob Agents. 2000;13:249–255. [PubMed]
135. Sejvar J, Bancroft E, Winthrop K, Bettinger J, Bajani M, Bragg S, Shutt K, Kaiser R, Marano N, Popovic T, Tappero J, Ashford D, Mascola L, Vugia D, Perkins B, Rosenstein N. Leptospirosis in "eco-challenge" athletes, Malaysian Borneo, 2000. Emerg Infect Dis. 2003;9:702–707. [PMC free article] [PubMed]
136. Self CA, Iskrzynska WI, Waitkins SA, Whicher JW, Whicher JT. Leptospirosis among British cavers. Cave Sci. 1987;14:131–134.
137. Shaw RD. Kayaking as a risk factor for leptospirosis. Mo Med. 1992;89:354–357. [PubMed]

138. Shenberg E, Torten M. A new leptospiral vaccine for use in man. I. Development of a vaccine from *Leptospira* grown on a chemically defined medium. J Infect Dis. 1973;128:642–646. [PubMed]
139. Signorini ML, Lottersberger J, Tarabla HD, Vanasco NB. Enzyme-linked immunosorbent assay to diagnose human leptospirosis: a meta-analysis of the published literature. Epidemiol Infect. 2013;141:22–32. [PubMed]
140. Silva HR, Tanajura GM, Tavares-Neto J, de Gomes Md ML, daLinhares Ad AC, Vasconcelos PF, Ko AI. Aseptic meningitis syndrome due to enterovirus and *Leptospira* sp in children of Salvador, Bahia. Rev Soc Bras Med Trop. 2002a;35:159–165. [PubMed]
141. Silva JJ, Dalston MO, Carvalho JE, Setubal S, Oliveira JM, Pereira MM. Clinicopathological and immunohistochemical features of the severe pulmonary form of leptospirosis. Rev Soc Bras Med Trop. 2002b;35:395–399. [PubMed]
142. Sitprija V, Evans H. The kidney in human leptospirosis. Am J Med. 1970;49:780–788. [PubMed]
143. Slack AT, Dohnt MF, Symonds ML, Smythe LD. Development of a multiple-locus variable number of tandem repeat analysis (MLVA) for *Leptospira interrogans* and its application to *Leptospira interrogans* serovar Australis isolates from far North Queensland. Australia. Ann Clin Microbiol Antimicrob. 2005;4:10. [PMC free article] [PubMed]
144. Smits HL, Hartskeerl RA, Terpstra WJ. International multi-centre evaluation of a dipstick assay for human leptospirosis. Trop Med Int Health. 2000a;5:124–128. [PubMed]
145. Smits HL, van Der Hoorn MA, Goris MG, Gussenhoven GC, Yersin C, Sasaki DM, Terpstra WJ, Hartskeerl RA. Simple latex agglutination assay for rapid serodiagnosis of human leptospirosis. J Clin Microbiol. 2000b;38:1272–1275. [PMC free article] [PubMed]
146. Smits HL, Chee HD, Eapen CK, Kuriakose M, Sugathan S, Gasem MH, Yersin C, Sakasi D, Lai AFRF, Hartskeerl RA, Liesdek B, Abdoel TH, Goris MG, Gussenhoven GC. Latex based, rapid and easy assay for human leptospirosis in a single test format. Trop Med Int Health. 2001a;6:114–118. [PubMed]
147. Smits HL, Eapen CK, Sugathan S, Kuriakose M, Gasem MH, Yersin C, Sasaki D, Pujianto B, Vestering M, Abdoel TH, Gussenhoven GC. Lateral-flow assay for rapid serodiagnosis of human leptospirosis. Clin Diagn Lab Immunol. 2001b;8:166–169. [PMC free article] [PubMed]
148. Smythe L, Dohnt M, Norris M, Symonds M, Scott J. Review of leptospirosis notifications in Queensland 1985 to 1996. Commun Dis Intell. 1997;21:17–20. [PubMed]
149. Smythe LD, Smith IL, Smith GA, Dohnt MF, Symonds ML, Barnett LJ, McKay DB. A quantitative PCR (TaqMan) assay for pathogenic *Leptospira* spp. BMC Infect Dis. 2002;2:13. [PMC free article] [PubMed]
150. Smythe LD, Wuthiekanun V, Chierakul W, Suputtamongkol Y, Tiengrim S, Dohnt MF, Symonds ML, Slack AT, Apiwattanaporn A, Chueasuwanchai S, Day NP, Peacock SJ. The microscopic agglutination test (MAT) is an unreliable predictor of infecting *Leptospira* serovar in Thailand. Am J Trop Med Hyg. 2009;81:695–697. [PubMed]
151. Spichler A, Spichler E, Moock M, Vinetz JM, Leake JA. Acute pancreatitis in fatal anicteric leptospirosis. Am J Trop Med Hyg. 2007;76:886–887. [PubMed]
152. Spichler A, Athanazio D, Seguro AC, Vinetz JM. Outpatient follow-up of patients hospitalized for acute leptospirosis. Int J Infect Dis. 2011;15:e486–e490. [PMC free article] [PubMed]
153. Steneroden KK, Hill AE, Salman MD. Zoonotic disease awareness in animal shelter workers and volunteers and the effect of training. Zoonoses Public Health. 2011;58:449–453. [PubMed]
154. Stern EJ, Galloway R, Shadomy SV, Wannemuehler K, Atrubin D, Blackmore C, Wofford T, Wilkins PP, Ari MD, Harris L, Clark TA. Outbreak of leptospirosis among adventure race participants in Florida, 2005. Clin Infect Dis. 2010;50:843–849. [PubMed]

155. Stoddard RA, Gee JE, Wilkins PP, McCaustland K, Hoffmaster AR. Detection of pathogenic *Leptospira* spp. through TaqMan polymerase chain reaction targeting the LipL32 gene. Diagn Microbiol Infect Dis. 2009;64:247–255. [PubMed]
156. Stoenner HG, Dodd T, Larsen C. Antigenic variation of *Borrelia hermsii*. J Exp Med. 1982;156:1297–1311. [PMC free article] [PubMed]
157. Sulzer CR, Jones WL. Leptospirosis: methods in laboratory diagnosis. U.S. Department of Health, Education and Welfare; Atlanta: 1978.
158. Takafuji ET, Kirkpatrick JW, Miller RN, Karwacki JJ, Kelley PW, Gray MR, McNeill KM, Timboe HL, Kane RE, Sanchez JL. An efficacy trial of doxycycline chemoprophylaxis against leptospirosis. N Engl J Med. 1984;310:497–500. [PubMed]
159. Thaipadungpanit J, Wuthiekanun V, Chierakul W, Smythe LD, Petkanchanapong W, Limpaiboon R, Apiwatanaporn A, Slack AT, Suputtamongkol Y, White NJ, Feil EJ, Day NP, Peacock SJ. A dominant clone of *Leptospira interrogans* associated with an outbreak of human leptospirosis in Thailand. PLoS Negl Trop Dis. 2007;1:e56. [PMC free article] [PubMed]
160. Thaipadunpanit J, Chierakul W, Wuthiekanun V, Limmathurotsakul D, Amornchai P, Boonslip S, Smythe LD, Limpaiboon R, Hoffmaster AR, Day NP, Peacock SJ. Diagnostic accuracy of real-time PCR assays targeting 16S rRNA and LipL32 genes for human leptospirosis in Thailand: a case-control study. PLoS ONE. 2011;6:e16236. [PMC free article] [PubMed]
161. Torten M. Leptospirosis. In: Stoenner HE, Torten M, Kaplan W, editors. CRC handbook series in zoonoses section a: bacterial, rickettsial and mycotic diseases. CRC press; Boca Raton: 1979. pp. 363–420.
162. Trevejo RT, Rigau-Perez JG, Ashford DA, McClure EM, Jarquin-Gonzalez C, Amador JJ, de los Reyes JO, Gonzalez A, Zaki SR, Shieh WJ, McLean RG, Nasci RS, Weyant RS, Bolin CA, Bragg SL, Perkins BA, Spiegel RA. Epidemic leptospirosis associated with pulmonary hemorrhage-Nicaragua, 1995. J Infect Dis. 1998;178:1457–1463. [PubMed]
163. Truccolo J, Serais O, Merien F, Perolat P. Following the course of human leptospirosis: evidence of a critical threshold for the vital prognosis using a quantitative PCR assay. FEMS Microbiol Lett. 2001;204:317–321. [PubMed]
164. Truccolo J, Charavay F, Merien F, Perolat P. Quantitative PCR assay to evaluate ampicillin, ofloxacin, and doxycycline for treatment of experimental leptospirosis. Antimicrob Agents Chemother. 2002;46:848–853. [PMC free article] [PubMed]
165. Tubiana S, Mikulski M, Becam J, Lacassin F, Lefevre P, Gourinat AC, Goarant C, D'Ortenzio E. Risk factors and predictors of severe leptospirosis in New Caledonia. PLoS Negl Trop Dis. 2013;7:e1991. [PMC free article] [PubMed]
166. Turner LH. Leptospirosis II. Serology. Trans R Soc Trop Med Hyg. 1968;62:880–889. [PubMed]
167. Turner LH. Leptospirosis III. Maintenance, isolation and demonstration of leptospires. Trans R Soc Trop Med Hyg. 1970;64:623–646. [PubMed]
168. Verma A, Kumar P, Babb K, Timoney JF, Stevenson B. Cross-reactivity of antibodies against leptospiral recurrent uveitis-associated proteins A and B (LruA and LruB) with eye proteins. PLoS Negl Trop Dis. 2010;4:e778. [PMC free article] [PubMed]
169. Vinetz JM, Wilcox BA, Aguirre A, Gollin LX, Katz AR, Fujioka RS, Maly K, Horwitz P, Chang H. Beyond disciplinary boundaries: leptospirosis as a model of incorporating transdisciplinary approaches to understand infectious disease emergence. EcoHealth. 2005;2:1–16.
170. Viriyakosol S, Matthias MA, Swancutt MA, Kirkland TN, Vinetz JM. Toll-like receptor 4 protects against lethal *Leptospira interrogans* serovar Icterohaemorrhagiae infection and contributes to in vivo control of leptospiral burden. Infect Immun. 2006;74:887–895. [PMC free article] [PubMed]

171. Wagenaar JF, Goris MG, Partiningrum DL, Isbandrio B, Hartskeerl RA, Brandjes DP, Meijers JC, Gasem MH, van Gorp EC. Coagulation disorders in patients with severe leptospirosis are associated with severe bleeding and mortality. Trop Med Int Health. 2010;15:152–159. [PubMed]
172. Waitkins S. Leptospirosis in man, British Isles: 1984. Brit Med J. 1986;292:1324. [PMC free article] [PubMed]
173. Wani H. Über die prophylaxe der pirochaetosis icterohaemorrhagica Inada (Weilschen Krankheit) durch Schutzimpfung. Zeitschr Immunforsch Exp Therap. 1933;79:1–26.
174. Watt G, Padre LP, Tuazon ML, Calubaquib C, Santiago E, Ranoa CP, Laughlin LW. Placebo-controlled trial of intravenous penicillin for severe and late leptospirosis. Lancet. 1988;1:433–435. [PubMed]
175. Weil A. Ueber eine eigenthümliche, mit Milztumor, Icterus und Nephritis einhergehende, acute Infectionskrankheit. Dtsch Arch Klin Med. 1886;39:209.
176. Werts C, Tapping RI, Mathison JC, Chuang T-H, Kravchenko V, Saint Girons I, Haake D, Godowski PJ, Hayashi F, Ozinsky A, Underhill D, Aderem A, Tobias PS, Ulevitch RJ. Leptospiral endotoxin activates cells via a TLR2-dependent mechanism. Nat Immunol. 2001;2:346–352. [PubMed]
177. Werts C. Leptospirosis: a Toll road from B lymphocytes. Chang Gung Med J. 2010;33:591–601. [PubMed]
178. WHO . Leptospirosis worldwide, 1999. Wkly Epidemiol Rec. 1999;74:237–242. [PubMed]
179. WHO. Report of the second meeting of the leptospirosis burden epidemiology reference group. 2011.
180. Wilkins E, Cope A, Waitkins S. Rapids, rafts, and rats. Lancet. 1988;2:283–284. [PubMed]
181. Wilson MR, Naccache SN, Samayoa E, Biagtan M, Bashir H, Yu G, Salamat SM, Somasekar S, Federman S, Miller S, Sokolic R, Garabedian E, Candotti F, Buckley RH, Reed KD, Meyer TL, Seroogy CM, Galloway R, Henderson SL, Gern JE, DeRisi JL, Chiu CY. Actionable diagnosis of neuroleptospirosis by next-generation sequencing. N Engl J Med. 2014;370:2408–2417. [PMC free article] [PubMed]
182. Winslow WE, Merry DJ, Pirc ML, Devine PL. Evaluation of a commercial enzyme-linked immunosorbent assay for detection of immunoglobulin M antibody in diagnosis of human leptospiral infection. J Clin Microbiol. 1997;35:1938–1942. [PMC free article] [PubMed]
183. Wolff JW. The laboratory diagnosis of leptospirosis. C.C. Thomas; Springfield, Illinois: 1954.
184. Yagupsky P, Nolte FS. Quantitative aspects of septicemia. Clin Microbiol Rev. 1990;3:269–279. [PMC free article] [PubMed]
185. Yang CW, Hung CC, Wu MS, Tian YC, Chang CT, Pan MJ, Vandewalle A. Toll-like receptor 2 mediates early inflammation by leptospiral outer membrane proteins in proximal tubule cells. Kidney Int. 2006;69:815–822. [PubMed]
186. Yersin C, Bovet P, Merien F, Clement J, Laille M, Van Ranst M, Perolat P. Pulmonary haemorrhage as a predominant cause of death in leptospirosis in Seychelles. Trans R Soc Trop Med Hyg. 2000;94:71–76. [PubMed]
187. Zaki SA, Shanbag P. Clinical manifestations of dengue and leptospirosis in children in Mumbai: an observational study. Infection. 2010;38:285–291. [PubMed]
188. Zaki SR, Shieh WJ. Leptospirosis associated with outbreak of acute febrile illness and pulmonary haemorrhage, Nicaragua, 1995. Lancet. 1996;347:535–536. [PubMed]

Postgraduate Medical Journal (1988) **64**, 163–164

Leptospirosis – can it be a sexually transmitted disease?

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Summary: **Person to person spread of leptospirosis has not been previously reported. We describe two cases occurring in a man and wife which tantalisingly raise this possibility.**

Introduction

Leptospirosis has until now been regarded as a zoonosis, and person to person spread has not been reported. We report on two cases occurring in a man and wife which raise both this possibility, and that of sexual transmission.

Case report

On 2nd September 1985, an airman was canoeing in rat infested waters, whilst his wife watched from the bank. Ten days later he developed a febrile illness with severe myalgia, anorexia and headache, and over the next 4 days became jaundiced. His illness lasted 18 days, and resolved spontaneously by 30 September. During his illness, liver function tests revealed a mild hepatitis: bilirubin 82 U/l, aspartate transaminase 189 U/l, alanine transaminase 62 U/l, alkaline phosphatase 284 U/l (normal 39–117), gamma-glutamyl transferase 81 U/l (normal 7–35). Blood was sent for leptospira titres. On 1 October, his wife developed a similar febrile illness with low back pain, severe myalgia, vomiting, diarrhoea and headache. She was admitted to Royal Air Force Hospital Wegberg with dehydration, and an initial diagnosis of gastroenteritis. Her liver function tests were also mildly abnormal (bilirubin 24 U/l, alkaline phosphatase 199 U/l, gamma-glutamyl transferase 37 U/l). She also recovered spontaneously without any specific treatment.

Serology was positive in both, for *Leptospira interrogans*, and indicated an identical serotype, icterohaemorrhagiae. Microscopic agglutination tests were strongly positive for icterohaemorrhagiae – husband 1/5120 (recovery), wife 1/5120 (acute and recovery). Enzyme linked immuno-sorbent assay IgM was positive at 1/5120 in both patients.

Discussion

Leptospirosis is a zoonosis, man being infected through his association with animals, or their environments. The icterohaemorrhagiae serotype is usually contracted following exposure to rat urine, the incubation period ranging from 2 days to 3 weeks, with an average of 10 days.[1] This is followed by a disease of varying severity, from a mild non-specific illness to classical Weil's disease. It characteristically runs a bi-phasic course,[2] with 'flu-like' symptoms in the first week coincident with a leptospiraemia. After 1–3 days without fever there follows the 'immune phase' when antibodies to leptospirae become detectable in the blood. Spirochaetes can be isolated from blood and cerebrospinal fluid during the leptospiraemic phase. In the immune phase leptospirae are said to disappear from all tissues except the aqueous humour and kidneys,[2] and leptospiriuria develops.[3]

How can the relationship between these two cases be explained? If identical serotypes of leptospira have similar incubation periods, it would indicate that the wife was *not* infected at the same time as her husband. If this is so, then how and when was she infected? Exposure to leptospirae in her immediate living environment was unlikely. She had no contact with animals during this period, and did not handle the wet canoeing clothing. The temporal relationship between the cases however, begs another possibility, that of person to person spread. Leptospirae have been isolated from blood, cerebrospinal fluid, sputum and urine.[4] It is not known whether they exist in other body fluids such

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Accepted: 3 September 1987

as semen or prostatic secretions. However, venereal transmission occurs in some rodents, and may occur in humans.[5]

In the latter stages of the husband's illness, intimate relations did occur between them. So assuming leptospiriuria to have been present during the husband's convalescence (not proven due to the mild nature of his illness), then the organism could have been present in the husband's urethra, and have been expelled by the ejaculate, thus gaining entry to the wife per vagina. If leptospirae occur in semen, then this would be even more tenable. That person to person spread has not been reported cannot deny its existence. The evidence is circumstantial, but appears to be the most likely explanation in this case.

Acknowledgement

We thank the Director General, Medical Services (RAF), for permission to publish.

References

1. Topley, W.W.G. & Wilson, Sir G. *Principles of Bacteriology, Virology and Immunology.* 7th Edition, Volume 3. Edward Arnold, London, 1983, p 532.
2. Maze, S.S. & Kirsch, R.E. Leptospirosis experience at Groote Schuur Hospital, 1969–1979. *SA Med Tydskr* 1981, 36.
3. Lawson, J.H. Infections from animals. *Medicine International* 1981, **1**: 179.
4. Mansson-Bahr, P. *Manson's Tropical Diseases.* 15th Edition. Cassell, London, 1961, p 191.
5. Turner, L.H. Leptospirosis. *Br Med J* 1969, **1**: 231–235.

(old view)

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PLoS Negl Trop Dis. 2016 Jan; 10(1): e0004405.
Published online 2016 Jan 28. doi: 10.1371/journal.pntd.0004405

PMCID: PMC4731082

Human Leptospirosis Infection in Fiji: An Eco-epidemiological Approach to Identifying Risk Factors and Environmental Drivers for Transmission

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The authors have read the journal's policy and have the following conflicts. An employee of WHO was involved in study design and preparation of the manuscript.

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Received 2015 Sep 21; Accepted 2016 Jan 4.

Abstract

Go to:

Leptospirosis is an important zoonotic disease in the Pacific Islands. In Fiji, two successive cyclones and severe flooding in 2012 resulted in outbreaks with 576 reported cases and 7% case-fatality. We conducted a cross-sectional seroprevalence study and used an eco-epidemiological approach to characterize risk factors and drivers for human leptospirosis infection in Fiji, and aimed to provide an evidence base for improving the effectiveness of public health mitigation and intervention strategies. Antibodies indicative of previous or recent infection were found in 19.4% of 2152 participants (81 communities on the 3 main islands). Questionnaires and geographic information systems data were used to assess variables related to demographics, individual behaviour, contact with animals, socioeconomics, living conditions, land use, and the natural environment. On multivariable logistic regression analysis, variables associated with the presence of *Leptospira* antibodies included male gender (OR 1.55), iTaukei ethnicity (OR 3.51), living in villages (OR 1.64), lack of treated water at home (OR 1.52), working outdoors (1.64), living in rural areas (OR 1.43), high poverty rate (OR 1.74),

living <100m from a major river (OR 1.41), pigs in the community (OR 1.54), high cattle density in the district (OR 1.04 per head/sqkm), and high maximum rainfall in the wettest month (OR 1.003 per mm). Risk factors and drivers for human leptospirosis infection in Fiji are complex and multifactorial, with environmental factors playing crucial roles. With global climate change, severe weather events and flooding are expected to intensify in the South Pacific. Population growth could also lead to more intensive livestock farming; and urbanization in developing countries is often associated with urban and peri-urban slums where diseases of poverty proliferate. Climate change, flooding, population growth, urbanization, poverty and agricultural intensification are important drivers of zoonotic disease transmission; these factors may independently, or potentially synergistically, lead to enhanced leptospirosis transmission in Fiji and other similar settings.

Author Summary

Go to:

Leptospirosis is a bacterial infection transmitted from animals to humans, and many outbreaks are associated with flooding. Globally, leptospirosis is responsible for at least a million cases of severe illness each year, and many deaths. The bacteria are excreted in the urine of infected animals; humans can become infected through direct contact with animals or through contaminated water and soil. In Fiji, two successive cyclones and severe flooding in 2012 resulted in 576 cases and 40 deaths. We conducted this study to improve our understanding of the factors that increase the risk of leptospirosis transmission, so that public health control measures can be improved. Our study found that infection risk is related to many factors including individual demographics and behaviour, contact with animals, living conditions, poverty, and flooding risk. With global climate change, flooding is expected to become a bigger problem in the South Pacific. Population growth could lead to more intensive livestock farming; and urbanization in developing countries is often associated with slums with high risk of infectious diseases. Climate change, flooding, population growth, urbanization, poverty and livestock farming are important factors for leptospirosis transmission; these factors may combine to increase the risk of leptospirosis in Fiji and other Pacific Islands in the future.

Introduction

Go to:

Leptospirosis is an emerging infectious disease worldwide, with particularly high incidence reported in the Pacific Islands [1,2]. Humans are infected through direct contact with infected animals, or through contact with water or soil that has been contaminated by urine of infected animals. Disease transmission is strongly driven by environmental factors including high rainfall, flooding, natural disasters, population growth, urbanisation, and poor sanitation and hygiene [2–4]. In addition, infection risk depends on individual behaviour (e.g. swimming in fresh water, working outdoors), and contact with animals including livestock, rodents, pets, & wildlife [2,4]. Risk factors for infections and drivers of outbreaks depend on interactions between humans, animals, and the environment, and vary significantly between locations based on environmental, cultural, and socio-demographic factors [4]. Transmission dynamics are therefore highly complex and variable, and likely to evolve with global environmental change of both natural and anthropogenic environments [2,3].

In Pacific island nations, important risk factors for human leptospirosis include outdoor activities, tropical climate, flooding secondary to extreme weather events, and exposure to livestock [5–8]. Subsistence livestock are commonly kept in backyards, and veterinary expertise is generally limited. In some Pacific Islands, rapid population growth and urbanization exacerbate problems with sanitation, access to clean water, and waste management. Most islands have limited human or financial resources for the management and mitigation of the health impacts of natural disasters and climate change [9,10]. In Fiji, leptospirosis was identified as one of the four priority climate-sensitive diseases of major public health concern [11]. A recent systematic review of the global morbidity and mortality of leptospirosis identified tropical islands as particularly high-risk settings [2]. Apart from the tropical climate and high

frequency of extreme weather events [3], factors that could contribute to the high risk of leptospirosis on tropical islands include the low biodiversity and delicate ecosystems that make islands vulnerable to invasive species such as rodents [12]; the outdoor lifestyle and associated intense exposure to the environment; and close contact with subsistence livestock animals [2,4].

Climate change is projected to increase the severity of extreme weather events including increased rainfall and flooding in the Pacific Islands [10], and such events have been associated with increased leptospirosis transmission and outbreaks around the world [3,7,13–16]. In 2012, two successive tropical depressions caused severe flooding and resulted in two outbreaks of leptospirosis in Fiji, with 576 reported cases and 40 deaths (7% case-fatality) (Fiji Ministry of Health and Medical Services [MHMS]). Cases were defined as positive reactions to *Leptospira* ELISA IgM (Panbio, Brisbane, Australia); this laboratory test was only available at the national reference laboratory, and it was likely that reported cases were an underestimate of the true scale of the outbreaks. In comparison, previous studies in Fiji reported a total of 576 cases during an 8-year period from 2000–2007 [17], and 487 cases during a 13-year period from 1969–1981 [18]. These studies identified a higher risk of infection in males, indigenous Fijians (iTaukei), young adults (aged 15 to 45 years), rural dwellers and abattoir workers; increase in reported cases in the rainy months and after a cyclone in 2001; and geographic variation in incidence.

Following the outbreaks in 2012, the Fiji MHMS and the World Health Organization convened a leptospirosis expert consultation to review the epidemiology of leptospirosis in Fiji and recommend priorities for control of endemic and epidemic disease. A key conclusion of the expert consultation was that significant knowledge gaps in the current epidemiology of leptospirosis in Fiji limited effective prevention and control. The study described in this paper was identified as one of several important steps to address the knowledge gaps. This study uses an eco-epidemiological approach and framework [19] to characterize the epidemiology and risk factors for human leptospirosis infection in Fiji, and aimed to provide an evidence base for improving the effectiveness and efficiency of public health mitigation and intervention strategies. Our findings would also be relevant to other countries with similar environments, particularly in the South Pacific.

Methods

Go to:

Study location and population

The Republic of the Fiji Islands is an archipelago of 322 islands with a population of 837,217 in 2007; indigenous Fijians (iTaukei) and Indo-Fijians (Fijians of Indian descent) account for 57% and 35% of the population respectively [20]. Fiji is considered a 'small island developing state' by the United Nations [21] with a per capita GDP of US$4,712 [22]. The main island of Viti Levu has a landmass of 10,349 square kilometers and is home to >70% of the population. Vanua Levu is the second largest island in both population and land area, followed by Taveuni. The largest urban centre is the Greater Suva Area (population ~180,000) on the southeast coast of Viti Levu. The largest administrative units in geographical size are Divisions (Central, Western, Northern, and Eastern) followed by Provinces (14 in total), Tikinas (86 in total), and Enumeration Areas (smallest unit for population census that typically include 80 to 120 households). Nursing zones are the smallest administrative unit of the MHMS; they are under the care of a single nursing station and form a contiguous network across the Fijian Islands. Communities are residential clusters used by MHMS for administrative purposes. The four main community types in Fiji are urban residential areas, villages, Indo-Fijian settlements, and mixed Indo-Fijian/iTaukei settlements.

Seroprevalence study and sampling design

Field data were collected from September to December 2013 (January to March being the wettest months), and included the Central Division (on the eastern side of Viti Levu), the Western Division (on the western side of Viti Levu), and the Northern Division (the islands of Vanua Levu and Taveuni). The Eastern Division, with a population of ~40,000 spread across multiple small islands groups, was not included in the study because of logistical reasons. Field data were collected for a sero-epidemiological study of typhoid as well as the leptospirosis study described here.

We conducted a cross-sectional seroprevalence study, with a four-stage sampling design. An overview of the sampling plan is shown in Fig 1. In the first stage, both population-proportionate sampling and purposeful sampling approaches were used. The former was used to select 28 nursing zones from the Central Division, 21 from the Western Division, 11 from the Northern Division and 4 from the Ba Province which lies within the Western Division. Due to high incidence of reported leptospirosis and post-flood outbreaks in 2012, the latter sampling approach was used to select 6 more nursing zones from the Ba Province. Similar to Ba Province, Taveuni Island (part of the Northern Division) was oversampled because of a high incidence of typhoid in 2008–2009. Consequently, 12 additional nursing zones were selected from this region, resulting in 82 zones in total being selected from the five regions in the first stage of sampling. At the second stage of sampling, one community was randomly selected from each of the 82 nursing zones. Headmen, health workers and other community leaders were consulted to seek agreement to participate in the study; no community leaders declined participation. At the third stage of sampling, 25 households were randomly selected from each community using health census records if available, or using a modified World Health Organization's Expanded Programme on Immunization (EPI) sampling method. For the fourth and final stage of sampling, household members (defined as a person who stayed at the house the previous night) were enumerated and one selected at random for inclusion. In Ba subdivision, up to three randomly selected household members were included. If a selected household member was absent but returning later that day, the survey team would await their return or made a repeat visit. Wholly absent household members were substituted from within the household. Empty households were substituted by selecting the nearest house to the right of the front door. The above sampling strategy aimed to include 25 households from each of 82 communities, with up to three participants per household in Ba, and one participant per households in other areas. We therefore aimed to recruit a total of 2050 to 2250 participants.

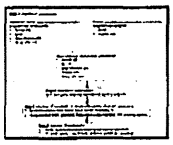

Fig 1

Overview of sampling strategy used in 2015 field study.

Participants were eligible for inclusion if they were aged 12 months or older. Exclusion criteria included clotting disorders or medical anticoagulation, severe underlying medical conditions, significant acute illness, and fear of needles.

The communities included in the study represented the general population and different environments in Fiji (urban, peri-urban, rural), with higher sampling density in Ba and Taveuni. The study successfully included a total of 81 communities, with 28 in Central Division, 10 in Ba, 21 in other parts of the Western Division, 11 in Taveuni, and 11 in Vanua Levu. These areas will be referred to as the five 'regions' in this paper.

Informed consent and ethics approvals

Ethics approvals were granted by the Fiji National Research Ethics Review Committee (2013 03), the Human Research Ethics Committee of The University of Queensland (2014000008) and the London School of Hygiene & Tropical Medicine (6344). Support was sought and obtained from divisional and

sub-divisional Ministry of Health officers for community visits. To ensure that research activities were culturally acceptable and local customs respected, community visits were conducted with field teams that included multilingual local Fijians. The study was explained to the heads of each of the randomly selected households, or another competent adult, and permission sought to include the household in the study. Written or thumb-printed informed consent was obtained from adult participants. The ethics committees specifically approved the use of thumbprint informed consent in illiterate participants. Parental/guardian consent and informed assent was obtained for child participants.

An overview of data sources and statistical methods is shown in Fig 2.

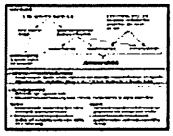

Fig 2

Overview of data sources and statistical methods.

Data collection during field study

The following were collected from each participant:

1. Venous blood samples, collected by trained phlebotomists under sterile conditions (5–8mL depending on the age of the participant).
2. Questionnaire data, using standard questionnaires administered by field research assistants, and conducted in English or other local languages depending on each participant's preference. Questions related to demographics, income, occupation, recreational activities, household environment, contact with animals, and other potential risk factors for leptospirosis.
3. Geographic Positioning System (GPS) coordinates of the place of residence, using handheld GPS devices.

Environmental, census, socio-demographic and livestock data

Environmental data on hydrology and roads were obtained from the Fiji Ministry of Lands and Mineral Resources [23]; and soils and land use/cover data from Fiji Ministry of Agriculture [24]. Climate (temperature and rainfall) and elevation data were obtained from the Landcare Research Institute [25]. Data on educational attainment, household construction, employment, ethnicity, and other socio-demographic variables were obtained from the 2007 Fiji National Census [20], and data on poverty rates from the 2011 World Bank Report [26]. Livestock data were provided by the Fiji Ministry of Agriculture's 2009 National Agricultural Census [27]. All geospatial data were georeferenced to the Fiji Map Grid 1986 coordinate system. Table 1 provides a summary of the environmental, census, socio-demographic and livestock data used in the study. The five geographic regions used in this study and examples of the geo-referenced data are shown in Fig 3.

Fig 3

Fiji geography, and examples of environmental and census data used: a) Divisions and 'regions' included in the study, major rivers; b) altitude; c) rainfall; d) total cattle density; e) poverty rate; f) proportion of households with metered ...

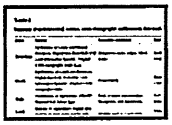

Table 1

Summary of environmental, census, socio-demographic and livestock data used.

Household GPS coordinates from the study were projected on to the Fiji Map Grid 1986 coordinate system. Attributes from the geospatial predictor layers were linked to each household location by intersecting points through polygons for vector GIS data, and sampling the raster GIS data in a similar fashion. As a result, GIS attributes for each predictor layer were obtained for each household location. Attributes for a community were obtained by first calculating the location of the median centre of sampled households, followed by an approach similar to that which was carried out for individual households. All GIS data preparation and analysis was performed using ArcGIS v10.1 (Environmental Systems Research Institute, Redlands, CA).

All GIS data preparation and analysis was performed using ArcGIS v10.1 (Environmental Systems Research Institute, Redlands, CA).

Stratification of independent variables

Independent variables were stratified according to the ecological level at which they could potentially influence the risk of leptospirosis transmission and infection. Individual-level data relate to risk factors that are specific to individual demographics or behaviour. Household-level and community-level data include risk factors that are common to all inhabitants of a household and community respectively.

- **Individual-level data.** Potential risk factors for leptospirosis were assessed using questionnaire-based interviews, including demographics, occupation, recreational activities, contact with animals, education, and knowledge about leptospirosis.
- **Household-level data.** Information on household income, house construction, access to utilities (toilets, water, sewage, electricity), and the presence of animals and crops around the home were obtained through questionnaires. In addition, data on environmental attributes (including rainfall, temperature, elevation, land cover, soil type, and distance to rivers) at household locations were extracted or calculated using geographic information systems (GIS) as described above.
- **Community-level data.** Community type, urban/rural settings, and the presence of animal species in each community were ascertained through questionnaires. Census and agricultural data were extracted or calculated using the geospatial databases described in Table 1. Data were available at the enumeration area resolution (~80–120 households) for a variety of socioeconomic and demographic measures, including the proportions of households with metered water, toilets, electricity, and sewage services; population ethnicity, level of educational attainment, and reliance on subsistence farming as the main source of income. At the Tikina level, data were available on World Bank estimates of poverty measures, and census of animal populations conducted by the Fiji Ministry of Agriculture.

Maps

Maps were produced to show the locations of communities that participated in the field study, and the observed community-level seroprevalence in 2013. Although all household GPS locations were recorded, only community-level seroprevalence were depicted on maps to protect the identity of participants. Locations of communities were mapped to their median centre, calculated as the location nearest to all sampled households in the community while minimizing the effects of outliers.

Serological analysis

Blood samples were processed in Fiji, and frozen sera transported to Australia for serological analysis. Microscopic agglutination tests (MAT) were used to detect anti-*Leptospira* antibodies, and determine the putative serogroups associated with infections. The MAT is the reference serological test recommended by the WHO and the International Committee on Systematic Bacteriology (Subcommittee on the Taxonomy of *Leptospira*) [28,29]. Serological analyses were conducted at the

WHO/FAO/OIE Collaborating Centre for Reference & Research on Leptospirosis in Brisbane, Australia. Based on the laboratory's knowledge of the epidemiology of leptospiral serovars in the South Pacific, 21 pathogenic serovars (see S1 Appendix) were selected for the initial MAT panel for this study, and samples were tested at dilutions from 1:50 to 1:3200. The 21-serovar panel was used to test a random selection of ~10% of total samples to determine the most common serogroups responsible for infections. In addition, the 21-serovar panel was used to test 199 *Leptospira* ELISA-positive samples collected from patients with suspected clinical leptospirosis in Fiji in 2012 and 2013 to ensure that the most common serogroups associated with clinical infections were included in the final panel. Based on the MAT results from the two sets of sera, six serovars were chosen for the final panel used to test the remaining samples from this study (S1 Appendix): *Leptospira interrogans* serovars Pohnpei (serogroup Australis), Australis (serogroup Australis), Canicola (serogroup Canicola), Copenhageni (serogroup Icterohaemorrhagiae), Hardjo (serogroup Sejroe), and *Leptospira borgpetersenii* serovar Ballum (serogroup Ballum).

The MAT assay is expensive and time-consuming, and the described strategy to limit the number of serovars included in the final panel resulted in reduced project costs. Considering that one dominant serovar was identified in the preliminary tests, it was determined that the smaller MAT panel was unlikely to have significant impact on the overall epidemiological findings. MAT titres of $\geq$1:50 were considered reactive or seropositive, and indicative of recent or past infection. For samples that reacted to multiple serovars within a serogroup, the serovar associated with the highest titre was considered to be the reacting serovar. Samples that reacted to serovars in more than one serogroup were recorded as reacting to multiple serovars. Although serogroups are no longer used in the taxonomic classification of serovars, they remain useful for laboratory purposes and epidemiological comparisons.

Statistical analysis

An overview of the statistical analyses is shown in Fig 2. The outcome measure used was seropositive reactions to any of the six serovars included in the final MAT panel. Firstly, crude associations between the independent variables and the outcome measure were obtained by univariable logistic regression. Independent variables associated with the outcome by a likelihood ratio test (LRT) p-value of <0.2 were then subjected to a stepwise backward elimination process ($p<0.05$) to select the final set of independent variables for the multivariable logistic regression models. In addition, the possible presence of effect modification in the multivariable modelling was investigated using the LRT. This was assessed using interaction terms, which consisted of all independent variables found to be significant in the univariable analysis. Interaction terms were added separately to the analyses to determine their joint effect on the outcome measure. Multilevel hierarchical modelling was used to take into account the clustering of participants, and allowed for correlation of observations by *region* (n = 5), *community* (n = 81), and *household* (up to 3 participants per household in Ba) as random effects. Intra-cluster correlation coefficients (ICCs) with corresponding 95% confidence intervals were obtained from final multivariable models. Biological plausibility and collinearity between variables were taken into account when selecting the variables to be retained in the final models. For example, if we observed strong collinearity between poverty rate and % of households in the community with electricity supply, poverty rate would be chosen for the final model because of more direct relationship to exposure risks.

Two multivariable models were built:

- **Model A.** Used independent variables where data could be ascertained by questioning an individual, and included primarily individual-level variables, but also some household-level and community-level variables. Model A was designed to assess the risk of infection for an individual, e.g. for producing predictive risk charts to graphically depict the combined effects of variables in determining overall seroprevalence. The charts are designed for use in clinical

settings, and are similar to cardiovascular risk charts used to predict the risk of cardiac events based on combinations of risk factors such as blood pressure, diabetes, smoking, and cholesterol levels.

- **Model B.** Used independent variables derived from geospatial and other national databases described in Table 1, without using the field data collected in this study. Model B included household-level and community-level variables only, and was designed to assess the risk of infection at geographic locations, e.g. estimating community-level seroprevalence, identifying hotspots and producing predictive risk maps.

Independent variables found to be statistically significant on multivariable regression analyses were reported. Adjusted odds ratios (OR) with 95% confidence intervals obtained from regression coefficients were used to quantify associations between the independent and outcome variables. In addition, univariate results of variables associated with animal exposure and contact were reported. Statistical significance was considered at $p < 0.05$ and two-sided. Data analysis was performed using STATA 13 (StataCorp, 2013). Model fit was assessed using the Hosmer-Lemeshow test [30], while relative predictive performance was undertaken using the area under the receiver operating curve (AUC) was calculated for each model and compared for statistical differences. An AUC of 0.7 was deemed to indicate an adequate predictive ability of the model. Akaike information criterion (AIC) and Bayesian information criterion (BIC) were reported for the final models.

Results

Go to:

Study population

A total of 2152 participants from 1922 households in 81 communities on the three main islands of Fiji were included in the study. The age of participants ranged from 1 to 90 years (mean 33.6, SD 19.8), and 985 (45.8%) were males. The age and sex distribution of participants are shown in Fig 4. The study included 662 participants from the Central Division (28 communities), 453 from Ba (10 communities), 520 from other parts of the Western Division (21 communities), 261 from Taveuni (11 communities), and 256 from Vanua Levu (11 communities) (Table 2). One of the selected communities in Taveuni was not included because of logistic constraints.

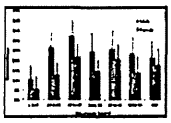

Fig 4

Seroprevalence by age group and gender.

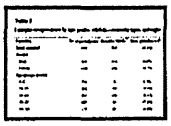

Table 2

***Leptospira* seroprevalence by age, gender, ethnicity, community types, and region.**

Seroprevalence and serovars

Details of the 21 serovars included in the screening panel and the 6 serovars included in the final panel are shown in S1 Appendix, together with the seroprevalence of the initial 198 randomly selected samples from this study and the 199 *Leptospira* ELISA-positive samples from patients with suspected leptospirosis from April 2012 to November 2013., The six serovars included in the final MAT panel accounted for 86.7% of reactive tests: *Leptospira interrogans* serovars Pohnpei, Australis, Canicola, Copenhageni, and Hardjo; and *Leptospira borgpetersenii* serovar Ballum. Using the 6-serovar panel, the overall seroprevalence was 19.4% (95% CI 17.7%–21.1%), with 417 participants having reactive MATs to at least one serovar. One predominant serovar, Pohnpei, accounted for 351 (84.2%; 95% CI

80.3%–87.5%) of reactive MATs. A total of 63 participants had MAT titres of ≥1:400 (47 for serovar Pohnpei, and 16 for other serovars), the cutoff used by our laboratory to indicate an acute infection. The distribution of MAT titres for Pohnpei and other serovars is shown in Fig 5.

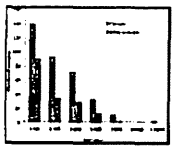

Fig 5

Distribution of MAT titres for serovar Pohnpei (blue) and other serovars (red); using the final panel of 6 serovars.

Table 2 shows that there were significant differences in seroprevalence by age, gender, ethnicity, community types, and region. Community-level seroprevalence ranged from 0% to 60%, and are shown on the maps in Fig 6a to 6d. Variations in seropositive reactions to each serovar by age groups and region of residence are shown in Fig 7a & 7b respectively.

Fig 6

Community-level seroprevalence at the 81 communities included in the study; a) prevalence varied from 0% to 60%; b) enlargement of the Suva area in eastern Viti Levu; c) enlargement of Taveuni and eastern Vanua Levu; and d) enlargement of northwestern ...

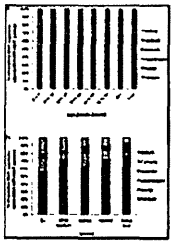

Fig 7

Percentage of positive MAT reactions associated with each of the 6 serovars included in the final panel by: a) age groups, and b) regions.

Risk factor analysis and multivariable models

A total of 118 independent variables were assessed on univariate analysis: 75 variables obtained from questionnaires, and 43 derived using GIS from the sources described in Table 1. Independent variables included 31 individual-level, 38 household-level, and 49 community-level risk factors described above. S2 Appendix provides a list of the independent variables assessed at the univariate level. Variables statistically significant on univariate analyses were considered for the multivariable models, and included 19 individual-level, 21 household-level, and 25 community-level risk factors. Due to statistical significance not be reached, no interaction effect was included in the multivariable modelling.

Multivariable Model A (using variables where data could be ascertained by questioning an individual) included five variables that were independently associated with the presence of *Leptospira* antibodies, with an AUC of 0.7 (Table 3) including: male gender (OR 1.55 compared to females), iTaukei ethnicity (OR 3.51 compared to Indo-Fijians), living in settlements and villages (OR 2.13 and 1.64 respectively compared to urban residential areas), not having metered water at home (OR 1.52), and working outdoors (OR 1.64 compared to working indoors). Of the 434 participants who worked outdoors, 378 (87%) were full- or part-time farmers, indicating that outdoor work in Fiji is predominantly related to farming.

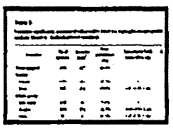

Table 3

Variables significantly associated with positive MAT for *Leptospira* on univariable and multivariable analysis–Model A^ (individual-level variables).

Multivariable Model B (using only variables derived from geospatial and other national databases) included six variables that were independently associated with the presence of *Leptospira* antibodies, with an AUC of 0.7 (Table 4) including: living in rural areas (OR 1.43 compared to living in urban or peri-urban areas), poverty rate ≥ 40% (OR 1.74), living <100m from a river or major creek (OR 1.41), presence of pigs in the community (OR 1.54), total cattle density in the Tikina (OR 1.04 per head of cattle per square km), and high maximum rainfall in the wettest month (OR 1.003 per mm of rain). Total cattle density (includes both commercial and subsistence livestock) ranged from 0.11 to 31.48 head of cattle per square km (mean 8.96, SD 5.31), and maximum rainfall in the wettest month ranged from 275 to 789mm (mean 375.02, SD 56.94). A similar multivariable model using total dairy farm density instead of total cattle density performed better than the final model, but data on dairy farm density were only available for 57 of the 81 (70.4%) communities included in our study, and this variable was therefore not selected for the final Model B.

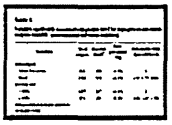

Table 4

Variables significantly associated with positive MAT for *Leptospira* on univariable and multivariable analyses–Model B^ (environmental and census variables).

Collection of biological samples from animals was outside of the scope of this study, but study questionnaires included detailed information about contact with animals (rodents, mongoose, pets, and livestock) at home and the presence of animals in the community. A number of animal-related exposures were significantly associated with the presence of *Leptospira* antibodies on univariable analysis (Table 5). The presence of rats, mice, and mongoose around the home was not significantly associated with seroprevalence, but higher infection rates were found in participants who reported physical contact with rats or mice (OR 1.58) and mongoose (OR 1.81). Table 5 shows that many Fijians have livestock animals at home and in the community. The presence of each livestock species was associated with a higher infection rates on univariable analysis, but only the presence of pigs in the community was significant on multivariable analysis, and included in Model B.

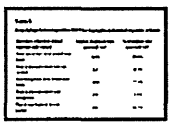

Table 5

Associations between positive MAT for *Leptospira* and animal exposure at home and in the community.

Multilevel hierarchical models were built to take into account spatial correlation of data: i) defining *region* and *community* as random effects, using the entire dataset, and ii) defining *community* and *household* as random effects, using Ba data only. The results of multilevel models were not statistically different to the results of multivariable Model A using all data (chi2 = 0.01, $p = 1.00$), Model A using Ba data (chi2 = 0.00, $p = 1.00$), Model B using all data (chi2 = 0.01, $p = 0.99$), or Model B using Ba data (chi2 = 0.00, $p = 1.00$). For all multilevel models, intra-class correlation coefficients were <0.01 and odds ratio estimates were very similar to the reported models. Results for the multilevel models were therefore not reported here.

Seroprevalence estimation chart using Model A

Fig 8 shows a seroprevalence estimation chart that incorporates individual-level variables to show the combined effects of multiple independent risk factors on the prevalence of infection. Estimated seroprevalence were based on the five variables used in Model A. For example, the chart shows a range of seroprevalence from 2.0% for female Indo-Fijians who live in urban residential areas, have metered water at home, and work indoors; to 39.4% for male iTaukei who live in mixed ethnic settlements, do not have metered water and home, and work outdoors. It is uncommon for Indo-Fijians to live in villages or for iTaukei to live in Indo-Fijian settlements, and results were therefore not shown for these scenarios.

Fig 8

Seroprevalence estimation chart based on Model A, a multivariable logistic regression model of individual-level variables for a) females and b) males.

Discussion

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Our study identified a high risk of human leptospirosis infection in Fiji, with an overall seroprevalence of 19.4% using a 6-serovar MAT panel. One dominant serovar, Pohnpei, was associated with 84.2% of reactive MATs. The serovar was originally isolated from rodents and pigs during an animal leptospirosis study in the island of Pohnpei in the Federated States of Micronesia [31], and has been found to be an important cause of human infections [32]. Seroprevalence varied significantly between the five regions in our study, and ranged from 16.2% in the Central Division to 29.3% in Vanua Levu in the Northern Division. Community-level seroprevalence also varied significantly from 0% to 60% in the 81 communities included in our study. These findings indicate marked geographic variation in infection risk in Fiji and the presence of hotspots where disease transmission is more intense.

Globally, reported leptospirosis seroprevalence vary significantly between and within countries, based on environmental settings, behavioural risk factors, and socio-demographics; our results corroborate these findings. To put the Fiji results into a global context, examples of seroprevalence reported from known high risk settings [2,4] such as urban slums, tropical islands, and flood risk areas include 15.4% in an urban slum in Brazil [33], 37% of healthy adult males in the Seychelles [34], 18.8% in the Mekong delta in Vietnam [35], and 23.9% and 38.2% in flood-prone areas in Laos and Bangladesh respectively [36,37]. As found in Fiji, small-scale variations in seroprevalence within countries and differences between occupational groups have been reported. In American Samoa (a group of remote islands in the south Pacific), a community-based study reported an overall seroprevalence of 15.5% [8] and significant variation between islands with different environments, and between areas with different population density [38]. In Peru, seroprevalence varied from 28.0% in the Amazonian city of Iquitos and 16.5% in the surrounding villages (wet tropics), to 0.7% in a desert shantytown near Lima [39]. In the Andaman Islands, a study of high-risk populations found seroprevalence of 62.5% in agricultural workers, 39.4% in sewage workers, 37.5% in animal handlers, and 30.0% in butchers [40]. In contrast, a study of healthy blood donors in an area of high leptospirosis incidence in northern Queensland in Australia found a seroprevalence of only 1.4% [41,42].

Our study found that individual-level factors were important predictors of leptospirosis infection risk in Fiji. Model A shows that gender, ethnicity, community type, availability of water at home, and work location were independently associated with the presence of *Leptospira* antibodies. Higher infection rates in males corroborates findings in the majority of leptospirosis studies around the world, and is likely to be associated with higher frequency of outdoor activities as well as higher risk occupational and recreational exposures. Reasons for the marked difference in seroprevalence between the two main ethnic groups in Fiji are unclear, but could be related to differences in genetic susceptibility or behaviours that were not elucidated by our questionnaire, e.g. differences in animal husbandry or

slaughtering practices related to religion or culture. Further studies are required to explain the disparate risk between ethnic groups. Seroprevalence in villages was significantly higher than in urban residential areas or settlements, and is likely the result of more intimate contact with the natural environment and domestic animals. In our study, working outdoors was associated with a higher risk of infection, and the majority of outdoor work in Fiji involves farming. Agriculture is an important part of Fiji's economy, and apart from the livestock industry, there is commercial farming of a range of crops include sugarcane, coconut, copra, and a wide variety of fruits and vegetables. Occupational exposure in the agricultural industry is therefore likely to be an important source of leptospirosis infection in Fiji.

Of note, three of the predictors included in our final multivariable models were related to water: the *availability of metered (treated) water at home* (Model A), *distance between the home and the closest river or major creek* (Model B), and *maximum rainfall in the wettest month* (Model B). Considering that *Leptospira* can survive for weeks to months in fresh water, and are efficiently carried and disseminated by water (e.g. flooding, flowing downstream in rivers), the findings were not unexpected. Lack of metered water at home and proximity to rivers are likely to be associated with higher levels of contact with untreated freshwater, e.g. using rivers for bathing, cleaning, swimming, and recreational activities. Furthermore, poor access to water at home is generally associated with poverty (discussed below), and also influences personal hygiene, e.g. the ability to clean and wash after working outdoors, or after contact with mud, contaminated water, or animals. Two of the water-related predictors (*distance to river or major creek* and *maximum rainfall in the wettest month*) are also proxy measures of flooding risk. As seen with the post-flood leptospirosis outbreaks in 2012, flooding is an important driver of transmission in Fiji, as it is in many parts of the world.

Two of the predictors in Model B relate to livestock exposure: *total cattle density in the Tikina* and *presence of pigs in the community*. Data on cattle density in Tikinas includes both commercial and subsistence farming, and varied from < 1 to over 30 heads of cattle per square km. Infection risk could be related to direct occupational contact with cattle, or through more general contamination of the environment (especially rivers) with cattle urine. As shown in Table 5, many households in Fiji keep subsistence livestock. Backyard piggeries are commonly found in communities in Fiji and other Pacific Islands, and are usually small pens with less than 10 pigs. The pens are often built on the edge of rivers and streams to allow convenient drainage of waste, but unfortunately also lead to contamination of freshwater at that community as well as further downstream. In American Samoa, similar backyard piggeries have been associated with the risk of human leptospirosis infection [8,43]. Dairy farmers are known to be at high risk for leptospirosis in many parts of the world because of close contact with cattle, and exposure to urine during milking. In our study, high density of dairy farms was strongly associated with infection risk, but was not included as a variable in the final model because data were only available for ~70% of the Tikinas in our study. As more data on dairy farms become available, associations with leptospirosis risk could be further explored and model performance potentially improved. Commercial dairy and beef farming could potentially intensify in the future with population growth, and increase the risk of leptospirosis.

Model B also shows that leptospirosis is a disease of poverty in Fiji and disproportionately affects the poorest. Leptospirosis has been associated with poverty in diverse settings around the world, including Brazilian and Indian urban slums [33,44,45], Peruvian Amazon [39], and areas of poor socioeconomic status in the USA and Europe [46,47]. Furthermore, the combination of poverty, livestock keeping, and global climate change are important drivers of zoonotic diseases transmission [48]. In our study, participants living in communities with high poverty rates (defined as ≥40% of households in the community) had almost twice the infection rate compared to other communities, independent of the other predictors in Model B. As discussed above, poor access to metered (treated) water at home was associated with a higher risk of infection for many reasons, and is also a proxy measure of socioeconomic status.

Although serovar Pohnpei was associated for 84.2% of reactive MATs, there were differences in serovar distribution by age and by region of residence, suggesting that the relative importance of animal species in disease transmission varies between subgroups. Variation in risk factors between age groups likely relates to age-specific behaviours, e.g. young children spend more time playing around the home, and have closer contact with pets and soil; teenagers have more frequent recreational freshwater contact from swimming in rivers and waterfalls; and adults have more intense contact with livestock through occupational exposure and managing animals at home. Variation in risk factors between regions likely relates to differences in environmental settings, with proportionately greater urbanization in the Central division, and more farming in the other regions. For example, rodents could be more important in transmission cycles in urban and peri-urban areas, and livestock more important in rural areas.

Many of the risk factors and environmental drivers identified in our study provide significant cause for concern about future risk of leptospirosis in Fiji, as well as other Pacific Islands with similar environments. Population growth is typically associated with agricultural intensification, leading to increase in livestock numbers (both commercial and subsistence) and occupational exposure. With global climate change, extreme weather events and flooding are predicted to become more frequent and intense in the Pacific Islands. Rapid population growth in developing countries is often associated with urban and peri-urban slums where diseases of poverty proliferate. Although our study found that leptospirosis seroprevalence was lower in urban areas, poverty rate was a significant risk factor independent of urban or rural settings. Climate change, flooding, population growth, urbanization, and agricultural intensification may independently, or potentially synergistically, lead to enhanced leptospirosis transmission in Fiji [3].

The findings should be considered in light of the study's limitations. Limitations of the MAT have been well documented; the test is considered to be serogroup rather than serovar specific, cross-reactions occur between serovars within a serogroup, and complex paradoxical reactions could occur in persons who have had previous infections [29]. Despite these limitations, the MAT is considered the gold standard test for identifying putative serogroups and serovars when isolates are not available [28]. Isolates of leptospires would be required to definitively confirm the serovars circulating in Fiji. Due to budgetary reasons, our study used a 6- rather than 21-serovar MAT panel to test the majority of samples. If the larger panel was used, additional less-common serovars may have been detected. However, the 6 serovars selected included the most reactive serovars when 198 randomly selected samples from this study were tested against the full 21- serovar panel; the 6 serovars selected accounted for 86.7% of the reactive samples, and one dominant serovar (Pohnpei) accounted for 65.9% of reactive samples. The reduced MAT panel size could have underestimated the overall seroprevalence by a factor of 0.13 compared to a 21-serovar panel, but unlikely to have significantly influenced the overall epidemiological patterns reported here because one serovar dominated the reactive MATs, and our data analyses in this paper were not stratified by serovars.

Our study measured antibodies to *Leptospira* to identify evidence of prior infection. However, many leptospirosis infections do not result in any apparent illness and are of no clinical significance. The severity of clinical disease depends on many factors including pathogen virulence and the individual's immune status, comorbidities, and age [49]. Serovar Pohnpei, the serovar associated with 84.2% of MAT-reactive cases, has been reported as an important cause of overt clinical disease in the Federated States of Micronesia [32], suggesting the findings in this study are applicable not only to infection risk but also clinical illness. However, there are currently no available data on the proportion of serovar Pohnpei infections that result in clinical disease or severe complications.

Future studies could further improve our understanding of leptospirosis transmission in Fiji by examining serovar-specific risk factors; identifying the most important exposures in different subgroups such as age groups, gender, ethnic groups, and community types; determining the relative importance of

livestock, rodents, pets and wildlife in transmitting leptospirosis to humans; and developing models to determine transmission (causal) pathways rather than just epidemiological links. For environmental and census variables, we used data at the place of residence, but infections could also have occurred at work or elsewhere. Future studies that focus specifically on work-related activities would provide more insight into the importance of occupational exposures in Fiji. The performances of models were partly determined by the accuracy of available environmental, census, and livestock data, and models could be updated and improved as more data become available. Models based on environmental factors, such as Model B, could be used to produce predictive risk maps for the whole of Fiji.

In summary, our study found that risk factors and drivers for human leptospirosis infection in Fiji are complex and multifactorial, and include climate, the natural environment, livestock (both subsistence and commercial), living conditions, socioeconomic status, demographics and individual behaviour. Some of these factors corroborate findings previously reported in different settings (e.g. male gender, working outdoors), but other factors appear to be specific to the cultural and environmental settings in Fiji, including ethnicity and presence of pigs in communities. By using an integrated eco-epidemiological approach and including a wide range of data sources in our analyses, we were able to quantify the relative importance of risk factors at different ecological scales. At the individual level, gender, ethnicity, and work location were strongly associated with infection risk. At the community level, important predictors of risk included rural setting, community type, poor access to clean water, close proximity to rivers, high rainfall in the wettest month, high poverty rate, presence of pigs, and high cattle density. From a wider perspective, significant geographic variations in risk and the ability to predict risk based only on environmental and census variables indicate that environmental factors play a crucial role in driving leptospirosis transmission in Fiji.

The above findings provide an important evidence base to guide the focus of public health and environmental health interventions at individual, community, and national levels. Health promotion activities and educational materials should be designed to reach the highest risk groups including males, farmers, and iTaukei. Public health and environmental health interventions should target the highest risk communities (villages, rural areas, those in hotspots and high-risk regions), and include advice on proper management of livestock, avoiding contact with floodwaters, and minimizing flooding risk (e.g. adequate garbage disposal to reduce the risk of flooding from blocked streams and drains). At high risk times, e.g. post-flooding, communities should also be reminded about the risk of leptospirosis, the use of protective measures, and the importance of seeking early medical care if unwell. In smaller communities in Fiji, where laboratory diagnostic tests are often not available, the predictive risk chart shown in Fig 8 could assist clinicians with determining the likelihood (pre-test probability) of leptospirosis infection based on a combination of individual-level variables. Broader environmental factors (both natural and anthropogenic) play a major role in leptospirosis transmission in Fiji, most of which are beyond the immediate control of individuals or small communities. Effective environmental health management at the public health and national level will therefore be crucial for the sustainable control of leptospirosis in Fiji and other countries with similar environmental and socio-demographic settings.

Supporting Information

Go to:

S1 Appendix

Initial 21 pathogenic serovars included in the microscopic agglutination test (MAT) panels, and the six serovars chosen for the final MAT panel.

(DOCX)

Click here for additional data file.(152K, docx)

S2 Appendix

Independent variables stratified by data source and scale of ecological influence.

(DOCX)

Click here for additional data file.(98K, docx)

S1 Checklist

STROBE statement for cross-sectional studies.

(DOC)

Click here for additional data file.(88K, doc)

Acknowledgments

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We would like to thank Lee Smythe, Mary-Anne Burns and Michael Dohnt at the WHO/FAO/OIE Collaborating Centre for Reference and Research on Leptospirosis in Brisbane, Australia for providing valuable technical expertise and laboratory support, and for reviewing the manuscript. We also thank the Fiji Ministry of Health and Medical Services for providing access to clinical serum samples collected in 2012–2013.

We extend our warmest thanks to all the many participants, communities, Ministry of Health staff and community health workers who generously contributed to the study. We gratefully acknowledge the work of the field team: Dr. Kitione Rawalai, Jeremaia Coriakula, Ilai Koro, Sala Ratulevu, Ala Salesi, Meredani Taufa, and Leone Vunileba; advice and local expertise from Dr. Viema Biaukula at the WHO Division of Pacific Technical Support, Fiji; and the assistance of Drs. Catherine Brock, Daisy Stevens and Frances Yarlett, then of Peninsula Medical School, United Kingdom.

Funding Statement

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Fieldwork was funded by the World Health Organization, Division of Pacific Technical Support. CLL was supported by a research grant from the Global Change Institute (607562) at The University of Queensland. http://www.gci.uq.edu.au. CHW was supported by the UK Medical Research Council (grant MR/J003999/1) and the Chadwick Trust. http://www.mrc.ac.uk. http://www.ucl.ac.uk/srs/our-services/academic-services/chadwick-trust. EJN is an employee of the WHO, and was involved in study design and preparation of the manuscript. The other funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Data Availability

Go to:

The study was conducted in small communities in Fiji, and participants could potentially be re-identifiable if the study data were fully available, e.g. by diagnosis of leptospirosis, demographics, occupation, and household GPS locations. Public deposition of the data would compromise participant privacy, and therefore breach compliance with the protocol approved by the research ethics committees.

Data can be requested via The University of Queensland's Human Research Ethics Committee for researchers who meet the criteria for access to confidential data. Email: humanethics@research.uq.edu.au Phone: + 61 (7) 3365 3924

References

Go to:

1. Victoriano AF, Smythe LD, Gloriani-Barzaga N, Cavinta LL, Kasai T, et al. Leptospirosis in the Asia Pacific Region. BMC Infect Dis. 2009;9: 147 doi: 10.1186/1471-2334-9-147 [PMC free article] [PubMed]

2. Costa F, Hagan JE, Calcagno J, Kane M, Torgerson P, et al. Global Morbidity and Mortality of Leptospirosis: A Systematic Review. PLoS Negl Trop Dis. 2015;9: e0003898 doi: 10.1371/journal.pntd.0003898 [PMC free article] [PubMed]

3. Lau CL, Smythe LD, Craig SB, Weinstein P. Climate Change, Flooding, Urbanisation and Leptospirosis: Fuelling the Fire? Trans R Soc Trop Med Hyg. 2010;104: 631–638. doi: 10.1016/j.trstmh.2010.07.002 [PubMed]

4. Mwachui MA, Crump L, Hartskeerl R, Zinsstag J, Hattendorf J. Environmental and Behavioural Determinants of Leptospirosis Transmission: A Systematic Review. PLoS Negl Trop Dis. 2015;9: e0003843 doi: 10.1371/journal.pntd.0003843 [PMC free article] [PubMed]

5. Berlioz-Arthaud A, Kiedrzynski T, Singh N, Yvon JF, Roualen G, et al. Multicentre Survey of Incidence and Public Health Impact of Leptospirosis in the Western Pacific. Trans R Soc Trop Med Hyg. 2007;101: 714–721. [PubMed]

6. Coudert C, Beau F, Berlioz-Arthaud A, Melix G, Devaud F, et al. [Human Leptospirosis in French Polynesia. Epidemiological, Clinical and Bacteriological Features]. Med Trop (Mars). 2007;67: 137–144. [PubMed]

7. Goarant C, Laumond-Barny S, Perez J, Vernel-Pauillac F, Chanteau S, et al. Outbreak of Leptospirosis in New Caledonia: Diagnosis Issues and Burden of Disease. Trop Med Int Health. 2009;14: 926–929. doi: 10.1111/j.1365-3156.2009.02310.x [PubMed]

8. Lau C, Dobson A, Smythe L, Fearnley E, Skelly C, et al. Leptospirosis in American Samoa 2010—Epidemiology, Environmental Drivers, and the Management of Emergence. Am J Trop Med Hyg. 2012;86: 309–319. doi: 10.4269/ajtmh.2012.11-0398 [PMC free article] [PubMed]

9. Gero A, Fletcher S, Rumsay M, Thiessen J, Kuruppu N, et al. Disasters and Climate Change in the Pacific: Adaptive Capacity of Humanitarian Response Organizations. Climate and Development. 2014;7: 35–46.

10. McIver L, Kim R, Woodward A, Hales S, Spickett J, et al. Health Impacts of Climate Change in Pacific Island Countries: A Regional Assessment of Vulnerabilities and Adaptation Priorities. Environ Health Perspect. 2015. [PubMed]

11. McIver L, Naicker J, Hales S, Singh S, Dawainavesi A. Climate Change and Health in Fiji: Environmental Epidemiology of Infectious Diseases & Potential for Climate-Based Early Warning Systems. Fiji J of Pub Health. 2012;1: 7–13.

12. Derne BT, Fearnley EJ, Lau CL, Paynter S, Weinstein P. Biodiversity and Leptospirosis Risk: A Case of Pathogen Regulation? Med Hypotheses. 2011;77: 339–344. doi: 10.1016/j.mehy.2011.05.009 [PubMed]

13. Vanasco NB, Schmeling MF, Lottersberger J, Costa F, Ko AI, et al. Clinical Characteristics and Risk Factors of Human Leptospirosis in Argentina (1999–2005). Acta Trop. 2008;107: 255–258. doi: 10.1016/j.actatropica.2008.06.007 [PubMed]

14. Ko AI, Galvao Reis M, Ribeiro Dourado CM, Johnson WD Jr., Riley LW. Urban Epidemic of Severe Leptospirosis in Brazil. Salvador Leptospirosis Study Group. Lancet. 1999;354: 820–825. [PubMed]

15. Amilasan AS, Ujiie M, Suzuki M, Salva E, Belo MC, et al. Outbreak of Leptospirosis after Flood, the Philippines, 2009. Emerg Inf Dis. 2012;18: 91–94. [PMC free article] [PubMed]

16. Wynwood SJ, Craig SB, Graham GC, Blair BR, Burns MA, et al. The Emergence of Leptospira Borgpetersenii Serovar Arborea as the Dominant Infecting Serovar Following the Summer of Natural Disasters in Queensland, Australia 2011. Trop Biomed. 2014;31: 281–285. [PubMed]

17. Ghosh A, Khan S, Kishore K. Leptospirosis in Fiji: Incidence between 2000 to 2007 and Review of Literature. Fiji Med J. 2010;29: 8–14.

18. Ram P, Collings DF. Further Observations on the Epidemiology of Leptospirosis in Fiji. Fiji Med J. 1982;10: 71–75.

19. Lau C, Jagals P. A Framework for Assessing and Predicting the Environmental Health Impact of Infectious Diseases: A Case Study of Leptospirosis. Rev Environ Health. 2012;27: 1–12. [PubMed]

20. Fiji Bureau of Statistics. Census of Population and Housing. 2007. Available: http://www.statsfiji.gov.fj/index.php/2007-census-of-population

21. United Nations. World Economic Situation and Prospects 2015. New York: United Nations; Available: http://www.un.org/en/development/desa/policy/wesp/

22. Economy Watch. Gdp Per Capita Data for All Countries. 2015. Available: http://www.economywatch.com/economic-statistics/economic-indicators/GDP_Per_Capita_Current_Prices_US_Dollars/

23. Republic of Fiji Islands. Ministry of Lands and Mineral Resources DoL. Digital Data from Fiji Land Information System (Flis). GIS Data of 1:50k Topographic Maps.

24. Republic of Fiji Islands. Ministry of Agriculture. National Soil Survey. GIS Data of Soils and Land Use/Cover.

25. Barker G, Price R. Environmental and Biogeographic Classifications as Spatial Frameworks for Assessing Representativeness in Island Archipelagos: A Fijian Case Study. Hamilton, New Zealand: Landcare Research.

26. Bank World. Republic of Fiji Poverty Trends, Profiles and Small Area Estimation (Poverty Maps) in Republic of Fiji (2003–2009). Washington, DC: World Bank.

27. Fiji Ministry of Agriculture. Fiji National Agricultural Census 2009. Available: http://catalog.ihsn.org/index.php/catalog/4370

28. World Health Organization. Human Leptospirosis: Guidance for Diagnosis, Surveillance and Control. 2003. Available: http://www.who.int/zoonoses/resources/Leptospirosis/en/

29. Haake DA, Levett PN. Leptospirosis in Humans. Curr Top Microbiol Immunol. 2015;387: 65–97. doi: 10.1007/978-3-662-45059-8_5 [PMC free article] [PubMed]

30. Hosmer DW, Lemeshow S, Sturdivant RX. Applied Logistic Regression: John Wiley & Sons; 2013.

31. Simms J. Animal Leptospirosis in the Federated States of Micronesia. Pac Health Dialog. 1997;5: 30–37.

32. Colt S, Pavlin BI, Kool JL, Johnson E, McCool JP, et al. Human Leptospirosis in the Federated States of Micronesia: A Hospital-Based Febrile Illness Survey. BMC Infect Dis. 2014;14: 186 doi: 10.1186/1471-2334-14-186 [PMC free article] [PubMed]

33. Reis RB, Ribeiro GS, Felzemburgh RD, Santana FS, Mohr S, et al. Impact of Environment and Social Gradient on Leptospira Infection in Urban Slums. PLoS Negl Trop Dis. 2008;2: e228 doi: 10.1371/journal.pntd.0000228 [PMC free article] [PubMed]

34. Bovet P, Yersin C, Merien F, Davis CE, Perolat P. Factors Associated with Clinical Leptospirosis: A Population-Based Case-Control Study in the Seychelles (Indian Ocean). Int J Epidemiol. 1999;28: 583–590. [PubMed]

35. Van CT, Thuy NT, San NH, Hien TT, Baranton G, et al. Human Leptospirosis in the Mekong Delta, Viet Nam. Trans R Soc Trop Med Hyg. 1998;92: 625–628. [PubMed]

36. Kawaguchi L, Sengkeopraseuth B, Tsuyuoka R, Koizumi N, Akashi H, et al. Seroprevalence of Leptospirosis and Risk Factor Analysis in Flood-Prone Rural Areas in Lao Pdr. Am J Trop Med Hyg. 2008;78: 957–961. [PubMed]

37. Morshed MG, Konishi H, Terada Y, Arimitsu Y, Nakazawa T. Seroprevalence of Leptospirosis in a Rural Flood Prone District of Bangladesh. Epidemiol Infect. 1994;112: 527–531. [PMC free article] [PubMed]

38. Lau C, Skelly C, Craig S, Smythe L, Weinstein P. Emergence of New Leptospiral Serovars in American Samoa—Ascertainment or Ecological Change? BMC Infect Dis. 2012;12: 19 doi: 10.1186/1471-2334-12-19 [PMC free article] [PubMed]

39. Johnson MA, Smith H, Joeph P, Gilman RH, Bautista CT, et al. Environmental Exposure and Leptospirosis, Peru. Emerg Infect Dis. 2004;10: 1016–1022. [PMC free article] [PubMed]

40. Sharma S, Vijayachari P, Sugunan AP, Natarajaseenivasan K, Sehgal SC. Seroprevalence of Leptospirosis among High-Risk Population of Andaman Islands, India. Am J Trop Med Hyg. 2006;74: 278–283. [PubMed]

41. Faddy H, Seed C, Lau C, Racloz V, Flower R, et al. Antibodies to Leptospira among Blood Donors in Higher-Risk Areas of Australia: Possible Implications for Transfusion Safety. Blood Transfus. 2015;13: 32–36. doi: 10.2450/2014.0012-14 [PMC free article] [PubMed]

42. Lau CL, Skelly C, Dohnt M, Smythe LD. The Emergence of *Leptospira Borgpetersenii* Serovar Arborea in Queensland, Australia, 2001 to 2013. BMC Infect Dis. 2015;15: 230 doi: 10.1186/s12879-015-0982-0 [PMC free article] [PubMed]

43. Lau C, Clements A, Skelly C, Dobson A, Smythe L, et al. Leptospirosis in American Samoa—Estimating and Mapping Risk Using Environmental Data. PLoS Negl Trop Dis. 2012;6: e1669 doi: 10.1371/journal.pntd.0001669 [PMC free article] [PubMed]

44. Oliveira DS, Guimaraes MJ, Portugal JL, Medeiros Z. The Socio-Demographic, Environmental and Reservoir Factors Associated with Leptospirosis in an Urban Area of North-Eastern Brazil. Ann Trop Med Parasitol. 2009;103: 149–157. doi: 10.1179/136485909X398221 [PubMed]

45. Karande S, Kulkarni H, Kulkarni M, De A, Varaiya A. Leptospirosis in Children in Mumbai Slums. Indian J Pediatr. 2002;69: 855–858. [PubMed]

46. Hotez PJ. Neglected Infections of Poverty in the United States of America. PLoS Negl Trop Dis. 2008;2: e256 doi: 10.1371/journal.pntd.0000256 [PMC free article] [PubMed]

47. Hotez PJ, Gurwith M. Europe's Neglected Infections of Poverty. Int J Infect Dis 2011;15: e611 –619. doi: 10.1016/j.ijid.2011.05.006 [PubMed]

48. International Livestock Research Institute. Mapping of Poverty and Likely Zoonoses Hotspots. 2012. Available: https://cgspace.cgiar.org/bitstream/handle/10568/21161/ZooMap_July2012_final.pdf?sequence=4

49. Adler B. Leptospira and Leptospirosis Adler B. Verlag Berlin Heidelberg: Springer; 2015.

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Toxocariasis FAQs

On this Page

What is toxocariasis?

Toxocariasis is an infection transmitted from animals to humans (zoonosis) caused by the parasitic roundworms commonly found in the intestine of dogs (*Toxocara canis*) and cats (*T. cati*).

Who is at risk for toxocariasis?

Anyone can become infected with *Toxocara.* Young children and owners of dogs or cats have a higher chance of becoming infected.

Approximately 13.9% of the U.S. population has antibodies to *Toxocara*. This suggests that tens of millions of Americans may have been exposed to the *Toxocara* parasite.

How can I get toxocariasis?

Dogs and cats that are infected with *Toxocara* can shed *Toxocara* eggs in their feces. You or your children can become infected by accidentally swallowing dirt that has been contaminated with dog or cat feces that contain infectious *Toxocara* eggs. Although it is rare, people can also become infected from eating undercooked meat containing *Toxocara* larvae.

What are the clinical manifestations of toxocariasis?

Many people who are infected with Toxocara do not have symptoms and do not ever get sick. Some people may get sick from the infection, and may develop:

- **Ocular toxocariasis:** Ocular toxocariasis occurs when *Toxocara* larvae migrate to the eye. Symptoms and signs of ocular toxocariasis include vision loss, eye inflammation or damage to the retina. Typically, only one eye is affected.
- **Visceral toxocariasis:** Visceral toxocariasis occurs when *Toxocara* larvae migrate to various body organs, such as the liver or central nervous system. Symptoms of visceral toxocariasis include fever, fatigue, coughing, wheezing, or abdominal pain.

How serious is infection with *Toxocara*?

In most cases, *Toxocara* infections are not serious, and many people, especially adults infected by a small number of larvae (immature worms), may not notice any symptoms. The most severe cases are rare, but are more likely to occur in young children, who often play in dirt, or eat dirt (pica) contaminated by dog or cat feces.

How is toxocariasis spread?

The most common *Toxocara* parasite of concern to humans is *T. canis*, which puppies usually contract from the mother before birth or from her milk. The larvae mature rapidly in the puppy's intestine; when the pup is 3 or 4 weeks old, they begin to produce large numbers of eggs that contaminate the environment through the animal's feces. Over a 2 to 4 week time period, infective larvae develop in the eggs. Toxocariasis is not spread by person-to-person contact like a cold or the flu.

What should I do if I think I have toxocariasis?

See your health care provider to discuss the possibility of infection and, if necessary, to be examined. Your provider may take a sample of your blood for testing.

What is the treatment for toxocariasis?

Visceral toxocariasis is treated with antiparasitic drugs. Treatment of ocular toxocariasis is more difficult and usually consists of measures to prevent progressive damage to the eye.

How do I prevent toxocariasis?

- Take your pets to the veterinarian to prevent infection with Toxocara. Your veterinarian can recommend a testing and treatment plan for deworming.

- Wash your hands with soap and water after playing with your pets or other animals, after outdoor activities, and before handling food.
- Teach children the importance of washing hands to prevent infection.
- Do not allow children to play in areas that are soiled with pet or other animal feces.
- Clean your pet's living area at least once a week. Feces should be either buried or bagged and disposed of in the trash. Wash your hands after handling pet waste.
- Teach children that it is dangerous to eat dirt or soil.

More on: Handwashing

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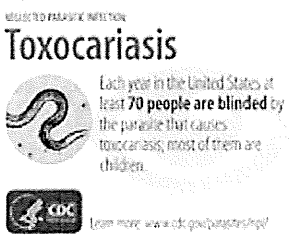
This information is not meant to be used for self-diagnosis or as a substitute for consultation with a health care provider. If you have any questions about the parasites described above or think that you may have a parasitic infection, consult a health care provider.

Read More



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PLoS NEGLECTED TROPICAL DISEASES

Editorial

Toxocariasis: America's Most Common Neglected Infection of Poverty and a Helminthiasis of Global Importance?

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New information indicates that toxocariasis is the most common human parasitic worm infection in the United States, affecting millions of Americans living in poverty. The infection is also highly prevalent in many developing countries and its global importance may be greatly underestimated.

Toxocariasis results from zoonotic transmission of the roundworms, *Toxocara canis* and *T. cati* from dogs and cats, respectively. Infection occurs when humans accidentally ingest the microscopic, oval and thick-shelled-embryonated eggs (shed in dog and cat feces) containing *Toxocara* larvae by hand-to-mouth contact. Children are particularly prone to infection because they are exposed to the eggs on sandboxes and playgrounds contaminated with dog and cat feces [1,2]. After ingestion of the eggs, the released larvae penetrate the intestine and migrate through the liver, lungs, and central nervous system (Figure 1). The resulting host inflammatory response ultimately overwhelms and either kills the migrating larvae or forces them into a state of arrested development, but not before they cause both mechanical and immunopathological damage to the issues (Figure 2).

There are two "classical" clinical syndromes resulting from infection [1,2]. Visceral larva migrans occurs most commonly in young children and results in hepatitis and pneumonitis as the larvae migrate through the liver and lungs, respectively. The full clinical presentation of toxocariasis includes hepatomegaly and pulmonary infiltrates or nodules accompanied by cough, wheezing, eosinophilia, lymphadenopathy, and fever. Larval entry into the central nervous system can also result in meningoencephalitis and cerebritis manifesting as seizures [3,4]. Ocular larva migrans occurs more frequently in older children and adolescents and may result from the migration of even a single larva in the eye. The resulting inflammation presents clinically as either a granuloma or a granulomatous larval track in the retina or as a condition of the vitreous that resembles endophthalmitis [5,6]. Neither visceral larva migrans nor ocular larva migrans are considered common conditions, although the incidence of the former has not been determined and it has been estimated at just under 1 per 10,000 annually for the ocular form [6]. Far more common is non-classic, or covert toxocariasis, which may manifest with only some of the clinical features found in visceral larva migrans, especially wheezing, pulmonary infiltrates, and eosinophilia [2]. Because these features are also the hallmark of childhood asthma, some investigators have hypothesized or in some cases have actually shown a link with *Toxocara* infection [2,7–14]. Similarly, some of the central nervous system features of toxocariasis have been implicated as a cause of occult seizures, mental retardation, and developmental delays [3,4,15]. Because pica is a risk factor for both toxocariasis and lead ingestion [16], it is possible that an element of the cognitive and mental deficits ascribed to toxocariasis may partially result from plumbism.

There are an estimated 73 million dogs and 90 million cats in the United States [17]. Many pups are born with congenital canine toxocariasis and large numbers of both dogs and cats are either stray animals or pets that are not routinely dewormed as recommended by the American Veterinary Medical Association [18]. Such huge numbers of *Toxocara*-infected dogs and cats serve as rich sources of eggs in the environment, which have been recovered in poor urban areas [16] as well as in rural areas, especially in the American South and Appalachia [19–21]. Most of the prevalence estimates for toxocariasis in the US are based on serological surveys with banked sera that detect *Toxocara*-specific antibodies [17,20,22]. The enzyme immunoassay (EIA) using *T. canis* excretory-secretory (TES) antigens from infective-stage larvae is the most useful diagnostic test for toxocaral visceral larva migrans and ocular larva migrans and is the assay used by most commercial reference laboratories in the US, including the reference laboratory at the US Centers for Disease Control and Prevention (CDC) [17,20,22–31]. Results from the CDC EIA measure total immunoglobulin antibodies and are reported as a titer; the assay detects infections caused by both *T. canis* and *T. cati*. For visceral larva migrans and some forms of covert toxocariasis, the sensitivity and specificity of the *Toxocara* EIA is estimated at 78% and 92%, respectively, at a titer of 1:32 [17,22,26,27]. The sensitivity of the EIA for ocular larva migrans, however, is considerably less [1,28]. Following initial infection, *Toxocara* larvae migrate through host tissues for several months, and ultimately generate a host granulomatous response, which

Citation: Hotez PJ, Wilkins PP (2009) Toxocariasis: America's Most Common Neglected Infection of Poverty and a Helminthiasis of Global Importance? PLoS Negl Trop Dis 3(3): doi:10.1371/journal.pntd.0000400

Published March 31, 2009

Funding: The authors received no specific funding for this article.

Competing Interests: The authors have declared that no competing interests exist.

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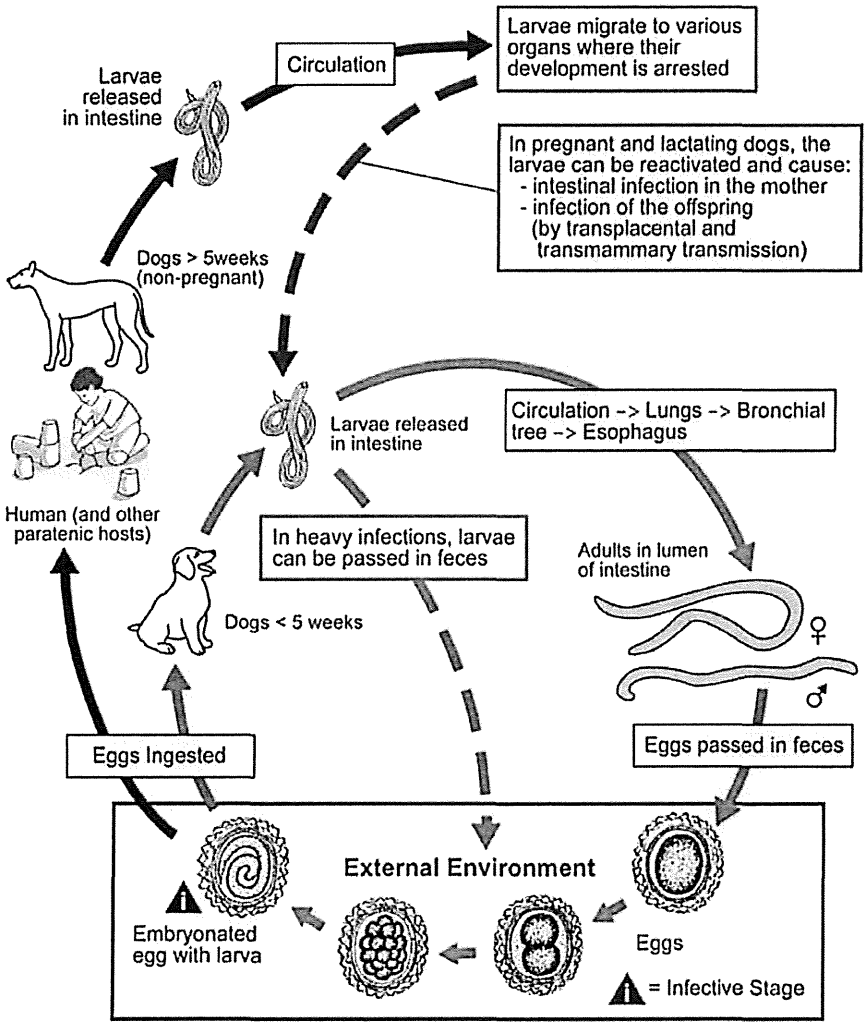

Figure 1. The Life Cycle of Human Infection with *Toxocara canis*. From the Public Health Image Library of the CDC, http://phil.cdc.gov.
doi:10.1371/journal.pntd.0000400.g001

blocks further larval migration. However, the larvae may remain alive within the host for months, and host antibody levels may remain strongly positive for 2 or 3 years or more [17,31]. Therefore, in the CDC EIA, the presence of antibody titers greater than 1:32 may be considered reflective of active infection, although we are not aware of careful studies that have determined the length of persistent toxocaral antibodies over long periods of time.

Using a nationally representative set of banked sera, the CDC has undertaken two major national surveys for toxocariasis [17,20,22]. The first was reported more than 20 years ago using sera from children aged 1 to 11 that were collected during the first Health and Nutrition Examination Survey (HANES I) of over 23,000 persons 1 to 74 years of age in 35 geographic regions from 1971 to 1973 [20]. Nationwide, the overall prevalence was found to vary between 4.6% and 7.3%, but ranged as high as 10% in the American South and over 30% for socioeconomically disadvantaged African American children [20]. Higher seroprevalence was also linked to markers of low socioeconomic status, including poverty and crowding and lower educational level for head of household [20]. In 2008, the CDC again reported on *Toxocara* seroprevalence from the Third National Health and Nutrition Examination Survey (NHANES III), a cross-sectional survey conducted between 1988 and 1994 [17,22]. The survey sampled at higher rates specific minority groups (e.g., non-Hispanic blacks and Mexican Americans) and age groups (young children and the elderly) [17]. Based on a representative sample of just over 20,000 in individuals over the age of 6, the overall seroprevalence was 13.9% [17,22], suggesting that tens of millions of Americans are infected with *Toxocara*. However, the seropreva-

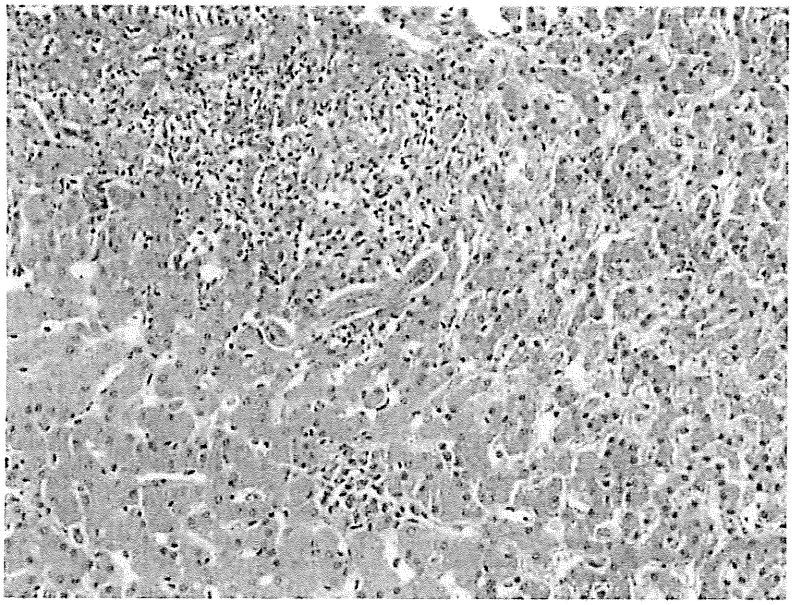

Figure 2. Toxocara Larva in Liver of Child Necropsied in New Zealand. Larva discovered at some distance from lesion. Image courtesy of CDC and DPDx.
doi:10.1371/journal.pntd.0000400.g002

lence was found to be considerably higher among non-Hispanic blacks and people living in poverty. Based on the number of African Americans living in poverty in the US, we calculated that as many as 2.8 million have toxocariasis, making this disease one of the most common infections among any underrepresented minority groups [32]. In a separate study conducted in the 1990s, high rates of toxocariasis were also found among inner city Hispanic populations in Bridgeport and New Haven, Connecticut, especially among Puerto Rican immigrants [14]. High rates of the infection were noted previously to occur in Puerto Rico [33]. Given its proposed links with asthma and developmental delays, human-toxocariasis may represent a health disparity of staggering proportions, possibly associated with the high frequency of asthma and developmental delays noted among African Americans and some Hispanic groups living in poverty [34–37]. The earlier association noted between toxocariasis and elevated lead levels observed in the HANES I study was confirmed in the NHANES III serum bank data, as was an interesting association between toxocariasis and co-infection with toxoplasmosis [17,22]. The health and developmental impact of these co-factors also warrants further investigation. Globally, high rates of toxocariasis has been noted in middle-income countries, with prevalence rates reaching 40% or higher in Indonesia and Brazil [30,38]. Although there are few reported studies from low-income countries, it is of great interest to determine whether infection rates with *Toxocara* may exceed some of the better known human soil-transmitted helminth infections such as ascariasis, trichuriasis, or hookworm infection.

While the NHANES studies indicate that toxocariasis continues to persist and is under-recognized as a health problem, a full appreciation of the US and global burden of disease caused by toxocariasis demands improved serodiagnostic tools. In the US, EIA testing is not widely available because of the limited capacity for parasitic disease diagnosis in the US and the limited availability of antigen made from *T. canis* larvae. In addition, the existing assays have a low sensitivity for detecting ocular larva migrans, so some true cases remain undiagnosed and the approximations of national seroprevalence are underestimated. These features, together with the observation that many physicians in the US are not knowledgeable about the infection, helps to preserve the neglected status of toxocariasis. In developing countries, survey results based on EIA with TES are confounded by high rates of co-infections with other soil-transmitted helminths, as antibodies to these other nematodes may cross-react to *T.canis* antigens [29,38]. In an effort to increase both the sensitivity and specificity of TES-based EIAs, some investigators have examined the advantages of measuring IgG subclass antibodies. At least one study has shown that sensitivity could be increased by measuring IgG2 subclass antibodies, presumably those that measure anti-carbohydrate antibodies against TES glycans, while specificity could be increased by measuring IgG3 or IgG4 antibodies [29,30]. In 2000, a 30-kDa recombinant TES antigen was cloned and expressed in bacteria [39]. The recombinant protein requires solubilization in urea (which may lessen its usability in an EIA format), but is undergoing evaluation as a potentially improved diagnostic reagent [38], as are other recombinant *T. canis* antigens [40]. Ultimately, further epidemiological studies and disease burden assessments of toxocariasis would benefit from the development of an immunodiagnostic assay that is both highly sensitive and specific for (and uses) the detection of antibodies to a chemically defined recombinant *T. canis* antigen, preferably one that is soluble in aqueous solution, and would be made widely available. Production of recombinant antigens may require expression in yeast or other low-cost eukaryotic expression vectors, which are often preferable to bacteria for producing soluble recombinant nematode antigens [41,42]. Alternatively, tests could be developed for measuring the presence of *Toxocara* antigen in the bloodstream, similar to the immunochromatographic test (ICT) developed for lymphatic filariasis [43] or tests for other helminth infections [44].

Further studies to improve diagnostic testing and expand epidemiologic surveillance should be conducted in parallel with control and prevention efforts. These include periodic deworming of dogs (especially after whelping) and hand-washing to prevent fecal oral contact [18], and case-detection and treatment with albendazole [45]. Given the high prevalence of toxocariasis in areas of poor urban and rural hygiene [16,21], improved sanitation and access to clean water may also have important roles. As a potential explanation for the high rates of asthma and developmental delays among disadvantaged children in poor urban and rural areas, there is an urgent need to fully explore the contribution of toxocariasis to these conditions, which in turn will require increased advocacy and resource mobilization. Recognition of toxocariasis as a common parasitic disease in the US and possibly an even greater health problem in developing countries is a first important step to national and international efforts to combat this neglected infection of poverty.

References

1. Despommier D (2003) Toxocariasis: Clinical aspects, epidemiology, medical ecology, and molecular aspects. Clin Microbiol Rev 16(2): 265–272.
2. Sharghi N, Schantz P, Hotez PJ (2000) Toxocariasis: An occult cause of childhood neuropsychological deficits and asthma? Seminars in Pediatric Infectious Diseases 11(4): 257–260.
3. Hotez PJ (1993) Visceral and ocular larva migrans. Semin Neurol 13(2): 175–179.
4. Marx C, Lin J, Masruha MR, Rodrigues MG, da Rocha AJ, et al. (2007) Toxocariasis of the CNS simulating acute disseminated encephalomyelitis. Neurology 69(8): 806–807. 10.1212/01.wnl.0000267664.53595.75.
5. Stewart JM, Cubillan LD, Cunningham ET Jr (2005) Prevalence, clinical features, and causes of vision loss among patients with ocular toxocariasis. Retina 25(8): 1005–1013.
6. Good B, Holland CV, Taylor MR, Larragy J, Moriarty P, et al. (2004) Ocular toxocariasis in schoolchildren. Clin Infect Dis 39(2): 173–178. 10.1086/421492.
7. Taylor MR, Keane CT, O'Connor P, Mulvihill E, Holland C (1988) The expanded spectrum of toxocaral disease. Lancet 1(8587): 692–695.
8. Buijs J, Borsboom G, van Gemund JJ, Hazebroek A, van Dongen PA, et al. (1994) Toxocara seroprevalence in 5-year-old elementary schoolchildren: relation with allergic asthma. Am J Epidemiol 140(9): 839–847.
9. Buijs J, Borsboom G, Renting M, Hilgersom WJ, van Wieringen JC, et al. (1997) Relationship between allergic manifestations and toxocara seropositivity: a cross-sectional study among elementary school children. Eur Respir J 10(7): 1467–1475.
10. Chan PW, Anuar AK, Fong MY, Debruyne JA, Ibrahim J (2001) Toxocara seroprevalence and childhood asthma among malaysian children. Pediatr Int 43(4): 350–353.
11. Feldman GJ, Parker HW (1992) Visceral larva migrans associated with the hypereosinophilic syndrome and the onset of severe asthma. Ann Intern Med 116(10): 838–840.
12. Kuk S, Ozel E, Oguzturk H, Kirkil G, Kaplan M (2006) Seroprevalence of toxocara antibodies in patients with adult asthma. South Med J 99(7): 719–722.
13. Oteifa NM, Moustafa MA, Elgozamy BM (1998) Toxocariasis as a possible cause of allergic diseases in children. J Egypt Soc Parasitol 28(2): 365–372.
14. Sharghi N, Schantz PM, Caramico L, Ballas K, Teague BA, et al. (2001) Environmental exposure to toxocara as a possible risk factor for asthma: a clinic-based case-control study. Clin Infect Dis 32(7): E111–6. 10.1086/319593.
15. Nelson S, Greene T, Ernhart CB (1996) Toxocara canis infection in preschool age children: risk factors and the cognitive development of preschool children. Neurotoxicol Teratol 18(2): 167–174.
16. Marmor M, Glickman L, Shofer F, Faich LA, Rosenberg C, et al. (1987) Toxocara canis infection of children: epidemiologic and neuropsychologic findings. Am J Public Health 77(5): 554–559.
17. Jones JL, Kruszon-Moran D, Won K, Wilson M, Schantz PM (2008) Toxoplasma gondii and toxocara spp. co-infection. Am J Trop Med Hyg 78(1): 35–39.
18. Harvey JB, Roberts JM, Schantz PM (1991) Survey of veterinarians' recommendations for treatment and control of intestinal parasites in dogs: public health implications. J Am Vet Med Assoc 199(6): 702–707.
19. Chorazy ML, Richardson DJ (2005) A survey of environmental contamination with ascarid ova, Wallingford, Connecticut. Vector Borne Zoonotic Dis 5(1): 33–39. 10.1089/vbz.2005.5.33.
20. Herrmann N, Glickman LT, Schantz PM, Weston MG, Domanski LM (1985) Seroprevalence of zoonotic toxocariasis in the United States: 1971–1973. Am J Epidemiol 122(5): 890–896.
21. Jones WE, Schantz PM, Foreman K, Smith LK, Witte EJ, et al. (1980) Human toxocariasis in a rural community. Am J Dis Child 134(10): 967–969.
22. Won KY, Kruszon-Moran D, Schantz PM, Jones JL (2008) National seroprevalence and risk factors for zoonotic toxocara spp. infection. Am J Trop Med Hyg 79(4): 552–557.
23. Cypess RH, Karol MH, Zidian JL, Glickman LT, Gitlin D (1977) Larva-specific antibodies in patients with visceral larva migrans. J Infect Dis 135(4): 633–640.
24. Glickman LT, Schantz PM (1981) Epidemiology and pathogenesis of zoonotic toxocariasis. Epidemiol Rev 3: 230–250.
25. Glickman LT, Schantz PM (1985) Do toxocara canis larval antigens used in enzyme-linked immunosorbent assay for visceral larva migrans cross-react with AB isohemagglutinins and give false positive results? Z Parasitenkd 71(3): 395–400.
26. Glickman L, Schantz P, Dombroske R, Cypess R (1978) Evaluation of serodiagnostic tests for visceral larva migrans. Am J Trop Med Hyg 27(3): 492–498.
27. Glickman L, Schantz P, Greive R (1986) Toxocaraiasis. In: Walls K, Schantz P, eds. Immunodiagnosis of parasitic diseases. New York: Academic Press. pp 201–231.
28. Schantz PM, Meyer D, Glickman LT (1979) Clinical, serologic, and epidemiologic characteristics of ocular toxocariasis. Am J Trop Med Hyg 28(1): 24–28.
29. Watthanakulpanich D, Smith HV, Hobbs G, Whalley AJ, Billington D (2008) Application of toxocara canis excretory-secretory antigens and IgG subclass antibodies (IgG1-4) in serodiagnostic assays of human toxocariasis. Acta Trop 106(2): 90–95. 10.1016/j.actatropica.2008.01.008.
30. Noordin R, Smith HV, Mohamad S, Maizels RM, Fong MY (2005) Comparison of IgG-ELISA and IgG4-ELISA for toxocara serodiagnosis. Acta Trop 93(1): 57–62. 10.1016/j.actatropica.2004.09.009.
31. Smith HV (1993) Antibody reactivity in human toxocariasis. In: Lewis S, Maizels RM, eds. Toxocara and Toxocariasis: Clinical, Epidemiological, and Molecular Perspectives. London: British Society for Parasitology/Institute of Biology. pp 91–109.
32. Hotez PJ (2008) Neglected infections of poverty in the United States of America. PLoS Negl Trop Dis 2(6): e256. 10.1371/journal.pntd.0000256.
33. Schantz PM (1989) Toxocara larva migrans now. Am J Trop Med Hyg 41(3 Suppl): 21–34.
34. Federico MJ, Liu AH (2003) Overcoming childhood asthma disparities of the inner-city poor. Pediatr Clin North Am 50(3): 655–75, vii.
35. LeNoir MA (1999) Asthma in inner cities. J Natl Med Assoc 91(8 Suppl): 1S–8S.
36. Smith DE, Ashiabi GS (2007) Poverty and child outcomes: A focus on jamaican youth. Adolescence 42(168): 837–858.
37. Bergen DC (2008) Effects of poverty on cognitive function: A hidden neurologic epidemic. Neurology 71(6): 447–451. 10.1212/01.wnl.0000324420.03960.36.
38. De Andrade Lima Coelho R, De Carvalho LB Jr, Perez EP, Araki K, Takeuchi T, et al. (2005) Prevalence of toxocariasis in northeastern Brazil based on serology using recombinant Toxocara canis antigen. Am J Trop Med Hyg 72(1): 103–107.
39. Yamasaki H, Araki K, Lim PK, Zasmy N, Mak JW, et al. (2000) Development of a highly specific recombinant toxocara canis second-stage larva excretory-secretory antigen for immunodiagnosis of human toxocariasis. J Clin Microbiol 38(4): 1409–1413.
40. Tetteh KK, Loukas A, Tripp C, Maizels RM (1999) Identification of abundantly expressed novel and conserved genes from the infective larval stage of Toxocara canis by an expressed sequence tag strategy. Infect Immun 67(9): 4771–4779.
41. Goud GN, Bottazzi ME, Zhan B, Mendez S, Deumic V, et al. (2005) Expression of the necator americanus hookworm larval antigen na-ASP-2 in pichia pastoris and purification of the recombinant protein for use in human clinical trials. Vaccine 23(39): 4754–4764. 10.1016/j.vaccine.2005.04.040.
42. Hancock K, Pattabhi S, Whitfield FW, Yushak ML, Lane WS, et al. (2006) Characterization and cloning of T24, a taenia solium antigen diagnostic for cysticercosis. Mol Biochem Parasitol 147(1): 109–117. 10.1016/j.molbiopara.2006.02.004.
43. Weil GJ, Lammie PJ, Weiss N (1997) The ICT filariasis test: A rapid-format antigen test for diagnosis of bancroftian filariasis. Parasitol Today 13(10): 401–404.
44. van Dam GJ, Wichers JH, Ferreira TM, Ghati D, van Amerongen A, et al. (2004) Diagnosis of schistosomiasis by reagent strip test for detection of circulating cathodic antigen. J Clin Microbiol 42(12): 5458–5461. 10.1128/JCM.42.12.5458–5461.2004.
45. Pawlowski Z (2001) Toxocariasis in humans: Clinical expression and treatment dilemma. J Helminthol 75(4): 299–305.

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Parasites & Vectors

REVIEW Open Access

Environmental contamination by canine geohelminths

Donato Traversa[1*], Antonio Frangipane di Regalbono[2], Angela Di Cesare[1], Francesco La Torre[3], Jason Drake[4] and Mario Pietrobelli[2]

Abstract

Intestinal nematodes affecting dogs, i.e. roundworms, hookworms and whipworms, have a relevant health-risk impact for animals and, for most of them, for human beings. Both dogs and humans are typically infected by ingesting infective stages, (i.e. larvated eggs or larvae) present in the environment. The existence of a high rate of soil and grass contamination with infective parasitic elements has been demonstrated worldwide in leisure, recreational, public and urban areas, i.e. parks, green areas, bicycle paths, city squares, playgrounds, sandpits, beaches. This review discusses the epidemiological and sanitary importance of faecal pollution with canine intestinal parasites in urban environments and the integrated approaches useful to minimize the risk of infection in different settings.

Keywords: *Toxocara canis*, *Ancylostoma caninum*, *Trichuris vulpis*, Faeces, Dog, Urban areas

Review

Soil-transmitted helminthoses affects more than 2 billion people worldwide [1]. Other than human-specific parasites, intestinal nematodes affecting dogs have a relevant health-risk impact for both animals and human beings. The importance of these pathogens is often minimized by veterinarians and the general public, although *Toxocara canis*, hookworms (i.e. *Ancylostoma* spp.) and whipworms (i.e. *Trichuris vulpis*) are the most relevant canine helminths in terms of geographic distribution and clinical importance [2,3].

The presence of infective eggs or larvae in the environment has a crucial role among the different routes of transmission of dog intestinal nematodes in both humans and animals. In fact, human beings become infected by canine *Toxocara* spp. and *Ancylostoma* spp. most frequently via contaminated soil [4-7].

Studies from various countries have demonstrated a high rate of soil and grass contamination with infective parasitic elements in leisure, recreational, public and urban areas, i.e. parks, green areas, bicycle paths, city squares, playgrounds, sandpits, beaches.

When using these areas, people often take their pets with them. Owned dogs and stray animals may defecate in public streets and areas, thus contaminating the environment with parasites and favoring zoonotic transmission and (re-) infection for other animals.

While readers interested in biology, pathology and general control of canine intestinal nematodes are referred to [2,3,7-9], the present article reviews the epidemiological importance of faecal pollution in urban environments with canine intestinal parasites in terms of veterinary and human health and discusses the integrated approaches useful to minimize the risk of infection.

The environment is incessantly contaminated

Toxocara canis and *Ancylostoma caninum* are, respectively, the primary species of roundworms and hookworms infecting dogs worldwide. Other species of ascarids and ancylostomatids may be present in particular areas, e.g. *Toxascaris leonina* in Europe and USA, *Uncinaria stenocephala* in colder areas of temperate and subarctic regions, and *Ancylostoma braziliense* in the southern hemisphere. Additionally, the whipworm *T. vulpis* is the ubiquitous whipworm inhabiting the large intestine of dogs [2,3].

Parasitic burdens and egg output are higher in puppies but patent intestinal infections may occur in dogs of all ages and categories [10-19], even when under regular control programs [15,20]. Bitches are a relevant source of infection for other animals and environmental contamination because they often harbor somatic larvae, which mobilize

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during pregnancies and infect subsequent litters even when re-infections do not occur. Puppies become infected *in utero* and *via* the milk, but a proportion of mobilized larvae reach adulthood in the intestine of the dam and cause a patent infection with a long-lasting high egg shedding [21,22]. The patent infection in the bitch can be re-enforced when suckling puppies defecate immature ascarids, which are ingested by the dam and become adults in her intestine [23]. Altogether these biological features make nursing bitches and puppies a very important source of environmental contamination by *T. canis*.

Remarkably, pre-vaccination confinement of puppies would often imply that eggs are shed into the home or private gardens and backyards, thus posing a potential health risk for the owners [24]. This is of great importance considering that virtually 100% of puppies acquire toxocarosis by transmammary and/or transplacental route/s and that they pass thousands of *T. canis* eggs per gram of feces every day (Figure 1).

Hookworm filariform larvae present in the soil infect a suitable host by actively penetrating the skin (especially for *Ancylostoma* spp.) and/or *via* the oral route (i.e. *Ancylostoma* spp., *Uncinaria* spp.) [3,23,25-27]. As with *T. canis*, hypobiotic larvae may survive for years in the tissues of adult dogs and when reactivated during oestrus and in the last 2–3 weeks of pregnancy, they are passed *via* the milk to the litter [27-30]. Adult dogs may suffer patent ancylostomosis when they become infected with environmental larvae or when hypobiotic stages are re-activated by drivers of stress [3]. Remarkably, dogs infected by *A. caninum* may shed millions of hookworm eggs for weeks [7].

The absence of a vertical transmission in *T. vulpis*, its long pre-patent period and a partial ability to stimulate a protective immune response [31,32], explain the high degree of intestinal trichurosis in adult dogs rather than in puppies. Hence, it could be erroneously argued that this parasite is not spread as easily as roundworms and that the environment is not as contaminated by whipworm eggs.

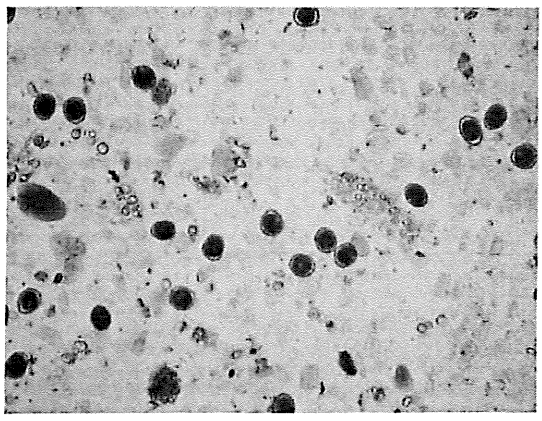

Figure 1 Copromicroscopic examination of a puppy: microscopic field (10×) showing a high shedding of *Toxocara canis* eggs.

It is estimated that the contamination of soil with *Toxocara* eggs may be more than the 90% of the investigated areas worldwide [33]. This is explained by the fact that mature eggs of ascarids (and *T. vulpis* as well) can survive in contaminated soil even in harsh conditions (e.g. they may resist to chemicals, broad temperature ranges and several degrees of moisture), thus are available for ingestion at any time by susceptible hosts [8,9,34]. Also, viability and infectivity of environmental larvated eggs persist for years, thus explaining the high number of chances that dogs have of becoming infected and the difficulties in controlling these intestinal parasitoses. As an example, eggs of *T. vulpis* survive from cold winter to hot summer, especially in wet and shady areas, which are widely distributed in green areas of metropolitan cities [9].

Larvated eggs of *T. canis* and larval ancylostomatids are an efficient environmental source of infection for various animals, which act as paratenic hosts. These animals greatly contribute to maintaining the biological cycle of toxocarosis and ancylostomosis everywhere. In fact, dogs can become infected by *Toxocara* by ingesting tissues of invertebrates (e.g. earthworms), ruminants (e.g. sheep), rodents, birds (e.g. chicken) [3,7,31].

The role of wildlife is another exogenous factor contributing to the environmental contamination. In fact, movements of wildlife to sub-urban and urban environments due to destruction or reduction of their habitat is another source of soil contamination by *T. canis* [35]. The key example is represented by synantropic fox populations, which reinforce environmental contamination and risk of infection for humans and stray and domestic dogs [36].

Thus, a combination of these factors is the basis for an extremely high environmental contamination and a life-long risk of infection for dogs living in contaminated areas.

The analysis of datasets from field investigations has recently described general principles and approaches useful to quantify levels of contamination with ascarid eggs and to prioritize control measures. In particular, the relative role of dogs, cats and foxes in disseminating parasite eggs in a given environment (i.e. the city of Bristol, UK) was investigated. This study, carried out in an urban setting in the absence of stray animals, showed that pet dogs are the source of most of the eggs that contaminate the environment [24]. Obviously, this study example would differ in terms of results and conclusions upon different localities, but in general it demonstrated that an estimation of egg density in urban settings is possible and provides local epidemiological models of egg outputs and sources of contamination. Also, this study illustrated that education of pet owners is crucial to minimize the risk of disease transmission to animals and humans and that stray dogs are not the culprits of faecal

urban pollution in every city. It is obvious that the number of eggs contaminating the environment is dependent on the amount of faeces eliminated by owned and stray dogs and on the extent of feces removal by the owners. However, there is a lack of information on rates of deposition and removal of dog faeces from public spaces in several areas [24]. In this regard, recent field studies conducted during summer 2012 by operators observing dogs and their owners in parks and green public areas located in the cities of Rome and Padua (Italy), showed that 15.6% pet-owners did not remove dog faecal deposits from the ground, with a few differences between the investigated cities (13.5% and 16.9%, respectively) (unpublished data).

Risk for humans

Human beings become infected by *T. canis* most commonly by ingesting embryonated eggs from the soil. Other sources of transmission with dog intestinal nematodes include ingestion of larvae resting in tissues of paratenic hosts, or hookworm larvae in contaminated soil, which can penetrate the skin of humans walking barefoot.

The presence of eggs on the ground is not only implicated with the direct infection for humans but could represent a source of contamination for pets' coats. Indeed, the role of embryonated ascarid eggs present on the fur of dogs has been evocated as a source of human infections via hand-to-mouth contact [6,37,38].

Indeed, infective eggs have been found on the coat of dogs in different studies suggesting that direct contact with these animals could be a potential risk for humans. Eggs of *T. canis* may be present on the hair of both stray and privately owned dogs, with the latter considered as a more important risk for human infection due to the frequent contact with people [39]. On the other hand, close-contact with a pet has been considered an unlikely risk of infection with intestinal parasites for humans because the strong adherence of eggs on the animal's fur, the relatively high number of eggs which should be ingested to establish an infection and the long time for the embryonation (i.e. minimum 2 weeks) [7,40]. Rather than a self-contamination (e.g. with self-grooming transmitting eggs from the peri-anal region to other parts of the body), dogs may pick up *Toxocara* eggs on their hair by the scent-rolling [6]. In any case, regarding the actual risk for human infection *via* touching or petting a pet, scent-rolling can be a relevant cause of contamination for the animals coat when a pet is taken out in contaminated areas. Interestingly, the presence of non-canine parasite eggs on the fur of dogs indicates that the contact with a contaminated environment plays a key role in the acquisition of eggs by the animals [41]. The presence of embryonated eggs on the fur of owned dogs in some studies [37,40,42,43] may account for a lack of care in terms of anthelmintic treatment programmes. Surveys in Ireland and in the Netherlands have shown the presence of eggs on the coat of owned dogs with a percentage of 8.8% [42] and 12.2% [40] respectively. However, eggs in both studies were not infective. Relatively old private dogs have been found with a higher percentage of eggs on their coats than puppies [37,40,42]. Additionally, the absence of a correlation between intestinal worm burden and intensity of coat contamination suggests that pick-up from a contaminate soil is the main reason for the presence of parasite eggs on the coat of a dog [6,40].

Dogs with patent toxocarosis do not represent an immediate risk for human infection for a variety of reasons [44-46] and direct contact with an infected dog is considered of minor importance in the zoonotic transmission of intestinal nematodes [47,48].

Canid ascarids can cause different syndromes (e.g. *visceral, neural* or *ocular larva migrans*, covert toxocarosis) in human beings, especially children and toddlers.

In fact, children are the subjects at highest risk of infection, due to exposure to areas (e.g. sandpits, green areas, gardens, playgrounds) potentially contaminated by *T. canis* eggs [44]. Children suffering by geophagic pica caused by mineral deficiency or behavior disorders are also at high risk [44,49]. For example, the impact of human infection by larval *Toxocara* in childhood is demonstrated by the hundreds of cases of blindness and eye damage calculated to occur yearly in the USA, which in the past has often led to eye enucleations due to misdiagnosis with retinoblastomas [3,48,50]. However, the role of migrating larvae of the feline ascarid *Toxocara cati* has been repeatedly also evocated in causing human syndromes [5]. Thus, the importance of environmental contamination by *T. cati* should not be neglected considering the likely absence of differences in terms of zoonotic potential between dog and cat roundworms [51]. People with a soil-related job (e.g. mechanics, gardeners, farmers, street cleaners) may be at more risk of infection with toxocarosis, as shown by their higher seroprevalence compared with values found in people with non-soil related occupations [52].

A survey from Ireland showed that garden soil contamination is not associated with the household presence of pets [53]. In general, ownership of companion animals is not definitively associated with seropositivity and seroprevalence for toxocarosis [52-54]. Contrariwise, human seropositivity to *Toxocara* spp. has been put in relation with the contamination of soil with parasite eggs in some US areas [55], although actual risk factors for human infections may change according to different geographical and epidemiological settings [56,57]. A study carried out in a city of Brazil showed that almost all seropositive children had the behavior disorder of geophagy and that they played nearly every day of the week in public squares with a minimum contamination of 1 *Toxocara* egg/gram of sand [58]. Additionally, it was also shown that contamination in

the neighborhood of domiciles in the same areas was again positively correlated with seropositivity in children in the presence of infected animals. Interestingly, seronegative children played infrequently in public squares [58].

Zoonotic hookworms may cause different pictures of skin, enteric and pulmonary diseases, being the *cutaneous larva migrans* the most important. Interested readers are referred to [7,8,59]. A relationship between the presence of *Ancylostoma* spp. larvae in soil of public squares and occurrence of *cutaneous larva migrans* in children has been demonstrated in Brazil [60].

It is obvious that tourists sunbathing on beaches in risky areas where zoonotic hookworms are endemic are at risk of infection with larval hookworms.

The dog whipworm *T. vulpis* is not included in zoonotic intestinal nematodes of pets [48] and its zoonotic potential is questioned although presumed cases of *visceral larva migrans* and of patent intestinal infections have been described in people. At the moment *T. vulpis* cannot be ultimately considered as a zoonotic canine parasite and readers interested may find more details in [9].

Despite its high zoonotic potential, few references are available on the presence of *Strongyloides stercoralis* in public areas. For instance, *S. stercoralis*-like larvae have been found in soil samples from Iran [61] and Nigeria [62].

Contamination and geography

Eggs of *Toxocara* spp., eggs and larvae of *Ancylostoma* spp. and eggs of *T. vulpis* have been found from soil and faecal samples in public areas from Europe, the Americas, Africa and Asia.

Table 1 reports key examples of surveys carried out in different countries to evaluate the frequency of canine parasites due to faecal pollution in various human settings.

In a recent survey, canine faecal deposits were collected from June 2012 to January 2013 in public green areas (e.g., historic gardens, children's playgrounds or green places for physical activities or fitness) in three different municipalities of Italy (i.e., Padua, Rome and Teramo). Out of a total of 677 collected samples, 38 (5.6%) scored positive upon copromicroscopical examination for at least one canine geo-helminth, i.e. 22/209 (10.6%) from Rome, 13/198 (6.6%) from Teramo, and 4/270 (1.5%) from Padua. Overall, the highest prevalence was detected for *T. vulpis* (30; 4.4%), followed by *T. canis* (13; 1.9%), and *A. caninum* (3; 0.4%), distinguished from *Uncinaria* based on the egg size differences reported in literature [91,92]. More specifically, prevalence values for *T. vulpis* and *T. canis* showed a similar trend in each municipality (7.7% and 1.9% in Rome, 5.1% and 3.6% in Teramo, 1.5% and 0.7% in Padua, respectively), whereas *A. caninum*-positive samples (1.4%) were observed solely in Rome (unpublished data).

Although parasite eggs may be found in several urban and industrialised settings, the risk of environmental contamination is particularly relevant in resource poor communities due to the fact that extensive worm control programs are limited by financial constraints. Also, in those poor settings the public health system is deficient, there is usually a high number of stray and feral animals and people lack awareness of health risks [93]. In these settings the bond between physicians, veterinarians and the whole community should be re-enforced to minimize as much as possible the risk of public hazard.

What can we do to reduce environmental contamination?

A reduction of the contamination of public areas by dog helminths can be achieved only with a combination of approaches, e.g. reliable worm control programs, awareness of veterinarian and behavior of pet owners and the general public.

No reliable methods exist to realistically eliminate eggs or larvae of intestinal nematodes of pets present on the ground. Therefore, preventing the initial contamination of the environment is of paramount importance. The individualized treatment of parasitized animals is mandatory to control infection in pets and environmental pollution. Unfortunately, negligence in performing diagnostic copromicroscopy in veterinary practices is frequent, due to the fallacy in considering an antiparasitic treatment powerful enough to "generically clear parasites".

Contrariwise, copromicroscopic examinations should be regular for pets, given that virtually all dogs are at risk of becoming infected by intestinal nematodes for all their life. The role of veterinarians is crucial, because pet owners should be convinced of the importance of periodic faecal examinations. Veterinarians have a plethora of parasiticides, which can be administered according to each individual possible *scenario* and both owner and animal compliance to treat infected animals [7,9]. Thorough indications for worm control programs have been released by the US Companion Animal Parasite Council (CAPC) and the European Scientific Counsel Companion Animal Parasites (ESCCAP) [7,94,95].

A key point for controlling pet parasites is the lifelong chemopreventative program. Using year-round treatment is of importance where there is the necessity to perform the annual chemoprophylaxis for other severe parasites and not only for intestinal nematodes, e.g. for the prevention of cardio-pulmonary nematodes, i.e. *Dirofilaria immitis* and *Angiostrongylus vasorum*. In addition, several formulations containing compounds effective against intestinal nematodes also contain cestocides which are powerful for controlling infections caused by tapeworms distributed worldwide (e.g. *Dipylidium caninum*) or hazardous for humans (e.g. *Echinococcus* spp.).

Broad-spectrum formulations with an easy mode of administration (e.g. chewy tablets, spot-on) fit particularly with year-long worm control programs. Faecal examinations

Table 1 Key examples of studies that evaluated the frequency (%) of soil contamination of public areas by roundworm, hookworm and whipworm eggs in different continents

Geographical area	Site	Frequency (%)			Reference
		Roundworms	Hookworms	Whipworms	
Africa					
Niger	Kaduna		9.0		[63]
Americas					
USA	Connecticut	14.4			[64]
Argentina	Buenos Aires	13.2			[65]
	Buenos Aires	1.7	20.5	2.6	[66]
Brazil	Fernandopolis	79.4	6.9		[67]
	Itabuna		47.9		[68]
	São Paulo	29.7			[69]
	Guarulhos, São Paulo	68.1	64.8		[70]
Chile	Santiago	66.7			[71]
Venezuela	Ciudad Bolívar		61.1		[72]
Asia					
Japan	Tokushima	63.3			[73]
Thailand	Bangkok	5.7			[74]
Turkey	Ankara	45.0			[75]
Europe					
Ireland	Dublin	15.0			[76]
Spain	Madrid	16.4	3.0		[77]
Italy	Marche region	33.6			[78]
	Milan	7.0	3.0	5.0	[79]
	Bari	2.5	1.6	2.5	[80]
	Naples	0.7-1.4	2.4	10.1	[81]
	Messina	3.6	2.6	1.3	[82]
	Alghero	0.5-8.0	4.0	1.9	[83]
Poland	Wroclaw	3.2	4.9	4.9	[84]
	Warsaw	26.1			[85]
	Kraków	15.6-19.8			[86]
Turkey	Erzurum	64.3			[87]
Czech Republic	Prague	20.4			[88]
Hungary	Eastern and northern areas	24.3-30.1	8.1-13.1	20.4-23.3	[89]
Slovak Republic	Bratislava	18.7			[90]

should be performed whether or not a monthly-based treatment program is used, even when the dog appears healthy, as there are parasites that may not be covered by the treatment program or there may be poor compliance with the program. In fact, owners may be not interested in paying for faecal examination if the animals are asymptomatic, because they are commonly considered parasite-free. While puppies and their thousands of eggs shed daily are the major source of contamination for the environment, a US study has shown that after young dogs, the most parasitized category of pets are > 10 years old [96]. This high degree of parasitism in old animals could reside in a lack of willingness of owners in chemopreventative and/or worm control programs in old pets [96]. Indeed, there is no reason to consider an old animal a less effective source of infection for pets, human beings and the environment.

Unfortunately, public risk perception and awareness may be poor in veterinarians, the general public and pet owners of several countries [97-100]. Interview- based studies have been conducted to understand how the risk perception is present in the human population and to implement awareness of the general public and of pet

owners. For example, a British survey has unveiled that less than the half of the participants (i.e. pet and non-pet owners) were aware of the potential for transmission of parasites *via* animal faeces with no differences between who had a pet and who did not [55].

Similarly, a recent Italian interview-based study carried out during summer 2012 in the cities of Rome and Padua illustrated that out of 469 participants, 246 (52.5%) were aware of the health risk associated with canine faecal pollution in urban settings, with no differences between pet and non-pet owners. In the same study, the awareness of the health risks was higher in Padua (205/339, corresponding to 60.4%) than in Rome (41/130; 31.5%), again with no differences between pet and non-pet owners (unpublished data).

Veterinarians should routinely inform clients about source of infections for both pets and humans and on reliable measures to prevent transmission to other animals and people. Regrettably, this is not a frequent behavior. As a key example, less than the half of interviewed veterinarians in a Canadian survey discussed the zoonotic risk of pet ownership with clients, while the remainder did this only in particular cases or not at all [100].

Given that public squares, sandpits, playgrounds, beaches are always at a high risk for heavy contamination by pet faeces and public parks and green areas are always contaminated by parasites of dogs [4,8,101-103], avoiding animal defecation in public areas or immediate collection of stool by the pet owner is crucial (Figure 2). Veterinarians should educate owners on regular removal and disposal of faeces, which is at the basis to minimize environmental contamination and risk of transmission [44,48]. When walking their pets in public areas, all owners should respect local indications and keep their animals in reserved areas, if present (Figure 3).

A very "creative" measure was recently adopted by the Municipality of Brunete (Spain), in which undercover volunteers were recruited to patrol the streets, and to confront dog-owners who did not remove the faeces of their pet. Approaching the guilty owner for a friendly conversation, volunteers swindled some useful information to identify his/her domicile by the preexisting pet-registration database. At the end of the conversation, when the dog-owner was out of sight, the volunteers picked up the dog's faeces, the excrements were boxed, and hand-delivered to the pet owner along with an official fine and warning [104].

Other than constant municipal cleaning and maintenance, controlled access of green areas and public parks by fences is an effective way of prevention of faecal contamination. A study in Japan has shown that placing vinyl plastic covers over sandboxes at night is able to discourage animals from defecating there [105]. An extreme measure chosen by some municipalities is the elimination of sandboxes from

Figure 2 Indication for dog-owners in New York City, USA.

parks and playgrounds [8]. It is important to note, however, that while *T. canis* eggs are most prevalent in public parks, sandboxes are mainly contaminated with eggs of *T. cati* due to the common behavior of cats during defecation [103].

Surveillance of the presence of parasite eggs in public soil is also important in this integrated approach to control intestinal parasites. In general, microscopic examination of soil samples is performed to identify *Toxocara* eggs, although this method may have low sensitivity and specificity [106,107]. DNA-based approaches have been

Figure 3 Beach area reserved for dogs in Broome, Australia.

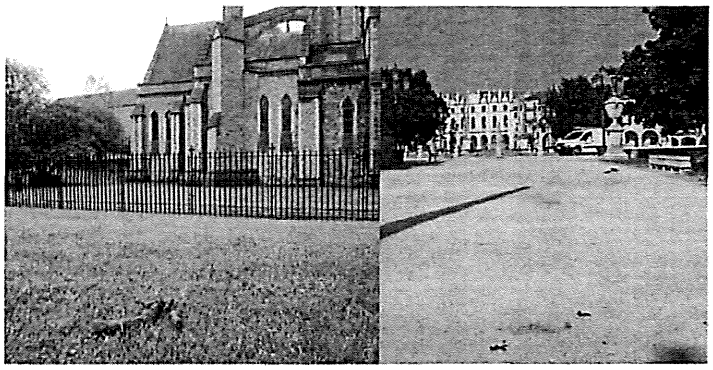

Figure 4 Dog faeces in a green park of Dublin, Ireland (left) and in a public square of Padua, Italy (right).

developed to discriminate eggs of ascarids in soil samples [107,108], although some pitfalls may impair a routine use, e.g. low throughput analysis and risk of carry-on contamination. A duplex Real-Time PCR has been recently validated for the detection and discrimination of *T. canis* and *T. cati* eggs in different samples, including soil. This assay is promising for the implementation of standardized methods able to evaluate the presence of roundworm eggs in contaminated soil on a large scale. In particular, this novel molecular tool can be used to investigate, with a high throughput, the occurrence and the level of contamination of eggs of *T. canis* (and *T. cati*) in urban parks, green areas, playgrounds and sandpits [109].

This is of importance because different investigations have shown that some urban environments may be heavily contaminated by *T. cati* rather than by *T. canis* [101].

Conclusion

Canine faeces in cities are an important source of pathogens for the pet population, for dog owners and for the community in general. Prevention of initial contamination is the most important way to avoid human and animal infections, given that no practical methods are available to actually minimize environmental egg contamination. The non-polite habit of dog owners of not removing feces of their pet from streets and green areas (Figure 4) represents a concern for hygiene and health of both animals and humans. Hence, polluted public environments represent the principle risk for human health with zoonotic intestinal nematodes of dogs [38]. Other than social responsibility in eliminating dog faeces from streets, parks and squares, appropriate worm control programs, especially in young dogs, are crucial to control faecal contamination and minimize the risk of infection for humans and other animals.

Unfortunately, public education in reducing the risk of exposure for both humans and companion animals is poor. In recent years sociological changes have influenced the relationships between physicians and veterinarians, towards the concept of the "One Health Program" (i.e. *"the collaborative work of multiple disciplines to help attain optimal health of people, animals, and our environment"*) [110]. Thus, there is the necessity for physicians, veterinarians and the general public to foster interest and efforts in appropriate control programs towards a reduction of pollution of the cities and of the risk of infection for both animals and people.

Competing interests
Studies whose unpublished results are reported in the review were financed by Novartis Animal Health, of which FLT and JD are employees.

Authors' contributions
DT, AFdR and MP conceived the article and all authors contributed to its drafting, preparation and intellectual content. AFdR and MP were scientifically responsible for the studies whose unpublished results are reported in the review. All authors read and approved the final manuscript.

Acknowledgements
The authors are grateful to all the co-workers who contributed in the studies whose unpublished results are presented in the review.

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Received: 17 December 2013 Accepted: 6 February 2014
Published: 13 February 2014

References

1. *World Health Organization and partners unveil new coordinated approach to treat millions suffering from neglected tropical disease.* 2006 [http://whqlibdoc.who.int/press_release/2006/PR]
2. Soulsby EJL: *Helminths, Arthropods and Protozoa of Domesticated Animals.* 7th edition. London, UK: Bailliere Tindall; 1982.
3. Bowman DD: *Georgi's Parasitology for Veterinarians.* 9th edition. Philadelphia, USA: Saunders Company; 2009.
4. Holland CV, Smith HV: *Toxocara: The Enigmatic Parasite.* Wallingford, UK: CABI Publishing; 2006.
5. Fisher M: ***Toxocara cati*: an underestimated zoonotic agent.** *Trends Parasitol* 2003, 19(4):167–170.
6. Roddie G, Stafford P, Holland C, Wolfe A: **Contamination of dog hair with eggs of *Toxocara canis*.** *Vet Parasitol* 2008, 152(1–2):85–93.
7. Traversa D: **Pet roundworms and hookworms: a continuing need for globalworming.** *Parasit Vectors* 2012, 5:91.
8. Despommier D: **Toxocariasis: clinical aspects, epidemiology, medical ecology, and molecular aspects.** *Clin Microbiol Rev* 2003, 16(2):265–272.

9. Traversa D: **Are we paying too much attention to cardio-pulmonary nematodes and neglecting old-fashioned worms like Trichuris vulpis?** *Parasit Vectors* 2011, **4**:32.
10. Visco RJ, Corwin RM, Selby LA: **Effect of age and sex on the prevalence ofintestinal parasitism in dogs.** *J Am Vet Med Assoc* 1977, **170**:835–837.
11. Visco RJ, Corwin RM, Selby LA: **Effect of age and sex on the prevalence of intestinal parasitism in cats.** *J Am Vet Med Assoc* 1978, **1978**(172):797–800.
12. Lloyd S: **Toxocarosis.** In *Zoonoses. Biology, Clinical Practice and Public Health Control.* Edited by Palmer SR, Soulsby EJL, Simpson DIH. Oxford: Oxford University Press; 1998:841–854.
13. Malloy WF, Embil JA: **Prevalence of *Toxocara* spp. and other parasites in dogs and cats in Halifax, Nova Scotia.** *Can J Comp Med* 1978, **42**:29–31.
14. Martínez-Barbabosa I, Vázquez Tsuji O, Cabello RR, Cárdenas EM, Chasin OA: **The prevalence of *Toxocara cati* in domestic cats in Mexico City.** *Vet Parasitol* 2003, **114**:43–49.
15. Fahrion AS, Staebler S, Deplazes P: **Patent *Toxocara canis* infections in previously exposed and in helminth-free dogs after infection with low numbers of embryonated eggs.** *Vet Parasitol* 2008, **152**:108–115.
16. Little SE, Johnson EM, Lewis D, Jaklitsch RP, Payton ME, Blagburn BL, Bowman DD, Moroff S, Tams T, Rich L, Aucoin D: **Prevalence of intestinal parasites in pet dogs in the United States.** *Vet Parasitol* 2009, **166**:144–152.
17. Scorza AV, Duncan C, Miles L, Lappin MR: **Prevalence of selected zoonotic and vector-borne agents in dogs and cats in Costa Rica.** *Vet Parasitol* 2011, **183**:178–183.
18. Savilla TM, Joy JE, May JD, Somerville CC: **Prevalence of dog intestinal nematode parasites in south central West Virginia, USA.** *Vet Parasitol* 2011, **178**:115–120.
19. Barutzki D, Schaper R: **Results of parasitological examinations of faecal samples from cats and dogs in Germany between 2003 and 2010.** *Parasitol Res* 2011, **109**(Suppl 1):S45–S60.
20. Sager H, Moret CS, Grimm F, Deplazes P, Doherr MG, Gottstein B: **Coprological study on intestinal helminths in Swiss dogs: temporal aspects of anthelminthic treatment.** *Parasitol Res* 2006, **98**:333–338.
21. Lloyd S, Amersinghe PH, Soulsby EJL: **Periparturient immunosuppression in the bitch and its influence on infection with *Toxocara canis*.** *J Small Anim Pract* 1983, **24**:237–247.
22. Lloyd S: ***Toxocara canis*: the dog.** In *Toxocara and Toxocariasis Clinical, Epidemiological and Molecular Perspectives.* Edited by Lewis JW, Maizels RM. London: British Society for Parasitology. Institute of Biology; 1993:11–24.
23. Epe C: **Intestinal nematodes: biology and control.** *Vet Clin North Am Small Anim Pract* 2009, **39**:1091–1107. vi-vii.
24. Morgan ER, Azam D, Pegler K: **Quantifying sources of environmental contamination with Toxocara spp. eggs.** *Vet Parasitol* 2013, **193**(4):390–397.
25. Anderson RC: **Nematode Parasites of Vertebrates.** In *Their development and transmission.* 2nd edition. Guilford: CABI; 2000.
26. Prociv P: **Zoonotic hookworm infections (Ancylostomosis).** In *In Zoonoses.* 1st edition. Edited by Palmer SR, Soulsby EJL, Simpson DIH. Oxford, UK: Oxford Medical Publications; 1998.
27. Bowman DD, Montgomery SP, Zajac AM, Eberhard ML, Kazacos KR: **Hookworms of dogs and cats as agents of cutaneous larva migrans.** *Trends Parasitol* 2010, **26**:162–167.
28. Stoye M: **Galactogenic and prenatal *Toxocara canis* infections in dogs (Beagle).** *Dtsch Tierarztl Woch* 1976, **83**:107–108. in German.
29. Stoye M: **Biology, pathogenicity, diagnosis and control of *Ancylostoma caninum*.** *Dtsch Tierarztl Woch* 1992, **99**:315–321. in German.
30. Bosse M, Manhardt J, Stoye M: **Epidemiology and Control of neonatal Helminth Infections of the dog.** *Fortschr Vet Med* 1980, **30**:247–256. in German.
31. Taylor MA, Coop RL, Wall RL: *Veterinary Parasitology.* 3rthth edition. Oxford, UK: Blackwell Publishing; 2007.
32. Fontanarrosa MF, Vezzani D, Basabe J, Eiras DF: **An epidemiological study of gastrointestinal parasites of dogs from Southern Greater Buenos Aires (Argentina): age, gender, breed, mixed infections, and seasonal and spatial patterns.** *Vet Parasitol* 2006, **136**:283–295.
33. Kirchheimer R, Jacobs DE: ***Toxocara* species egg contamination of soil from children's play areas in southern England.** *Vet Rec* 2008, **163**(13):394–395.
34. Parsons JC: **Ascarid infections of cats and dogs.** *Vet Clin North Am Small Anim Pract* 1987, **17**:1307–1339.
35. Brochier B, De Blander H, Hanosset R, Berkvens D, Losson B, Saegerman C: ***Echinococcus multilocularis* and *Toxocara canis* in urban red foxes (*Vulpes vulpes*) in Brussels, *Belgium.*** *Prev Vet Med* 2007, **80**(1):65–73.
36. Antolová D, Reiterová K, Miterpáková M, Stanko M, Dubinský P: **Circulation of *Toxocara* spp. in suburban and rural ecosystems in the Slovak Republic.** *Vet Parasitol* 2004, **126**:317–324.
37. Wolfe A, Wright IP: **Human toxocariasis and direct contact with dogs.** *Vet Rec* 2003, **152**:419–422.
38. Deplazes P, van Knapen F, Schweiger A, Overgaauw PA: **Role of pet dogs and cats in the transmission of helminthic zoonoses in Europe, with a focus on echinococcosis and toxocarosis.** *Vet Parasitol* 2011, **182**(1):41–53.
39. El-Tras WF, Holt HR, Tayel AA: **Risk of *Toxocara canis* eggs in stray and domestic dog hair in Egypt.** *Vet Parasitol* 2011, **178**(3–4):319–323.
40. Overgaauw PA, van Zutphen L, Hoek D, Yaya FO, Roelfsema J, Pinelli E, van Knapen F, Kortbeek LM: **Zoonotic parasites in fecal samples and fur from dogs and cats in The Netherlands.** *Vet Parasitol* 2009, **163**:115–122.
41. Holland C, O'Connor P, Taylor MR, Hughes G, Girdwood RW, Smith H: **Families, parks, gardens and toxocariasis.** *Scand J Infect Dis* 1991, **23**(2):225–231.
42. Keegan JD, Holland CV: **Contamination of the hair of owned dogs with the eggs of *Toxocara* spp.** *Vet Parasitol* 2010, **173**:161–164.
43. Aydenizöz-Ozkayhan M, Yağci BB, Erat S: **The investigation of *Toxocara canis* eggs in coats of different dog breeds as a potential transmission route in human toxocariasis.** *Vet Parasitol* 2008, **152**(1–2):94–100.
44. Overgaauw PAM: **Aspects of *Toxocara* epidemiology: human toxocarosis.** *Crit Rev Microbiol* 1997, **23**:215–231.
45. Overgaauw PAM, Van Knapen F: **Dogs and nematodes zoonoses.** In *Dogs Zoonoses and Public Haelth.* 1st edition. Edited by MacPhersom CNL, Melsin FX, Wandeler A. New York: CABI Publishing Oxon; 2000:213–245.
46. Overgaauw PAM, Van Knapen F: **Negligible risk of visceral or ocular larva migrans from petting a dog.** *Ned Tijdschr Geneeskd* 2004, **148**:1600–1603.
47. Overgaauw PAM: **Aspects of *Toxocara* epidemiology: toxocarosis in dogs and cats.** *Crit Rev Microbiol* 1997, **23**:233–251.
48. Robertson ID, Thompson RC: **Enteric parasitic zoonoses of domesticated dogs and cats.** *Microbes Infect* 2002, **4**:867–873.
49. Schantz PM: **Toxocara larva migrans now.** *Am J Trop Med Hyg* 1989, **41**:21–34.
50. Glickman LT, Schantz PM: **Epidemiology and pathogenesis of zoonotic toxocariasis.** *Epidemiol Rev* 1981, **3**:230–250.
51. Overgaauw PA, van Knapen F: **Veterinary and public health aspects of *Toxocara* spp.** *Vet Parasitol* 2013, **193**(4):398–403.
52. Raschka C, Haupt W, Ribbeck R: **Studies on endoparasitization of stray cats.** *Mon Vet* 1994, **49**:307–315.
53. Jenkins DJ: **Hydatidosis a zoonosis of unrecognised increasing importance?** *J Med Microbiol* 1998, **47**:1–3.
54. Tenter AM, Heckeroth AR, Weiss LM: **Toxoplasma gondii: from animals to humans.** *Int J Parasit* 2000, **30**:1217–1258.
55. Won KY, Kruszon-Moran D, Schantz PM, Jones JL: **National seroprevalence and risk factors for Zoonotic Toxocara spp. infection.** *Am J Trop Med Hyg* 2008, **79**(4):552–557.
56. Andrade C, Alava T, De Palacio IA, Del Poggio P, Jamoletti C, Gulletta M, Montresor A: **Prevalence and intensity of soil-transmitted helminthiasis in the city of Portoviejo (Ecuador).** *Mem Inst Oswaldo Cruz* 2001, **96**(8):1075–1079.
57. Matsuo J, Nakashio S: **Prevalence of fecal contamination in sandpits in public parks in Sapporo City, Japan.** *Vet Parasitol* 2005, **128**(1–2):115–119.
58. Manini MP, Marchioro AA, Colli CM, Nishi L: **Falavigna-Guilherme AL: Association between contamination of public squares and seropositivity for Toxocara spp. in children.** *Vet Parasitol* 2012, **188**(1–2):48–52.
59. Hotez PJ, Wilkins PP: **Toxocariasis: America's most common neglected infection of poverty and a helminthiasis of global importance?** *PLoS Negl Trop Dis* 2009, **3**(3):e400.
60. Santarém VA, Giuffrida R, Zanin GA: **Larva *migrans* cutânea: ocorrência de casos humanos e identificação de larvas de *Ancylostoma* spp. em parque público do município de Taciba, São Paulo.** *Rev Soc Bras Med Trop* 2004, **37**:179–181.
61. Motazedian H, Mehrabani D, Tabatabaee SH, Pakniat A, Tavalali M: **Prevalence of helminth ova in soil samples from public places in Shiraz.** *East Mediterr Health J* 2006, **12**:562–565.
62. Ogbolu DO, Alli OA, Amoo AO, Olaosun II, Ilozavbie GW, Olusoga-Ogbolu FF: **High-level parasitic contamination of soil sampled in Ibadan metropolis.** *Afr J Med Med Sci* 2011, **40**:321–325.
63. Maikai BV, Umoh JU, Ajanusi OJ, Ajogi I: **Public health implications of soil contaminated with helminth eggs in the metropolis of Kaduna, Nigeria.** *J Helminthol* 2008, **82**(2):113–118.
64. Chorazy ML, Richardson DJ: **A survey of environmental contamination with ascarid ova, Wallingford, Connecticut.** *Vector Borne Zoonotic Dis* 2005, **5**(1):33–39.

65. Fonrouge R, Guardis MV, Radman NE, Archelli SM: **Soil contamination with *Toxocara* sp. eggs in squares and public places from the city of La Plata. Buenos Aires, Argentina.** *Bol Chil Parasitol* 2000, 55(3–4):83–85.
66. Rubel D, Wisnivesky C: **Dog fouling and helminth contamination in parks and sidewalks of Buenos Aires City, 1991–2006.** *Medicina (B Aires)* 2010, 70(4):355–363.
67. Cassenote AJ, Pinto Neto JM, Lima-Catelani AR, Ferreira AW: **Soil contamination by eggs of soil-transmitted helminths with zoonotic potential in the town of Fernandópolis, State of São Paulo, Brazil, between 2007 and 2008.** *Rev Soc Bras Med Trop* 2011, 44(3):371–374.
68. Campos Filho PC, Barros LM, Campos JO, Braga VB, Cazorla IM, Albuquerque GR, Carvalho SM: **Zoonotic parasites in dog feces at public squares in the municipality of Itabuna, Bahia, Brazil.** *Rev Bras Parasitol Vet* 2008, 17(4):206–209.
69. Muradian V, Gennari SM, Glickman LT, Pinheiro SR: **Epidemiological aspects of Visceral Larva Migrans in children living at São Remo Community, São Paulo (SP), Brazil.** *Vet Parasitol* 2005, 134(1–2):93–97.
70. Marques JP, Guimarães Cde R, Boas AV, Carnaúba PU, Moraes J: **Contamination of public parks and squares from Guarulhos (São Paulo State, Brazil) by Toxocara spp. and Ancylostoma spp.** *Rev Inst Med Trop Sao Paulo* 2012, 54(5):267–271.
71. Castillo D, Paredes C, Zañartu C, Castillo G, Mercado R, Muñoz V, Schenone H: **Environmental contamination with *Toxocara* sp. eggs in public squares and parks from Santiago, Chile, 1999.** *Bol Chil Parasitol* 2000, 55(3–4):86–91.
72. Devera R, Blanco Y, Hernández H, Simoes D: ***Toxocara* spp. and other helminths in squares and parks of Ciudad Bolivar, Bolivar State (Venezuela).** *Enferm Infecc Microbiol Clin* 2008, 26(1):23–26.
73. Shimizu T: **Prevalence of *Toxocara* eggs in sandpits in Tokushima city and its outskirts.** *J Vet Med Sci* 1993, 55(5):807–811.
74. Wiwanitkit V, Waenlor W: **The frequency rate of *Toxocara* species contamination in soil samples from public yards in a urban area "Payathai", Bangkok, Thailand.** *Rev Inst Med Trop Sao Paulo* 2004, 46(2):113–114.
75. Avcioglu H, Burgu A: **Seasonal prevalence of *Toxocara* ova in soil samples from public parks in Ankara, Turkey.** *Vector Borne Zoonotic Dis* 2008, 8(3):345–350.
76. O'Lorcain P: **Prevalence of Toxocara canis ova in public playgrounds in the Dublin area of Ireland.** *J Helminthol* 1994, 68(3):237–241.
77. Dado D, Izquierdo F, Vera O, Montoya A, Mateo M, Fenoy S, Galván AL, García S, García A, Aránguez E, López L, del Águila C, Miró G: **Detection of zoonotic intestinal parasites in public parks of Spain. Potential epidemiological role of microsporidia.** *Zoonoses Public Health* 2012, 59(1):23–28.
78. Habluetzel A, Traldi G, Ruggieri S, Attili AR, Scuppa P, Marchetti R, Menghini G, Esposito F: **An estimation of *Toxocara canis* prevalence in dogs, environmental egg contamination and risk of human infection in the Marche region of Italy.** *Vet Parasitol* 2003, 113(3–4):243–252.
79. Genchi M, Ferroglio E, Traldi G, Passera S, Mezzano G, Genchi C: **Fecalizzazione ambientale e rischio parassitario nelle città di Milano e Torino.** *Professione Veterinaria* 2007, 41:15–17.
80. Lia R, La Montanara C, Leone N, Pantone N, Llazari A, Puccini V: **Canine helminthic fauna and environmental faecalization in the town of Bari (Apulia region, Southern Italy).** *Parassitologia* 2002, 44(1):92.
81. Rinaldi L, Biggeri A, Carbone S, Musella V, Catelan D, Veneziano V, Cringoli G: **Canine faecal contamination and parasitic risk in the city of Naples (southern Italy).** *BMC Vet Res* 2006, 2:29.
82. Risitano AL, Brianti E, Gaglio G, Ferlazzo M, Giannetto S: **Environmental contamination by canine feces in the city of Messina: parasitological aspects and zoonotic hazards.** In *Proceedings of LXI Congress of the Italian Society for Veterinary Science (S.I.S.Vet.).* Salsomaggiore Terme, Italy; 2007:135–136.
83. Scala A, Garippa G, Pintus D: **Environmental contamination by canine feces in the city of Alghero (SS): parasitological aspects and zoonotic hazards.** In *Proceedings of LXIII Congress of the Italian Society for Veterinary Science (S.I.S.Vet.).* Udine, Italy; 2009:180–182.
84. Perec-Matysiak A, Hildebrand J, Zaleśny G, Okulewicz A, Fatula A: **The evaluation of soil contamination with geohelminth eggs in the area of Wrocław. Poland.** *Wiad Parazytol* 2008, 54(4):319–323.
85. Borecka A, Gawor J: **Prevalence of *Toxocara canis* infection in dogs in the Warszawa area.** *Wiad Parazytol* 2000, 46(4):459–462.
86. Mizgajska H: **Soil contamination with *Toxocara* spp. eggs in the Kraków areaand two nearby villages.** *Wiad Parazytol* 2000, 46(1):105–110.
87. Avcioglu H, Balkaya I: **The relationship of public park accessibility to dogs to the presence of *Toxocara* species ova in the soil.** *Vector Borne Zoonotic Dis* 2011, 11(2):177–180.
88. Dubná S, Langrová I, Nápravník J, Jankovská I, Vadlejch J, Pekár S, Fechtner J: **The prevalence of intestinal parasites in dogs from Prague, rural areas, and shelters of the Czech Republic.** *Vet Parasitol* 2007, 145(1–2):120–128.
89. Fok E, Szatmári V, Busák K, Rozgonyi F: **Prevalence of intestinal parasites in dogs in some urban and rural areas of Hungary.** *Vet Q* 2001, 23(2):96–98.
90. Totková A, Klobusický M, Holková R, Friedová L: **Current prevalence of toxocariasis and other intestinal parasitoses among dogs in Bratislava.** *Epidemiol Mikrobiol Imunol* 2006, 55(1):17–22.
91. Ehrenford FA: **Differentiation of the ova of Ancylostoma caninum and Uncinaria stenocephala in dogs.** *Am J Vet Res* 1953, 14(53):578–580.
92. Sloss MW, Kemp RL, Zajac AM: *Veterinary Clinical Parasitology.* Veterinary Clinical Parasitology: Iowa State University Press; 1994.
93. Heukelbach J, Mencke N, Feldmeier H: **Editorial: cutaneous larva migrans and tunglasis: the challenge to control zoonotic ectoparasitoses associated with poverty.** *Trop Med Int Health* 2002, 7(11):907–910.
94. *Companion Animal Parasite Council.* [http://www.capcvet.org]
95. *European Scientific Counsel Companion Animal Parasites.* [http://www.esccap.org]
96. Gates MC, Nolan TJ: **Endoparasite prevalence and recurrence across different age groups of dogs and cats.** *Vet Parasitol* 2009, 166:153–158.
97. Rubinstensky-Elefant G, Hirata CE, Yamamoto JH, Ferreira MU: **Human toxocariasis: diagnosis, worldwide seroprevalences and clinical expression of the systemic and ocular forms.** *Ann Trop Med Parasitol* 2010, 104:3–23.
98. Harvey JB, Roberts JM, Schantz PM: **Survey of veterinarians' recommendations for treatment and control of intestinal parasites in dogs: public health implications.** *J Am Vet Med Assoc* 1991, 199:702–707.
99. Overgaauw PAM: **Effect of a government educational campaign in the Netherlands on awareness of *Toxocara* and toxocarosis.** *Prev Vet Med* 1996, 28:165–174.
100. Stull JW, Carr AP, Chomel BB, Berghaus RD, Hird DW: **Small animal deworming protocols, client education, and veterinarian perception of zoonotic parasites in western Canada.** *Can Vet J* 2007, 48:269–276.
101. Lee CY, Schantz PM, Kazacos KR, Montgomery SP, Bowman DD: **Epidemiologic and zoonotic aspects of ascarid infections in dogs and cats.** *Trends Parasitol* 2010, 26:155–161.
102. Tharaldsen J: **Parasitic organisms from dogs and cats in sandpits from nursery schools in Oslo.** *Norsk Veterinaertidsskrift* 1982, 94:251–254.
103. Jansen J, Van Knapen F: ***Toxocara* eggs in public parks and sandboxes in Utrecht.** *Tijdschr Diergeneeskd* 1993, 118:611–614.
104. **Brunete entrega a domicilio las cacas de perro 'extraviadas' por sus dueños.** [http://www.elmundo.es/elmundo/2013/06/03/madrid/1370257901.html]
105. Uga S, Kataoka N: **Measures to control *Toxocara* egg contamination in sandpits of public parks.** *Am J Trop Med Hyg* 1995, 52:21–24.
106. Uga S, Matsuo J, Kimura D, Rai SK, Koshino Y, Igarashi K: **Differentiation of Toxocara canis and T. cati eggs by light and scanning electron microscopy.** *Vet Parasitol* 2000, 92(4):287–294.
107. Borecka A, Gawor J: **Modification of gDNA extraction from soil for PCR designed for the routine examination of soil samples contaminated with *Toxocara* spp. eggs.** *J Helminthol* 2008, 82(2):119–122.
108. Fogt-Wyrwas R, Jarosz W, Mizgajska-Wiktor H: **Utilizing a polymerase chain reaction method for the detection of Toxocara canis and T. cati eggs in soil.** *J Helminthol* 2007, 81(1):75–78.
109. Durant JF, Irenge LM, Fogt-Wyrwas R, Dumont C, Doucet JP, Mignon B, Losson B, Gala JL: **Duplex quantitative real-time PCR assay for the detection and discrimination of the eggs of Toxocara canis and Toxocara cati (Nematoda, Ascaridoidea) in soil and fecal samples.** *Parasit Vectors* 2012, 7(5):288.
110. Paul M, King L, Carlin EP: **Zoonoses of people and their pets: a US perspective on significant pet-associated parasitic diseases.** *Trends Parasitol* 2010, 26:153–154.

doi:10.1186/1756-3305-7-67
Cite this article as: Traversa *et al.*: Environmental contamination by canine geohelminths. *Parasites & Vectors* 2014 7:67.

OPEN ACCESS Freely available online

PLOS | NEGLECTED TROPICAL DISEASES

Viewpoints

Neglected Parasitic Infections and Poverty in the United States

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The neglected tropical diseases (NTDs) are a group of chronic and disabling infections that occur primarily in settings of extreme poverty and affect over 1,000,000,000 people globally [1]. A selected group of neglected parasitic infections, including some which overlap with the World Health Organization's new list of recognized NTDs [2], are also common in the United States, where they disproportionately affect the poor. The major neglected parasitic infections in the US include Chagas disease, cysticercosis, toxocariasis, toxoplasmosis, and trichomoniasis. These five parasitic infections are considered "neglected" based on their high prevalence, chronic and disabling features, and their strong links with poverty [1,2]. In contrast, the major intestinal parasitic infections found in the US—cryptosporidiosis, cyclosporiasis, and giardiasis—are mostly acute diarrheal illnesses without significant links to poverty or neglected populations. This review highlights new information (mostly from the last five years) on the major neglected parasitic infections affecting impoverished Americans, with respect to their distribution and unique clinical presentations as well as their surprising links to cardiovascular, respiratory, and neuropsychiatric conditions ordinarily thought of as noncommunicable diseases. Key diagnostic and therapeutic challenges and urgent needs for active surveillance and prevention are also presented.

Determinants of Neglected Parasitic Infections in the US

NTDs have been shown to flourish in settings of warm climate and extreme poverty found in global subtropical regions similar to the southern US. Indeed, new information suggests that many of the world's NTDs occur predominantly among the extreme poor living in the group of 20 (G20) countries, mostly in in the subtropics, including Brazil, Indonesia, India, China, Saudi Arabia, and Mexico [3]. It is now recognized that several neglected parasitic infections are also widespread in the southern US [4]. This finding is consistent with new US census data indicating that 20 million Americans now live in "extreme poverty" [5], with 1.65 million households (with 3.55 million children) living on less than US$2 per day in a given month [6]—a standard benchmark for global poverty. Today, the states with the highest poverty rates are all in the southern part of the country (Table 1) [7], and the nation's poorest large metropolitan area (McAllen-Edinburg-Mission, Texas) and the eight most impoverished smaller metropolitan areas are located in this region [8]. While there are noncash safety-net programs, including food stamps and public health insurance, that blunt some of the hardship of those living in extreme poverty, there has still been a clear increase in the number of Americans living in poverty over the last 40 years [5]. The underlying basis for why poverty promotes neglected parasitic infections in the southern US is unknown, although factors such as poor housing and sanitation and environmental contamination are likely contributors [5], while so far the links to ethnicity appear to be mainly socioeconomic. Through their chronic and disabling effects on worker productivity, child development, and maternal health [9], it is plausible that neglected parasitic infections could also help perpetuate generational poverty among people of color in the US.

Initial efforts to assess the prevalence or incidence of the neglected parasitic infections were hampered by a dearth of available data on these conditions [5,10]. In response, legislation known as the "Neglected Infections of Impoverished Americans Act" (HR 528) was drafted to raise awareness of these diseases among the general public and subsequently introduced as a bill in 2010 and 2011 [11]. While efforts have since stalled in the US Congress, over the last five years additional information about the neglected parasitic infections has accumulated so that it is possible to begin making more informed statements on their status with regards to prevalence, geographic distribution, and novel associations with illnesses that resemble noncommunicable diseases (Table 2) and on the initial steps required for prevention.

Neglected Helminthic Infections

Toxocariasis and cysticercosis are two of the most common parasitic worm infections. Toxocariasis occurs disproportionately in the southern US. Less is known about the distribution of cysticercosis, but it also tends to concentrate in southern and southwestern states with large Hispanic American populations [12].

Toxocariasis

Toxocariasis is a soil-transmitted helminth infection and zoonosis that results from accidental ingestion of *Toxcara canis* or *T. cati* eggs found in soil contaminated with dog or cat feces, respectively. The resulting larval migrations through the lungs, liver, and eyes continue for months or even years to produce several different inflammatory conditions that include visceral toxocariasis, ocular toxocariasis, and covert toxocariasis with elevated serum

Citation: Hotez PJ (2014) Neglected Parasitic Infections and Poverty in the United States. PLoS Negl Trop Dis 8(9): e3012. doi:10.1371/journal.pntd.0003012

Editor: Serap Aksoy, Yale School of Public Health, United States of America

Published September 4, 2014

Funding: No direct funding was supplied for this article.

Competing Interests: The author has declared that no competing interests exist.

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Table 1. Geographic distribution of poverty in the United States.

State	Poverty Rate	Rank among US States
Mississippi	21.0	1
New Mexico	19.9	2
Arizona	19.1	3
District of Columbia	19.1	3
Louisiana	18.9	5
Georgia	18.5	6
Texas	17.7	7

Percentage of people in poverty by state using 3-year averages: 2009–11. http://www.census.gov/hhes/www/poverty/data/incpovhlth/2011/tables.html.
doi:10.1371/journal.pntd.0003012.t001

immunoglobulin E (IgE) and eosinophilia [13]. Toxocariasis is widespread and is likely our nation's most common helminthiasis [13]. Much of what we currently know about the prevalence and risk factors for toxocariasis are from data on over 20,000 serum samples collected from the Third National Health and Nutrition Examination Survey (NHANES III) and tested by the Centers for Disease Control and Prevention (CDC) for *Toxocara* antibodies [14]. The age-adjusted seroprevalence was highest among non-Hispanic blacks (21.2%) and associated with low education, poverty, elevated lead concentrations, dog ownership, and living in the South or Northeast areas of the country [14,15]. Toxocariasis and toxoplasmosis

Table 2. Neglected parasitic infections in the United States.

Neglected Parasitic Infection	Selected Prevalence Data	Major Risk Factors	Clinical Sequelae In Adults and Children 1	Clinical Sequelae In Adults and Children 2	Congenital Clinical Sequelae	References
Toxocariasis	>21% Seroprevalence among African-Americans (up to 2.8 million African-Americans); >17% seroprevalence in the American South	African-American race, male sex, poverty, low education level, lead ingestion, contact with dogs, coinfection with *Toxoplasma*	Neurologic and psychiatric: cognitive delays; epilepsy; ocular manifestations	Pulmonary: diminished lung function asthma	Not well established	[13–22]
Cysticercosis	Up to 41,000–169,000 infected persons; likely widely under-recognized, with only an estimated 1,000 hospitalized cases diagnosed	Hispanic immigrants	Neurologic: epilepsy; chronic headaches	None	Not well established	[24–28]
Chagas Disease	300,167 cases	Hispanic-Americans	Cardiovascular: cardiomyopathy neurysms; conduction disturbances; sudden death	Gastrointestinal: megaviscera	Congenital: congenital Chagas disease syndrome	[29–38]
Toxoplasmosis	1.1 million new cases annually, including 21,505 cases of ocular toxoplasmosis and up to 4,000 cases of congenital toxoplasmosis	African-American ethnicity and poverty	Neurologic and psychiatric: cerebritis; schizophrenia; bipolar and other mood disorders	Ocular: retinitis and retinal scars and other ocular findings	Congenital toxoplasmosis syndrome: hydrocephalus; chorioretinitis; intracranial calcifications; cognitive deficits; hearing loss	[39–47]
Trichomoniasis	7.4 million new cases annually	African-American ethnicity—10 times more common	Genitourinary: vaginitis; pelvic inflammatory disease; pregnancy complications	HIV coinfections	Neonatal infections	[48–52]
Total	Approximately 12 million incident or prevalent infections, including some people living with chronic sequelae					

Features that produce clinical manifestations and sequelae similar to selected noncommunicable diseases.
doi:10.1371/journal.pntd.0003012.t002

coinfections were also noted to be common [16]. Based on these data and the number of at-risk African Americans living in poverty, one estimate suggested that up to 2.8 million African Americans may have *Toxocara* infections [4], but there is a need to refine those estimates through further research. Among the compelling reasons to collect additional information about toxocariasis are potential links between the covert form and important pulmonary and neurologic sequelae [13]. An expanded NHANES III analysis recently linked toxocariasis to significantly diminished lung function [17] and reduced cognitive function in children [18]. Still other new studies support a link to epilepsy [19] but with conflicting evidence on the association with bronchial asthma [20]. Ocular toxocariasis is associated with vision loss, especially among children living in the South [21]. Given the high rates of toxocariasis among people living in the southern US and its association with cognitive delays, epilepsy, vision loss, and reduced pulmonary function, there is an urgent need to conduct prospective studies to confirm these links as well as to evaluate potential therapies with albendazole and other anthelminthic drugs [22]. The extent to which other soil-transmitted helminth infections still occur in the US is not known. As recently as 1982, ascariasis, trichuriasis, hookworm infection, and strongyloidiasis were prevalent in impoverished areas of the southern US and Appalachia, but over the last 30 years, there have been few if any high-quality studies to assess whether these infections still occur in historically endemic areas [23].

Cysticercosis

Cysticercosis is a human infection with the larval form of the pork tapeworm, *Taenia solium*, which results from the accidental ingestion of eggs passed in feces from another person, typically a family member or household contact. The disease is widely distributed in Latin America as well as in Asian and African developing countries, where it is an important global cause of epilepsy and manifestations of hydrocephalus, but it has also emerged as a public health problem among Hispanic-Americans living in the US. One estimate suggests that tens of thousands of cases are present at any given time [4]. The clinical presentation of cysticercosis in Houston, Texas, is typical of that seen in other regions of the US, with neurologic manifestations ("neurocysticercosis"), especially seizures and headaches, the most common presenting signs [24]. In the patient population seen at one of Houston's two major public hospitals, more than half have parenchymal disease, which is usually a solitary cyst (often with a scolex) seen on neuroimaging [24]. However, a significant percentage of cases also show intraventricular (20%) or subarachnoid disease (12%) or calcifications (12%) [24]. In southern California, where neurocysticercosis also causes a significant health and economic burden, most of the cases require hospitalization (at which point they are most frequently diagnosed), and men were more likely to suffer from severe disease including hydrocephalus, which was more costly and required longer hospital stays [25]. The American Academy of Neurology recently issued evidence-based guidelines for the treatment of neurocysticercosis and currently recommends specific anthelminthic therapy with albendazole either with our without corticosteroids in order to decrease the number of active lesions on brain imaging studies or to reduce long-term seizure frequency [26]. Such guidelines focus on parenchymal neurocysticercosis, so additional guidelines may be required to address more complicated disease. Substance P was identified as a possible factor responsible for seizures in neurocysticercosis [27], a finding which could provide new avenues for therapy. Importantly, cysticercosis can also be acquired in the US, but the actual extent to which this occurs is unknown [25,28]. Pilot surveillance systems in California have screened contacts of neurocysticercosis cases for tapeworm carriage, identifying a source in up to 21% of US-born cases [28]. Treatment of these tapeworm carriers can prevent disease in their contacts.

Neglected Protozoan Infections

Three major protozoan infections have been linked to poverty in the US.

Chagas disease

Chagas disease (American trypanosomiasis) is a chronic parasitic infection caused by *Trypanosoma cruzi*, transmitted by triatomine vectors ("kissing" bugs), and associated with severe cardiomyopathy and other life-threatening sequelae in approximately one-third of infected individuals [29]. The CDC estimates that 300,167 people live with *T. cruzi* infection in the US, including up to 45,000 with undiagnosed Chagasic cardiomyopathy [29,30]. A new economic analysis projects the healthcare and other costs of Chagas disease in the US to be almost US$900 million annually, placing it on a similar footing with other better-known infections such as Lyme disease and methicillin-resistant *Staphylococcus aureus* infection [31]. An important gap in our knowledge of the burden of Chagas disease in the US is an accurate estimate of the number of infants being infected through mother-to-child transmission [32]. The first case of congenital Chagas disease was confirmed in 2012 [33], but it has been estimated that up to 315 infants annually (in the same order of magnitude as phenylketonuria or other inborn errors of metabolism) are born in the US with congenital Chagas disease [29]. Information on the number of infected infants and highest-risk groups could inform policies on targeted screening. Limited data suggest that up to 13% of patients with dilated cardiomyopathy in at-risk Latino populations in the US may be due to Chagas disease [34]. More accurate estimates of the burden, risk groups, and costs would inform treatment guidelines and prevent disease progression. Another major unknown is the proportion of cases in the US due to immigration from endemic areas of Latin America versus those attributable to autochthonous transmission. The triatomine vectors are widely distributed in the southern US [30], especially in Texas, where canine Chagas disease is also widespread [35]. However, to date only 23 cases of autochthonous Chagas disease have been confirmed [36]. Reasons for the lack of clarity on disease burden include limited public health surveillance and targeted surveys and poor disease-related knowledge among American physicians [37]. Few obstetricians know about the risk of congenital Chagas disease [38]. Finally, the diagnostic tests for Chagas are not easily accessible, and the antitrypanosomal drugs are highly toxic, often of limited efficacy, and contraindicated in pregnancy [30].

Toxoplasmosis

Toxoplasmosis is a parasitic infection of humans and numerous animal species. Transmission of *Toxoplasma gondii* to humans occurs through either ingestion of cysts found in meat or oocysts found in water or soil contaminated by cat feces [39]. Based on NHANES 1999–2004 and the 2009 US census data, almost 1.1 million people are infected each year, including more than 21,505 people who develop ocular lesions [40]. While overall the prevalence of toxoplasmosis has declined from the prior decade, the disease still occurs disproportionately among

non-Hispanic blacks and people living in poverty [39]. Up to 4,000 cases of congenital toxoplasmosis also occur annually [41]. Interestingly, a new test that detects antibodies to sporozoites has recently suggested that most congenital infections result from ingestion of *T. gondii* oocysts (zoonotically transmitted from cats) during pregnancy [42]. There are also data to indicate that severe disease resulting from congenital toxoplasmosis is more common in the US than in Europe [43]. However, despite this new information, there is a low level of awareness among obstetricians about toxoplasmosis and how to prevent infection [44]. The burden of *T. gondii* infection may extend beyond well-known manifestations that include ocular disease, congenital defects, and severe disease in the immunocompromised host. Some recent studies have also indicated an association between seropositivity and various psychiatric conditions, including schizophrenia, bipolar and other mood disorders, and suicide attempts [45–47]. Addressing important gaps in our understanding of this disease, such as estimates of incidence of congenital toxoplasmosis and cost/benefit of screening; elucidation of the association between *T. gondii* infection and mental illness; and improved diagnostic tests and treatments, would enable better prevention and control in the US.

Trichomoniasis

Trichomoniasis is a common sexually transmitted parasitic infection with more than 7 million cases annually in the US, where it is a leading cause of vaginitis, preterm labor, and pelvic inflammatory disease [48]. Data collected over the last decade has also revealed an important link with HIV/AIDS, as women with *Trichomonas vaginalis* infection exhibit increased HIV viral shedding, which has been shown to decrease following antiparasitic chemotherapy [49]. Indeed, a significant number of HIV transmission events from HIV-infected women may be attributable to trichomoniasis coinfections [50]. The prevalence of trichomoniasis is more than ten times higher among black women than non-Hispanic white women [51]. Other factors associated with Trichomonas infection in this national sample included poverty, low educational level, increasing age, high number of sex partners, being born in the US, douching, and having a concurrent chlamydial infection [51]. Nitroimidazoles (metronidazole or tinidizole) are the only class of drug available in the US for treatment. Low levels of metronidazole resistance are now widespread among *T. vaginalis* isolates in US urban centers [52]. The CDC has also received isolates that have been resistant to tinidazole. In addition, some women are allergic to the nitroimidazoles and require desensitization, so other drugs are urgently needed. Addressing important gaps in the epidemiology of this disease, including the role of asymptomatic infections in disease transmission and the role of male infections, is needed to inform prevention policies.

Other protozoan infections

Two other intestinal protozoan infections, i.e., cryptosporidiosis and giardiasis, are also common in the US, where they are neither linked to neglect nor poverty. Both diseases were reviewed recently with respect to their epidemiology in the US [53,54]. Briefly, both infections are more prevalent in the northern US and exhibit their highest incidence during the summer months with links to recreational water use [53,54]. Cyclosporiasis is a parasitic infection linked to food-borne illness, also with high incidence during the summer months [55].

Urgent Needs and Future Directions

The neglected parasitic infections are not rare conditions in the US. Instead, they affect at least 12 million Americans, either through new infections (e.g., trichomoniasis) or from prevalent persistent infections resulting in chronic sequelae. However, these diseases typically go undiagnosed because of poor awareness among health care providers as well as the relative inaccessibility or unavailability of the diagnostic tests. Confirmatory diagnostic testing for these parasitic infections requires serologic testing that detects antibody against antigens obtained from whole parasites that are typically unavailable in most clinical laboratories. Therefore, there is an urgent need to develop improved diagnostic reagents (including recombinant antigens) and point-of-care tests. The chronic and disabling features of neglected parasitic infections can resemble selected noncommunicable diseases, which further compounds diagnostic difficulties and their lack of recognition. As examples, few health care providers might recognize toxocariasis and toxoplasmosis as underlying causes of pediatric cognitive deficits and developmental delays, toxocariasis as a cause of asthma, toxocariasis and cysticercosis as etiologies of epilepsy, or Chagas disease as a cause of heart disease. In addition to the lack of diagnostic tools, better drugs are urgently needed to either overcome resistance or have a better safety profile. There is no Food and Drug Administration (FDA)-approved drug for Chagas disease in the US, which limits the ability to scale up a treatment program for thousands of people in the US. The drugs, however, are available under investigational protocols from the CDC. Almost all of our current estimates of disease prevalence, incidence, or disease burden are based on limited testing or NHANES surveys. While blood products are screened for *T. cruzi* antibody [36,56], such activities underestimate the true prevalence and geographic distribution of an infection like Chagas disease that occurs mostly among the poor [30]. For some diseases like Chagas disease or neurocysticercosis, for which there is a potential public health response such as screening children of infected mothers or screening household contacts of neurocysticercosis for taeniasis, public health surveillance is appropriate but seldom conducted except in a few states. Currently, Chagas disease is reportable in only four states and neurocysticercosis in five states, but even in these states there are few if any programs of active surveillance. Such limited surveillance activities hinder efforts to assess disease burdens, identify at-risk populations, and elucidate modes of transmissions. Finally, we need programs of health education and advocacy to promote awareness for the neglected parasitic infections and to shape policies for control and prevention. Pediatricians and obstetricians in particular can play a major role in advising families how to prevent these diseases. Communities need to enact and enforce regulations that prohibit pet access to children's play areas in public parks and programs to prevent *T. canis*, *T. cati*, and *T. gondii* zoonotic transmission from dog and cat feces. Directing attention and resources to the neglected parasitic infections would provide a cornerstone for a broader approach to help the most impoverished and marginalized Americans.

Acknowledgments

PJH wishes to thank Capt. Monica E. Parise, MD, and Dana Woodhall, MD, from the Parasitic Disease Branch of the CDC for their input and advice on the manuscript.

References

1. Hotez PJ, Molyneux DH, Fenwick A, Kumaresan J, Sachs SE, et al. (2007) Control of neglected tropical diseases. N Engl J Med 357: 1018–1027.
2. World Health Organization (2010) Working to overcome the global impact of neglected tropical diseases: First WHO report on neglected tropical diseases. Geneva: World Health Organization. Available: http://www.who.int/neglected_diseases/2010report/en/. Accessed 2 August 2013.
3. Hotez PJ (2013) NTDs V.2.0: "Blue Marble Health"—Neglected Tropical Disease Control and Elimination in a Shifting Health Policy Landscape. PLoS Negl Trop Dis 7: e2570.
4. Hotez PJ (2008) Neglected infections of poverty in the United States of America. PLoS Negl Trop Dis 2: e256.
5. Denavas-Walt C, Proctor BD, Smith JC, US Census Bureau (2011) Income, Poverty, and Health Insurance Coverage in the United States: 2010. Available: http://www.census.gov/prod/2011pubs/p60-239.pdf. Accessed 25 August 2012.
6. Shaefer HL, Edin K (2013) Rising extreme poverty in the United States and the response of federal means-tested transfer programs. National Poverty Center Working Paper Series #13-06, May 2013. Available: http://npc.umich.edu/publications/u/2013-06-npc-working-paper.pdf. Accessed 28 March 2014.
7. US Census Bureau (2012) Poverty: Income, Poverty and Health Insurance in the United States: 2011—Tables & Figures. Available: http://www.census.gov/hhes/www/poverty/data/incpovhlth/2011/tables.html. Accessed 6 April 2013.
8. Gabe T (2013) Poverty in the United States: 2012. Congressional Research Service, Report for Congress, November 13, 2013. Available: http://www.fas.org/sgp/crs/misc/RL33069.pdf. Accessed 6 April 2013.
9. Hotez PJ, Fenwick A, Savioli L, Molyneux DH (2009) Rescuing the bottom billion through control of neglected tropical diseases. Lancet 373: 1570–1575.
10. Hotez P, Stillwaggon E, McDonald M, Todman L, DiGrazia L (2010) National summit on neglected infections of poverty in the United States. Emerg Infect Dis 16: e1.
11. Sunlight Foundation, OpenCongress (2011) H.R. 528 – Neglected Infections of Impoverished Americans Act of 2011. Available: https://www.opencongress.org/bill/112-h528/show. Accessed 6 April 2013.
12. Pew Research Center (2014) Hispanic Trends Project. Statistical Portrait of the Hispanic Population in the United States, 2001. Hispanic Population, by State: 2011. Available: http://www.pewhispanic.org/files/2013/02/Statistical-Portrait-of-Hispanics-in-the-United-States-2011_FINAL.pdf. Accessed 2 August 2013.
13. Hotez PJ, Wilkins PP (2009) Toxocariasis: America's most common neglected infection of poverty and a helminthiasis of global importance. PLoS Negl Trop Dis 3: e400.
14. Won KY, Kruszon-Moran D, Schantz PM, Jones JL (2008) National seroprevalence and risk factors for zoonotic Toxocara spp. Infection. Am J Trop Med Hyg 79: 552–557.
15. Congdon P, Lloyd P (2011) Toxocara infection in the United States: the relevance of poverty, geography and demography as risk factors, and implications for estimating county prevalence. Int J Publ Health 56: 15–24.
16. Jones JL, Kruszon-Moran D, Won K, Wilson M, Schantz PM (2008) Toxoplasma gondii and Toxocara spp. Co-infection. Am J Trop Med Hyg 78: 35–39.
17. Walsh MG (2011) Toxocara infections and diminished lung function in a nationally representative sample from the United States population. Int J Parasitol 41: 243–247.
18. Walsh MG, Haseeb MA (2012) Reduced cognitive function in children with toxocariasis in a nationally representative sample of the United States. Int J Parasitol 42: 1159–1163.
19. Quattrocchi G, Nicoletti A, Marin B, Bruno E, Druet-Cabanac M, Preux P-M (2012) Toxocariasis and epilepsy: systematic review and meta-analysis. PLoS Negl Trop Dis 6: e1775.
20. Pinelli E, Aranzamendi C (2012) Toxocara infection and its association with allergic manifestations. Endocrin Metabol Immun Disorders Drug Targets 12: 33–44.
21. Woodhall D, Starr MC, Montgomery SP, Jones JL, Lum F, et al. (2012) Ocular toxocariasis: epidemiologic, anatomic, and therapeutic variations based on a survey of ophthalmic subspecialists. Ophthalmology 119: 1211–1217.
22. Othman AA (2012) Therapeutic battle against larval toxocariasis: are we still far behind? Acta Trop 124: 171–178.
23. Starr MC, Montgomery SP (2011) Soil-transmitted helminthiases in the United States: a systematic review – 1940–2010. Am J Trop Med Hyg 85: 680–684.
24. Serpa JA, Graviss EA, Kass JS, White AC Jr (2011) Neurocysticercosis in Houston, Texas. Medicine 90: 81–86.
25. Croker C, Redelings M, Reporter R, Sorvillo F, Mascola L, et al. (2012) The impact of neurocysticercosis in California: a review of hospitalized cases. PLoS Negl Trop Dis 6: e1480.
26. Baird RA, Wiebe S, Zunt JR, Halperin JJ, Gonseth G, et al. (2013) Evidence-based guideline: treatment of parenchymal neurocysticercosis. Report of the Guideline Development Subcommittee of the American Academy of Neurology. Neurology 80: 1424–1429.
27. Robinson P, Garza A, Weinstock J, Serpa JA, Goodman JC, et al. (2012) Substance P causes seizures in neurocysticercosis. PLoS Pathog 8: e1002489.
28. Sorvillo F, Wilkins P, Shafir S, Eberhard M (2011) Public health implications of cysticercosis acquired in the United States. Emerg Infect Dis 17: 1–6.
29. Bern C, Montgomery SP (2009) An estimate of the burden of Chagas disease in the United States. Clin Infect Dis 49: e52–e54.
30. Bern C, Kjos S, Yabsley MJ, Montgomery SP (2011) Trypanosoma cruzi and Chagas' disease in the United States. Clin Microbiol Rev 24: 655–681.
31. Lee BY, Bacon KM, Bottazzi ME, Hotez PJ (2013) Global economic burden of Chagas disease: a computational simulation model. Lancet Infect Dis 13: 342–348.
32. Buekens P, Almendares O, Carlier Y, Dumonteil E, Eberhard M, et al. (2008) Mother-to-child transmission of Chagas disease in North America: why don't we do more? Matern Child Health J 12: 283–286.
33. Centers for Disease Control and Prevention (2012) Congenital transmission of Chagas disease – Virginia, 2010. MMWR Morb Mortal Wkly Rep 61: 477–479.
34. Kapelusznik L, Varela D, Montgomery SP, Shah AN, Steurer FJ, et al. (2013) Chagas disease in Latin American immigrants with dilated cardiomyopathy in New York City. Clin Infect Dis 57: e7.
35. Sarkar S, Strutz SE, Frank DM, Rivaldi C-L, Sissel B, et al. (2010) Chagas disease risk in Texas. PLoS Negl Trop Dis 4: e836.
36. Cantey PT, Stramer SL, Townsend RL, Kamel H, Ofafa K, et al. (2012) The United Trypanosoma cruzi infection Study: evidence for vector-borne transmission of the parasite that causes Chagas disease among United States blood donors. Transfusion 52: 1922–1930.
37. Stimpert KK, Montgomery SP (2010) Physician awareness of Chagas disease, USA. Emerg Infect Dis 16: 871–872.
38. Verani JR, Montgomery SP, Schulkin J, Anderson B, Jones JL (2010) Survey of obstetrician-gynecologists in the United States about Chagas disease. Am J Trop Med Hyg 83: 891–895.
39. Jones JL, Kruszon-Moran D, Sanders-Lewis K, Wilson M (2007) Toxoplasma gondii infection in the United States, 1999–2004, decline from the prior decade. Am J Trop Med Hyg 77: 405–410.
40. Jones JL, Holland GN (2010) Short report: annual burden of ocular toxoplasmosis in the United States. Am J trop Med Hyg 82: 464–465.
41. Lopez A, Dietz V, Wilson M, Navin TR, Jones JL (2000) Preventing congenital toxoplasmosis. MMWR Recomm Rep 49: 57–75.
42. Boyer K, Hill D, Mui E, Wroblewski K, Karrison T, Dubey JP, et al. (2011) Unrecognized ingestion of Toxoplasma gondii oocysts leads to congenital toxoplasmosis and causes epidemics in North America. Clin Infect Dis 53: 1081–1089.
43. Olariu TR, Remington JS, McLeod R, Alam A, Montoya JG (2011) Severe congenital toxoplasmosis in the United States: clinical and serologic findings in untreated infants. Pediatr Infect Dis J 30: 1056–1061.
44. Jones JL, Krueger A, Schulkin J, Schantz PM (2010) Toxoplasmosis in prevention and testing in pregnancy, survey of obstetrician-gynaecologists. Zoonoses Publ Health 57: 27–33.
45. Torrey EF, Bartko JJ, Yolken RH (2012) Toxoplasma gondii and other risk factors for schizophrenia: an update. Schizophr Bull 38: 642–647.
46. Arling TA, Yolken RH, Lapidus M, Langenberg P, Dickerson FB, et al. (2009) Toxoplasma gondii antibody titers and history of suicide attempts in patients with recurrent mood disorders. J Nerv Ment Dis 197: 905–908.
47. Hamdani N, Daban-Huard C, Lajnef M, Richard JR, Delavest M, et al. (2013) Relationship between Toxoplasma gondii infectiona nd bipolar disorder in a French sample. J Affect Disord 148: 444–448.
48. Coleman JS, Gaydos CA, Witter F (2013) Trichomonas vaginalis vaginitis in obstetrics and gynecology practice: new concepts and controversies. Obstet Gynecol Surv 68: 43–50.
49. Kissinger P, Amedee A, Clark RA, Dumestre J, Theall KP, et al. (2009) Trichomonas vaginalis treatment reduces vaginal HIV-1 shedding. Sex Transm Dis 36: 11–16.
50. Quinlivan EB, Patel SN, Grodensky CA, Golin CE, Tien HC, et al. (2012) Modeling the impact of Trichomonas vaginalis infection on HIV transmission in HIV-infected individuals in medical care. Sex Transm Dis 39: 671–677.
51. Sutton M, Sternberg M, Koumans EH, McQuillan G, Berman S, et al. (2007) The prevalence of Trichomonas vaginalis infection among reproductive-age women in the United States, 2001–2004. Clin Infect Dis 45: 1319–1326.
52. Kirkcaldy RD, Augostini P, Asbel LE, Bernstein KT, Kerani RP, et al. (2012) Trichomonas vaginalis antimicrobial drug resistance in 6 US cities, STD Surveillance Network, 2009–2010. Emerg Infect Dis 18: 939–943.
53. Yoder JS, Wallace RM, Collier SA, Beach MJ, Hlavasa MC (2012) Cryptosporidiosis surveillance – United States, 2009–2010. MMWR Surveill Summ 61: 1–12.
54. Yoder JS, Gargano JW, Wallace RM, Beach MJ (2012) Giardiasis surveillance - United States, 2009–2010. MMWR Surveill Summ 61: 13–23.
55. Hall RL, Jones JL, Hurd S, Smith G, Mahon BE, et al. (2012) Population-based active surveillance for Cyclospora infection – United States, Foodborne Diseases Active Surveillance Network (FoodNet), 1997–2009. Clin Infect Dis 54 Suppl 5: S411–S417.
56. Kessler DA, Shi PA, Avecilla ST, Shaz BH (2013) Results of lookback for Chagas disease since the inception of donor screening at New York Blood Center. Transfusion 53: 1083–1087.

Veterinary World
Open access and peer reviewed journal

Vet World. 2017 Jul; 10(7): 798–802. PMCID: PMC5553150
Published online 2017 Jul 20. doi: 10.14202/vetworld.2017.798-802

Identification of *Toxocara* spp. eggs in dog hair and associated risk factors

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Received 2017 Mar 6; Accepted 2017 Jun 12.

Abstract

Go to:

Aim:

The aim of the study was to identify the presence of eggs of *Toxocara*spp. in dog hair and to identify any risk factors associated with this.

Materials and Methods:

A total of 96 dogs were sampled collecting hair from the head, perianal and hindquarters. Epidemiologic data from each animal were recorded to identify risk factors. The samples of hair were washed with solutions of distilled water, phosphate-buffered saline and Tween 20 detergent. Microscopic analysis was subsequently performed for the identification of eggs.

Results:

Out of the total dogs, 41.7% were positive for the presence of parasite egg in their hair. *Toxocara* eggs were found in hair from the head (14.5%), tail (20.8%), and limbs (10.4%). Dogs, younger than 12 months old, showed higher values (4.7%) of egg presence in the perianal area ($p<0.05$). The principal risk factors for the presence of eggs in hair were not deworming (odds ratio [OR]=3.60, $p<0.004$) and not brushing (OR=2.26, $p<0.12$).

Conclusion:

These results show that in the state of Mexico there is a high percentage of dogs contaminated with *Toxocara* spp. eggs in their hair. This should be seriously considered due to the potential problems of toxocariasis and the risk to public health.

Keywords: dog hair, public health, risk factors, *Toxocara* eggs

Introduction

Go to:

Toxocara canis is a nematode parasite commonly found in the intestines of dogs [1]. It can be excreted as eggs in feces, and under appropriate conditions, they become infective in the environment during a period of 2-5 weeks [2]. Moreover, the parasite has the potential to invade other paratenic hosts such as humans [3]. The dog is the most common companion animal of humans, and this close contact exposes humans to possible zoonotic diseases like toxocariasis, which can cause mild symptoms or severe manifestations (slight fever, recurrent vomiting, headache, and abdominal pain) and in rare cases can sometimes lead to death [4,5]. Infection by *Toxocara* spp. is initiated through the ingestion of embryonated eggs and once the larvae in the eggs hatch they can migrate to different organs, causing syndromes such as visceral larva migrans, characterized by hepatic, pulmonary compromise, anemia, and eosinophilia; ocular larva migrans, in which the effects of pathological toxocariasis on the host are restricted to the eye and optic nerve, causing a significant decreased visual acuity and even total loss of the same [2,5].

A number of different risk factors for toxocariasis have been described. Among these are bad hygiene habits, ingestion of undercooked meat, food preparation and pica and specifically geophagia [6-9]. An important factor that has not been fully addressed is infection through human contact with dog hair [10]. Amaral *et al.* [6], however, have described how dog hair contaminated with *T. canis* at different stages of development is a source of infection and furthermore, higher densities of *Toxocara* eggs than detected in the soil have also been reported in dog hair [11].

Most studies of risk factors have been conducted in humans [1,4,8,12]; few have been conducted in dogs. Moreover, studies of risk factors for the presence of *Toxocara* eggs in dog hair have not been conducted in México. Considering this, the objective of this study was to evaluate the presence of *Toxocara* spp. eggs in dog hair and to evaluate risk factors for the presence of the eggs.

Materials and Methods

Go to:

Ethical approval

With the consent of the pet owners, 96 dogs were sampled in the Southeast region of the State of Mexico from March 2013 to October 2013. This non-invasive collection method did not present any threat to these dogs. The sampling procedure required no specific permissions for the Southeast region of the State of Mexico.

Sample collection and questionnaires

From each of the 96 dogs, samples were collected by trichotomy, with three samples of hair from different anatomical regions: Head, perianal area, and hindquarters. This resulted in a total of 288 hair samples being obtained which could be used to identify the presence of eggs of *Toxocara* spp. The samples were kept in a polyethylene bag with a zipper, labeled with the corresponding data (dog

number and anatomical region from which the sample was collected) and kept frozen until analyses. A survey was completed by each of the dog owners, including questions related to epidemiological history and risk factors: Dog data (height, age, and hair length), health conditions, health habits (worming, flea presence, frequency of bathing, and hair cut service), and living conditions of the animals (stray habits, living with other dogs, wearing clothes, brushing, and type of floor where they live).

Egg recovery technique

Samples were processed using the modified method of Overgaauw *et al.* [13]. They were weighed using an analytical scale (Velab™ model ES.1000H) and those samples weighing <2.5 mg were excluded. The samples were next washed with vigorous shaking in the presence of 0.2 ml of Tween 20 (label CIVEQ) and 40 ml of distilled water (JT. Baker®). After 10 min, the floating hair was transferred to another tube for a second wash with 40 ml of phosphate-buffered saline. After 10 min, the hair was discarded. The first and second washes were centrifuged at 800 ×*g* for 10 min and then the supernatant was decanted until approximately 1 ml. Once the sediments were re-suspended, these were transferred into a single tube to mix them. This was centrifuged at 800 ×*g* for 10 min and 1 ml of the supernatant retained. The re-suspended sediment was moved to an Eppendorf tube for a last centrifugation at 800 ×*g* for 10 min and approximately 100 µl of the supernatant was kept. Finally, each sample processed was observed under an optical microscope at 40× magnification to search for eggs of *Toxocara* spp.

Statistical analysis

The degree of contamination of dog hair with eggs of *Toxocara* spp. was expressed as a percentage for the anatomical region and also per gram of hair. The percentages were also compared using the Kruskal –Wallis test [14]. The information obtained in the survey (age, breed, gender, etc.) was used to identify risk factors for *Toxocara* eggs in hair by calculating the odds ratio (OR) [15].

Results

Go to:

Of the sampled dogs, 41.7% (n=40) contained eggs of *Toxocara* spp. in their hair. In total, 67 eggs (not embryonated) were recovered from dogs' hair; 19 from head, 38 from perianal region, and 10 from limbs. The perianal region showed a higher percentage of positive tests (20.8%) compared with the head (14.6%) and limbs (10.4%), however, the anatomical region was not found to be a predisposing factor in the overall population (Table-1). The mean number of eggs per gram (EPG) of hair was 1.4 EPG. Among anatomical regions, the mean number of EPG of hair was similar ($p>0.05$), with 1.23 in the head, 1.90 in the perianal region, and 1.0 in the limbs. Eggs density (per gram of hair) was not different ($p>0.05$) by gender (female 1.28 and males 1.60) or by the size of the dog (small 1.0; medium 1.45; and large 1.5). When the dogs were grouped by age (Table-2), younger dogs showed a higher density of *Toxocara* eggs in their perianal region ($p<0.05$).

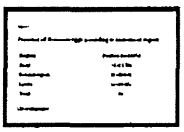

Table-1
Presence of *Toxocara* eggs according to anatomical region.

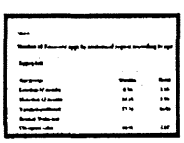

Table-2
Number of *Toxocara* eggs by anatomical region according to age group.

Regarding the possible risk factors associated with the presence of *Toxocara* spp. eggs, the size of dog, age or hair length was not significant (Table-3). Deworming of dogs was, however, a protective factor (OR=0.27) and not deworming was a considerable risk factor (OR=3.60). The presence of fleas, bathing frequency, and grooming activities were not significant risk factors (Table-3).

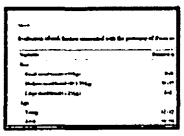

Table-3
Evaluation of risk factors associated with the presence of *Toxocara* eggs in dog hair.

In regard to the stray habits, dressing, and the type of floor, these were not risk factors. However, there was a tendency (p=0.12) to indicate that not brushing could be a risk factor (OR=2.26) or brushing a protective factor (OR=0.44) (Table-3).

Discussion

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The presence of *Toxocara* spp. in dogs represents a risk for public health due to the repercussions such as zoonotic diseases [5]. The results obtained in this study indicate that beside reports of *Toxocara* spp. eggs being recovered from feces and in public places, their presence in the hair of canines is a potential risk factor for the transmission of this parasite to other animals and humans. Furthermore, this data are supported by other studies; for example, Amaral *et al.* [6] and Öge *et al.* [16] also found that contamination of dog hair with *T. canis* at different stages of development represented a potential source of infection for humans.

Overgaauw *et al.* [13] analyzed 148 domestic dogs aged between 0.5 and 13 years of age, collecting hair samples from the lumbar region and flanks, however, they found eggs in 18 dogs (12.2%), which contrasts with our data presented here (40 positives, 41.7%). Both in the reported by Overgaauw *et al.* [13], as in this study embryonated eggs were not detected, however, Keegan *et al.* [3] reported that *Toxocara* eggs have the potential to become infective on dog which should not be ignored [17]. In another study, Keegan *et al.* [3] investigated 182 dogs, with 65 younger than a year and 117 older than a year, and hair samples were taken from the head, neck, back, and perianal region; 16 (8.8%) dogs were positive for *Toxocara* eggs. Again, the percentage of positive dogs here is lower than that in our present study.

Amaral *et al.* [6] analyzed hair from 100 stray dogs and found that 67% of dogs were positive for *T. canis* eggs. Of these, 95% were puppies, which indicates that stray dogs, and especially puppies, carry the eggs in their hair at higher densities than that reported from on the soil or in the environment [6,18]. Nevertheless, according to Overgaauw *et al.* [13] and Keegan *et al.* [3] a higher percentage of positive animals were found in adult dogs, similar to the results of this study, where 9 out of 10 geriatric dogs were positive for *Toxocara* spp. in the hair. Previous investigations of Keegan and Holland [3] and Overgaauw *et al.* [13] found similar results to those obtained in this study, indicating that age is an important factor for the presence of eggs in dog hair, associated with the *Toxocara* spp. biological cycle and the condition of the animal.

El-Tras *et al.* [19] compared the hair of 56 domestic dogs and 64 stray dogs: 6 domestic dogs (10.7%) and 17 stray dogs (26.6%) were positive for *Toxocara*. Although stray habits were not detected as a significant risk factor, numerically, hair from dogs with stray habits was more contaminated (26.0%) than hair from dogs without these habits (15.6%), which could be associated with the better care provided by the owner and a permanent home.

As with the mean number of EPG of hair reported by Overgaauw *et al.* (3.8 EPG) [13] and Keegan *et al.* (0.1 EPG) [3], the value found in this study (1.4 EPG) is considerably lower than the value obtained by Roddie *et al.* [11] (584 EPG), which is higher than that reported for soil. These differences may be explained by the fact that Roddie *et al.* [11] only used samples from stray dogs where the lack of attention and hygiene likely influenced in the high number of eggs in hair compared with the number found in animals with an owner. It is also possible that there is a relationship between the degree of contamination in the soil and in the hair. In places where the soil has a density of 0.0016-1.1 EPG [20-22], low concentrations of eggs in the hair were found, as in this study. This is important because soil has been considered as the principal source of infestation of *Toxocara* spp. to humans [23].

Conclusions

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A significant proportion of the dogs sampled in the Southeast region in the State of Mexico was contaminated with eggs of *Toxocara* spp. in their hair. The main risk factor for egg contamination was a lack of deworming, and the foremost protective measure against egg contamination was performing deworming. Although other factors were not found to be statistically significant risk factors, brushing dogs, avoiding stray habits, and grooming dogs at least every 4 months may contribute to the reduction of further contamination and infections.

Authors' Contributions

Go to:

TOR and CR performed the experiments and prepared manuscript. LGB, GS, and RH interpreted the data and participated in draft and revision of the manuscript. All authors read and approved the final manuscript.

Acknowledgments

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The authors are thankful to the staff of the Hospital of Small Animals of University Center UAEM Amecameca, for their help and providing samples under the study. The authors funded this study.

Competing Interests

Go to:

The authors declare that they have no competing interests.

References

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1. Nava C.N, Romero N.C, Bautista G.L, Hernández G.P, Heredia C.R. Presence of anti-*Toxocara canis* antibodies and risk factors in children from the Amecameca and Chalco regions of México. BMC Pediatr. 2015;15:65. [PMC free article] [PubMed]

2. Despommier D. Toxocariasis: Clinical aspects, epidemiology, medical ecology, and molecular aspects. Clin. Microbiol. Rev. 2003;16(2):265–272. [PMC free article] [PubMed]

3. Keegan J.D, Holland C.V. Contamination of the hair of owned dogs with the eggs of *Toxocara* spp. Vet. Parasitol. 2010;173(1-2):161–164. [PubMed]

4. Cong W, Meng Q, You H, Zhou N, Dong X, Dong W, Wang X, Qian A, Zhu X. Seroprevalence and risk factors of *Toxocara* infection among children in Shandong and Jilin provinces, China. Acta Trop. 2015;152:215–219. [PubMed]

5. Macpherson C.N. The epidemiology and public health importance of toxocariasis: A zoonosis of global importance. Int. J. Parasitol. 2013;43(12-13):999–1008. [PubMed]

6. Amaral H.L, Rassier G.L, Pepe M.S, Gallina T, Villeta M.M, Nobre M.O, Scaini C.J, Berne M.E. Presence of *Toxocara canis* eggs on the hair of dogs: A risk factor for visceral larva migrans. Vet. Parasitol. 2010;174(1-2):115–118. [PubMed]

7. Romero N.C, Mendoza M.G, Yañez A.S, Ponce M.M, Bustamante M, Ramírez D.N. Prevalence and risk factors associated with *Toxocara canis* infection in children. Sci. World J. 2013;2013:4. Article ID:572089. [PMC free article] [PubMed]

8. Negri E.C, Alvares S.V, Rubinski-Elefant G, Giuffrida R. Anti-*Toxocara* spp. antibodies in an adult healthy population: Serosurvey and risk factors in Southeast Brazil. Asian Pac. J. Trop. Biomed. 2013;3 (3):211–216. [PMC free article] [PubMed]

9. Cassenote A.J, Abreu L.A, Pinto N.J, Rubinski-Elefant G. Seroprevalence and modifiable risk factors for *Toxocara* spp. in Brazilian school children. PLoS Negl. Trop. Dis. 2014;8(5):e2830. [PMC free article] [PubMed]

10. Aydenizöz Ö.M, Yagci B, Erat S. The investigation of *Toxocara canis* eggs in coats of different dog breed as a potential transmissions route in human toxocariosis. Vet. Parasitol. 2008;152(1-2):94–100. [PubMed]

11. Roddie G, Stafford P, Holland C.V, Wolfe A. Contamination of dog hair with eggs of *Toxocara canis*. Vet. Parasitol. 2008;152(1-2):85–93. [PubMed]

12. Mendonça L.R, Figuereido C.A, Esquivel R, Fiaccone R.L, Pontes-de-Carvalho L, Cooper P, Barreto M.L, Alcantara N.M. Seroprevalence and risk factors for *Toxocara* infection in children from an urban large setting in Northeast Brazil. Acta Trop. 2013;128:90–95. [PubMed]

13. Overgaauw P.A, van Zutphen L, Hoek D, Yaya F.O, Roelfsema J, Pinelli E, van Knapen F, Kortbeek L.M. Zoonotic parasites in fecal samples and fur from dogs and cats in the Netherlands. Vet. Parasitol. 2009;163(1-2):1–8. [PubMed]

14. Hollander M, Wolfe D.A, Chicken E. Nonparametric Statistical Methods. 3rd ed. New York: John Wiley & Sons; 2013.

15. Merril R.M. Statistical Methods in Epidemiologic Research. Sudbury, MA: Jones & Bartlett Publishers; 2015.

16. Öge S, Öge H, Gönenç B, Özbakiş G, Yildiz C. Presence of *Toxocara* eggs on the hair of dogs and cats. Ankara Üniv. Vet. Fak. 2013;60:171–176.

17. Keegan J.D, Holland C.V. A comparison of *Toxocara canis* embryonation under controlled conditions in soil and hair. J. Helminthol. 2013;87(1):78–84. [PubMed]

18. Wolf A, Wright I.P. Human toxocariasis and direct contact with dogs. Vet. Rec. 2003;152(14):419 –422. [PubMed]

19. El-Tras W.F, Holt H.R, Tayel A.A. Risk of *Toxocara canis* eggs in stray and domestic dog hair in Egypt. Vet. Parasitol. 2011;178(3-4):319–323. [PubMed]

20. Jarosz W. Soil contamination with *Toxocara* spp. eggs in the Elblag area. Wiad. Parazytol. 2001;47 (1):143–149. [PubMed]

21. Ruiz de Ybáñez M.R, Garijo M, Goyena M, Alonso F.D. Improved methods for recovering eggs of *Toxocara canis* from soil. J. Helminthol. 2000;74(4):349–353. [PubMed]

22. Mizgajska H. Soil contamination with *Toxocara* spp. eggs in the Krakow area and two nearby villages. Wiad. Parazytol. 2000;46(1):105–110. [PubMed]

23. Maraghi S, Mazhab J.K, Mahmoud S.S, Mahmoud L.S, Mohammad Z. Study on the contamination of Abadan public parks soil with *Toxocara* spp. Eggs. J. Environ. Health Sci. Eng. 2014;12:86. [PMC free article] [PubMed]

Rev. Inst. Med. trop. S. Paulo
47(4):223-226, July-August, 2005

ENVIRONMENTAL CONTAMINATION BY *Toxocara* sp. EGGS IN RIBEIRÃO PRETO, SÃO PAULO STATE, BRAZIL

Divani Maria CAPUANO(1) & Gutemberg de Melo ROCHA(2)

SUMMARY

Toxocariasis is a zoonosis mainly caused by *Toxocara canis*, an intestinal nematode of dogs. Man acquires the infection through accidental ingestion of viable eggs, and the toxocariasis clinical manifestations may vary from an asymptomatic infection up to the Visceral Larva *Migrans* syndrome. Seventy eight public squares of Ribeirão Preto, São Paulo, Brazil, including Bonfim Paulista district were visited aiming to evaluate the soil contamination by *Toxocara* eggs. The squares were divided in five different areas corresponding to the Sanitary Districts of the city. From May to December 2003, soil samples weighting about 250 g each were collected from five distinct sites of each public square. The laboratorial analysis was done by centrifugal-flotation techniques in magnesium sulphate solutions with 5% of potassium iodide (d = 1.33) and zinc sulphate (d = 1.20), and by the sedimentation-flotation in conic chalices with zinc sulphate (d = 1.20). *Toxocara* sp. eggs were found on 16 (20.5%) squares, with the lowest prevalence (12%) at the central area. From these results, it is expected that the legal authority will adopt protection measures for the city public areas, reducing thus the contamination risk by *Toxocara* sp. eggs.

KEYWORDS: *Toxocara* sp.; Soil contamination; Zoonosis.

INTRODUCTION

Human toxocariasis, a zoonosis caused by intestinal nematodes of dogs and cats, is an important public health issue in developed and developing countries[21,22]. Human infection occurs by the accidental ingestion of infective eggs, usually from *Toxocara canis*, present in the soil, vegetables or other contaminated surfaces. Children are the most susceptible group, due to contact with the soil while playing. The clinical manifestations of toxocariasis may vary from an asymptomatic infection till the most severe one, the Visceral Larva *Migrans* (VLM)[16,21,22], caused by the inflammatory response to the larvae migration through different vital organs and tissues of the body, including the Central Nervous System (CNS). Ocular Larva *Migrans* (OLM) can lead to partial or total loss of vision[21].

It is assumed that VML cases are underestimated because the clinical signs are frequently nonspecific. Nevertheless, serological assays performed in several Brazilian municipalities and in different population groups have demonstrated that this zoonosis is widespread, with prevalence from 3 to 39%[1,3,4,5,9,10,18]. Several studies performed in Brazil[2,7,11,12,13,20] and in other countries[6,14,16,17,19,23,24,25], have evidenced soil contamination of public areas by *Toxocara* sp. eggs, varying from 5.71 to 92%, thus calling attention to the risk of human contamination.

The present work aimed to evaluate the soil contamination of public areas in the city of Ribeirão Preto, by *Toxocara* sp. eggs.

MATERIAL AND METHODS

Sample choice: There are 207 public squares in the city of Ribeirão Preto, including Bonfim Paulista district. According to the city Parks and Gardens Sector, 160 of them are partly or fully urbanized. The study sample was made of 78 squares, 67 of which where randomly chosen and 11 included in the research for having children's playground. The sample size was calculated, considering a confidence level of 95%, normal distribution, prevalence of 25% and worst acceptable of 7.5%. The distribution of the squares corresponded to the areas comprised by the five Health Districts of the city: southwest, northwest, north, southeast and central, with boundaries established by the Municipal Health Secretariat.

Period and collection of the samples: From May to December, 2003, soil samples from five equidistant points (four at the sides and one central) were collected from each public square, weekly and in the morning, and stocked in brand new plastic bags. After eliminating the soil superficial dirt, about 250 g of soil were removed down to 5 cm deep. As a precaution, a minimum distance of two meters was established for the soil collection, whenever feces were visible.

Processing of the soil samples: The samples were kept under refrigeration and processed within a maximum period of 24 hours from the collection. After careful homogenization, 30 g were taken from each soil sample, divided into three aliquots of 10 g each. Two aliquots

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were placed into 50 mL tubes, washed with a 1.0% Tween 80 solution, and left to rest for 20 minutes. After that time, the sediments were resuspended, and the tubes were filled up with saturated magnesium sulphate solution, added with 5% potassium iodide (d = 1.33) and zinc sulphate (d = 1.20). After centrifugation, the supernatant material of each tube was removed onto microscopic slides, covered by coverslips and examined for *Toxocara* eggs. The remaining 10 g aliquot was placed into a sedimentation chalice, washed with a 0.5% solution of sodium hypochloride, and after 10 minute rest, the sediment was resuspended in a zinc sulphate saturated solution (d = 1.20). After 30 minutes, surface samples were analyzed under light microscope.

Statistical analysis: Student's "t" test for ratios was used to evaluate the differences in square contamination prevalence between city areas, using a confidence interval of 95%. The analysis of the performance of the diagnostic techniques employed was done using the Chi-square test.

RESULTS

Eggs of *Toxocara* sp. were found in 16 (20.5%) of the 78 public squares researched, by any of the laboratorial technique. Table 1 shows the distribution of contamination prevalence on public squares, according to the different city areas.

Among the soil samples analyzed individually, the number of *Toxocara* sp. eggs recovered varied from one to a maximum of three. Regarding the evolutionary stage of the eggs, they were embryonated in five (31.2%) of the contaminated public squares, being either in the morula (60%) or in the larva phase (30%). The presence of strayed dogs was observed in 22 (28%) squares, at the moment of the research.

Table 1
Distribution of public squares contaminated by *Toxocara* sp. eggs, according to the geographic areas of Ribeirão Preto city, São Paulo, Brazil

	Public squares				
	Existent	Researched	Contaminated		
Area	N	N	N	%	(CI = 95%)
Southwest	13	07	02	28.5	29.5 (0 - 58.0)
Northwest	17	10	03	30.0	25.1 (4.9 - 55.1)
North	22	10	03	30.0	25.1 (4.9 - 55.1)
Southeast	53	26	05	19.2	13.3 (5.9 - 32.5)
Central	55	25	03	12.0	11.2 (0.8 - 23.2)
Total	160	78	16	20.5	

Table 2 shows the presence of *Toxocara* sp. eggs at the 16 public squares investigated, according to the diagnostic technique used.

DISCUSSION

The positivity of 20.5% observed in this study was similar to those reported by COSTA-CRUZ *et al.*[12] in Uberlândia, MG (23.07%) and by ALCÂNTARA *et al.*[2] in Salvador, BA (24.8%), and a slightly higher than that obtained by SANTARÉM *et al.*[20] in Botucatu, SP (17.5%). However, it was lower than those reported by CHIEFFI & MULLER[7]

Table 2
Presence of *Toxocara* sp. eggs in the soil of 16 public squares in the city of Ribeirão Preto, SP, Brazil, according to the diagnostic technique used

		Diagnostic technique		
		Centrifugation		Flotation
Area	Square number*	$MgSO_4$	$ZnSO_4$	$ZnSO_4$
Southwest	135	+	+	(-)
	205	+	(-)	+
	1	+	+	+
Northwest	10	(-)	+	(-)
	67	+	(-)	(-)
	25	+	+	+
North	108	(-)	+	(-)
	149	+	+	+
	3	+	(-)	(-)
	103	+	+	(-)
Southeast	122	+	(-)	(-)
	158	+	+	+
	183	+	+	+
	56	+	+	+
Central	61	+	(-)	(-)
	173	+	(-)	(-)
Total	Positive	14 (87.5%)	10 (62.5%)	07 (43.7%)
	Negative	02 (12.5%)	06 (37.5%)	09 (56.3%)

+ : presence (-): absence; * Source: Cadastre of the Public Parks Sector, Municipality of Ribeirão Preto.

in Londrina, PR (60%), COELHO *et al.*[11] in Sorocaba, SP (53.3%) and FERREIRA *et al.*[13], in Rio de Janeiro, RJ (41.6%). Several factors may have contributed to this inconsistency, such as climatic and environmental conditions, texture of the soil analyzed, diagnostic methodology employed, presence of dogs, etc. In our study it was possible to observe the presence of different environmental conditions among the squares, because we researched not only squares with plenty of vegetation, favoring shadowing and soil humidity preservation, but also squares with poor vegetation and a dry soil, due to prolonged exposure to the sun, which causes quick disintegration of the *Toxocara* eggs. Another factor observed for keeping the soil humidity in some squares was the presence of a gardener responsible for its maintenance. The presence of strayed dogs in 28% of the squares may have contributed to the soil contamination, not only by *Toxocara canis*, but by several other parasites with zoonotic potential, as these dogs had not been dewormed.

The results obtained in this study allowed us to draw the profile of soil contamination distribution among the different city areas. The lowest prevalence in the central area (12%), represented by the city center, neighboring regions and also Bonfim Paulista district, is probably related to the fact that its inhabitants have a better socioeconomic and cultural level, thus facilitating veterinary assistance and routine anthelmintic treatments of the dogs. In addition, a lot of them live in buildings, where dogs are not allowed.

Although this was a study performed mostly along the dry season (May to September), the fact of embryonated eggs have been found in

31.2% of the contaminated squares, indicates that there were favorable environmental conditions to their development, representing thus a public health risk. The presence of viable eggs was also observed by several authors in studies performed in Japan[23], Peru[15], Mexico[24] and Cuba[19]. In Brazil, CHIEFFI & MULLER[8] analyzing soil samples in the city of Londrina for 15 months, found *Toxocara* sp. eggs every month, but the presence of viable eggs was only observed from May to June and from September to December, leading to the assumption that the rain season did not have a big influence in the eggs viability. Regarding the diagnostic techniques employed in this study, the flotation method in magnesium sulphate with 5% of potassium iodide proved to be the most efficient in the recovery of *Toxocara* eggs, detecting the presence of eggs in 87.5% of the contaminated squares ($\chi^2 = 6.740$; $p = 0.0344$)

This study represents the first written report on the contamination of *Toxocara* eggs in public areas of the city of Ribeirão Preto. We hope that on the basis of these data prevention and control measures will be taken by the local health authorities, such as making citizens aware of the importance of preventing dog defecation in public areas, controlling strayed dogs and promoting laws to protect public areas, mainly children's playgrounds.

RESUMO

Contaminação ambiental por ovos de *Toxocara* sp. no município de Ribeirão Preto, Estado de São Paulo, Brasil

A toxocaríase é uma zoonose causada principalmente pelo *Toxocara canis*, nematóide intestinal de cães. O homem adquire a infecção através da ingestão acidental de ovos viáveis, sendo que as manifestações clínicas da toxocaríase podem variar desde uma infecção assintomática à síndrome da Larva *Migrans* Visceral. Com o objetivo de avaliar a contaminação do solo por ovos de *Toxocara* sp, foram visitadas 78 praças públicas de Ribeirão Preto, incluindo o distrito de Bonfim Paulista. As praças foram distribuídas em cinco áreas diferentes correspondentes as mesmas dos Distritos Sanitários do município. Entre maio a dezembro de 2003 foram coletadas de cinco pontos de cada praça amostras de solo de aproximadamente 250 gramas. A análise laboratorial foi realizada pelas técnicas da centrífugo-flutuação em soluções de sulfato de magnésio com 5% de iodeto de potássio (d = 1.33) e de sulfato de zinco (d = 1.20) e da flotação-sedimentação em cálice cônico com sulfato de zinco (d = 1.20). Foram encontrados ovos de *Toxocara* sp. em 16 (20,5%) praças, sendo que a região central apresentou a mais baixa prevalência (12,0%). Espera-se que a partir destes resultados as autoridades competentes adotem medidas de proteção das áreas públicas do município, reduzindo o risco da contaminação por ovos de *Toxocara* sp.

ACKNOWLEDGEMENTS

To Dr. Amaury Lelis Dal Fabbro, from the Department of Social Medicine in the Faculty of Medicine of Ribeirão Preto, for the statistical help; to Drs. Pedro Paulo Chieffi and Suzanna A. Zevallos Lescano, from the Institute of Tropical Medicine of University of São Paulo, for the methological orientation and to Ms Eloísa Fonseca Del Tedesco, from the Adolfo Lutz Institute of Ribeirão Preto, for the support given to this study.

REFERENCES

1. AGUIAR-SANTOS, A.M.; ANDRADE, L.D.; MEDEIROS, Z. *et al.* - Human toxocariasis: frequency of anti-*Toxocara* antibodies in children and adolescents from an outpatient clinic for lymphatic filariasis in Recife, Northeast Brazil. Rev. Inst. Med. trop. S. Paulo, 46: 81-85, 2004.

2. ALCÂNTARA, N.; BAVIA, E.; SILVÃO, R.M. & CARVALHO, E. - Environmental contamination by *Toxocara* sp eggs in public areas of Salvador, Bahia State, Brazil. Rev. Soc. bras. Med. trop., 22: 187-190, 1989.

3. ALDERETE, J.M.S.; JACOB, C.M.A.; PASTORINO, A.C. *et al.* - Prevalence of *Toxocara* infection in schoolchildren from the Butantã region, São Paulo, Brazil. Mem. Inst. Oswaldo Cruz, 98: 593-597, 2003.

4. ANAMURA FILHO, F.; CHIEFFI, P.P.; CORREA, C.R.S. *et al.* - Human toxocariasis: incidence among residents in the outskirts of Campinas, State of São Paulo, Brazil. Rev. Inst. Med. trop. S. Paulo, 45: 293-294, 2003.

5. CAMPOS Jr., D.C.; ELEFANT, G.R.; MELO E SILVA, E.O. *et al.* - Freqüência de soropositividade para antígenos de *Toxocara canis* em crianças de classes sociais diferentes. Rev. Soc. bras. Med. trop., 36: 509-513, 2003.

6. CANESE, A.; DOMINGUEZ, R.; OTTO, C.; OCAMPOS, C. & MENDONÇA, E. - Huevos infectivos de *Toxocara*, en arenas de plazas y parques de Asunción, Paraguay. Rev. chil. Pediat., 74: 611-616, 2003.

7. CHIEFFI, P.P. & MÜLLER, E.E. - Prevalência de parasitismo por *Toxocara canis* em cães e presença de ovos de *Toxocara* sp. no solo de localidades públicas da zona urbana do município de Londrina, Estado do Paraná, Brasil. Rev. Saúde públ. (S. Paulo), 10: 367-372, 1976.

8. CHIEFFI, P.P. & MÜLLER, E.E. - Estudo da variação mensal na contaminação do solo por ovos de *Toxocara* sp. (*Nematoda, Ascaroidea*), na zona urbana do município de Londrina, Estado do Paraná, Brasil. Rev. Inst. Adolfo Lutz, 38: 13-16, 1978.

9. CHIEFFI, P.P; UEDA, M.; CAMARGO, E.D. *et al.* - Contato domiciliar e profissional com cães como fatores de risco para infecção humana por larvas de *Toxocara*. Rev. Inst. Med. trop. S. Paulo, 30: 379-382, 1988.

10. CHIEFFI, P.P; UEDA, M.; CAMARGO, E.D. *et al.* - Visceral larva migrans: a seroepidemiological survey in five municipalities of São Paulo State, Brazil. Rev. Inst. Med. trop. S. Paulo, 32: 204-210, 1990.

11. COELHO, L.M.P.S.; DINI, C.Y.; MILMAN, M.H.S.A. & OLIVEIRA, S.M. - *Toxocara* spp. eggs in public squares of Sorocaba, São Paulo State, Brazil. Rev. Inst. Med. trop. S. Paulo, 43: 189-191, 2001.

12. COSTA-CRUZ, J.M.; NUNES, R.S. & BUSO, A. G. - Presença de ovos de *Toxocara* spp em praças públicas da cidade de Uberlândia, Minas Gerais, Brasil. Rev. Inst. Med. trop. S. Paulo, 36: 39-42, 1994.

13. FERREIRA, L.F.; OLIVEIRA, E.L. & CAMILO-COURA, L. - Sobre a presença de ovos de *Toxocara*, em praças da cidade do Rio de Janeiro. Rev. Soc. bras. Med. trop., 10: 51-54, 1976.

14. FONROUGE, R.; GUARDIS, M.V.; RADMAN, N.E. & ARCHELLI, S.M. - Contaminación de suelos con huevos de *Toxocara* sp. en plazas y parques públicos de la ciudad de La Plata, Buenos Aires, Argentina. Bol. chil. Parasit., 55: 83-85. 2000.

15. LESCANO, S.A.; CHIEFFI, P.P.; PERES, B.A. *et al.* - Soil contamination and human infection by *Toxocara* sp. in the urban area of Lima, Peru. Mem. Inst. Oswaldo Cruz, 93: 733-734, 1998.

16. MAGNAVAL, J.F.; GALINDO, V.; GLICKMAN, L.T. & CLANET, M. - Human *Toxocara* infection of the central nervous system and neurological disorders: a case-control study. Parasitology, 115: 537-543, 1997.

17. MIZGAJSKA, H. - Eggs of *Toxocara* spp. in the environment and their public health implications. **J. Helminth., 75:** 147-151, 2001.

18. MOREIRA-SILVA, S.F.; LEÃO, M.E.; MENDONÇA, H.F.S. & PEREIRA, F.E.L. - Prevalence of anti-*Toxocara* antibodies in a random sample of inpatients at a children's hospital in Vitória, Espírito Santo, Brazil. **Rev. Inst. Med. trop. S. Paulo, 40:** 259-261, 1998.

19. PÉREZ, R.M.L.; ARRIETA, D.C.; ZAMORA, E.M.R.; ROCHE, R.G. & DÍAZ, V.P. - *Toxocara* sp. en parques y zonas públicas de Ciudad de la Habana, 1995. **Rev. cuba. Hig. Epidem., 38:** 112-116, 2000.

20. SANTARÉM, V.A.; SARTOR, I.F. & BERGAMO, F.M.M. - Contaminação por ovos de *Toxocara* spp. de parques e praças públicas de Botucatu, São Paulo, Brasil. **Rev. Soc. bras. Med. trop., 31:** 529-532, 1998.

21. SCHANTZ, P.M. - *Toxocara* larva migrans now. **Amer. J. trop. Med. Hyg., 41(3) (suppl.):** 21-34, 1989.

22. TAN, J.S. - Human zoonotic infections transmitted by dogs and cats. **Arch. Intern. Med., 157:** 1933-1943, 1997.

23. UGA, S. - Prevalence of *Toxocara* eggs and number of faecal deposits from dogs and cats in sandpits of public parks in Japan. **J. Helminth., 67:** 78-82, 1993.

24. VÁSQUEZ-TSUJI, O.; RUIZ-HERNÁNDEZ, A. ; MARTÍNEZ-BARBABOSA, I. *et al.* - Contaminación de suelos por huevos de *Toxocara* sp. en parques públicos y jardines de casas-habitación de la ciudad de México. **Bol. chil. Parasit., 51:** 54-58, 1996.

25. WIWANITKIT, V. & WAENLOR, W. - The frequency rate of *Toxocara* species contamination in soil samples from public yards in a urban area "Payathai", Bangkok, Thailand. **Rev. Inst. Med. trop. S. Paulo, 46:** 113-114, 2004.

Received: 26 January 2005
Accepted: 2 May 2005

OPEN ACCESS Freely available online

PLOS | NEGLECTED TROPICAL DISEASES

Toxocariasis in North America: A Systematic Review

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Abstract

Toxocariasis is an important neglected tropical disease that can manifest as visceral or ocular larva migrans, or covert toxocariasis. All three forms pose a public health problem and cause significant morbidity in areas of high prevalence. To determine the burden of toxocariasis in North America, we conducted a systematic review of the literature following PRISMA guidelines. We found 18 articles with original prevalence, incidence, or case data for toxocariasis. Prevalence estimates ranged from 0.6% in a Canadian Inuit community to 30.8% in Mexican children with asthma. Commonly cited risk factors included: African-American race, poverty, male sex, and pet ownership or environmental contamination by animal feces. Increased prevalence of *Toxocara spp.* infection was linked in a group of case control studies conducted in Mexico to several high risk groups including waste pickers, asthmatic children, and inpatient psychiatry patients. Further research is needed to determine the true current burden of toxocariasis in North America; however the prevalence estimates gathered in this review suggest that the burden of disease is significant.

Citation: Lee RM, Moore LB, Bottazzi ME, Hotez PJ (2014) Toxocariasis in North America: A Systematic Review. PLoS Negl Trop Dis 8(8): e3116. doi:10.1371/journal.pntd.0003116

Editor: Robin B. Gasser, University of Melbourne, Australia

Received May 13, 2014; **Accepted** July 14, 2014; **Published** August 28, 2014

Data Availability: The authors confirm that all data underlying the findings are fully available without restriction. All relevant data are within the paper and its Supporting Information files.

Funding: The authors received no specific funding for this work.

Competing Interests: The authors have declared that no competing interests exist.

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Introduction

Toxocariasis is an important neglected tropical disease caused by the roundworms *Toxocara canis* and *Toxocara cati*, which are transmitted when eggs in canine or feline feces, respectively, are ingested by humans [1]. Larvae penetrate the walls of the intestine then travel through the circulatory system to various organs throughout the body. *Toxocara spp.* do not multiply within the human host, but exist in host tissues in a state of arrested development [2,3]. When larvae die, the inflammatory reaction produced by the body causes the symptoms of toxocariasis [1]. Toxocariasis can manifest in three ways; visceral larva migrans results from larvae migrating to major organs including the brain, lungs, and liver, causing a variety of signs and symptoms (usually in young children) including eosinophilia, abdominal pain, headache, cognitive and behavioral disturbances, pneumonitis, hepatitis with hepatomegaly, while ocular toxocariasis more commonly occurs in older children and results from larvae migrating to the eye and can cause irreversible vision loss due to scarring of the retina or retinal detachment. Covert toxocariasis, the most difficult form to diagnose, is believed to be caused by chronic exposure and can manifest as eosinophilia with cognitive disturbances, or nonspecific symptoms that resemble asthma, i.e., coughing, and wheezing [1,2,4,5]. Both visceral larva migrans and covert toxocariasis, and often ocular larva migrans, are associated with enzyme immunoassay antibody titers that can remain elevated for months or years [6].

Toxocariasis causes significant morbidity and loss of productivity and poses an important, yet largely unaddressed public health problem in areas of high prevalence. Most notably, infection with *Toxocara spp.* has been associated with reduced cognitive function in children; Walsh and colleagues found that infected children scored significantly lower on math, reading, digit span, and block design tests than uninfected children [7]. Infected children were significantly more likely to be in a lower socioeconomic class than uninfected children, though the difference in performance persisted when socioeconomic class was controlled for, suggesting that toxocariasis, like many other neglected tropical diseases, may play a role in perpetuating poverty and contribute to health disparities in endemic areas [7]. It has been suggested that toxocariasis may even partially account for the achievement gap noted among socioeconomically disadvantaged students [8]. Medically, toxocariasis has also been proposed to be a potential cause of asthma, has been associated with seizures, and is an important cause of blindness [4,9].

The current burden of disease due to toxocaraisis in North America is largely unknown. We conducted this review in order to determine the both the prevalence of and the amount of available data measuring the burden of toxocariasis in North America, and to identify needed areas of future research.

Methods

Search strategy and selection criteria

Searches were completed in February 2014 using PubMed and were restricted to English articles, articles published in the last 10 years, and articles pertaining to humans. Search terms included

Author Summary

Toxocariasis is a parasitic worm infection that is transmitted by the ingestion of parasite eggs present in the feces of dogs and cats. After ingestion of the Toxocara eggs, the released larvae migrate through human organs, including the lungs, liver, brain, and eyes, where they cause inflammation and organ damage. The different larval migrans syndromes associated with toxocariasis are diagnosed by detecting Toxocara antibodies in sera of infected individuals. This study is a systematic review to in order assess the prevalence of toxocariasis in North America and whether selected groups are at risk for infection. The prevalence was highest among Mexican children with asthma, but also African Americans living in poverty exhibited high rates of infection. Among the major risk factors for infection were African-American ethnicity, male sex, pet ownership, and environmental contamination by animal species. Additional high risk groups in Mexico included waste pickers, asthmatic children, and inpatient psychiatry patients. Further research is needed to determine the true current burden of toxocariasis in North America; however, the prevalence estimates gathered in this review suggest that the burden of disease is significant. Thus, toxocariasis may be emerging as an important health disparity in North America

"toxocara", "toxocariasis", "visceral larva migrans", and "ocular larva migrans" and the country name: "United States", "Mexico", and "Canada". Searches were also performed using the country names and disease variations as MeSH terms. Non-English articles, dead links, and duplicate results were excluded from abstract review. Abstracts were reviewed by two reviewers and were excluded from full-text review if they were definitively about a different disease or about the relevant disease in an animal population.

Assessment and data extraction

Documents in the full-text review were classified as containing (1) original prevalence data/estimates for toxocariasis, (2) original incidence or outbreak data, (3) reports of individual cases of toxocariasis, or (4) no original prevalence, incidence, outbreak, or case report information. Citations for unoriginal prevalence, incidence, or case report data were followed up and included in the review if they were English articles published within the past 10 years. Each paper was reviewed independently by two reviewers for figures, tables, or text containing original prevalence, incidence, and case data as well as descriptions of persons or populations affected and reported risk factors for disease. Articles using the same source data but reporting prevalence estimates for different age groups were considered to contain original estimates. Discrepancies between reviewers to resolved by discussion and consensus. This review is compliant with the PRISMA checklist for systematic reviews. Full bibliography available on request.

Results

We found 18 articles with original prevalence, incidence, or case data for toxocariasis (Figure 1). Of these articles, 5 reported data in Canada, 8 reported data in the United States, and 5 reported data in Mexico. One article reported only prevalence data, 0 articles reported incidence or outbreak data, 5 articles reported only affected cases, and 12 articles reported both prevalence data and number of affected cases. Table 1 summarizes the prevalence and reported cases data for toxocariasis in North America. 63% (5/8) of articles with seroprevalence estimates for the United States tested blood samples collected during the 1988–1994 National Health and Nutrition Examination Survey (NHANES).

Data for the United States was the most robust, as many articles analyzed blood samples collected for NHANES, which collects data from a population representative of that of the United States. However, blood samples for the most recent NHANES were collected between 1988 and 1994 and thus prevalence data may not be representative of the current burden of disease 20 years later. Prevalence estimates for the United States ranged from 8.6% for ages 1–5 to 15.1% for ages 6–11, with an overall prevalence rate of 13.9% reported for all adults and children ages 6 and older [3,10]. Data for Canada focused mainly on indigenous communities living in rural areas. Prevalence estimates ranged from 0.6% to 13.4%, indicating that for some communities toxocara infection causes a significant burden; however, the overall burden of disease in Canada as a whole is still unknown [11,12]. Reported seroprevalence estimates in Mexico tended to be higher than those reported for the United States and Canada and the data available focused on comparing prevalence rates in high-risk groups, such as psychiatry patients, waste pickers, and asthmatic children to controls (Table 2) [9,13,14]. Seroprevalence rates in these high-risk groups ranged from 4.7% in inpatient psychiatric patients to 30.8% in children with asthma [9,14].

The most commonly cited risk factors for toxocariasis included male sex, canine or feline pet ownership, particularly if the animals are allowed to live primarily outdoors and eat other animals or otherwise unconventional pet food, African American race, age less than 18, low level of education, being foreign born, living in the southern United States, and playing at parks or in sandboxes where dogs and cats have defecated (Figure 2).

Twelve studies reported prevalence of *Toxocara spp.* antibodies by gender [2,3,7,10,11,12,15,16,17,18,19,20]. Eight (73%) of these studies found a significantly greater prevalence in males compared to females, while 27% of studies found no significant differences between genders. No studies found females to have significantly higher prevalence of toxocara infection than males. Three studies from Canada reported prevalence by gender; two of these studies reported no significant difference in prevalence between genders and one study reported an increased prevalence in males [11,12,17]. Seven studies from the United States reported prevalence by gender; 7 of these studies reported an increased prevalence in males and one study reported no difference between genders [2,3,7,10,15,16,19,20,21]. One study from Mexico reported prevalence by gender; this study found a significantly higher prevalence in males than females [18].

Canine and feline ownership is a somewhat controversial yet commonly cited risk factor. Infected cats and dogs pass eggs in feces, which can contaminate their environment and be ingested by humans [1]. Puppies in particular are commonly infected in utero and must be dewormed after birth [1,2]. Uninfected cats and dogs are at a high risk for becoming infected if they live outside or eat a diet of other animals or otherwise unconventional pet food [1]. These findings indicate that perhaps ownership itself is not an independent risk factor for toxocariasis, but that type of pet care can increase or decrease the risk of infection. Three studies from Canada indicated that pet ownership was not a risk factor, citing that dogs that are owned are more likely to be dewormed than feral dogs, which are more likely to eat an unconventional diet and live exclusively outside [11,12,17]. Additionally, environmental contamination by infected animals is an important risk factor; contamination rates of up to 40% have been found in urban playgrounds and children who play in public areas that are not kept clear of animal feces are at a higher risk for *Toxocara* infection [2,20,22].

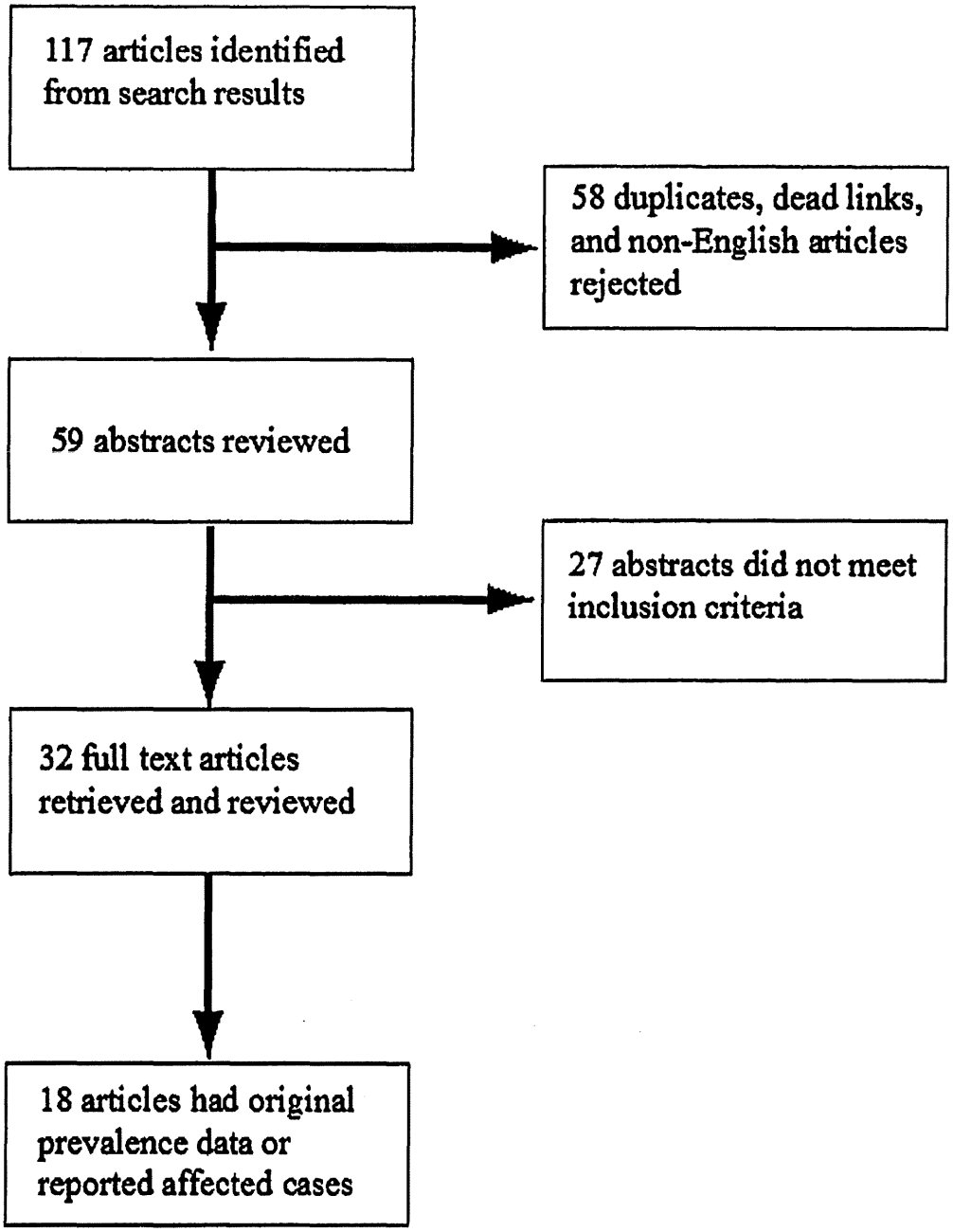

Figure 1. Systematic review process.
doi:10.1371/journal.pntd.0003116.g001

All eight studies included in this review from the United States provided prevalence estimates by race. Five of these studies cited African American race as a risk factor [3,7,10,16,19]. Jones and colleagues found the odds ratio of Non-Hispanic Blacks being infected compared to Non-Hispanic Whites to be 1.7 (95% CI 1.4–2.0), while the odds ratio for Mexican-Americans compared to white was found to be 0.6 (95% CI 0.4–0.6) [3]. The national seroprevalence for ages 6 and over was estimated to be 22.8% in African Americans, 12.6% in Mexican-Americans, and 10.6% in Whites using the 1988–1994 NHANES data [10]. This disparity in prevalence between races has interesting public health implications. Hotez et al. estimated that as many as 2.8 million African-Americans living in poverty in the United States were infected with *Toxocara spp.* making toxocariasis one of the most common infections in an underrepresented minority group [23]. The three studies that reported an increased prevalence of toxocariasis in

Table 1. Summary of *Toxocara spp.* seroprevalence estimates in published literature.

Country	Article	Year	Location	Seroprevalence (IgG)	N	Demographics
Canada						
	Campagna 2011 [26]	2007	Eastmain Village, James Bay, Northern Quebec	5.4%	6	Indiginous Cree community, volunteers ages 15 and older, 37% male
			Wemindji Village, James Bay, Northern Quebec	1.4%	2	Indiginous Cree community, volunteers ages 15 and older, 46% male
	Levesque 2007 [17]	2005	Mistissini Village, Lake Mistissini, Quebec	4.2%	2	Indiginous Cree community, active hunter/trappers and their spouses, 44% male
	Ota 2009 [27]	2008	Alberta	-	1	14 month old boy with history of retinoblastoma
	Sampasa-Kayinga 2012 [11]	2008	Chisasibi Village, James Bay, Northern Quebec	0.6% (95% CI 0.02–3.4%)	1	Indiginous Cree community, volunteers ages 18 and older, 41% male
			Waskaganish Village, James Bay, Northern Quebec	10% (95% CI 4.9–17.5%)	10	Indiginous Cree community, volunteers ages 18 and older, 42% male
	Schurer 2013 [12]	2011	Saskatchewan	13.4%	27	Indiginous Dene community, volunteers ages 4 and older, 62% male
United States						
	Congdon 2011 [16]	1988–1994	Countrywide	14.6% males, 12.6% females	2862	NHANES data, adults and children ages 6 and over
	Jones 2008 [3]	1988–1994	Countrywide	8.6%	63	NHANES data, children ages 1–5
				15.1%	240	NHANES data, children ages 6–11
	MMWR 2011 [15]	Sept 2009–Sept 2010	23 states, DC, and Puerto Rico *	-	68	Newly diagnosed ocular toxocariasis cases reported by ophthalmologists, median age 8.5 years (range 1–60 years), 56% male
	Stewart 2005 [21]	1977–1996	San Francisco, California	-	22	Ocular toxocariasis patients seen for uveitits, median age 14 years (range 1–37 years), 45.5% male
	Walsh 2011 [19]	1988–1994	Countrywide	14.2% (95% CI 12.7–15.9%)	1898	NHANES data, adults age 17–64
	Walsh 2012 [7]	1988–1994	Countrywide	-	688	NHANES data, children ages 6–16
	Won 2008 [10]	1988–1994	Countrywide	13.9% (95% CI 12.5–15.3%)	2835	NHANES data, adults and children over 6 years, 48.4% male
	Woodhall 2012 [20]	Sept 2009–Sept 2010	33 states, DC, and Puerto Rico**	-	159	Ocular toxocariasis patients reported by ophthalmologists, median age 11.5 years (range 1–66 years), 54% male
Mexico						
	Alvarado-Esquivel 2013 [13]	-	Durango City	13%	12	Waste pickers, mean age 36 years (range 14–76 years), 38% male
	Alvarado-Esquivel 2013 [14]	-	Durango City	4.7%	6	Inpatient psychiatric patients, mean age 43.6 (range 16–83), 69% e s
	Jimenez-Balderas 2012 [28]	Mexico City	16.6%	4	Ankylosing spondylitis patients, mean age 44 years, 38% male	
	Munoz-Guzm an 2010 [9]	-	Mexico City	30.80%	-	Asthmatic children from the Traumatology Service of Federico Gomez Children's Hospital, mean age 6.9 years (range 4–12 years), 65% male
	Romero Nunez 2013 [18]	Aug 2010–Sept 2010	Municipality of Ecatepec of Morelos	22.2%	26	Volunteers age 2–16 years, 48% male

*Alabama (5), Arkansas (2), California (6), Connecticut (2), District of Columbia (3), Florida (8), Georgia (9), Illinois (3), Indiana (2), Iowa (1), Louisiana (1), Maryland (2), Nevada (2), New York (3), North Carolina (1), Ohio (1), Oklahoma (1), Oregon (1), Pennsylvania (1), Puerto Rico (1), South Carolina (3), Tennessee (1), Texas (16), Virginia (2), West Virginia (1).

**Alabama (6), Arkansas (2), California (7), Colorado (3), Connecticut (3), District of Columbia (8), Florida (10), Georgia (9), Hawaii (1), Idaho (2), Illinois (13), Indiana (6), Iowa (2), Louisiana (2), Maryland (4), Massachusetts (2), Michigan (2), Minnesota (2), Missouri (1), Nevada (3), New York (14), North Carolina (2), Ohio (10), Oklahoma (5), Oregon (1), Pennsylvania (6), Puerto Rico (1), Rhode Island (1), South Carolina (3), Tennessee (3), Texas (13), Utah (1), Virginia (3), Washington (7), West Virginia (1).

doi:10.1371/journal.pntd.0003116.t001

Table 2. Case control studies of *Toxocara spp.* seroprevalence.

Article	Case Group	Case Group Seroprevalence (N)	Control Group	Control Group Seroprevalnce (N)	P value
Alvarado-Esquivel 2013 [13]	90 waste pickers aged 14–76 years, 38% male	13% (12)	90 non-waste pickers of various occupations matched by age and gender	1% (1)	<0.01
Alvarado-Esquivel 2013 [14]	128 psychiatric inpatients aged 16–83 years, 69% male	4.7% (6)	276 volunteers aged 16–91 years, 40% male	1.1% (3)	0.03
Jimenez-Balderas 2012 [28]	24 ankylosing spondylitis patients mean age 44±14 years, 38% male	16.6% (4)	77 healthy volunteers mean age 30±15 years, 40% male	2.6% (2)	0.027
Munoz-Guzman 2010 [9]	285 asthmatic children aged 4–12 years, 65% male	30.8% (88)	152 non-asthmatic children aged 4–12 years	19.7% (30)	<0.05

All case control studies identified were conducted in Mexico.

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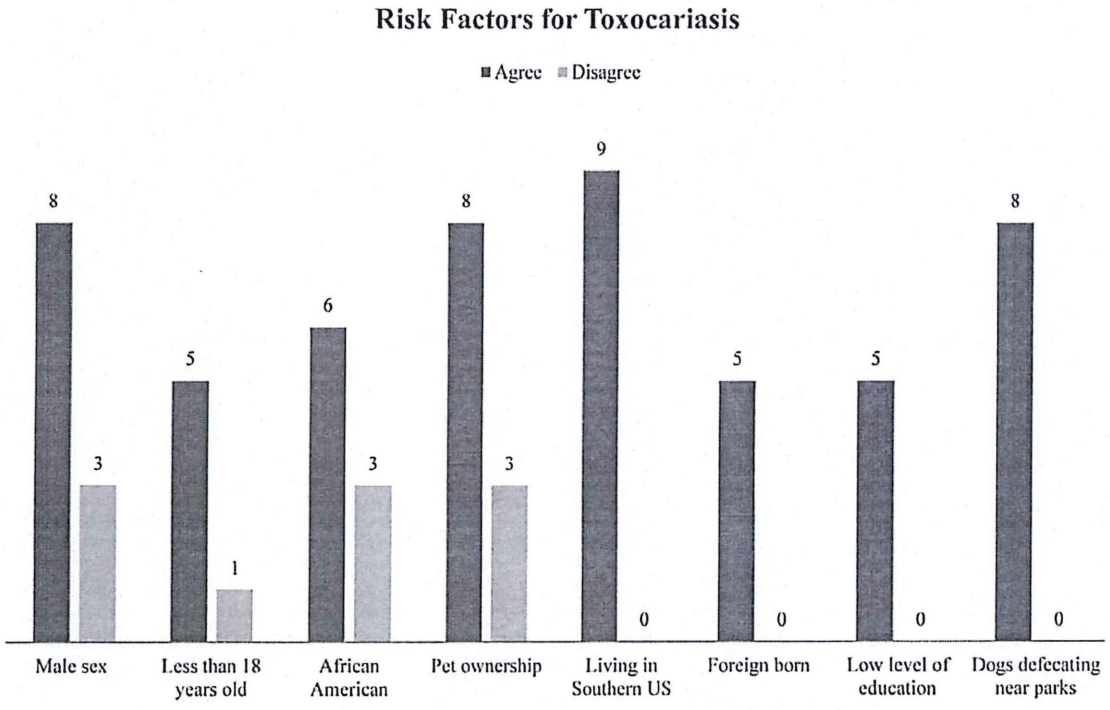

Figure 2. Articles citing risk factors for *Toxocara spp.* infection.

doi:10.1371/journal.pntd.0003116.g002

Whites compared to African-Americans were studies that involved surveying clinicians treating patients with uveitis and asking them to report cases presented with ocular toxocariasis [15,20,21]. The discrepancy between these two sets of studies may point to an issue of access to healthcare in the African-American community.

Discussion

The findings in this review indicate that the seroprevalence of human toxocariasis is high in North America, frequently exceeding 10 percent in the United States and Mexico and among some populations in Canada. Moreover, they suggest that toxocariasis may be emerging as a North American health disparity, with the highest seroprevalence found among African Americans and children in the southern U.S. and some indigenous communities in Canada, as well as populations with low education levels those living in areas where dog feces are found, indicative of environmental degradation.

The detection of antibodies against Toxocara antigens in sera is associated with past exposure to *T. canis* and *T. cati* eggs and the resulting parasite larval migrations. However, because Toxocara larvae can remain in a developmentally arrested state and live with minimal metabolic activity in mammalian tissues for years [24], seropositivity – enzyme immunoassay titers can remain positive for years [6] - may also be indicative of active infection. This finding can help explain the associations noted in case-control studies conducted in Mexico between Toxocara seropositivity and conditions such as asthma and ankylosing spondylitis, and psychiatric illness [7,11,12,24], and US NHANES data linking toxocariasis to pulmonary disease and cognitive deficits [6,17].

Limitations of this study include restrictions used in our search methodology, in particular only including articles published within the past 10 years. An additional limitation was that the studies conducted in the US mostly relied on the same NHANES data. Another was the lack of harmonization of study populations between the different North American countries. For example all of the Canadian studies were conducted in adults only.

Our overall purpose was to assess the available literature on the current burden of disease and to discern the amount of evidence available to inform current or future public health measures to control and prevent toxocariasis. Ultimately, more recent and widespread research is needed to determine the true current burden of toxocariasis in North America, but the prevalence estimates gathered during this review preliminarily indicate that the burden of disease is not insignificant. Other particularly salient points identified by this review that would benefit from further research efforts include differences in prevalence by race in the United States, especially with how these differences are confounded by or related to income and education levels, the relationship between toxocariasis and asthma in children and between toxocariasis and psychiatric diagnoses in both children and adults, and the cognitive effects of toxocara infection in childhood. Links between arthritis and toxocariasis also require further investigation.

Recently the US Centers for Disease Control and Prevention (CDC) announced an initiative to prioritize five neglected parasitic infections in the US, including toxocariasis [25]. Among the major priorities outlined by the CDC are efforts to better define risk factors, and research to elucidate the natural history of toxocariasis and its provocative associations with pulmonary and neurologic diseases [6]. There is also urgency to develop improved and more widely accessible diagnostic tests for detecting active infection, and studies to optimize current treatment regimens using anthelmintic drugs [6]. Thus, we are still in the nascent stages of understanding the full extent of human toxocariasis in the US and North America and how to best diagnose, manage and treat, and ultimately prevent this illness and emerging health disparity.

Supporting Information

Text S1 PRISMA checklist.
(DOC)

Text S2 PRISMA flowchart.
(DOC)

Text S3 Full reference list.
(XLSX)

Author Contributions

Analyzed the data: RML LBM MEB PJH. Contributed to the writing of the manuscript: PJH RML LBM. Reviewed and finalized the manuscript: RML LBM MEB PJH.

References

1. Global Health - Division of Parasitic Diseases and Malaria (2013) Parasites - Toxocariasis (also known as Roundworm Infection). Atlanta, GA: Centers for Disease Control and Prevention.
2. Clinton RM, Carabin H, Little SE (2010) Emerging zoonoses in the southern United States: toxocariasis, bovine tuberculosis and southern tick-associated rash illness. The American journal of the medical sciences 340: 187–193.
3. Jones JL, Kruszon-Moran D, Won K, Wilson M, Schantz PM (2008) Toxoplasma gondii and Toxocara spp. co-infection. The American journal of tropical medicine and hygiene 78: 35–39.
4. Barry MA, Weatherhead JE, Hotez PJ, Woc-Colburn L (2013) Childhood parasitic infections endemic to the United States. Pediatric clinics of North America 60: 471–485.
5. Hotez PJ (2008) Neglected infections of poverty in the United States of America. PLoS neglected tropical diseases 2: e256.
6. Woodhall DM, Eberhard ML, Parise ME (2014) Review Article: Neglected parasitic infections in the United States: Toxocariasis. Am J Trop Med Hyg 90: 810–813.
7. Walsh MG, Haseeb MA (2012) Reduced cognitive function in children with toxocariasis in a nationally representative sample of the United States. International journal for parasitology 42: 1159–1163.
8. Hotez PJ (2014) Neglected infections of poverty and the American brain. JAMA Psychiatry: In press.
9. Munoz-Guzman MA, del Rio-Navarro BE, Valdivia-Anda G, Alba-Hurtado F (2010) The increase in seroprevalence to Toxocara canis in asthmatic children is related to cross-reaction with Ascaris suum antigens. Allergologia et immunopathologia 38: 115–121.
10. Won KY, Kruszon-Moran D, Schantz PM, Jones JL (2008) National seroprevalence and risk factors for Zoonotic Toxocara spp. infection. The American journal of tropical medicine and hygiene 79: 552–557.
11. Sampasa-Kanyinga H, Levesque B, Anassour-Laouan-Sidi E, Cote S, Serhir B, et al. (2012) Zoonotic infections in native communities of James Bay, Canada. Vector borne and zoonotic diseases 12: 473–481.
12. Schurer JM, Ndao M, Skinner S, Irvine J, Elmore SA, et al. (2013) Parasitic zoonoses: one health surveillance in northern Saskatchewan. PLoS neglected tropical diseases 7: e2141.
13. Alvarado-Esquivel C (2013) Toxocariasis in waste pickers: a case control seroprevalence study. PloS one 8: e54897.
14. Alvarado-Esquivel C (2013) Toxocara infection in psychiatric inpatients: a case control seroprevalence study. PloS one 8: e62606.
15. (2011) Ocular toxocariasis–United States, 2009–2010. MMWR Morbidity and mortality weekly report 60: 734–736.
16. Congdon P, Lloyd P (2011) Toxocara infection in the United States: the relevance of poverty, geography and demography as risk factors, and implications for estimating county prevalence. International journal of public health 56: 15–24.
17. Levesque B, Messier V, Bonnier-Viger Y, Couillard M, Cote S, et al. (2007) Seroprevalence of zoonoses in a Cree community (Canada). Diagnostic microbiology and infectious disease 59: 283–286.
18. Romero Nunez C, Mendoza Martinez GD, Yanez Arteaga S, Ponce Macotela M, Bustamante Montes P, et al. (2013) Prevalence and risk factors associated with Toxocara canis infection in children. TheScientificWorldJournal 2013: 572089.

19. Walsh MG (2011) Toxocara infection and diminished lung function in a nationally representative sample from the United States population. International journal for parasitology 41: 243–247.
20. Woodhall D, Starr MC, Montgomery SP, Jones JL, Lum F, et al. (2012) Ocular toxocariasis: epidemiologic, anatomic, and therapeutic variations based on a survey of ophthalmic subspecialists. Ophthalmology 119: 1211–1217.
21. Stewart JM, Cubillan LD, Cunningham ET, Jr. (2005) Prevalence, clinical features, and causes of vision loss among patients with ocular toxocariasis. Retina 25: 1005–1013.
22. Hotez PJ (2007) Neglected diseases and poverty in "The Other America": the greatest health disparity in the United States? PLoS neglected tropical diseases 1: e149.
23. Hotez PJ, Wilkins PP (2009) Toxocariasis: America's most common neglected infection of poverty and a helminthiasis of global importance? PLoS neglected tropical diseases 3: e400.
24. Loukas A, Maizels RM (1998) Cloning and characterisation of a prohibitin gene from infective larvae of the parasitic nematode Toxocara canis. DNA sequence : the journal of DNA sequencing and mapping 9: 323–328.
25. Parise ME, Hotez PJ, Slutsker L (2014) Neglected parasitic infections in the United States: Needs and opportunities. Am J Trop Med Hyg 90: 783–785.
26. Campagna S, Levesque B, Anassour-Laouan-Sidi E, Cote S, Serhir B, et al. (2011) Seroprevalence of 10 zoonotic infections in 2 Canadian Cree communities. Diagnostic microbiology and infectious disease 70: 191–199.
27. Ota KV, Dimaras H, Heon E, Babyn PS, Yau YC, et al. (2009) Toxocariasis mimicking liver, lung, and spinal cord metastases from retinoblastoma. The Pediatric infectious disease journal 28: 252–254.
28. Jimenez-Balderas FJ, Camargo-Coronel A, Gargia-Jaimes J, Zonana-Nacach A, Alcantara-Anguianoi I, et al. (2012) A study on parasites in Mexican rheumatic disease patients. Journal of the Egyptian Society of Parasitology 42: 271–280.

Am. J. Trop. Med. Hyg., 79(4), 2008, pp. 552–557

National Seroprevalence and Risk Factors for Zoonotic *Toxocara* spp. Infection

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Abstract. To estimate the prevalence of *Toxocara* spp. infection in a representative sample of the United States population ≥ 6 years of age, sera from participants in the Third National Health and Nutrition Examination Survey (1988–1994) were tested for antibodies to *Toxocara*. Among the 30,930 persons selected for the survey, 82% (N = 25,733) were interviewed, and 91% (N = 23,527) of those interviewed underwent physical examination of which 87% (N = 20,395) were tested. The age adjusted *Toxocara* seroprevalence was 13.9% (95% confidence intervals [CI] 12.5, 15.3), and was higher in non-Hispanic blacks (21.2%) than non-Hispanic whites (12%) or Mexican Americans (10.7%; $P < 0.001$). Increased *Toxocara* seropositivity was associated with head of household level of education (low versus high) (odds ratio [OR]: 2.2; CI: 1.8, 2.8), poverty (OR: 1.5; CI: 1.3, 1.8), elevated blood lead concentrations (OR: 1.4; CI: 1.1, 1.9), and dog ownership (OR: 1.2; CI: 1.1, 1.4). *Toxocara* infection is widespread and associated with specific risk groups.

INTRODUCTION

Larval stages of *Toxocara canis* and *Toxocara cati*, common intestinal roundworms of dogs and cats, respectively, frequently infect humans worldwide. *Toxocara* eggs are passed unembryonated in the feces of these animals, become infectious in suitable environments, and can remain infective in the soil for many years. Human infection can result in a variety of syndromes with different clinical manifestations. Two commonly described syndromes, visceral larva migrans and ocular larva migrans, can include abdominal pain, hepatomegaly, persistent eosinophilia, visual impairment, and retinal scarring.[1] A condition known as covert toxocariasis may be the most common form of the disease and can include symptoms such as headache, cough, fever, and wheezing. Individuals with covert toxocariasis may or may not have elevated eosinophil counts.[2] However, many *Toxocara* infections remain asymptomatic and therefore remain underdiagnosed and underappreciated.

High human seroprevalence has been shown in areas with documented soil contamination, and the risk for transmission may be increased in proportion to the degree of environmental contamination.[3–5] In studies of clinical cases of *Toxocara* infections in humans, pet ownership and geophagia or pica have consistently been identified as important risk factors for zoonotic transmission of *Toxocara*.[1,6–8] Factors such as age, sex, and geographic location may also be important risk factors, and reports have shown that toxocariasis disproportionately affects socioeconomically disadvantaged populations.[9,10] Because only limited information is available on the persistence of *Toxocara* antibodies after infection, it is difficult to determine whether high human seroprevalence is indicative of persistent antibodies or re-infection.[11,12] However, because *Toxocara* infection can cause human morbidity, it is valuable to understand the level of *Toxocara* exposure among populations.

In this study, serum samples collected as part of the Third National Health and Nutrition Examination Survey (NHANES III) were tested for *Toxocara* antibodies to estimate the seroprevalence of *Toxocara* infection in the United States and to identify characteristics associated with human infection. Examining seroprevalence differences will aid in identifying groups to target for health education messages.

METHODS

The NHANES III was a nationally representative, cross-sectional survey conducted by the National Center for Health Statistics (NCHS) of the Centers for Disease Control and Prevention (CDC) between 1988 and 1994. It was designed to obtain health statistics of the United States population through household interviews, standardized physical exams, and collection of blood samples in mobile examination centers. The NHANES III consisted of a stratified, multistage, probability cluster sample of 33,994 people older than two months of age, representative of the civilian, non-institutionalized general U.S. population. People between two months and five years of age, those older than 60 years of age, Mexican Americans, and non-Hispanic blacks were oversampled to assure adequate sample size for these groups. A more detailed description of the survey design, informed consent procedure and the sample has been published elsewhere.[13] For our study, only surplus sera were examined, and there was no link between existing study data, biological specimens, and patient identifiers. Our evaluation was determined to be exempt from human subjects review.

Race/ethnicity was based on self-reported information and classified as non-Hispanic white, non-Hispanic black, or Mexican American. Those who were not classified into one of these three categories were placed in the "other" racial/ethnic group and were only analyzed within the total population. Although children as young as two months of age were included in NHANES III, many of the serum samples from young children were not available for testing. For our study, surplus sera from individuals ≥ 6 years of age were tested for antibodies to *Toxocara*. Multivariate analyses were conducted for persons ≥ 6 years of age with the exception of one predictor variable (occupation) that was only applicable to individuals ≥ 20 years of age. Age was grouped into eight categories (6–11, 12–19, 20–29, 30–39, 40–49, 50–59, 60–69, and 70+ years) and was entered into logistic regression models accordingly. Poverty index was calculated by dividing total

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family income by the U.S. poverty threshold and adjusted for family size. A crowding index was calculated by dividing the total number of residents in a household by the number of rooms in the household. This index was expressed as number of persons per room and grouped into three categories (< 0.5, 0.5–0.99, and ≥ 1 persons per room). Head of household education was measured as the last year of schooling completed by the head of household and was initially grouped into four categories (no high school, some high school, high school graduate, and some college). In the logistic regression models, education was collapsed into three categories (less than high school, high school graduate, and some college). Blood lead levels were considered above normal at concentrations of 10 μg/dL or higher. Metropolitan residence was defined as living in an area with a population of ≥ 1 million persons. All other areas, including rural areas, were considered to be nonmetropolitan. Pet ownership, as well as dog and cat ownership specifically, was defined as ownership of an animal(s) at the time of the survey and did not reflect ownership in the past. Working in a soil-related occupation was according to the longest held job and included farm workers, farm operators, farm managers, and related agricultural occupations. Regions were defined as Northeast (Connecticut [CT], Massachusetts [MA], Maine [ME], New Hampshire [NH], New Jersey [NJ], New York [NY], Pennsylvania [PA], Rhode Island [RI], Vermont [VT]); South (Alabama [AL], Arkansas [AR], Delaware [DE], Washington [DC], Florida [FL], Georgia [GA], Kentucky [KY], Louisiana [LA], Maryland [MD], Mississippi [MS], North Carolina [NC], Oklahoma [OK], South Carolina [SC], Tennessee [TN], Texas [TX], Virginia [VA], West Virginia [WV]); Midwest (Iowa [IA], Illinois [IL], Indiana [IN], Kansas [KS], Michigan [MI], Montana [MN], Missouri [MO], North Dakota [ND], Nebraska [NE], Ohio [OH], South Dakota [SD], Wisconsin [WI]); West (Arkansas [AK], Arizona [AZ], California [CA], Connecticut [CO], Hawaii [HI], Idaho [ID], Montana [MT], New Mexico [NM], Nevada [NV], Oregon [OR], Utah [UT], Washington [WA], Wyoming [WY]).

Laboratory Testing. All specimens were tested during the years 2005–2006 using a one dilution, enzyme immunoassay (EIA) with sensitivity and specificity of 78% and 92%, respectively.[14] Specimens were tested using 96-well Immulon II HB flat bottom plates sensitized with *Toxocara canis* excretory-secretory (TES) antigen at a dilution of 1:2000 using a 0.1M $NaHCO_3/Na_2CO_3$ buffer. Although this assay used a *T. canis* antigen, it was not able to distinguish between *T. canis* and *T. cati* infections. All sera were diluted 1:100 with a phosphate buffered saline (PBS) + 0.05% Tween solution. An anti-IgG enzyme conjugate was used to detect antigen-antibody complexes, and 3,3′, 5,5′-tetra-methylbenzidine (TMB) was the substrate used to visualize any reaction. The plates were read at 450 nm using a vMax microplate reader (Molecular Devices Corp., Menlo Park, CA) with a computer equipped with SOFTmax software (Molecular Devices Corp., Menlo Park, CA) for reader control and data analysis. For each individual test run, a positive cutoff ratio value was calculated. Because results from the standard EIA are considered positive at a titer of ≥ 1:32, four low-positive control specimens with this titer and a high positive control, run in duplicate, were used as quality control for each run. The cutoff ratio was calculated by averaging the optical density (O.D.) readings for the low positive controls and dividing this value by the mean of the high positive control O.D. values. Each sample ratio was calculated by dividing the specimen O.D. value by the mean of the high positive control and was compared with the cutoff ratio to determine if the sample was positive or negative.

A battery of 49 specimens (25 positive, 24 negative) originally tested using the standard titration EIA were evaluated using the one dilution EIA. Comparative sensitivity and specificity were 96% and 100%, respectively. Reproducibility of results was also evaluated midway through the study. Eighty-one samples (11 positive, 70 negative) were randomly chosen for retesting. Upon retesting, results were consistent with the initial results except for one of the 11 positive samples that tested negative, and one of the 70 negative samples that tested positive. Both of the samples with inverted results were close to the respective positive cutoff ratios when initially tested.

Statistical Analysis. Estimates were weighted to represent the total U.S. population and to account for oversampling and nonresponse to the household interview and physical examination.[15] Statistical analyses were conducted using SUDAAN, a family of statistical procedures for analysis of data from complex sample surveys.[16] Standard error estimates were calculated using the Taylor Series Linearization method in SUDAAN to account for the complex sample design, and prevalence estimates were age-adjusted by the direct method to the 2000 U.S. population when seroprevalence was compared across population subgroups.

Screening for possible predictors for *Toxocara* seropositivity was done by evaluating differences in seroprevalence without correcting for multiple comparisons and examining 95% confidence intervals (CI) generated by SUDAAN. The *P* values were determined from a univariate *t*-statistic generated from a general linear contrast procedure in SUDAAN. Multivariate logistic regression was used to further determine independent predictors. Modeling was conducted for the combined population and variables that had a Satterthwaite-adjusted F statistic with a P value < 0.05 from the logistic model were considered significant.

RESULTS

Of the 30,930 persons 6 years of age and older selected for the NHANES III survey, 83% (N = 25,733) were interviewed and 91% of those interviewed (N = 23,527) underwent physical examination. Of those examined, 87% (N = 20,395) were tested for *Toxocara* antibodies. The percentage of sera tested for *Toxocara* within designated age categories ranged from 84–95% except for the 6 to 11 year old group, for which 74% were available and tested. The availability of a specimen for antibody testing among those examined varied by age, race, sex, region, foreign birth, residence in a metropolitan area, poverty, crowding index, and head of household education (P < 0.05). It did not vary by dog or cat ownership or blood lead concentration.

The overall age adjusted *Toxocara* seroprevalence for individuals ≥ 6 years of age as 13.9% (95% CI: 12.5, 15.3) and varied significantly among racial/ethnic groups. *Toxocara* seropositivity was significantly higher for non-Hispanic blacks 21.2% (95% CI: 19.7, 22.8) than any other racial/ethnic group (Table 1). Seroprevalence differed by sex, foreign birth, poverty level, household crowding, education, and blood lead levels. Persons ≥ 20 years of age who reported being involved

TABLE 1
Age adjusted *Toxocara* seroprevalence for all persons ≥ 6 years of age, NHANES III, 1988–1994

Characteristic	Total no.*	Entire study population (*N* = 20,395)		Race/ethnicity: Non-Hispanic white (*N* = 7,754)		Non-Hispanic black (*N* = 5,874)		Mexican-American (*N* = 5,929)	
		%	95% CI†	%	95% CI	%	95% CI	%	95% CI
Race	20,395	13.9	12.5, 15.3	12.0	10.2, 13.8	21.2	19.7, 22.8	10.7	9.5, 11.9
Sex									
Male	9,876	15.6‡	13.8, 17.5	14.0‡	11.8, 16.2	24.6‡	22.1, 27.1	11.0	9.7, 12.3
Female (Ref)	10,519	12.4	10.9, 13.8	10.1	8.5, 11.8	18.5	17.0, 20.0	10.4	8.9, 11.8
Poverty level									
Below	5,091	22.9‡	19.7, 26.2	22.9‡	17.6, 28.2	25.0‡	22.4, 27.6	12.6‡	10.9, 14.3
At or above (Ref)	13,439	12.3	10.9, 13.6	11.1	9.6, 12.6	19.0	16.9, 21.0	8.3	6.8, 9.8
Crowding index (persons per room)									
≥ 1	5,626	20.5‡	16.7, 24.2	17.1§	10.7, 23.5	24.2‡	21.1, 27.2	13.8‡	11.6, 16.1
0.50–0.99	8,263	14.2‡	12.5, 15.8	12.0	9.8, 14.1	22.8‡	20.6, 25.1	8.7	6.9, 10.4
< 0.5 (Ref)	6,457	10.5	8.4, 12.5	9.9	7.6, 12.2	17.1	14.1, 20.0	7.4	5.1, 9.8
Head of household education									
None or elementary school	5,221	21.6‡	18.1, 25.0	22.0‡	15.3, 28.8	25.5¶	22.5, 28.6	11.9§	10.4, 13.5
Some high school	3,465	21.8‡	19.1, 24.5	22.2‡	18.3, 26.2	22.9¶	20.0, 25.9	13.6¶	10.4, 16.8
High school graduation	5,865	14.1‡	12.3, 15.9	12.6‡	10.4, 14.8	20.1	18.2, 22.0	7.4	5.1, 9.7
Some college (Ref)	5,668	9.0	7.8, 10.3	7.7	6.3, 9.1	18.0	15.3, 20.6	8.3	5.2, 11.3
Blood lead level									
High	940	26.9‡	21.0, 32.8	26.7¶	15.1, 38.3	30.1¶	24.6, 35.5	19.2‡	13.8, 24.5
Normal (Ref)	19,365	13.5	12.0, 15.0	11.7	9.9, 13.5	20.7	19.1, 22.4	10.3	9.1, 11.4
Dog ownership									
Yes	4,242	13.8	12.1, 15.5	13.1	11.0, 15.2	23.4	19.3, 27.4	10.9	8.8, 13.0
No (Ref)	16,135	13.9	12.3, 15.4	11.4	9.5, 13.3	20.8	19.2, 22.4	10.5	9.2, 11.7
Cat ownership									
Yes	2,834	14.1	11.6, 16.6	13.6	10.8, 16.4	21.6	16.0, 27.3	8.5	4.4, 12.6
No (Ref)	17,543	13.9	12.4, 15.4	11.4	9.6, 13.3	21.2	19.7, 22.8	10.9	9.6, 12.2
Residence in a metropolitan area									
Population of < 1 million or rural	10,476	15.3	12.5, 18.1	14.4¶	11.0, 17.8	25.1‡‖	22.4, 27.8	9.4‖	7.8, 11.1
Population of ≥ 1 million (Ref)	9,919	12.4	10.8, 13.9	8.8	7.3, 10.4	18.2	15.7, 20.6	11.7	9.8, 13.5
Place of birth									
Non-United States	3,769	22.2‡	18.1, 26.2	16.9	10.4, 23.5	30.9‡‖	25.0, 36.9	14.3‡	12.4, 16.1
United States (Ref)	16,571	12.7	11.2, 14.2	11.7	9.9, 13.6	20.5	18.9, 22.2	8.4	6.8, 9.9
Employment in an occupation involving soil exposure**									
Yes	786	25.5‡	18.0, 33.0	23.4§	12.0, 34.8	38.8¶	27.4, 50.3	11.2	8.0, 14.4
No (Ref)	14,438	13.5	12.2, 14.9	12.0	10.5, 13.5	19.8	18.2, 21.3	10.0	8.4, 11.7
Region††									
Northeast	2,638	15.6‡‖	14.2, 17.0						
Midwest	3,920	11.4‡	8.7, 14.1						
South	8,888	17.4‡	14.4, 20.5						
West (Ref)	4,949	9.4‡	6.5, 12.3						

* The total for each category may not equal the overall sample size because total includes the "other" race/ethnic group.
† CI = confidence interval.
‡ $P < 0.001$.
§ $P < 0.05$.
¶ $P < 0.01$.
‖ Unstable estimate; degrees of freedom < 16.
** Data for persons ≥ 20 years of age were used for this factor.
†† Sample size was not sufficient to assess this factor by race/ethnicity.
Ref = reference group.

in a soil occupation, such as farming and agriculture, had a seroprevalence of 25.5% (95% CI: 18.0, 33.0) compared with 13.5% (95% CI: 12.2, 14.9; $P < 0.001$) for those persons not involved in these types of occupations (Table 1). *Toxocara* seroprevalence varied by age among racial/ethnic groups as shown in Figure 1.

In the overall multivariate model for persons 6 years of age or older, *Toxocara* seroprevalence was significantly higher for non-Hispanic blacks (compared with non-Hispanic whites), and significantly lower for Mexican Americans (compared with non-Hispanic whites). Seroprevalence was higher for persons 12–19, 20–29, and 30–39 years of age as compared with 6–11 years of age, was higher among males, those living in poverty, individuals born outside of the United States, those living in non-metropolitan areas, those with above normal blood lead concentrations, dog owners, and those living in the three geographic regions outside of the West. Seroprevalence was also higher for those persons whose head of household had less than or at least a high school education compared with those with more than a high school education. Although crowding index was found to be a significant factor on the univariate analysis, it was no longer significant in the multivariate analysis (Table 2).

DISCUSSION

In our national population-based study, the overall age-adjusted *Toxocara* seroprevalence for individuals ≥ 6 years of age was 13.9%. Reported seroprevalence estimates vary worldwide, but many of these estimates were determined from convenience samples of various populations.[17–20] The

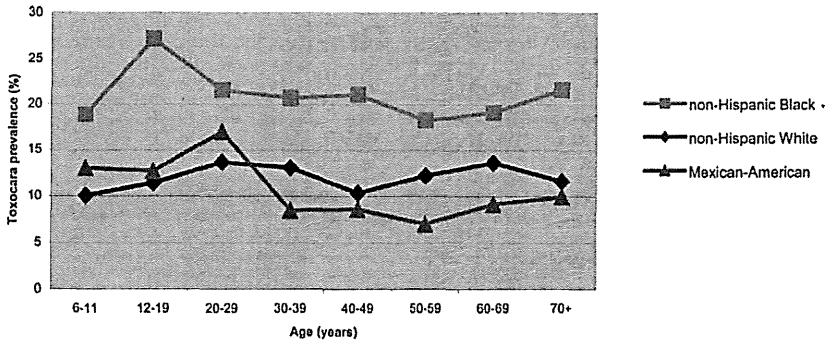

FIGURE 1. *Toxocara* seroprevalence by age and race/ethnicity, 6 years of age and older, NHANES III, 1988–1994. *Seroprevalence in this racial/ethnic group differed significantly ($P < 0.05$) from the other two racial/ethnic groups in each age category.

TABLE 2

Risk factors for *Toxocara* seropositivity, as estimated with a full logistic regression model for all persons ≥ 6 years of age, NHANES III, 1988–1994

Factor	OR	95% CI
Age (years)		
6–11	Ref	
12–19	1.2	1.0, 1.5
20–29	1.4*	1.1, 1.8
30–39	1.4*	1.1, 1.9
40–49	1.1	0.9, 1.5
50–59	1.2	0.8, 1.7
60–69	1.3	1.0, 1.8
≥ 70	1.1	0.9, 1.4
Race		
Non-Hispanic White	Ref	
Non-Hispanic Black	1.5*	1.2, 1.9
Mexican-American	0.6*	0.4, 0.7
Sex		
Male	1.5*	1.3, 1.7
Female	Ref	
Poverty level		
Below	1.5*	1.3, 1.8
At or above	Ref	
Crowding index (persons per room)		
≥ 1	1.3	0.9, 1.8
0.50–0.99	1.0	0.8, 1.3
< 0.5	Ref	
Head of household education		
< High school	2.2*	1.8, 2.8
High school	1.5*	1.2, 1.8
> High school	Ref	
Blood lead level		
High	1.4*	1.1, 1.9
Normal	Ref	
Dog ownership		
Yes	1.2*	1.1, 1.4
No	Ref	
Cat ownership		
Yes	1.2	0.9, 1.4
No	Ref	
Residence in a metropolitan area		
Population of < 1 million or rural	1.3*	1.1, 1.7
Population of ≥ 1 million	Ref	
Place of birth		
Non-United States	1.9*	1.4, 2.6
United States	Ref	
Region		
Northeast	2.2*	1.5, 3.2
Midwest	1.6*	1.1, 2.4
South	2.2*	1.5, 3.2
West	Ref	

* P value < 0.05 from Satterthwaite adjusted F statistic.
OR = odds ratio; CI = confidence interval; Ref = reference group.

wide range of seroprevalence estimates may be a reflection of a difference in characteristics (i.e., socioeconomic status, race, soil exposure, pet density, etc.) of the sampled populations. Because of the different serologic assays used to determine these figures and the potential bias introduced when using convenience samples, it is difficult to compare seroprevalence estimates. However, estimates from the NHANES III data were determined from a nationally representative sample, and confirm that there is substantial exposure to *Toxocara* in the United States.

Studies have reported age, both young and old, as a risk factor for *Toxocara* infection.[21–24] However, young age is widely considered to be a particularly vulnerable time for acquiring infection because children are likely to play in contaminated environments and then put their fingers and hands in their mouths either incidentally or intentionally. In a case control study conducted in Bogotá, Columbia, children between one and four years of age were found to have higher *Toxocara* seropositivity compared with older children.[25] In the NHANES III population sample, seroprevalence in children 6–11 years of age ranged from a low of 10.1% among non-Hispanic whites to a high of 18.9% among non-Hispanic blacks. In a study conducted by Hermann and others,[10] sera from children 6 to 11 years of age who had participated in the first Health and Nutrition Examination Survey (HANES I), 1971–1973, were tested for *Toxocara* antibodies. Seroprevalence was > 20% for black children, but < 5% for white children. This earlier report noted that the evaluated group was not representative of the U.S. population because sera of more white children were available for testing when compared with the entire HANES I population of the same age. The absolute differences in the number of children tested were relatively small, but reached statistical significance because of the large sample size. A similar study conducted with the same HANES I population reported *Toxocara* seroprevalence > 15% for black female children and > 20% for black male children between 6 and11 years of age, but < 5% for white children in the same age category.[26]

Among the entire NHANES III population, *Toxocara* seroprevalence was significantly higher for non-Hispanic blacks than other race/ethnicity groups. Prevalence among non-Hispanic blacks was greatest during adolescence, whereas prevalence among non-Hispanic whites and Mexican Americans did not show the same pattern. These differences may reflect behaviors and/or environmental factors, which placed

these different groups at risk of exposure to *Toxocara* at different stages of life. Because there is limited information on the duration of persistence of *Toxocara* antibodies and the incidence of reinfection, it is difficult to determine whether seroprevalence in older age groups is a reflection of previous infections with persistent antibodies or newly acquired infections. A few reports suggest that *Toxocara* antibodies can persist for years, even after anthelmintic treatment, but most of these reports are based on relatively small sample sizes. These reports also show that antibody levels tend to decrease over time.[27–29] In the NHANES III population, *Toxocara* seroprevalence did not increase with age as was seen with *Toxoplasma* seroprevalence.[30] Both *Toxocara* and *Toxoplasma* can be transmitted through soil, but *Toxoplasma* can also be transmitted through undercooked contaminated meat and antibodies to *Toxoplasma* are long lasting. However, the relatively stable seroprevalence across age groups may suggest that individuals are susceptible to reinfection with *Toxocara*.

Many studies have evaluated pet (most commonly dog and cat) ownership as a potential risk factor for *Toxocara* infection, and have shown associations with human *Toxocara* seroprevalence.[1,26] In contrast, other studies worldwide have not shown the same association.[25,31–33] The different findings may be indicative of the incidence of infection relative to the time the testing was done. Differences may also be related to the type of population evaluated. Individuals tested as part of a clinical case-control study may be significantly different than individuals in population-based studies. In our study, dog ownership was associated with *Toxocara* seropositivity in the multivariate analysis.

In the NHANES III population, head of household education was also found to be associated with *Toxocara* seroprevalence. Individuals who had not completed high school had the highest seroprevalence and seropositivity decreased steadily with higher levels of completed education. Lower education levels are often associated with lower socioeconomic status and may also be associated with occupations involving soil exposure, which was also associated with *Toxocara* seropositivity in the overall population. Those persons with lower education may also be more likely to live in areas with high environmental contamination. In addition, individuals with high blood lead levels had significantly higher *Toxocara* seropositivity. Elevated blood lead levels in children have often been associated with pica, a widely accepted risk factor for *Toxocara* infection.[34–37] Although increased lead exposure in adults may result from routes of exposure different from those associated with *Toxocara* infection, it may be indicative of lower socioeconomic status, which has been shown to be associated with *Toxocara* infection. The findings of this study confirm that *Toxocara* infection is widespread in the United States. Although the sensitivity of the assay used was not perfect, and the positive predictive value was relatively low (61%), even a 39% reduction of the overall seroprevalence would still yield a *Toxocara* seroprevalence of > 8% in the general population. Furthermore, when this assay is used in conjunction with a presumptive diagnosis to confirm *Toxocara* infection, the predictive values are raised.[26] Although this assay does not distinguish between acute and chronic *Toxocara* infections and progress has been made on the development of highly specific recombinant antigen tools,[38–40] this test is still the most widely used serologic tool in non-tropical settings. Because NHANES III was not designed specifically to evaluate risk factors associated with *Toxocara* infection, further studies should be conducted to confirm results obtained in this study.

Despite the limitations encountered in this study, NHANES III has provided the best national estimates to date of the prevalence of *Toxocara* infection in the United States. With an estimated 72 million dogs and 82 million cats in the United States,[41] there is potential for widespread environmental contamination with *Toxocara* spp. eggs. Further studies under controlled conditions are necessary to further define potential morbidity associated with *Toxocara* infection. Prevention efforts such as hand washing after soil contact, prevention of soil contamination in public areas by dog and cat feces, and preventive anthelmintic treatment of puppies and kittens can help minimize exposure to *Toxocara* spp.[42,43] and help control potential morbidity associated with *Toxocara* infection. Continued monitoring of *Toxocara* seroprevalence could be done through future NHANES surveys.

Received May 14, 2008. Accepted for publication July 6, 2008.

Acknowledgments: We are grateful for the interest and assistance of Dr. David Stansfield from Novartis Animal Health USA, Inc. We also thank Marianna Wilson, Kolby Sanders-Lewis, Abigail Babin, and Christopher Oh from CDC for their technical assistance in the laboratory.

Financial support: This study was funded in part by a grant from Novartis Animal Health USA, Inc.

Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Department of Health and Human Services or the Centers for Disease Control and Prevention.

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REFERENCES

1. Schantz PM, 1989. *Toxocara* larva migrans now. *Am J Trop Med Hyg 41:* 21–34.
2. Taylor MR, Keane CT, O'Connor P, Girdwood RW, Smith H, 1987. Clinical features of covert toxocariasis. *Scand J Infect Dis 19:* 693–696.
3. Alderete JM, Jacob CM, Pastorino AC, Elefant GR, Castro AP, Fomin AB, Chieffi PP, 2003. Prevalence of *Toxocara* infection in schoolchildren from the Butanta region, Sao Paulo, Brazil. *Mem Inst Oswaldo Cruz 98:* 593–597.
4. Conde Garcia L, Muro Alvarez A, Simon Martin F, 1989. Epidemiological studies on toxocariasis and visceral larva migrans in a zone of western Spain. *Ann Trop Med Parasitol 83:* 615–620.
5. Zacharasiewicz A, Auer H, Brath H, Stohlhofer B, Frank W, Aspock H, Zwick H, 2000. *Toxocara* and bronchial hyperreactivity–results of a seroprevalence study. *Wien Klin Wochenschr 112:* 922–926.
6. Fan CK, Liao CW, Kao TC, Li MH, Du WY, Su KE, 2005. Sero-epidemiology of *Toxocara* canis infection among aboriginal schoolchildren in the mountainous areas of north-eastern Taiwan. *Ann Trop Med Parasitol 99:* 593–600.
7. Kenny V, Allwright SP, 1987. Seroprevalence of toxocariasis in a hospital based sample in Ireland. *Ir J Med Sci 156:* 361–363.
8. Luzna-Lyskov A, 2000. Toxocarosis in children living in a highly

contaminated area. An epidemiological and clinical study. *Acta Parasitol 45:* 40–42.
9. Campos Junior D, Elefant GR, de Melo e Silva EO, Gandolfi L, Jacob CM, Tofeti A, Pratesi R, 2003. Frequency of seropositivity to *Toxocara* canis in children of different socioeconomic strata. *Rev Soc Bras Med Trop 36:* 509–513.
10. Hermann N, Glickman LT, Schantz PM, Weston MG, Domanski LM, 1985. Seroprevalence of zoonotic toxocariasis in the United States: 1971–1973. *Am J Epidemiol 122:* 890–896.
11. Fenoy S, Cuellar C, Aguila C, Guillen JL, 1992. Persistence of immune response in human toxocariasis as measured by ELISA. *Int J Parasitol 22:* 1037–1038.
12. Zarnowska H, Borecka A, Gawor J, Marczynska M, Dobosz S, Basiak W, 2008. A serological and epidemiological evaluation of risk factors for toxocariasis in children in central Poland. *J Helminthol 82:* 1–5.
13. Statistics NCfH, 1992. *Sample Design: Third National Health and Nutrition Examination Survey, 1988–94.* Vital and Health Statistics.
14. Glickman L, Schantz P, Greive R, 1986. *Toxocariasis.* Walls K, Schantz, P, eds. *Immunodiagnosis of Parasitic Diseases.* Volume 1. New York: Academic Press, 201–231.
15. Ezzati T, Khare M, 1993. Nonresponse adjustment in a national health survey. *1992 Proceedings of the Section on Survey Research Methods,* 339–344.
16. Shah BB, Hurt P, 1996. *SUDAAN User's Manual,* Release 5.50. Research Triangle Park, NC: Research Triangle Institute.
17. Cilla G, Perez-Trallero E, Gutierrez C, Part C, Gomariz M, 1996. Seroprevalence of *Toxocara* infection in middle-class and disadvantaged children in northern Spain (Gipuzkoa, Basque Country). *Eur J Epidemiol 12:* 541–543.
18. Guerra A, Navarro C, de Guevara CL, 1995. Seroprevalence of toxocariasis in children and a case of VLM. *Eur J Epidemiol 11:* 701–702.
19. Jones WE, Schantz PM, Foreman K, Smith LK, Witte EJ, Schooley DE, Juranek DD, 1980. Human toxocariasis in a rural community. *Am J Dis Child 134:* 967–969.
20. Holland CV, O'Lorcain P, Taylor MR, Kelly A, 1995. Seroepidemiology of toxocariasis in school children. *Parasitology 110:* 535–545.
21. Garcia-Pedrique ME, Diaz-Suarez O, Estevez J, Cheng-Ng R, Araujo-Fernandez M, Castellano J, Araujo J, Cabrera L, 2004. Prevalence of infection by *Toxocara* in schoolchildren in the community of El Mojan, Zulia state, Venezuela. *Invest Clin 45:* 347–354.
22. Logar J, Kraut A, Likar M, 1993. *Toxocara* antibodies in patients with visceral or ocular disorder in Slovenia. *Infection 21:* 27–29.
23. Radman NE, Archelli SM, Fonrouge RD, del V Guardis M, Linzitto OR, 2000. Human toxocarosis. Its seroprevalence in the city of La Plata. *Mem Inst Oswaldo Cruz 95:* 281–285.
24. Abo-Shehada MN, Sharif L, el-Sukhon SN, Abuharfeil N, Atmeh RF, 1992. Seroprevalence of *Toxocara* canis antibodies in humans in northern Jordan. *J Helminthol 66:* 75–78.
25. Agudelo C, Villareal E, Caceres E, Lopez C, Eljach J, Ramirez N, Hernandez C, Corredor A, 1990. Human and dogs *Toxocara* canis infection in a poor neighborhood in Bogota. *Mem Inst Oswaldo Cruz 85:* 75–78.
26. Glickman LT, Schantz PM, 1981. Epidemiology and pathogenesis of zoonotic toxocariasis. *Epidemiol Rev 3:* 230–250.
27. Malafiej E, Spiewak E, 2001. The significance of the level of antibodies in the evaluation of the effects of treatment of toxocariasis. *Wiad Parazytol 47:* 805–810.
28. Bass JL, Mehta KA, Glickman LT, Blocker R, Eppes BM, 1987. Asymptomatic toxocariasis in children. A prospective study and treatment trial. *Clin Pediatr (Phila) 26:* 441–446.
29. Elefant GR, Shimizu SH, Sanchez MC, Jacob CM, Ferreira AW, 2006. A serological follow-up of toxocariasis patients after chemotherapy based on the detection of IgG, IgA, and IgE antibodies by enzyme-linked immunosorbent assay. *J Clin Lab Anal 20:* 164–172.
30. Jones JL, Kruszon-Moran D, Won K, Wilson M, Schantz PM, 2008. Toxoplasma gondii and *Toxocara* spp. co-infection. *Am J Trop Med Hyg 78:* 35–39.
31. Genchi C, Di Sacco B, Gatti S, Sangalli G, Scaglia M, 1990. Epidemiology of human toxocariasis in northern Italy. *Parassitologia 32:* 313–319.
32. Buijs J, Borsboom G, van Gemund JJ, Hazebroek A, van Dongen PA, van Knapen F, Neijens HJ, 1994. *Toxocara* seroprevalence in 5-year-old elementary schoolchildren: relation with allergic asthma. *Am J Epidemiol 140:* 839–847.
33. Ajayi OO, Duhlinska DD, Agwale SM, Njoku M, 2000. Frequency of human toxocariasis in Jos, Plateau State, Nigeria. *Mem Inst Oswaldo Cruz 95:* 147–149.
34. De la Burde B, Reames B, 1973. Prevention of pica, the major cause of lead poisoning in children. *Am J Public Health 63:* 737–743.
35. Laraque D, McCormick M, Norman M, Taylor A, Weller SC, Karp J, 1990. Blood lead, calcium status, and behavior in preschool children. *Am J Dis Child 144:* 186–189.
36. Greene T, Ernhart CB, Boyd TA, 1992. Contributions of risk factors to elevated blood and dentine lead levels in preschool children. *Sci Total Environ 115:* 239–260.
37. Khan AH, Khan A, Ghani F, Khurshid M, 2001. Low-level lead exposure and blood lead levels in children: a cross-sectional survey. *Arch Environ Health 56:* 501–505.
38. Fong MY, Lau YL, 2004. Recombinant expression of the larval excretory-secretory antigen TES-120 of *Toxocara* canis in the methylotrophic yeast Pichia pastoris. *Parasitol Res 92:* 173–176.
39. Gems D, Ferguson CJ, Robertson BD, Nieves R, Page AP, Blaxter ML, Maizels RM, 1995. An abundant, trans-spliced mRNA from *Toxocara canis* infective larvae encodes a 26-kDa protein with homology to phosphatidylethanolamine-binding proteins. *J Biol Chem 270:* 18517–18522.
40. Yamasaki H, Araki K, Lim PK, Zasmy N, Mak JW, Taib R, Aoki T, 2000. Development of a highly specific recombinant *Toxocara canis* second-stage larva excretory-secretory antigen for immunodiagnosis of human toxocariasis. *J Clin Microbiol 38:* 1409–1413.
41. U.S. Pet Ownership–2007 Market Research Statistics. *American Veterinary Medical Association 2007 U.S. Pet Ownership & Demographics Sourcebook.*
42. Guidelines for Veterinarians, *Prevention of Zoonotic Transmission of Ascarids and Hook worms of Dogs and Cats.* Centers for Disease Control and Prevention/American Association of Veterinary Practitioners.
43. *Companion Animal Parasite Council Ascarid (Roundworm) Guidelines,* Companion Animal Parasite Council.

PLOS | NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

Prevalence of *Toxocara* species infection in the U.S.: Results from the National Health and Nutrition Examination Survey, 2011-2014

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OPEN ACCESS

Citation: Farmer A, Beltran T, Choi YS (2017) Prevalence of *Toxocara* species infection in the U. S.: Results from the National Health and Nutrition Examination Survey, 2011-2014. PLoS Negl Trop Dis 11(7): e0005818. https://doi.org/10.1371/journal.pntd.0005818

Editor: Alessandra Morassutti, PUCRS, BRAZIL

Received: April 18, 2017

Accepted: July 19, 2017

Published: July 31, 2017

Data Availability Statement: All data are available at: https://www.cdc.gov/nchs/nhanes/continuousnhanes/default.aspx?BeginYear=2011 (NHANES 2011-2012) and https://www.cdc.gov/nchs/nhanes/continuousnhanes/default.aspx?BeginYear=2013 (NHANES 2013-2014).

Funding: The authors report no external funding or financial relationships relevant to this article.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

Toxocariasis is one of the most common neglected infections of poverty in the U.S. with a reported National Health and Nutrition Examination Survey (NHANES) III (1988–1994) seroprevalence of 13.9% based on enzyme immunoassay testing. We reviewed NHANES data from 2011–2014 to assess current levels. Sera collected from NHANES 2011–2014 participants six years and older were tested for exposure using rTc-CTL-1 antigen, a more sensitive and specific recombinant antigen for IgG antibodies for *Toxocara* spp. These results were subdivided into children (age 6–17) and adults (age $\geq$ 18) and then compared between various sociodemographic characteristics. Given prior associations of *Toxocara* exposure with atopic disease and lead exposure, we also reviewed laboratory values including complete blood counts and blood and urine lead levels. Data from 13,509 individuals with *Toxocara* antibody results were examined including 3337 children (15.2%) and 10172 adults (84.8%). Overall seroprevalence was 5.1%. In adults increased antibody positivity occurred with non-White ethnicity, male gender, less than college-level education and lower income. Among children, increased antibody positivity was solely related to a lack of health insurance. Additionally, seropositivity was associated with increased blood lead and eosinophil levels in adults and both blood and urine lead levels in children. Relative to NHANES III (1988–1994), current data suggest an overall decrease in *Toxocara* spp. seroprevalence from 13.9% to 5.1%, however this may be artificially lowered due to difference in testing methods used. Persistent disparities appear to be associated with at-risk populations such as minority ethnicity and low socioeconomic status.

Author summary

Toxocariasis is a pervasive helminth infection transmitted to humans via embryonated eggs from soil contaminated by the feces of dogs and cats (*Toxocara canis* and *T. cati*, respectively). The most recent seroprevalence study of *Toxocara* infection in the United States used the National Health and Nutrition Examination Survey (NHANES) III data

collected from 1988 to 1994. At-risk groups identified included male gender, non-White ethnicity and low education and socioeconomic status. Since the original study, dogs and cats have increased in number and an improved testing platform has been created using rTc-CTL-1 antigen to *Toxocara* spp. with a sensitivity and specificity of 90% and 99%, respectively. In this study, we assessed current seroprevalence based on the NHANES data from 2011 to 2014 using the more specific testing platform. Although overall seroprevalence appears to have decreased from 13.9% to 5.1%; persistent disparities remain associated with at-risk populations including minority ethnicity and low socioeconomic status. Continued work is needed to improve awareness of disease transmission and treatment amongst the general population as well as veterinarians who may care for dogs and cats within the United States and take responsibility for education of the public.

Introduction

Toxocariasis has been identified as the most common human parasitic worm infection in the world and one of the most common neglected infections of poverty in the U.S. [1–3]. Spread through contact with soil contaminated by embryonated eggs from dog and cat feces, the etiologic agents, *Toxocara canis* and *T. cati*, have classically been associated with two major clinical syndromes: visceral larva migrans and ocular larva migrans [4, 5]. Following accidental ingestion, *Toxocara* spp. larvae migrate to various organs including the lungs, liver and eyes producing an inflammatory response leading to manifestations of disease. Subclinical infection (also called "covert" or "common" toxocariasis) can occur, with some studies suggesting associations with decreased cognitive function, asthma and atopic disease [6–9].

As soil contamination with embryonated eggs can persist for years and remain infective, human exposure often results from contact with feces-contaminated soil from dogs and cats [10]. The potential for exposure is increased further through various reservoirs for *Toxocara* spp. including intestinal infections and somatic larvae in definitive hosts as well as larvae in paratenic hosts. There is also evidence of eggs found on the hair of definitive hosts such as dogs and cats which may then be transferred to their owners, albeit rarely [11]. Currently, control efforts are focused on removal of pet feces and covering sand pits in recreational areas such as parks and playgrounds and elimination of adult worms in these companion animals to limit risk of transmission from pets to humans [12–16].

To better define the extent of the problem, serological data from over 20,000 samples were tested via enzyme immunoassay in the Third National Health and Nutrition Examination Survey (NHANES III; 1988–1994) and revealed a seroprevalence of 13.9% for *Toxocara* antibodies [17]. Higher rates of antibody positivity were noted in children, non-Hispanic Blacks, low education/socioeconomic status and in those living in the South or Northeast areas of the U.S.; the primary influences were non-Hispanic Black ethnicity and county poverty [18]. Additional studies have also confirmed the disproportionate burden in those of lower education and socioeconomic status [2, 19, 20]. Given an increase in the numbers of dogs and cats of approximately 60 and 50%, respectively, from NHANES III (1988–1994) to prior to the current study time-period (2011–2014) as well as the availability of an improved testing platform using a Luminex based assay to rTc-CTL-1 antigen for *Toxocara* spp., we sought to use the latest NHANES data from 2011–2014 to reassess the seroprevalence of *Toxocara* antibody positivity in the U.S. as well as trends in exposure risk groups [17, 21–24].

Materials and methods

Study design and population

The NHANES is a series of ongoing cross-sectional surveys conducted by the Centers for Disease Control and Prevention (CDC) and is designed to assess the health and nutritional status of Americans through physical examinations and interviews [25]. Individuals in the survey participate in a household interview followed by physical examination in a mobile examination center. The NHANES sampling procedure is a complex multistage probability cluster design which oversamples specific populations such as Hispanics, non-Hispanic blacks, non-Hispanic Asians, older adults, and low income persons to obtain both adequate samples for meaningful subgroup analyses and more reliable parameter estimates [26].

The survey's design and weighting methodology have previously been described [27]. To account for unequal selection probabilities among participants and adjustments for non-response, all estimates were weighted using multi-year sampling weights calculated from those provided by the National Center for Health Statistics (NCHS) to account for the two combined 2011–2012 and 2013–2014 NHANES cycles [28]. The data collection protocol was approved by the NCHS institutional review board.

Demographic data and characteristics

Demographic information was obtained during the household interview. Self-reported socio-demographic characteristics included age at the time of the survey. Participants aged 6 to 17 were classified as children and those 18 years and older were classified as adults. Additional factors include gender, race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, or other including multiracial), marital status (married or member of an unmarried couple; divorced, widowed, or separated; and never married), education level (did not graduate high school, graduated high school or attained a GED, some college or technical school, and graduated from college or technical school), health insurance coverage status, history of asthma, and family Poverty to Income Ratio (PIR). Pooling of demographic categories was conducted.

Specimen collection and laboratory methods

Sera collected from NHANES 2011–2012 and 2013–2014 participants 6 years and older were tested for *Toxocara* spp. by a Luminex assay using recombinant rTc-CTL-1 antigen that detects IgG antibodies against *Toxocara* spp. The antibody test was classified as positive if the antibody responses to rTc-CTL-1 was greater than the cut-off point value of 23.1 mean fluorescence intensity [29, 30]. The assay itself has been validated against reference serum and has a sensitivity of 90% and a specificity of 99% [23].

Statistical analysis

Analyses were conducted using NHANES provided statistical weights to account for the complex survey design. Weighted prevalence estimates are reported as percentages with 95% Wald confidence intervals (CI). Categorical variables were analyzed using Rao-Scott adjusted chi-square tests. Multivariable analysis was performed using logistic and linear regression models to determine predictors of *Toxocara* spp. infection. All statistical tests were performed by using a $P < 0.05$ level of significance. Data analyses were conducted using the complex sample package for SPSS 23 (IBM, Armonk, NY, USA).

Ethics statement

Per NCHS standards, all adult participants provide written informed consent and for children <18 years of age, written informed consent by both the child and the parent/guardian was required. For this study, a waiver was obtained from the Womack Army Medical Center Institutional Review Board in accordance with the use of publicly available de-identified data.

Results

A total of 13,509 individuals with valid *Toxocara* spp. antibody results participated in both the interview and examination portion of the 2011–2014 NHANES. The sample included 15.2% (N = 3,337) children and 84.8% (N = 10,172) adults. Overall, 858 (5.1%; 95% CI 4.4–5.9) participants tested positive for the *Toxocara* spp. antibody, representing over 12 million people in the greater non-institutionalized US population. Demographic and socioeconomic characteristics of children and adults are shown in Tables 1 and 2 respectively.

The prevalence of *Toxocara* spp. antibody for adults revealed no significant change in prevalence between the data collection cycles. In contrast, children experienced a significant reduction between 2011–2012 and 2013–2014 ($P < 0.01$). This was primarily associated with a decrease in prevalence among children with health insurance ($P < 0.01$, see Table 3); there was no difference in health care utilization.

Table 1. Characteristics associated with *Toxocara* spp. infection in children.

		Toxocara spp. Positive		*Toxocara* spp. Negative		OR (95% CI)[1]
		n	% Estimate (95% CI)	n	% Estimate (95% CI)	
Sample Size		131	3.6 (2.7–4.7)	3206	96.4 (95.3–97.3)	
Age group, yrs						
	6–9	37	2.6 (1.6–4.0)	1112	97.4 (96.0–98.4)	NS[2]
	10–13	45	3.9 (2.7–5.6)	1079	96.1 (94.4–97.3)	
	14–17	49	4.1 (2.8–5.9)	1015	95.9 (94.1–97.2)	
Gender						
	Male	75	4.0 (2.8–5.5)	1644	96.0 (94.5–97.2)	NS[2]
	Female	56	3.2 (2.2–4.6)	1562	96.8 (95.4–97.8)	
Race/Ethnicity						
	Non-Hispanic white	29	3.1 (2.0–4.7)	804	95.9 (95.3–98.0)	NS[2]
	Non-Hispanic black	38	4.7 (3.2–6.8)	832	95.3 (93.2–96.8)	
	Hispanic	48	4.5 (2.8–7.0)	1061	95.5 (93.0–97.2)	
	Other race, including multicultural	16	2.7 (1.7–4.2)	509	97.3 (95.8–98.3)	
Health Insurance						
	Covered	107	3.2 (2.4–4.3)	2890	96.8 (95.7–97.6)	1 (Reference)
	No Health Insurance	23	7.3 (4.6–11.6)	313	92.7 (88.4–95.4)	2.4 (1.4–4.2)
Asthma History						
	Yes	26	3.4 (2.1–5.6)	631	96.6 (94.4–97.9)	NS[2]
	No	105	3.6 (2.7–4.8)	2574	96.4 (95.2–97.3)	
Ratio of family income to poverty[3]						
	Below federal poverty level	55	4.9 (3.1–7.7)	1034	95.1 (92.3–96.9)	NS[2]
	At or above federal poverty level	66	2.9 (2.1–4.2)	1985	97.1 (95.8–97.9)	

[1] Odds Ratio based on regression analysis of 3333 participants with complete data.

[2] NS: Non Significant.

[3] Subsample does not sum to total sample due to non-responses.

https://doi.org/10.1371/journal.pntd.0005818.t001

Table 2. Characteristics associated with *Toxocara* spp. infection in adults.

		Toxocara spp. Positive		*Toxocara* spp. Negative		aOR (95% CI)[1]
		n	% Estimate (95% CI)	n	% Estimate (95% CI)	
Sample Size		727	5.3 (4.6–6.2)	9445	94.7 (93.8–95.4)	
Age group, yrs						
	18–29	107	4.4 (3.4–5.8)	2053	95.6 (94.2–96.6)	NS[2]
	30–39	92	4.8 (3.8–6.0)	1594	95.2 (94.0–96.2)	
	40–49	116	5.4 (4.3–6.8)	1529	94.6 (93.2–95.7)	
	50–59	130	6.4 (4.8–8.6)	1462	93.6 (91.4–95.2)	
	60+	282	5.7 (4.5–7.0)	2807	94.3 (93.0–95.5)	
Gender						
	Male	442	6.8 (5.9–7.9)	4489	93.2 (92.1–94.1)	1.9 (1.5–2.3)
	Female	285	4.0 (3.2–4.9)	4956	96.0 (95.1–96.8)	1 (Reference)
Race/Ethnicity						
	Non-Hispanic white	194	3.9 (3.1–4.9)	3945	96.1 (95.1–96.9)	1 (Reference)
	Non-Hispanic black	181	7.2 (5.8–8.8)	2063	92.8 (91.2–94.2)	1.5 (1.1–2.1)
	Hispanic	226	9.8 (7.6–12.6)	2001	90.2 (87.4–92.4)	1.6 (1.1–2.4)
	Other race, including multicultural	126	7.0 (5.7–8.7)	1436	93.0 (91.3–94.3)	1.8 (1.3–2.5)
Marital Status[3]						
	Married or living with partner	411	5.2 (4.4–6.1)	5209	94.8 (93.9–95.6)	
	Widowed, divorced, or separated	178	6.4 (5.0–8.0)	1940	93.6 (92.0–95.0)	NS[2]
	Never married	114	5.2 (3.9–6.9)	1793	94.8 (93.1–96.1)	
Education[3]						
	Less than high school graduate	279	11.5 (9.6–13.6)	1859	88.5 (86.4–90.4)	2.7 (1.9–3.7)
	High school graduate or GED	176	7.2 (5.9–8.8)	1891	92.8 (91.2–94.1)	2.1 (1.5–2.9)
	Some college	145	3.8 (2.9–4.9)	2830	96.2 (95.1–97.1)	NS[2]
	College graduate	103	2.9 (2.3–3.7)	2359	97.1 (96.3–97.7)	1 (Reference)
Health Insurance[3]						
	Covered	475	4.3 (3.7–5.0)	7410	95.7 (95.0–96.3)	1 (Reference)
	No Health Insurance	252	9.9 (7.9–12.2)	2024	90.1 (87.8–92.1)	1.5 (1.1–1.9)
Asthma History[3]						
	Yes	89	4.7 (3.6–6.0)	1469	95.3 (94.0–96.4)	NS[2]
	No	638	5.5 (4.7–6.4)	7968	94.5 (93.6–95.3)	
Ratio of family income to poverty[3]						
	Below federal poverty level	251	10.8 (8.9–13.0)	2034	89.2 (87.0–91.1)	1.8 (1.4–2.3)
	At or above federal poverty level	408	4.2 (3.6–4.8)	6685	95.8 (95.2–96.4)	1 (Reference)

[1] Adjusted Odds Ratio based on multiple regression analysis of 8901 participants with complete data.

[2] NS: Non Significant.

[3] Subsample does not sum to total sample due to non-responses.

https://doi.org/10.1371/journal.pntd.0005818.t002

Multivariable analysis of socio-demographic factors in adults identified the following risk factors for infection: gender ($P < 0.001$), race/ethnicity ($P = 0.01$), education ($P < 0.001$), healthcare coverage ($P < 0.01$), and PIR ($P < 0.001$). There was no significant difference between infection groups with regard to age category, marital status, or asthma diagnosis. Infection prevalence was higher among males compared to females and among non-White ethnicities. Additionally, the lack of health insurance and education was also associated with higher levels of infection among adults. Among children, only health insurance status distinguished seropositivity between the two groups ($P < 0.01$).

Table 3. *Toxocara* spp. antibody prevalence by time.

		2011–2012			2013–2014	P^1
		n	% Estimate (95% CI)	n	% Estimate (95% CI)	
Children with Positive Antibody Result						
	With Health Insurance	81	4.5 (3.0–6.5)	26	1.7 (1.1–2.7)	<0.01
	Without Health Insurance	15	8.7 (5.0–14.8)	8	5.3 (2.1–12.4)	0.32
	Total	96	4.9 (3.4–7.0)	34	2.0 (1.4–2.9)	<0.01
Adults with Positive Antibody Result						
	With Health Insurance	248	4.6 (3.8–5.6)	227	4.0 (3.2–5.0)	0.30
	Without Health Insurance	142	10.5 (8.2–13.3)	110	9.1 (6.2–13.3)	0.54
	Total	390	5.8 (4.8–7.0)	337	4.9 (3.8–6.2)	0.27

[1] P value based on Rao-Scott adjusted chi-square statistic.

https://doi.org/10.1371/journal.pntd.0005818.t003

Table 4 shows laboratory results by *Toxocara* spp. seroprevalence and age category. Seropositive and seronegative children had a median blood lead concentrations of 0.98 (IQR 0.50–1.46) ug/dL and 0.53 (IQR 0.40–0.76) ug/dL, respectively ($P < 0.01$); median urinary lead concentrations were 0.50 (IQR 0.26–0.83) ug/dL and 0.26 (IQR 0.14–0.47) ug/dL, respectively ($P < 0.01$).

Seropositive adults also had elevated serum lead levels compared to non-infected adults with a median of 1.24 (IQR 0.81–1.96) ug/dL vs. 0.98 (0.63–1.54) ug/dL ($P < 0.01$), but urinary lead levels were not significantly different. Additionally, eosinophilia (defined as $\geq$ 500 eosinophils/uL) was present in 8.8% (95% CI, 6.1–12.5) of seropositive adults versus 5.2% (95% CI, 4.6–5.9) of seronegative adults (OR 1.7; 95% CI 1.2–2.5). No similar difference in eosinophilia was noted among children.

Table 4. Laboratory values by *Toxocara* antibody results.

		Toxocara Positive		Toxocara Negative		P^1
		n	Median (IQR)	n	Median (IQR)	
Lead, urine (ug/L)						
	Adults	234	0.40 (0.20–0.66)	3082	0.33 (0.18–0.59)	0.37
	Children	37	0.50 (0.26–0.83)	1025	0.26 (0.14–0.47)	<0.01
Lead, blood (ug/dL)						
	Adults	560	1.24 (0.81–1.96)	7091	0.98 (0.63–1.54)	<0.01
	Children	120	0.98 (0.50–1.46)	2831	0.53 (0.40–0.76)	<0.01
Hemoglobin (g/dL)						
	Adults	727	14.40 (13.40–15.20)	9436	14.10 (13.20–15.10)	0.10
	Children	131	13.50 (12.90–14.50)	3204	13.50 (12.80–14.20)	0.75
Hematocrit (%)						
	Adults	727	42.20 (39.20–44.60)	9436	41.4 (38.70–44.20)	0.01
	Children	131	39.50 (37.60–42.10)	3204	39.40 (37.40–41.60)	0.90
RBC Folate (ng/mL)						
	Adults	390	411.00 (312.10–512.10)	4712	476.80 (359.40–618.10)	<0.001
	Children	97	4445.90 (312.10–525.40)	1663	450.30 (368.20–547.50)	0.18
TSH (mIU/L)						
	Adults	125	1.49 (1.16–2.00)	1563	1.58 (1.10–2.28)	0.16
	Children	16	1.16 (0.93–1.62)	252	1.49 (0.99–1.95)	0.08

[1] P value based on adjusted Rao-Scott chi-square statistic.

https://doi.org/10.1371/journal.pntd.0005818.t004

Discussion

Relative to NHANES III (1988–1994), current U.S. data show an overall decrease in *Toxocara* spp. seroprevalence from 13.9% to 5.1%. This may be related to changes in exposure, a more specific testing platform, and perhaps modifications in veterinary practices and awareness [17, 31]. Seroprevalence remains elevated in certain groups such as minority ethnic groups, male gender and those with low education and socioeconomic status. Despite an overall decrease, this study of a nationally representative sample highlights the continued exposure of the U.S. population to this disease and the persistent disparities associated with at-risk populations.

Similar to the previous seroprevalence study utilizing data obtained over 20 years ago, we showed a persistent elevation in odds of antibody positivity in adults among minority ethnicity, male gender and low education level [17]. These findings overall mirror those found by Congdon et al. demonstrating minority ethnicity and poverty were important drivers of higher levels of *Toxocara* antibody positivity [8, 18]. Conversely, with non-Hispanic white as the reference, we found Hispanic and other race/multicultural ethnicities to have a higher risk of exposure than non-Hispanic Black. This is different than the studies by Won et al. and Congdon et al., which suggested lower rates of exposure in the Hispanic population [17, 18]. It has been hypothesized that the lower rates in Hispanic populations may be related to residence in more arid Western states with less likelihood for exposure to viable *Toxocara* eggs [19]. Unfortunately, no data were available in our study for region of the country or rural versus urban environment. Non-college level education (surrogate for lower socioeconomic status) and male gender have also previously been associated with increased *Toxocara* antibody positivity and were identified in this study as well [17, 32]. Although previously reported, age did not appear to be a risk factor for this cohort [17, 33].

For children, no difference was found in demographic data despite looking at multiple variables including age, gender, socioeconomic status and ethnicity. Additional factors that have previously been associated such as a diagnosis of asthma and eosinophilia were also not significantly different [8, 34]. Interestingly, a protective effect of health insurance was noted. Typically, antibody positivity might reflect lower socio-economic status, however in our study, a child's family PIR was not a significant determinant of infectivity [35]. Furthermore, health care utilization rates did not differ between those with and without *Toxocara* infection.

From a testing standpoint, an additional consideration between NHANES III and the current data is the change in the testing platform itself. For NHANES III, seroprevalence was determined using *Toxocara canis* excretory secretory antigen enzyme immunoassay testing (TES-Ag EIA). Although this test has been the standard worldwide, the sensitivity is as low as 78% (for visceral larva migrans) and specificity has been limited by cross-reactivity, particularly in areas with other soil-transmitted helminthes such as *Ascaris* spp. [36]. Based on a review from 2014, prevalence of potential cross- reacting parasites such as *Ascaris* spp. may be as high as 49.4%, with higher rates in the southern U.S. and Appalachian regions [37]. Given this, results from prior seroprevalence studies may overestimate *Toxocara* exposure risk [1, 38]. For the most recent data, a newer assay based on rTc-CTL-1 antigen to *Toxocara* spp. was used with a sensitivity and specificity of 90% and 99%, respectively [23]. Thus, the current NHANES data likely is a better reflection of *Toxocara* exposure.

For the risk of exposure itself, the overall number of dogs and cats is an important factor. As mentioned previously, U.S. pet ownership has steadily increased from approximately 52 million dogs and 54 million cats at the time of NHANES III (1988–1994) to an estimated 70–83 million dogs and 74–96 million cats just prior to the current study time-period (2011–2014) [21, 22, 24]. As *Toxocara* spp. eggs are transmitted to the soil through dog and cat feces, the CDC encourages routine evaluation and treatment of house pets to limit human exposure as

well as removal of any pet feces from recreational areas [39]. Household gardens have also been shown to have high levels of *Toxocara* ova, suggesting use of gloves during gardening activities may help decrease risk for exposure [40]. In addition, the Companion Animal Parasite Council suggests year round treatment for control of intestinal parasites [41]. At least one study suggests this therapy can reduce intestinal parasites by up to 91% in pet dogs when compared to shelter dogs, although there was no direct analysis of treated versus untreated dogs to rule out confounders such as decreased exposure between these two groups [31]. Prior studies have shown treatment must be provided at least four times per year for efficacy [42]. Unfortunately, as was demonstrated in a Dutch cohort of pet owners, as few as 16% of pet dogs and 24.5% of pet cats were at this goal; no similar studies were found in the U.S. [42, 43]. Additionally, in a study performed in Ireland, only 51.7% of pet owners believed pet feces could pose a risk to human health and only 4.3% had heard of *Toxocara* infection, potentially accounting for the low adherence to treatment recommendations [44]. With regards to prevention of environmental exposure, a study from Poland revealed continued high levels of soil contamination with *Toxocara* spp. eggs, particularly in backyards of rural and urban areas highlighting the fact that current preventive efforts may have limited efficacy; no similar studies were found in the U.S. [45].

Finally, as exposure to environmental lead and *Toxocara* spp. can both occur from exposure to contaminated soil, elevated lead levels are frequently found in *Toxocara* seropositive patients [17, 46]. In this study, blood lead levels showed a statistically significant difference between seropositive and seronegative adults. For adults, occupational exposure (particularly construction and manufacturing work) accounts for over 60% of lead exposure in those found to have very high blood levels [47]. As these occupations may be associated with increased dust, soil and outdoor exposure this may provide a link with infection. However, the data are conflicting on occupational exposure with at least one study of gardeners failing to show an increased risk whereas increased seroprevalence has been noted in waste pickers and those engaged in agricultural work [17, 20, 48, 49]. Occupational information was not available in the 2011–2014 cycle so further comparison could not be made in this study. It is also important to note that while statistically significant differences occurred, blood lead levels noted in those seropositive were considerably less than the CDC reference value of 5 ug/dL.

Other laboratory findings in adults included a lower RBC folate ($P < 0.001$) and higher eosinophil count ($P < 0.01$) in seropositive samples. Review of the hemoglobin and hematocrit levels between seropositive and seronegative adults did not differ so the significance of a lower RBC folate is likely negligible. Also, although *Toxocara* infection has been associated with atopic diseases (such as asthma) and eosinophilia, no difference was noted for asthma and the absolute difference in eosinophil count noted was minimal and thus not likely to be clinically significant [50, 51].

For children, blood and urine lead levels were elevated in those with *Toxocara* antibody positivity, which has been identified in previous studies [7, 17]. The concurrent elevation of both *Toxocara* antibody and lead levels is concerning as both have been independently associated with decreased cognitive function in children [7, 17, 52]. In our study, the mean serum level of 1.43 ug/dl in seropositive children, though statistically higher than those seronegative, was well below the CDC reference level of 5 ug/dL. Despite this cut-off, no "safe" level of blood lead has been identified in children and thus interventions aimed at decreasing the common route of exposure (i.e. geophagy) may help decrease rates of both [53–55].

Several limitations of this study should be noted. This study did not include children younger than 6 years old. Also, we could not assess the extent to which higher seroprevalence might have reflected birth, prior residence outside the U.S. or current residence. In addition, antibody testing does not distinguish between current and prior infection so higher rates in adults

may be related to antibody persistence from infection at a younger age. Although little research has been done, most studies show that antibody positivity tends to wane with time and thus this is unlikely to have impacted our findings [56]. Finally, the use of a different testing platform with increased *Toxocara* sensitivity and specificity may limit the ability to make comparisons between prior reports and current data.

In conclusion, *Toxocara* spp. continues to affect a considerable portion of the U.S. non-institutionalized population and risk for exposure may increase as dogs and cats enter more homes. Significant gaps in research remain, particularly for *Toxocara* spp. contamination in various areas of the U.S. such as public parks, recreational areas and urban vs. rural locales. Studies assessing current CDC recommendations in reducing exposure such as disposal of pet waste and hand washing are also limited. Infection could be decreased with improved awareness in both the general population and U.S. veterinarians of exposure risks and knowledge of the effectiveness of anthelminthic treatment of dogs and cats.

Supporting Information

S1 Checklist. STROBE checklist.
(PDF)

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References

1. Hotez PJ, Wilkins PP. Toxocariasis: America's most common neglected infection of poverty and a helminthiasis of global importance? PLoS Negl Trop Dis. 2009; 3(3):e400. https://doi.org/10.1371/journal.pntd.0000400 PMID: 19333373
2. Hotez PJ. Neglected parasitic infections and poverty in the United States. PLoS Negl Trop Dis. 2014; 8 (9):e3012. https://doi.org/10.1371/journal.pntd.0003012 PMID: 25188455
3. Traversa D, Frangipane di Regalbono A, Di Cesare A, La Torre F, Drake J, Pietrobelli M. Environmental contamination by canine geohelminths. Parasit Vectors. 2014; 7:67. https://doi.org/10.1186/1756-3305-7-67 PMID: 24524656
4. Despommier D. Toxocariasis: clinical aspects, epidemiology, medical ecology, and molecular aspects. Clin Microbiol Rev. 2003; 16(2):265–72. https://doi.org/10.1128/CMR.16.2.265-272.2003 PMID: 12692098
5. Fisher M. Toxocara cati: an underestimated zoonotic agent. Trends Parasitol. 2003; 19(4):167–70. PMID: 12689646
6. Erickson LD, Gale SD, Berrett A, Brown BL, Hedges DW. Association between toxocariasis and cognitive function in young to middle-aged adults. Folia Parasitol (Praha). 2015;62.
7. Walsh MG, Haseeb MA. Reduced cognitive function in children with toxocariasis in a nationally representative sample of the United States. Int J Parasitol. 2012; 42(13–14):1159–63. https://doi.org/10.1016/j.ijpara.2012.10.002 PMID: 23123274

8. Walsh MG, Haseeb MA. Toxocariasis and lung function: relevance of a neglected infection in an urban landscape. Acta Parasitol. 2014; 59(1):126–31. https://doi.org/10.2478/s11686-014-0221-7 PMID: 24570059

9. Kanobana K, Vereecken K, Junco Diaz R, Sariego I, Rojas L, Bonet Gorbea M, et al. Toxocara seropositivity, atopy and asthma: a study in Cuban schoolchildren. Trop Med Int Health. 2013; 18(4):403–6. https://doi.org/10.1111/tmi.12073 PMID: 23397907

10. Holland CV. Knowledge gaps in the epidemiology of Toxocara: the enigma remains. Parasitology. 2017; 144(1):81–94. https://doi.org/10.1017/S0031182015001407 PMID: 26670118

11. Keegan JD, Holland CV. Contamination of the hair of owned dogs with the eggs of Toxocara spp. Vet Parasitol. 2010; 173(1–2):161–4. https://doi.org/10.1016/j.vetpar.2010.06.010 PMID: 20609527

12. Castillo D, Paredes C, Zanartu C, Castillo G, Mercado R, Munoz V, et al. Environmental contamination with Toxocara sp. eggs in public squares and parks from Santiago, Chile, 1999. Bol Chil Parasitol. 2000; 55(3–4):86–91. PMID: 11338980

13. Nooraldeen K. Contamination of public squares and parks with parasites in Erbil city, Iraq. Ann Agric Environ Med. 2015; 22(3):418–20. https://doi.org/10.5604/12321966.1167705 PMID: 26403106

14. Bojar H, Klapec T. Contamination of soil with eggs of geohelminths in recreational areas in the Lublin region of Poland. Ann Agric Environ Med. 2012; 19(2):267–70. PMID: 22742799

15. Macpherson CN. The epidemiology and public health importance of toxocariasis: a zoonosis of global importance. Int J Parasitol. 2013; 43(12–13):999–1008. https://doi.org/10.1016/j.ijpara.2013.07.004 PMID: 23954435

16. Mizgajska H. Eggs of Toxocara spp. in the environment and their public health implications. J Helminthol. 2001; 75(2):147–51. PMID: 11520438

17. Won KY, Kruszon-Moran D, Schantz PM, Jones JL. National seroprevalence and risk factors for Zoonotic Toxocara spp. infection. Am J Trop Med Hyg. 2008; 79(4):552–7. PMID: 18840743

18. Congdon P, Lloyd P. Toxocara infection in the United States: the relevance of poverty, geography and demography as risk factors, and implications for estimating county prevalence. Int J Public Health. 2011; 56(1):15–24. https://doi.org/10.1007/s00038-010-0143-6 PMID: 20422250

19. Jones JL, Kruszon-Moran D, Won K, Wilson M, Schantz PM. Toxoplasma gondii and Toxocara spp. coinfection. Am J Trop Med Hyg. 2008; 78(1):35–9. PMID: 18187782

20. Lee RM, Moore LB, Bottazzi ME, Hotez PJ. Toxocariasis in North America: a systematic review. PLoS Negl Trop Dis. 2014; 8(8):e3116. https://doi.org/10.1371/journal.pntd.0003116 PMID: 25166906

21. U.S. pet ownership & demographics sourcebook. 2012.

22. Ferrante A. 2013–2014 Appa National Pet Owners Survey: American Pet Products Association; 2013.

23. Anderson JP, Rascoe LN, Levert K, Chastain HM, Reed MS, Rivera HN, et al. Development of a Luminex Bead Based Assay for Diagnosis of Toxocariasis Using Recombinant Antigens Tc-CTL-1 and Tc-TES-26. PLoS Negl Trop Dis. 2015; 9(10):e0004168. https://doi.org/10.1371/journal.pntd.0004168 PMID: 26485145

24. Clancy EA, Rowan AN, 2003. Companion animal demographics in the United States: A historical perspective. In Salem D.J. & Rowan A.N. (Eds.), The state of the animals II: pp 9–26. Washington, DC: Humane Society Press.

25. US Department of Health and Human Services, Centers for Disease Control and Prevention. National Health and Nutrition Examination Survey, 2011–2012: Overview. Available at: https://www.cdc.gov/nchs/data/nhanes/nhanes_11_12/2011-12_overview_brochure.pdf. Accessed January 30, 2017.

26. Johnson CL, Dohrmann SM, Burt VL, Mohadjer LK. 2014. National Health and Nutrition Examination Survey: sample design, 2011–2014. National Center for Health Statistics. Vital Health Stat 2:162.

27. Parsons VL, Moriarity C, Jonas K, Moore TF, Davis KE, Tompkins L. Design and estimation for the national health interview survey, 2006–2015. Vital Health Stat 2. 2014(165):1–53.

28. US Department of Health and Human Services, Centers for Disease Control and Prevention. National Health and Nutrition Examination Survey, 2012–2012: Analytic Guidelines. Available at: https://www.cdc.gov/nchs/data/nhanes/analytic_guidelines_11_12.pdf. Accessed January 30, 2017.

29. National Health and Nutrition Examination Survey. (2017 JRM, 2017, from https://wwwn.cdc.gov/Nchs/Nhanes/2011-2012/SSTOCA_G.htm [

30. National Health and Nutrition Examination Survey. (2017 JRM, 2017, from https://wwwn.cdc.gov/Nchs/Nhanes/2013-2014/SSTOCA_H.htm.

31. Little SE, Johnson EM, Lewis D, Jaklitsch RP, Payton ME, Blagburn BL, et al. Prevalence of intestinal parasites in pet dogs in the United States. Vet Parasitol. 2009; 166(1–2):144–52. https://doi.org/10.1016/j.vetpar.2009.07.044 PMID: 19716659

32. Herrmann N, Glickman LT, Schantz PM, Weston MG, Domanski LM. Seroprevalence of zoonotic toxocariasis in the United States: 1971–1973. Am J Epidemiol. 1985; 122(5):890–6. PMID: 4050776

33. Overgaauw PA, van Knapen F. Veterinary and public health aspects of Toxocara spp. Vet Parasitol. 2013; 193(4):398–403. https://doi.org/10.1016/j.vetpar.2012.12.035 PMID: 23305972

34. Pinelli E, Aranzamendi C. Toxocara infection and its association with allergic manifestations. Endocr Metab Immune Disord Drug Targets. 2012; 12(1):33–44. PMID: 22214330

35. Population Health: Behavioral and Social Science Insights. AHRQ—Agency for Healthcare Research and Quality: Advancing Excellence in Health Care. U.S. HHS: Agency for Healthcare Research and Quality, 14 Sept. 2015. Available at: http://www.ahrq.gov/professionals/education/curriculum-tools/population-health/zimmerman.html. Accessed 10 April 2017.

36. Fillaux J, Magnaval JF. Laboratory diagnosis of human toxocariasis. Vet Parasitol. 2013; 193(4):327–36. https://doi.org/10.1016/j.vetpar.2012.12.028 PMID: 23318165

37. Starr MC, Montgomery SP. Soil-transmitted Helminthiasis in the United States: a systematic review—1940-2010. Am J Trop Med Hyg. 2011; 85(4):680–4. https://doi.org/10.4269/ajtmh.2011.11-0214 PMID: 21976572

38. Smith H, Holland C, Taylor M, Magnaval JF, Schantz P, Maizels R. How common is human toxocariasis? Towards standardizing our knowledge. Trends Parasitol. 2009; 25(4):182–8. https://doi.org/10.1016/j.pt.2009.01.006 PMID: 19269251

39. Prevention & Control. (2013, January 10). Retrieved February 22, 2017, from https://www.cdc.gov/parasites/toxocariasis/prevent.html

40. Holland C, O'Connor P, Taylor MR, Hughes G, Girdwood RW, Smith H. Families, parks, gardens and toxocariasis. Scand J Infect Dis. 1991; 23(2):225–31. https://doi.org/10.3109/00365549109023405 PMID: 1853172

41. Ascarid (also Roundworm, also Toxocara). (n.d.). Retrieved February 22, 2017, from https://www.capcvet.org/capc-recommendations/ascarid-roundworm

42. Nijsse R, Ploeger HW, Wagenaar JA, Mughini-Gras L. Toxocara canis in household dogs: prevalence, risk factors and owners' attitude towards deworming. Parasitol Res. 2015; 114(2):561–9. https://doi.org/10.1007/s00436-014-4218-9 PMID: 25468379

43. Nijsse R, Ploeger HW, Wagenaar JA, Mughini-Gras L. Prevalence and risk factors for patent Toxocara infections in cats and cat owners' attitude towards deworming. Parasitol Res. 2016; 115(12):4519–25. https://doi.org/10.1007/s00436-016-5242-8 PMID: 27637227

44. Wells DL. Public understanding of toxocariasis. Public Health. 2007; 121(3):187–8. https://doi.org/10.1016/j.puhe.2006.10.016 PMID: 17223143

45. Mizgajska-Wiktor H, Jarosz W, Fogt-Wyrwas R, Drzewiecka A. Distribution and dynamics of soil contamination with Toxocara canis and Toxocara cati eggs in Poland and prevention measures proposed after 20 years of study. Vet Parasitol. 2017; 234:1–9. https://doi.org/10.1016/j.vetpar.2016.12.011 PMID: 28115175

46. Marmor M, Glickman L, Shofer F, Faich LA, Rosenberg C, Cornblatt B, et al. Toxocara canis infection of children: epidemiologic and neuropsychologic findings. Am J Public Health. 1987; 77(5):554–9. PMID: 3565646

47. Centers for Disease Control and Prevention. Very high blood lead levels among adults—United States, 2002–2011. MMWR Morb Mortal Wkly Rep. 2013; 62(47):967–71. PMID: 24280917

48. Alvarado-Esquivel C, Hernandez-Tinoco J, Sanchez-Anguiano LF. Toxocara infection in gardeners: a case control seroprevalence study. Asian Pac J Trop Med. 2014; 7S1:S79–81. https://doi.org/10.1016/S1995-7645(14)60207-8 PMID: 25312196

49. Lotsch F, Obermuller M, Mischlinger J, Mombo-Ngoma G, Groger M, Adegnika AA, et al. Seroprevalence of Toxocara spp. in a rural population in Central African Gabon. Parasitol Int. 2016; 65(6 Pt A):632–4.

50. Li L, Gao W, Yang X, Wu D, Bi H, Zhang S, et al. Asthma and toxocariasis. Ann Allergy Asthma Immunol. 2014; 113(2):187–92. https://doi.org/10.1016/j.anai.2014.05.016 PMID: 24934109

51. Dattoli VC, Freire SM, Mendonca LR, Santos PC, Meyer R, Alcantara-Neves NM. Toxocara canis infection is associated with eosinophilia and total IgE in blood donors from a large Brazilian centre. Trop Med Int Health. 2011; 16(4):514–7. https://doi.org/10.1111/j.1365-3156.2010.02719.x PMID: 21410848

52. Lidsky TI, Schneider JS. Lead neurotoxicity in children: basic mechanisms and clinical correlates. Brain. 2003; 126(Pt 1):5–19. PMID: 12477693

53. Cassenote AJ, Lima AR, Pinto Neto JM, Rubinsky-Elefant G. Seroprevalence and modifiable risk factors for Toxocara spp. in Brazilian schoolchildren. PLoS Negl Trop Dis. 2014; 8(5):e2830. https://doi.org/10.1371/journal.pntd.0002830 PMID: 24874504

54. Lee AC, Schantz PM, Kazacos KR, Montgomery SP, Bowman DD. Epidemiologic and zoonotic aspects of ascarid infections in dogs and cats. Trends Parasitol. 2010; 26(4):155–61. https://doi.org/10.1016/j.pt.2010.01.002 PMID: 20172762

55. Raymond J, Brown MJ. Childhood Blood Lead Levels in Children Aged <5 Years—United States, 2009–2014. MMWR Surveill Summ. 2017; 66(3):1–10. https://doi.org/10.15585/mmwr.ss6603a1 PMID: 28103215

56. Fenoy S, Cuellar C, Aguila C, Guillen JL. Persistence of immune response in human toxocariasis as measured by ELISA. Int J Parasitol. 1992; 22(7):1037–8. PMID: 1459782

Urine containing sperm

Biomedicine,Microbes

Urine is not sterile, and neither is the rest of you

By Erika Engelhaupt 4:00pm, May 22, 2014

DON'T DO IT Urinating on a wound to clean it in an emergency has become fodder for urban legend, but new research debunks the idea that urine is sterile.

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Let's say you find yourself lying at the bottom of a ravine with a dirt-filled gash in your leg. According to the Internet, the first thing you want to do is pee on your wound. After all, the common wisdom holds, urine is sterile.

Wrong again, Internet.

Article follows

Urine is not sterile, even before it comes out of you and gets contaminated by your skin. Bacteria are present at low levels in the urine of healthy people not suffering from a urinary tract infection, Evann Hilt of Loyola University of Chicago reported May 18 at a conference of the American Society for Microbiology. Now, Hilt and her colleagues are figuring out what bacteria make up the normal bladder community and whether a change in that community might trigger urinary problems.

"Now that we know they're there, the question is what are they doing?" Hilt says. Most likely, she says, "it's like any other niche on our body. You have a good flora that keeps you healthy."

It appears that the urban legend about urine being sterile has its roots in the 1950s, Hilt says, when epidemiologist Edward Kass was looking for a way to screen patients for urinary tract infections before surgery. Kass developed

the midstream urine test (still used when you pee in a cup) and set a numerical cutoff for the number of bacteria in normal urine: not more than 100,000 colony-forming units (cell clusters on a culture dish) per milliliter of urine. A person tests "negative" for bacteria in their urine as long as the number of bacteria that grow in a lab dish containing the urine falls below this threshold. "It appears that the dogma that urine is sterile was an unintended consequence," Hilt says.

Hilt and her colleagues used a more sensitive growth-culture technique to detect the low levels of bacteria in normal urine, reasoning that maybe some urinary bacteria don't grow readily under the conditions of the standard test. Having already found bacterial genetic material in urine (as did another team), in their latest work they used catheters to collect urine directly from the bladders of 84 women, half of whom had overactive bladder syndrome, which causes patients to have to urinate frequently. They put samples of the urine in lab dishes and let the urine bacteria grow under friendlier conditions. More than 70 percent of the urine samples contained bacteria, including at least 33 types of bacteria (at the genus level) in normal urine. Women with overactive bladders had more types of bacteria in their urine (77 genera), including four species found only in overactive bladder patients.

This finding might provide hope for the 15 percent of women who suffer from overactive bladder; many aren't helped by the standard therapy that treats the condition as purely a muscular problem.

Learning that urine is not sterile also changes the way we think about infection. It had generally been assumed that if there are bacteria in your urinary tract, you have an infection and that's a bad thing. But if there is a normal community of bacteria, we may need to think about the bladder more in the way we have recently learned to think of the gut microbiome, in terms of "healthy" and "unhealthy" mixes of bacteria.

It's not clear anymore *what* body parts are actually sterile. The placenta was long thought to be, but scientists have just learned that's not true: They found bacteria on the baby's side of the placenta. There's also some evidence that babies are born with bacteria already in their guts, which must have gotten through the placenta.

And what about brains? Surely the braincase is last bacteria-free bastion, protected by the blood-brain barrier.

Sadly, that's not the case either. When I asked neuroscience writer Laura Sanders if our brains are sterile, she promptly said, "Oh, no. The brain's full of all kinds of junk." That includes viruses.

Last year, in fact, researchers reported finding soil bacteria in people's brains. (Before making a dirty-mind joke, these were alpha-proteobacteria normally found in soil, but there's no reason to think soil got into anyone's brain.) The researchers were studying whether people with a compromised immune system from HIV/AIDS might be prone to brain infections. Instead, they found that *all* the brains they looked at contained bacteria, regardless of HIV status. No one knows how the bacteria get in there, or when. Could they be leftovers from fetal development? Lucky tricksters that make it through the blood-brain barrier? We don't know, and just like for bladders, we don't even know what to consider "normal" yet.

So back to our original question: If urine isn't sterile, does that mean you shouldn't pee on a wound? Well, that was probably never a great idea anyway. If you don't have clean water, you're generally better off letting blood flow flush a wound, bathing it in infection-fighting white blood cells.

But if knowing there are bacteria in urine helps you talk a well-meaning friend out of peeing on you in an emergency, well, you're welcome.

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Urine Is Not Sterile: Use of Enhanced Urine Culture Techniques To Detect Resident Bacterial Flora in the Adult Female Bladder

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Our previous study showed that bacterial genomes can be identified using 16S rRNA sequencing in urine specimens of both symptomatic and asymptomatic patients who are culture negative according to standard urine culture protocols. In the present study, we used a modified culture protocol that included plating larger volumes of urine, incubation under varied atmospheric conditions, and prolonged incubation times to demonstrate that many of the organisms identified in urine by 16S rRNA gene sequencing are, in fact, cultivable using an expanded quantitative urine culture (EQUC) protocol. Sixty-five urine specimens (from 41 patients with overactive bladder and 24 controls) were examined using both the standard and EQUC culture techniques. Fifty-two of the 65 urine samples (80%) grew bacterial species using EQUC, while the majority of these (48/52 [92%]) were reported as no growth at 10^3 CFU/ml by the clinical microbiology laboratory using the standard urine culture protocol. Thirty-five different genera and 85 different species were identified by EQUC. The most prevalent genera isolated were *Lactobacillus* (15%), followed by *Corynebacterium* (14.2%), *Streptococcus* (11.9%), *Actinomyces* (6.9%), and *Staphylococcus* (6.9%). Other genera commonly isolated include *Aerococcus*, *Gardnerella*, *Bifidobacterium*, and *Actinobaculum*. Our current study demonstrates that urine contains communities of living bacteria that comprise a resident female urine microbiota.

Overactive bladder (OAB) is a highly prevalent syndrome characterized by urinary urgency with or without urge urinary incontinence and is often associated with frequency and nocturia (1). The etiology of OAB is often unclear and antimuscarinic treatments aimed at relaxing the bladder are ineffective in a large percentage of OAB sufferers, thereby suggesting etiologies outside neuromuscular dysfunction (2). One possibility is that OAB symptoms are influenced by microbes that inhabit the lower urinary tract (urinary microbiota).

The microbiota of the female urinary tract has been poorly described; primarily, because a "culture-negative" status has been equated with the dogma that normal urine is sterile. Yet, emerging evidence indicates that the lower urinary tract can have a urinary microbiota (3–8). For example, our group previously reported the use of 16S rRNA gene sequencing to identify bacterial DNA (urinary microbiome) in culture-negative urine specimens collected from women diagnosed with pelvic prolapse and/or urinary incontinence, as well as from urine of women without urinary symptoms (4). Other investigators also have used culture-independent 16S rRNA gene sequencing to obtain evidence of diverse bacteria that are not routinely cultivated by clinical microbiology laboratories in the urine of both women and men (3, 6, 9, 10).

Most of our previously sequenced urine specimens underwent standard clinical urine cultures that were reported as "no growth" at a 1:1000 dilution by our diagnostic microbiology laboratory (4). On the basis of this sequence-based evidence, which supports the presence of a urinary microbiome, we hypothesized that bacterial members of the urinary microbiota are not reported in routine urine cultures either because the number of organisms present is below the culture threshold of 10^3 CFU/ml or because growth of these bacteria required special culture conditions, such as anaerobic atmosphere, incubation in an increased CO_2 environment, or prolonged incubation time.

Furthermore, sequencing cannot determine whether the bacterial sequences observed in these culture-negative patients represent live bacterial species. Therefore, we conducted this study to determine whether the urinary microbiome was the product of living microbiota that consisted largely of organisms that would be missed by routine clinical microbiology culture practices. To address this question, we altered the routine urine culture conditions to include the plating of a greater volume of urine, incubation in varied atmospheric conditions, and the use of extended incubation times. Using these modified culture and incubation tactics (expanded quantitative urine culture [EQUC]), we demonstrated that many of the organisms identified in urine by 16S rRNA gene sequencing are in fact cultivable.

MATERIALS AND METHODS

Patients and sample collection. Following Loyola institutional review board (IRB) approval for all phases of this project, participants gave verbal and written consent for the collection and analysis of their urine for research purposes. Participants were women undergoing OAB treatment and a comparison group of women undergoing benign gynecologic surgery (controls). Participants' symptoms were characterized with the Pelvic Floor Distress Inventory (PFDI), a self-completed, validated symptom questionnaire. All participants were without clinical evidence of urinary tract infection (i.e., urine culture negative and absence of clinical urinary tract infection [UTI] diagnosis). Urine was collected via transurethral catheter from participants for the period March 2013 to July 2013 at the

Received 15 October 2013 Returned for modification 10 November 2013
Accepted 22 December 2013
Published ahead of print 26 December 2013
Editor: E. Munson
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doi:10.1128/JCM.02876-13

Female Pelvic Medicine and Reconstructive Surgery center of Loyola University Medical Center. A portion of each urine sample was placed in a BD Vacutainer Plus C&S preservative tube (Becton, Dickinson and Co, Franklin Lakes, NJ) and sent to the clinical microbiology lab for quantitative culture. A separate portion of the urine sample, to be used for sequencing, was placed at 4°C for no more than 4 h following collection. To this portion, 10% AssayAssure (Thermo Scientific, Waltham, MA) was added before freezing at −80°C.

Standard urine culture. Standard urine culture was performed by inoculating 0.001 ml of urine onto a 5% sheep blood agar plate (BAP) and MacConkey agars (BD BBL prepared plated media; Becton, Dickinson and Co., Sparks, MD) and streaking the entire plate surface to obtain quantitative colony counts. The plates were incubated aerobically at 35°C for 24 h. Each separate morphological colony type was counted and identified in any amount. The detection level was 10^3 CFU/ml, represented by 1 colony of growth on either plate. If no growth was observed, the culture was reported as "no growth" (of bacteria at lowest dilution, i.e., 1:1000).

Expanded quantitative urine culture. Each catheterized urine sample was processed following the standard urine culture procedure by the clinical microbiology lab and was also processed using the EQUC procedure. For EQUC, 0.1 ml of urine was inoculated onto BAP, chocolate and colistin, and nalidixic acid (CNA) agars (BD BBL prepared plated media), streaked for quantitation, and incubated in 5% CO_2 at 35°C for 48 h. For a second set of BAPs, each was inoculated with 0.1 ml of urine and incubated in room atmosphere at 35°C and 30°C for 48 h. Next, 0.1 ml of urine was inoculated onto each of two CDC anaerobe 5% sheep blood agar plates (BD BBL prepared plated media) and incubated in either a Campy gas mixture (5% O_2, 10% CO_2, 85% N) or under anaerobic conditions at 35°C for 48 h. The detection level was 10 CFU/ml, represented by 1 colony of growth on any of the plates. Finally, to detect any bacterial species that may be present at quantities lower than 10 CFU/ml, 1.0 ml of urine was placed in thioglycolate medium (BD BBL prepared tubed media) and incubated aerobically at 35°C for 5 days. If growth was visually detected in the thioglycolate medium, the medium was mixed and a few drops were plated on BAP and CDC anaerobe 5% sheep blood agars for isolation and incubated aerobically and anaerobically at 35°C for 48 h. Each morphologically distinct colony type was isolated on a different plate of the same media to prepare a pure culture that was used for identification.

Matrix assisted laser desorption ionization–time of flight mass spectrometry. Matrix assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) was performed using the direct colony method. Using toothpicks, we applied a small portion of a single isolated colony to the surface of a 96-spot, polished, stainless steel target plate (Bruker Daltonik GmbH, Leipzig, Germany) in a manner that created a thin bacterial film. The spot was left to dry at room temperature for 1 min., whereupon 1.0 μl of 70% formic acid was applied to each sample and allowed to dry at room temperature for 10 min. Then, 1.0 μl of the matrix solution, comprised of saturated α-cyano-4-hydrocinnamic acid (Bruker Daltonik) in an organic solvent (high-pressure liquid chromatography-mass spectrometry [HPLC-MS]-grade water, 100% trifluoroacetic acid, and acetonitrile; Fluka) was then applied to each sample and allowed to cocrystallize at room temperature for 10 min. The prepared sample target was placed in the MicroFlex LT mass spectrometer (Bruker Daltonik), and the results were analyzed by MALDI Biotyper 3.0 software (Bruker Daltonik). A bacterial quality control strain (*Escherichia coli* DH5α) was included in each analysis. A single measurement was performed once for each culture isolate.

Data analyses. MALDI Biotyper 3.0 software Realtime Classification was used to analyze the samples. In the Realtime Classification program, log score identification criteria are used as follows. A score between 2.000 and 3.000 is species-level identification, a score between 1.700 and 1.999 is genus-level identification, and a score that is below 1.700 is an unreliable identification. A Realtime Classification log score was given for each bacterial isolate sample for every condition from which it was isolated.

DNA isolation, PCR amplification and 16S rRNA amplicon sequencing. Genomic DNA was extracted from urine using previously validated protocols (4, 11). Briefly, 1 ml of urine was centrifuged at 13,500 rpm for 10 min and the resulting pellet was resuspended in 200 μl of filter-sterilized buffer consisting of 20 mM Tris-Cl (pH 8), 2 mM EDTA, 1.2% Triton X-100, and 20 μg/ml lysozyme and supplemented with 30 μl of filter-sterilized mutanolysin (5,000 U/ml; Sigma-Aldrich; St. Louis, MO). The mixture was incubated for 1 h at 37°C and the lysates were processed through the DNeasy blood and tissue kit (Qiagen, Valencia, CA) according to the manufacturer's protocol. The DNA was eluted into 50 μl of AE buffer (pH 8.0) and stored at −20°C.

The variable region 4 (V4) of the bacterial 16S rRNA gene in each DNA sample was amplified and sequenced using a custom protocol developed for the Illumina MiSeq. Briefly, the 16S rRNA V4 region was amplified in a two-step nested PCR protocol using the universal 515F and 806R primers, which were modified to contain the Illumina adapter sequences. Amplicons were analyzed by gel electrophoresis and purified using the QIAquick gel extraction kit (Qiagen). Extraction- and PCR-negative controls were included in all steps to assess potential DNA contamination. DNA samples were diluted to 10 nM, pooled, and sequenced using the MiSeq personal sequencer platform using a paired-end 2× 251-bp reagent cartridge. Raw sequences were processed using the open-source program mothur, v1.31.2 (12). Paired ends were joined and contigs of incorrect length (<285 bp or >300 bp) and/or contigs that contained ambiguous bases were removed. Sequences were aligned using the SILVA database, and chimeric sequences were removed with UCHIME (13). Sequences were classified using a naive Bayesian classifier and the RDP 16S rRNA gene training set (v9). Sequences that could not be classified to the bacterial genus level were removed from analysis.

RESULTS

Culture results. Sixty-five urine specimens (from 41 OAB patients and 24 controls) were examined using both the standard and EQUC techniques. Most (52/65 [80%]) grew bacterial species, with the majority of these (48/52 [92%]) reported as no growth at 10^3 by the clinical microbiology laboratory using the standard urine culture protocol.

Using the EQUC technique, we isolated 35 different genera (Fig. 1) and 85 different species (Table 1), as identified by MALDI-TOF. To isolate these species, a combination of different culture and incubation conditions were required. Most of the bacteria isolated required either increased CO_2 or anaerobic conditions for growth, along with prolonged incubation, and they often were present in numbers below the threshold of detection used in routine diagnostic urine culture protocols. With few exceptions, most bacteria were recovered on at least one of the primary plating media (data not shown). One case each of *Enterococcus faecalis*, *Rothia dentocariosa*, *Staphylococcus aureus*, and *Staphylococcus epidermidis* were recovered from thioglycolate broth only. A breakdown of the culture media and conditions for isolation of the 85 species is given in Table 1.

The most prevalent genera isolated were *Lactobacillus* (15%), followed by *Corynebacterium* (14%), *Streptococcus* (11.9%), *Actinomyces* (6.9%), and *Staphylococcus* (6.9%) (Fig. 1). Within each genus, the most frequently isolated species were *Lactobacillus gasseri*, *Corynebacterium coyleae*, *Streptococcus anginosus*, *Actinomyces neuii*, and *Staphylococcus epidermidis*. Other genera commonly isolated include *Aerococcus*, *Gardnerella*, *Bifidobacterium*, and *Actinobaculum*. The numbers of isolated species within each genus are listed in Table 1.

Lactobacillus, *Streptococcus*, *Corynebacterium*, *Staphylococcus*, *Actinomyces*, and *Bifidobacterium* spp. were isolated from both

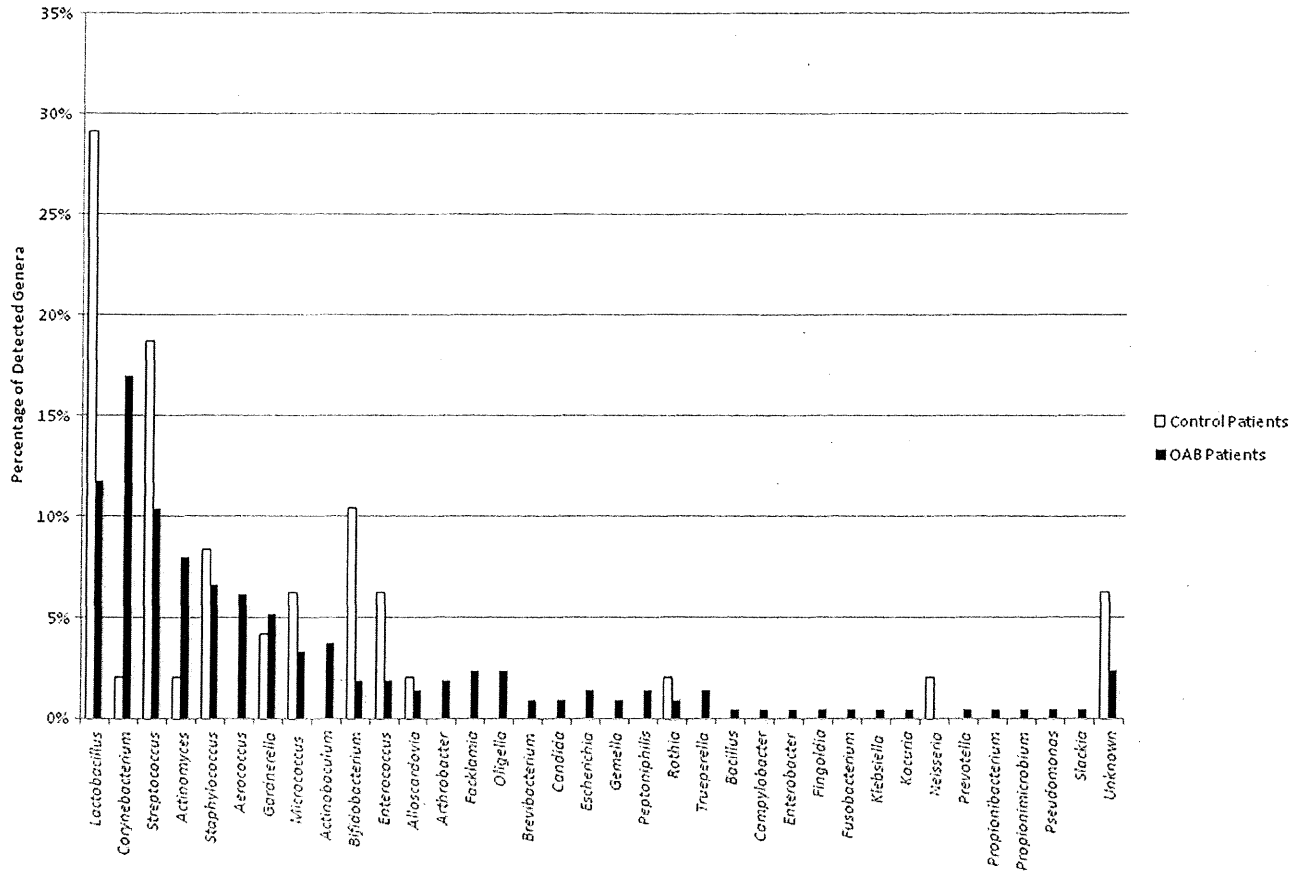

FIG 1 Percentages of detection of each genus normalized to the total organism isolated from the OAB patients (black bars, $n = 212$ isolates from 34 of 41 participants) or the controls (white bars, $n = 48$ isolates from 18 of 24 participants).

OAB and control cohorts. In contrast, *Aerococcus* and *Actinobaculum* were isolated only from OAB patients (Fig. 1).

16S rRNA amplicon sequencing results. To determine if the bacteria grown in culture (the microbiota) matched the bacterial DNA sequences (the microbiome) obtained from analysis of the same urine specimens, four OAB urine samples underwent 16S rRNA amplicon sequencing. The majority of the bacterial species that were detected by the EQUC procedure also were detected by 16S rRNA gene sequencing of the same urine from the same patient (Table 2). For example, in the urine sample of patient OAB18, the EQUC detected *Lactobacillus jensenii* and *Gardnerella vaginalis* at $>10^3$ CFU/ml. The 16S rRNA sequencing of that same urine sample found that 86.6% of the sequences were classified as *Lactobacillus* and 13.0% of the sequences were classified as *Gardnerella*. In each urine sample, the cultured genera represent >80% of the sequences obtained. Sequencing detected additional genera that were not detected by culture (data not shown), suggesting that those organisms were not viable and/or were not cultivable under the conditions tested.

DISCUSSION

In our previous report (4), we presented evidence of bacterial DNA (microbiome) in the bladders of adult women with and without lower urinary tract symptoms. In the present study, we have provided evidence of live bacteria in the adult female bladder and, importantly, demonstrated a correlation in matched urine samples between the bacteria isolated using our EQUC protocol and the bacterial sequences identified by 16S rRNA gene sequencing. These findings support our contentions that the urinary microbiome exists and that it is a reflection of living bacterial species that make up the resident flora (microbiota) in the adult female bladder.

These data support those of Khasriya and colleagues, who also found that expanded culture conditions detect diverse urinary bacteria missed by routine diagnostic laboratory testing methods (7). Furthermore, our results often parallel those of their previous study, which found *Staphylococcus*, *Streptococcus*, and *Lactobacillus* to be commonly isolated genera, results that closely match our findings. However, our results were not identical. For example, they isolated *Actinomyces*, *Aerococcus*, *Bifidobacterium*, and *Gardnerella* less frequently than we did. These differences might result from the relatively small sample sizes of both studies or from procedural differences. For example, we plated urine directly without centrifugation, while Khasriya and coauthors isolated bacteria from centrifuged urine sediments, which had been prepared from catheterized and voided urine specimens collected from patients with chronic lower urinary tract symptoms and healthy controls. Since the sedimentation procedure was enriched for bacteria associated with urothelial cells, the differences in results could reflect differences in the ability of certain bacteria to associate tightly

TABLE 1 List of bacterial species cultured in different conditions[a]

Organism (no. isolated) (n = 260)	Culture conditions				
	Aerobic, 35°C	Aerobic, 30°C	CO_2, 35°C	Anaerobic, 35°C	Campy (6% O_2, 10% CO_2) 35°C
Actinobaculum schaalii (7)	+		+	+	+
Actinobaculum urinae (1)				+	
Actinomyces europaeus (2)			+		
Actinomyces graevenitzii (1)					+
Actinomyces naeslundii (1)		+			
Actinomyces neuii (5)	+		+	+	
Actinomyces odontolyticus (2)	+		+	+	
Actinomyces oris (1)			+		
Actinomyces turicensis (3)	+	+	+	+	
Actinomyces urogenitalis (3)	+	+			
Aerococcus sanguinicola (3)	HD	HD	+		
Aerococcus urinae (9)	+	+	+	+	+
Aerococcus viridans (1)			+		
Alloscardovia omnicolens (4)	+		+	+	
Arthrobacter cumminsii (4)	+	+	+	+	
Bacillus subtilis (1)	HD	+			
Bifidobacterium bifidum (1)			+	+	
Bifidobacterium breve (6)			+	+	+
Bifidobacterium dentium (1)			+	+	
Bifidobacterium longum (1)				+	
Brevibacterium ravenspurgense (2)			+	+	
Campylobacter ureolyticus (1)	HD			+	
Candida glabrata (2)	+		+	+	
Corynebacterium afermentans (2)	+			+	
Corynebacterium amycolatum (3)		+	+	+	+
Corynebacterium aurimucosum (3)	+	+	+	+	
Corynebacterium coyleae (7)	+	+	+	+	+
Corynebacterium freneyi (2)	+		+	+	
Corynebacterium imitans (2)	+	+	+		
Corynebacterium lipophile group F1 (4)	+	+	+	+	+
Corynebacterium matruchotii (1)			+		
Corynebacterium minutissimum (1)		+		+	
Corynebacterium riegelii (4)	+	+	+	+	
Corynebacterium tuberculostearicum (2)	+				
Corynebacterium tuscaniense (3)	+		+		
Corynebacterium urealyticum (3)	+		+		
Enterobacter aerogenes (1)	+	+	+	+	+
Enterococcus faecalis (7)	+	+	+	+	+
Escherichia coli (3)	+	+	+	+	+
Facklamia hominis (5)	+	+		+	
Finegoldia magna (1)	HD			+	
Fusobacterium nucleatum (1)	HD			+	
Gardnerella vaginalis (10)	+	+	+	+	
Gardnerella spp. (3)	+		+	+	+
Gemella haemolysans (1)		+			
Gemella sanguinis (1)		+			
Klebsiella pneumoniae (1)	+	+	+	+	+
Kocuria rhizophila (1)		+			
Lactobacillus crispatus (6)			+	+	
Lactobacillus delbrueckii (1)			+	+	
Lactobacillus fermentum (1)					+
Lactobacillus gasseri (12)	+	+	+	+	+
Lactobacillus iners (6)	+		+	+	+
Lactobacillus jensenii (10)	+		+	+	+
Lactobacillus johnsonii (1)			+		
Lactobacillus rhamnosus (2)			+	+	
Micrococcus luteus (9)	+	+	+		+
Micrococcus lylae (1)	+		+		
Neisseria perflava (1)			+		

(Continued on following page)

TABLE 1 (Continued)

Organism (no. isolated) (n = 260)	Culture conditions				
	Aerobic, 35°C	Aerobic, 30°C	CO_2, 35°C	Anaerobic, 35°C	Campy (6% O_2, 10% CO_2) 35°C
Oligella urethralis (5)					+
Peptoniphilus harei (3)				+	
Prevotella bivia (1)				+	+
Propionibacterium avidum (1)				+	
Propionimicrobium lymphophilum (1)				+	
Pseudomonas aeruginosa (1)			+		
Rothia dentocariosa (1)	+		+		
Rothia mucilaginosa (2)	+		+		+
Slackia exigua (1)				+	
Staphylococcus capitis (1)	+		+	+	
Staphylococcus epidermidis (7)	+	+	+	+	+
Staphylococcus haemolyticus (2)	+		+		
Staphylococcus hominis (2)	+		+		
Staphylococcus lugdunensis (2)	+	+	+	+	+
Staphylococcus simulans (2)	+			+	+
Staphylococcus warneri (2)	+				+
Streptococcus agalactiae (1)	+	+		+	+
Streptococcus anginosus (15)	+	+	+	+	+
Streptococcus gallolyticus (1)	+	+	+	+	+
Streptococcus gordonii (1)		+	+	+	
Streptococcus parasanguinis (1)	+				
Streptococcus pneumonia/mitis/oralis (6)	+	+	+	+	+
Streptococcus salivarius (3)				+	
Streptococcus sanguinis (2)			+		
Streptococcus vestibularis (1)	HD		+	+	
Trueperella bernardiae (3)	+		+		
Unclassified #1 (1)	+	+			
Unclassified #2 (1)	+	+			+
Unclassified #3 (1)			+		
Unclassified #4 (1)	+	+			
Unclassified #5 (1)				+	
Unclassified #6 (1)	+		+		
Unclassified #7 (1)	+				
Unclassified #8 (1)	+		+	+	

[a] The + symbol designates the environmental conditions from which the organism was isolated and identified; HD indicates that only high-dilution (1 μl) urine was plated for that condition and that no growth was observed. A blank space designates no growth in that condition for that particular organism using a low-dilution (100-μl) inoculum.

TABLE 2 Comparison of cultured isolates to genera detected by 16S rRNA sequence in urine samples

Urine	Isolate cultured	CFU/ml	% of sequences per sample (genus level classification)
OAB18	*Lactobacillus jensenii*	>1,000	86.6
	Gardnerella vaginalis	>1,000	13.0
OAB21	*Lactobacillus jensenii*	140	92.4[a]
	Lactobacillus iners	120	
	Gardnerella vaginalis	40	4.9
OAB23	*Gardnerella vaginalis*	>1,000	80.0
	Rothia dentocariosa	Broth[b]	Not detected
	Streptococcus anginosus	60	0.07
	Aerococcus urinae	50, 60	0.13
	Enterococcus faecalis	Broth[b]	0.001
OAB26	*Gardnerella vaginalis*	300	97.2

[a] Sequence data cannot distinguish between species.
[b] Cultured in thioglycolate broth; therefore, unable to determine starting CFU/ml.

with urothelial cells. Further studies are required to determine if this is true. Finally, Khasriya and coworkers did not report the recovery of *Actinobaculum*, an emerging uropathogen (14). Since these authors did not perform 16S rRNA gene sequencing of their sedimented urine samples, they could not know if *Actinobaculum* might have been present in their samples but could not grow in culture using their protocol.

Similar results might be found in men. Using 16S rRNA gene sequencing to identify bacteria in first-catch urine specimens collected from asymptomatic men, Nelson and colleagues showed that the overwhelming majority of the urine sequences corresponded to a few abundant genera. In their analyses, 72 genera were detected in total, with four genera (namely, *Lactobacillus*, *Corynebacterium*, *Streptococcus*, and *Sneathia*) accounting for approximately 50% of the total urine sequences. They concluded that these organisms represented the urinary microbiome of men (9). However, culture-based studies of the urine samples were not included in their analyses. EQUC procedures could be used to determine if the sequences identified in the urine samples represent living bacteria.

In our previously reported study (4), we used 16S rRNA gene

sequencing to demonstrate evidence of uncultivated bacteria in the adult female bladder and we questioned the "sterile urine" dogma. Our current study demonstrates that urine contains communities of living bacteria that comprise a resident female urine microbiota. More specifically, we have shown that the bacterial sequences detected in the adult female bladder by 16S rRNA sequencing represent living organisms that can be grown when culture conditions are modified to include plating of larger quantities of urine, incubating in an atmosphere of increased CO_2, and extending incubation to 48 h.

Further studies are needed to elucidate the role of the urinary microbiota in health and disease, and the complementary features of EQUC and 16S rRNA sequencing will facilitate those efforts. Each technique possesses distinct advantages and disadvantages. Deep 16S rRNA sequencing is a high-throughput technology that facilitates rapid screening at great depth, permitting researchers to obtain a broad and deep overview of the microbiota without the need to cultivate. However, the advantage provided by the speed and depth of the high-throughput technology is balanced by the inability to reliably identify bacteria below the genus level. In contrast, EQUC can capture the bacterium, but only if it can be cultivated. However, any isolated bacterium can be identified to the species level by MALDI-TOF. With the bacterium in hand, full-scale characterization is now possible, including the ability to sequence its genome, which could identify the organism to the strain level.

ACKNOWLEDGMENTS

We gratefully acknowledge Bozena Zemaitaitis for her help in processing the urine samples.

We acknowledge the following unrestricted research funding sources: Falk Foundation Research Award, Loyola University Chicago Stritch School of Medicine Research Funding Committee, and Doyle Family Philanthropic Support (to L. Brubaker and A. J. Wolfe). This study was supported in part by Astellas Medical and Scientific Affairs and is registered at www.clinicaltrials.gov as NCT01642277. The MicroFlex LT mass spectrometer used in this study was furnished by Bruker Daltonik GmbH, Leipzig, Germany.

REFERENCES

1. Stewart W, Van Rooyen J, Cundiff G, Abrams P, Herzog A, Corey R, Hunt T, Wein A. 2003. Prevalence and burden of overactive bladder in the United States. World J. Urol. 20:327–336. http://dx.doi.org/10.1007/s00345-002-0301-4.
2. Nitti VW, Kopp Z, Lin AT, Moore KH, Oefelein M, Mills IW. 2010. Can. we predict which patient will fail drug treatment for overactive bladder? A think tank discussion. Neurourol. Urodyn. 29:652–657. http://dx.doi.org/10.1002/nau.20910.
3. Nelson DE, Dong Q, Van der Pol B, Toh E, Fan B, Katz BP, Mi D, Rong R, Weinstock GM, Sodergren E, Fortenberry JD. 2012. Bacterial communities of the coronal sulcus and distal urethra of adolescent males. PLoS One 7:e36298. http://dx.doi.org/10.1371/journal.pone.0036298.
4. Wolfe AJ, Toh E, Shibata N, Rong R, Kenton K, Fitzgerald M, Mueller ER, Schreckenberger P, Dong Q, Nelson DE, Brubaker L. 2012. Evidence of uncultivated bacteria in the adult female bladder. J. Clin. Microbiol. 50:1376–1383. http://dx.doi.org/10.1128/JCM.05852-11.
5. Fouts DE, Pieper R, Szpakowski S, Pohl H, Knoblach S, Suh MJ, Huang ST, Ljungberg I, Sprague BM, Lucas SK, Torralba M, Nelson KE, Groah SL. 2012. Integrated next-generation sequencing of 16S rDNA and metaproteomics differentiate the healthy urine microbiome from asymptomatic bacteriuria in neuropathic bladder associated with spinal cord injury. J. Transl. Med. 10:174. http://dx.doi.org/10.1186/1479-5876-10-174.
6. Siddiqui H, Nederbragt AJ, Lagesen K, Jeansson SL, Jakobsen KS. 2011. Assessing diversity of the female urine microbiota by high throughput sequencing of 16S rDNA amplicons. BMC Microbiol. 11:244. http://dx.doi.org/10.1186/1471-2180-11-244.
7. Khasriya R, Sathiananthamoorthy S, Ismail S, Kelsey M, Wilson M, Rohn JL, Malone-Lee J. 2013. Spectrum of bacterial colonization associated with urothelial cells from patients with chronic lower urinary tract symptoms. J. Clin. Microbiol. 51:2054–2062. http://dx.doi.org/10.1128/JCM.03314-12.
8. Lewis DA, Brown R, Williams J, White P, Jacobson SK, Marchesi JR, Drake MJ. 2013. The human urinary microbiome; bacterial DNA in voided urine of asymptomatic adults. Front. Cell. Infect. Microbiol. 3:41. http://dx.doi.org/10.3389/fcimb.2013.00041.
9. Nelson DE, Van Der Pol B, Dong Q, Revanna KV, Fan B, Easwaran S, Sodergren E, Weinstock GM, Diao L, Fortenberry JD. 2010. Characteristic male urine microbiomes associate with asymptomatic sexually transmitted infection. PLoS One 5:e14116. http://dx.doi.org/10.1371/journal.pone.0014116.
10. Dong Q, Nelson DE, Toh E, Diao L, Gao X, Fortenberry JD, Van der Pol B. 2011. The microbial communities in male first catch urine are highly similar to those in paired urethral swab specimens. PLoS One 6:e19709. http://dx.doi.org/10.1371/journal.pone.0019709.
11. Yuan S, Cohen DB, Ravel J, Abdo Z, Forney LJ. 2012. Evaluation of methods for the extraction and purification of DNA from the human microbiome. PLoS One 7:e33865. http://dx.doi.org/10.1371/journal.pone.0033865.
12. Kozich JJ, Westcott SL, Baxter NT, Highlander SK, Schloss PD. 2013. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. Appl. Environ. Microbiol. 79:5112–5120. http://dx.doi.org/10.1128/AEM.01043-13.
13. Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R. 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27:2194–2200. http://dx.doi.org/10.1093/bioinformatics/btr381.
14. Cattoir V. 2012. *Actinobaculum schaalii*: review of an emerging uropathogen. J. Infect. 64:260–267. http://dx.doi.org/10.1016/j.jinf.2011.12.009.

PLOS | ONE

RESEARCH ARTICLE

Clinical Background of Patients with Sperm in Their Urinary Sediment

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Citation: Tomita M, Kikuchi E, Maeda T, Kabeya Y, Katsuki T, Oikawa Y, et al. (2015) Clinical Background of Patients with Sperm in Their Urinary Sediment. PLoS ONE 10(9): e0136844. doi:10.1371/journal.pone.0136844

Editor: Harald Mischak, University of Glasgow, UNITED KINGDOM

Received: April 28, 2015

Accepted: August 10, 2015

Published: September 11, 2015

Data Availability Statement: All data underlying the findings in this study are freely available as the supporting information.

Funding: The authors have no support or funding to report.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

Introduction

The detection rate and associated factors of at least one sperm in urinary sediment is not well-known in real clinical practice.

Aims

The aim of the present study was to evaluate the clinical features associated with the presence of sperm in urinary sediment in a large number of samples.

Methods

We conducted a cross-sectional study at Tokyo Saiseikai Central Hospital. We identified 5,005 males who were aged ≥20 years in whom urinary sedimentation had been performed at least twice between May 2011 and June 2012. The sperm group included patients in whom at least one urinary sediment test performed under a microscope had detected at least one sperm. We evaluated the associations between the presence of at least one sperm in urinary sediment and clinical parameters such as various diseases and the use of particular oral medicines.

Main Outcomes

In total, 1.6% (339/20,937) of urinary sediment samples contained at least one sperm. The sperm group consisted of 282 subjects (5.6%), and the no-sperm group included 4,723 subjects (94.3%).

Results

Multivariate analysis demonstrated that younger age (<65) (odds ratio [OR]: 1.71, 95% confidence interval [CI]: 1.32–2.21), the total number of examinations (≥4) (OR: 1.46, 95%CI: 1.11–1.92), diabetes (OR: 1.72, 95%CI: 1.31–2.25), a history of pelvic surgery for colon cancer (OR: 4.89, 95%CI: 2.38–10.02), alpha-1 blocker use (OR: 1.55, 95%CI: 1.16–2.08), a history of trans-urethral resection of the prostate (OR: 2.77, 95%CI: 1.46–5.13), and selective serotonin reuptake inhibitor use (OR: 2.12, 95%CI: 1.07–4.19) were independent predictors of the presence of at least one sperm in urinary sediment.

Conclusion

There is considerable overlap between the factors associated with the presence of at least one sperm in urinary sediment and those that are strongly associated with ejaculatory disorders.

Introduction

Urinary sedimentation by centrifugal separation followed by a microscopic examination of the components of the sediment is routinely used to evaluate the general condition of urine and to detect kidney and urinary tract diseases in a timely and non-invasive manner. Most of the cellular components found in urinary sediment originate from the urinary tract, but sperm are occasionally detected. Sperm in urinary sediment are usually derived from the first post-ejaculatory voiding [1], and in older men sperm are sometimes found in urinary sediment due to reduced contraction of the internal urethral sphincter [2]. Furthermore, retrograde ejaculation (RE) causes a large number of sperm to be present in urinary sediment [2,3]. Although the only presence of sperm in urine does not imply RE [4], the presence of sperm in urinary sediment is an important factor in the diagnosis of RE [3]. However, there is no consensus as to defining of RE [5] and the rate of RE is subjectively evaluated by not-validated self-reported questionnaires in most of the studies [5–9]. Meanwhile, to the best of our knowledge there have not been any studies about the detection rate of at least one sperm in urinary sediment samples subjected to microscopic examinations, nor have any studies evaluated the associations between such a finding and clinical factors such as the presence of, or a history of, certain conditions or the use of particular medications. In fact, medical-staff often conduct routine urinary tests without paying particular attention to the presence/absence of sperm. Therefore, in the present study we evaluated 1) the detection rate of at least one sperm in urinary sediment in a large number of samples, 2) the associations between such a finding and clinical background factors, and 3) independent predictors for the presence of at least one sperm in urinary sediment.

Materials and Methods

Urinalysis and urinary sediment were tested in 8,509 patients at Tokyo Saiseikai Central Hospital during the May 2011 to June 2012. We excluded the patients in whom urinalysis and urinary sedimentation tests had been performed only once (n = 3,504), which left 5,005 males aged ≥20 years (total number of measurements: 20,937) patients in whom the tests were performed at least twice. Among the 5,005 subjects, urinalysis and urinary sedimentation test were performed due to routine work-up for urological disease (N = 2,002), general check-ups for

disorders of internal medicine (N = 2,600), health medical check-ups (N = 305), and unknown reasons (N = 98). The patients' first urinary samples were discarded, and their second urinary samples were collected. The urine samples were submitted promptly after micturition and were analyzed using a fully automated urine element analyzer (UF-1000i, Sysmex Corporation, Kobe, Japan). If the analyzer detected the presence of a foreign body, trained medical technicians visually examined the sample under a microscope for the presence of at least one sperm under high magnification (400×, HPF). The sperm group included patients whose urine contained at least one sperm according to at least one urinary sediment test performed under a microscope, while the no-sperm group included patients in whom sperm was not detected in any urinary sediment test. We evaluated the associations between the presence of at least one sperm in urinary sediment and clinical background factors such as hypertension, dyslipidemia, diabetes, a history of pelvic surgery due to colorectal cancer, cardiovascular disease, prostatitis or transurethral resection of the prostate (TURP); or the use of selective serotonin reuptake inhibitors (SSRI), proton pump inhibitors (PPI), H2 blockers, or alpha-1 blockers by performing comparisons between the two groups **(Data in** S1 File).

The Student's unpaired *t* test was used to compare continuous variables, while the χ^2 test was used for comparisons between categorical variables. Logistic regression analysis was performed to calculate odds ratios (OR) for the presence of at least one sperm in urine. First, univariate analysis was performed to identify variables that were significantly associated with the presence of at least one sperm in urinary sediment. Variables for which $P < 0.01$ were then included in the multiple logistic regression analysis. The multivariate model was tested for goodness of fit using the Hosmer-Lemeshow test, which showed that the fit of the model was acceptable ($P > 0.05$). All analyses were performed using the STATA software program (version 10; StataCorp, Texas, USA). This study was a retrospective observation fashion and it was difficult to receive informed consents from the past participants. This study was approved as the following contents by the ethics committee of Saiseikai Central Hospital (No. 220). We displayed study contents inside the hospital and exclude the data from the study cohort if the patients hope to exclude it.

Results

Comparison of clinical features between the sperm group and no-sperm group

The study population consisted of 5,005 males (mean age: 66.0 ± 12.9 years) in whom urinary sedimentation was performed at least twice during the study period. The patients' clinical background data are shown in Table 1. The mean number of examinations per patient was 4.2. In total, 1.6% (339/20,937) of the urinary sediment samples contained at least one sperm.

The sperm group consisted of 282 people (5.6%), and the no-sperm group included 4,723 people (94.3%). Significant differences in age; the total number of urinary sediment examinations; and the frequencies of hypertension, dyslipidemia, diabetes, alpha-1 blocker use, SSRI use, TURP, and pelvic surgery due to colorectal cancer were observed between the sperm and no-sperm groups.

Independent indicators for the presence of at least one sperm in urinary sediment

Table 2 shows the results of the uni- and multivariate analyses, which aimed to identify independent factors for the presence of at least one sperm in urinary sediment. In the univariate analysis, younger age (<65), the total number of urinary sediment examinations (≥4),

Table 1. Background characteristics of participants.

Characteristics	All subjects N = 5005	Presence of sperm		p value
		Yes N = 282(5.6%)	No N = 4723(94.3%)	
Age(years)	66.0±12.9	63.8±11.5	66.1±12.9	0.003
The number of measurement	4.2±2.5	4.7±2.4	4.2±2.4	<0.001
Hypertension (%)	1953(39.0)	132(46.8)	1821(38.6)	0.006
Dyslipidemia (%)	1216(24.3)	86(30.5)	1130(23.9)	0.012
Diabetes (%)	1663(33.2)	135(47.8)	1528(32.3)	<0.001
Past history of cardio-vascular disease (%)	788(15.7)	55(19.5)	733(15.5)	0.074
The use of alpha-1 blocker agent (%)	1015(20.3)	72(25.5)	943(19.9)	0.024
Past history of intrapelvic surgery for colon cancer (%)	53(1.1)	10(3.5)	43(0.9)	<0.001
Past history of TUR-P (%)	113(2.3)	12(4.3)	101(2.1)	0.020
Prostatitis (%)	108(2.2)	5(1.8)	103(2.2)	0.640
The use of PPI or H2-bloker (%)	970(19.4)	59(20.9)	911(19.3)	0.500
The use of SSRI(%)	88(1.8)	10(3.6)	78(1.6)	0.020

Data are n(%) or mean±SD, TUR-P:trans-urethral resection of the prostate, PPI: proton pump inhibitors, SSRI: selective serotonin reuptake Inhibitors
The study population consisted of 5,005 males and their clinical background data are shown in this table

doi:10.1371/journal.pone.0136844.t001

hypertension, dyslipidemia, diabetes, a history of pelvic surgery for colon cancer, alpha-1 blocker use, a history of TURP, and SSRI use were significantly associated with the presence of at least one sperm in urinary sediment. In the multivariate analysis adjusted for these factors, younger age (<65), total number of urinary sediment examinations (≥4), diabetes, a history of pelvic surgery for colon cancer, alpha-1 blocker use, a history of TURP, and SSRI use were demonstrated to be independent indicators for the presence of at least one sperm in urinary sediment. The detection rate of at least one sperm in urinary sediment was 1.6% (11/678), 4.1% (72/1,737), 6.2% (109/1,755), 10.6% (81/763) and 12.9% (9/70) in patients who had no, one, two, three and four independent associated factors.

The association between types of alpha-1 blocker and the presence of at least one sperm in urinary sediment

Of the 1,015 patients treated with an alpha-1 blocker, 574 (56.6%), 278 (27.4%), 162 (16.0%), and 1 patient were treated with tamsulosin, silodosin, naftopidil, and urapidil, respectively. The detection rate of at least one sperm in the urinary sediment in patients treated with tamsulosin, silodosin, and naftopidil was 5.6% (32/574), 10.4% (29/278), and 6.8% (11/162), respectively. There were significant differences in the detection rate of at least one sperm in the urinary sediment between patients treated with and without silodosin ($p = 0.011$).

The association between therapeutic type for diabetes mellitus and the presence of at least one sperm in urinary sediment

Among the diabetic patients, 1621 (97.5%) had type 2 diabetes and 42 (2.5%) type 1 diabetes. Their medical regimens were as follows: diet therapy alone, 35 (2.1%); oral hypoglycemic agent user, 1,007 (60.6%); and insulin user, 621 (37.3%).

The detection rate of at least one sperm in urinary sediment was 19.1% in type 1 diabetes patients, which was significantly higher than that in type 2 (7.8%, $p<0.05$). In addition the detection rate of at least one sperm in urinary sediment was 10.3% in patients treated with

Table 2. Associated factors for at least one sperm in urinary sediment.

Variables		All subjects (N = 5005)		Univariate	Multivariate	
		No sperm group N = 4723	Sperm group N = 282	Pvalue	OR (95% CI)	Pvalue
Age (years)	<65	1967	140	0.008	1.71 (1.32–2.21)	<0.001
	≥65	2756	142		1	
Total number of measurement of urinary sediment	2-<4	2196	92	<0.001	1	0.006
	≥4	2597	190		1.46 (1.11–1.92)	
Hypertension	NO	2902	150	0.009		0.390
	YES	1821	132			
Dyslipidemia	NO	3593	196	0.013		0.620
	YES	1130	86			
Diabetes mellitus	NO	3195	147	<0.001	1	<0.001
	YES	1528	135		1.72 (1.31–2.25)	
Past history of cardio-vascular disease	NO	733	55	0.070		0.350
	YES	3990	227			
Prostatitis	NO	4620	277	0.640		0.780
	YES	103	5			
The use of PPI or H2-bloker	NO	3812	223	0.500		0.860
	YES	911	59			
History of pelvic surgery for colon cancer	NO	4680	272	<0.001	1	<0.001
	YES	43	10		4.89 (2.38–10.02)	
The use of alpha- 1 blocker agent	NO	3780	210	0.024	1	0.003
	YES	943	72		1.55 (1.16–2.08)	
Past history of TURP	NO	4622	270	0.009	1	0.002
	YES	101	12		2.77 (1.46–5.13)	
The use of SSRI	NO	4645	272	0.022	1	0.030
	YES	78	10		2.12 (1.07–4.19)	

TUR-P, Trans-urethral resection of the prostate; PPI, proton pump inhibitor; SSRI, selective serotonin reuptake inhibitors;
OR, odds ratio; CI, confidence interval

The results of the uni- and multivariate analyses identified independent factors of the presence of at least one sperm in urinary sediment.

doi:10.1371/journal.pone.0136844.t002

insulin, which was significantly higher than those treated with an oral hypoglycemic agent (6.3%, p = 0.001).

Discussion

To the best of our knowledge, this is the first study to determine the incidence of at least one sperm in urinary sediment using a large number (more than 5,000) of patients and to evaluate the association between such a finding and clinical background factors. To date, the presence of sperm in urinary sediment has been treated as having little clinical significance and is usually seen in physiological conditions in which sperm has been incorporated into urine due to the mixing of semen components retained in the urethra following sexual activity or masturbation [2]. The present study demonstrated that 1.6% (339/20,937) of urinary sediment samples contained at least one sperm and that 5.6% (282/5,005) of general clinical practice patients who undergo urinary sediment examinations submitted samples that contain at least one sperm. Furthermore, the presence of at least one sperm in urinary sediment was found to be

independently associated with diabetes, a history of pelvic surgery for colon cancer, a history of TURP, and alpha-1 blocker or SSRI use, which are known risk factors for ejaculation disorders [3,10]. We found that there is considerable overlap between the factors associated with the presence of at least one sperm in urinary sediment and those that are strongly associated with RE [11]. There have been many studies evaluating the medical factors associated with ejaculatory disorder such as RE. However, the problem is the definition of RE in the literature is not standardized and the rate of RE is subjectively evaluated by not-validated self-reported questionnaires in most of the studies [5,9]. For instance, in regard to the use of alpha-1 blockers, which is known to be a strong associated factor for RE, the incidence of RE has been reported to range widely from 0.9% to 28.1% due to different definitions for RE and the use of different methods for the evaluation of RE [6–8]. The primary goal of the present study was not to determine the incidence and risk factors of RE, but rather to identify what clinical background may have an association with "the presence of at least one sperm in urinary sediment" in real clinical practice. Interestingly, we found that associated factors in the case of "the presence of at least one sperm in urinary sediment" are similar to those related with so-called RE.

Accordingly, two hypotheses can be proposed for the close association between the presence of at least one sperm in urinary sediment and the abovementioned clinical factors. The first possibility is that chronic neurogenic impairments or direct damage to nerve paths results in the dysfunction of the internal sphincter of the urethra, leading to insufficient closure of the internal urethral orifice and the leakage of sperm into the posterior urethra [12,13]. In our study, the incidence of the presence of at least one sperm in urinary sediment was 8.1% (135/1,663) and 18.9% (10/53) in patients with diabetes and those who had undergone pelvic surgery for colorectal cancer, respectively. Diabetic autonomic neuropathy contributes to a wide spectrum of clinical disorders including ejaculation disorders [14–16] and is reported to affect about one-third of men with diabetes [15]. Furthermore, damage to the nerve paths involved in ejaculation is the main reason for ejaculation disorders in patients with a history of pelvic surgery for colorectal cancer [11]. Especially in diabetic patients, the type of treatment for diabetes is significantly associated with the detection rate of at least one sperm in urinary sediment in our present study. In fact, patients treated with insulin therapy had a significantly higher detection rate than those treated with an oral hypoglycemic agent. This might be explained in part by the speculation that the severity of diabetes could be associated with the detection rate of at least one sperm in urinary sediment. We plan to evaluate whether various factors, such as HbA1c level, duration of diabetes mellitus, and presence of diabetes-related complications could be associated with at least one sperm in urinary sediment in a future study.

The second explanation involves traumatic or drug-induced impairments that directly affect the closure of the internal urethral orifice of the bladder neck during ejaculation [10,11,17]. Our study demonstrated that the incidence rate of the presence of at least one sperm in urinary sediment was 7.1% (72/1,015), 11.4% (10/88), and 10.6% (12/113) in patients who used alpha-1 blockers, those who used SSRI, and those with a history of TURP, respectively. It has been reported that RE occurs in 0.9–28.1% of alpha-1 blocker users [6,7,18], and psychotropic drugs such as SSRI are associated with sexual dysfunction including erectile dysfunction, anorgasmia, and RE [19]. We further evaluated whether type of alpha-1 blocker could affect the incidence of at least one sperm in urinary sediment in our study population. We found that patients treated with silodosin had a significantly higher detection rate of at least one sperm in the urinary sediment, as compared to other types of alpha-1 blocker such as tamsulosin and naftopidil. Interestingly, the detection rate of at least one sperm in the urinary sediment in patients treated with silodosin was 10.4% in our study, which was lower than the incidence rate of RE (14.2%-28.1%) due to silodosin in previous reports [6–8]. Furthermore, RE is one of the main

complications of TURP [11,20], and the incidence of RE after TURP varies from 36% to 100% depending on the degree of bladder neck resection [20,21].

The present study has several strengths. Firstly, it involved a large sample size (more than 5,000 patients), which reduced the risk of selection bias. Secondly, the sperm detection method used in this study was highly accurate; i.e., sperm was detected using a urinary element analyzer, and positive findings were confirmed by trained medical technicians who were unaware of the purpose of the study. Previously we compared the detection rate for the presence of sperm in urine examined using the automatic analyzer to that evaluated by a laboratory technician. With a sample size of 150 patients, 4 patients were found to have at least one sperm in urinary sediment by a laboratory technician and of these 4 the automatic analyzer could detect sperm in one patient, so the false negative rate by the analyzer was 75%. One hundred and forty-six patients were found to have no sperm in urinary sediment by the laboratory technician and the automatic analyzer could detect no sperm in these 146 patients, so the false positive rate by the analyzer was 0%. The overall concordance rate was 98%. However, the limitations of the present study should also be mentioned. Firstly, the total number of urinary sediment examinations differed among the subjects. Interestingly, among the patients in the sperm group, not all of their samples were found to contain sperm. Therefore, we only included patients who underwent urinalysis and urinary sedimentation testing at least twice during the observation period. The mean number of examinations per patient was 4.2, and 30.1±15.4% of the urinary sediment examinations included assessments of the presence/absence of sperm. Second, of seven independent indicators for the presence of at least one sperm in urinary sediment, four factors (younger age (<65), the total number of examinations (≥ 4), diabetes, and alpha-1 blocker use) were weak independent factors with odds ratio lower than 2. We cannot deny the possibility that these factors were extracted with some biases [22]. Thirdly, we do not have any data about the patients' sexual activity such as whether the urine samples they provided were collected just after sexual intercourse or masturbation, which is strongly associated with the presence of sperm in urinary sediment. We routinely instructed the patients to discard their first urine samples and hand in their second urine samples because there is a high incidence of contamination due to debris normally present at the urethral opening in the first few drops. This could also minimize the chances of their samples being contaminated by sperm that had remained in the urethra.

Conclusion

In conclusion, approximately 1.6% of all urinary sediment samples examined in daily clinical practice contain at least one sperm. There is considerable overlap between the factors associated with the presence of at least one sperm in urinary sediment and those that are strongly associated with ejaculatory disorders. These findings could help physicians to understand the clinical background of patients whose urinary sediment contains at least one sperm.

Supporting Information

S1 File. Background characteristics of participants. The patitens' number (de-identified) and clinical parameters were described.
(PDF)

Author Contributions

Conceived and designed the experiments: MT EK TM M. Ohashi. Performed the experiments: MT EK TM YK TK. Analyzed the data: MT EK TM. Contributed reagents/materials/analysis

tools: MT EK TM YK TK. Wrote the paper: MT EK TM M. Ohashi. Final approval of the complete article: MT EK TM YK TK YO KK M. Ohashi SN M. Oya AS.

References

1. Sivananthan T, Bathur F, Jimenez M, Conway A, Idan A, Handelsman D (2012) Objective non-intrusive markers of sperm production and sexual activity. Asian J Androl 14: 476–480. doi: 10.1038/aja.2012.2 PMID: 22522506
2. Examinaton of Urinary Sediment 2010, JCCLS Standard Guideline for Urinary Sediment Examinaton, JCCLS Document GP1-P4 Proposed Guideline(2010). Japanese Association of Medical Technologists.
3. Colpi G, Weidner W, Jungwirth A, Pomerol J, Papp G, Hargreave T, et al. (2004) EAU guidelines on ejaculatory dysfunction. Eur Urol 46: 555–558. PMID: 15474262
4. Ariagno JI, Mendeluk GR, Pugliese MN, Sardi SL, Acuna C, Repetto HE, et al. (2005) The only presence of sperm in urine does not imply retrograde ejaculation. Arch Androl 51: 431–436. PMID: 16214728
5. Fedder J, Kaspersen MD, Brandslund I, Hojgaard A (2013) Retrograde ejaculation and sexual dysfunction in men with diabetes mellitus: a prospective, controlled study. Andrology 1: 602–606. doi: 10.1111/j.2047-2927.2013.00083.x PMID: 23606485
6. Chapple CR, Montorsi F, Tammela TL, Wirth M, Koldewijn E, Fernandez Fernandez E, et al. (2011) Silodosin therapy for lower urinary tract symptoms in men with suspected benign prostatic hyperplasia: results of an international, randomized, double-blind, placebo- and active-controlled clinical trial performed in Europe. Eur Urol 59: 342–352. doi: 10.1016/j.eururo.2010.10.046 PMID: 21109344
7. Kawabe K, Yoshida M, Homma Y, Silodosin Clinical Study G (2006) Silodosin, a new alpha1A-adrenoceptor-selective antagonist for treating benign prostatic hyperplasia: results of a phase III randomized, placebo-controlled, double-blind study in Japanese men. BJU Int 98: 1019–1024. PMID: 16945121
8. Marks LS, Gittelman MC, Hill LA, Volinn W, Hoel G (2009) Rapid efficacy of the highly selective alpha1A-adrenoceptor antagonist silodosin in men with signs and symptoms of benign prostatic hyperplasia: pooled results of 2 phase 3 studies. J Urol 181: 2634–2640. doi: 10.1016/j.juro.2009.02.034 PMID: 19371887
9. Rosen RC, Catania JA, Althof SE, Pollack LM, O'Leary M, Seftel AD, et al. (2007) Development and validation of four-item version of Male Sexual Health Questionnaire to assess ejaculatory dysfunction. Urology 69: 805–809. PMID: 17482908
10. Yee CL, Pal RP, Batchelder A, Khan MA (2012) Risk of erectile dysfunction and retrograde ejaculation associated with thulium laser vaporesection of the prostate for bladder outflow obstruction: a retrospective study. Urol Int 88: 165–169. doi: 10.1159/000333046 PMID: 22237486
11. Ohl DA, Quallich SA, Sonksen J, Brackett NL, Lynne CM (2008) Anejaculation and retrograde ejaculation. Urol Clin North Am 35: 211–220, viii. doi: 10.1016/j.ucl.2008.01.014 PMID: 18423241
12. Jefferys A, Siassakos D, Wardle P (2012) The management of retrograde ejaculation: a systematic review and update. Fertil Steril 97: 306–312. doi: 10.1016/j.fertnstert.2011.11.019 PMID: 22177462
13. Fode M, Krogh-Jespersen S, Brackett NL, Ohl DA, Lynne CM, Sonksen J (2012) Male sexual dysfunction and infertility associated with neurological disorders. Asian J Androl 14: 61–68. doi: 10.1038/aja.2011.70 PMID: 22138899
14. Isidro ML (2012) Sexual dysfunction in men with type 2 diabetes. Postgrad Med J 88: 152–159. doi: 10.1136/postgradmedj-2011-130069 PMID: 22282735
15. Vinik AI, Freeman R, Erbas T (2003) Diabetic autonomic neuropathy. Semin Neurol 23: 365–372. PMID: 15088257
16. Ebiko M, Yoshizumi M, Shin-nosuke, Sakurada S (2006) Changes in ejaculatory capacity in the type 1 diabetes rats. The japanese journal of Impotence Research 2006: 221–232.
17. Kobayashi K, Masumori N, Hisasue S, Kato R, Hashimoto K, Itoh N, et al. (2008) Inhibition of Seminal emission is the main cause of anejaculation induced by a new highly selective alpha1A-blocker in normal volunteers. J Sex Med 5: 2185–2190. doi: 10.1111/j.1743-6109.2008.00779.x PMID: 18399947
18. Hisasue S, Furuya R, Itoh N, Kobayashi K, Furuya S, Tsukamoto T (2006) Ejaculatory disorder caused by alpha-1 adrenoceptor antagonists is not retrograde ejaculation but a loss of seminal emission. Int J Urol 13: 1311–1316. PMID: 17010010
19. Schmidt HM, Hagen M, Kriston L, Soares-Weiser K, Maayan N, Berner MM (2012) Management of sexual dysfunction due to antipsychotic drug therapy. Cochrane Database Syst Rev 11: CD003546. doi: 10.1002/14651858.CD003546.pub3 PMID: 23152218

20. Madersbacher S, Marberger M (1999) Is transurethral resection of the prostate still justified? BJU Int 83: 227–237. PMID: 10233485

21. Schulman CC (2003) Lower urinary tract symptoms/benign prostatic hyperplasia: minimizing morbidity caused by treatment. Urology 62: 24–33.

22. Grimes DA (2015) Epidemiologic research with administrative databases: red herrings, false alarms and pseudo-epidemics. Hum Reprod 30: 1749–1752. doi: 10.1093/humrep/dev151 PMID: 26113658

Journal of Medicine and Life

J Med Life. 2015; 8(Spec Iss 4): 1–5. PMCID: PMC5319273

Review of bacterial and viral zoonotic infections transmitted by dogs

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Received 2015 Jul 28; Accepted 2015 Dec 15.

Abstract

Go to:

Dogs are a major reservoir for zoonotic infections. Dogs transmit several viral and bacterial diseases to humans. Zoonotic diseases can be transmitted to human by infected saliva, aerosols, contaminated urine or feces and direct contact with the dog. Viral infections such as rabies and norovirus and bacterial infections including Pasteurella, Salmonella, Brucella, Yersinia enterocolitica, Campylobacter, Capnocytophaga, Bordetella bronchiseptica, Coxiella burnetii, Leptospira, Staphylococcus intermedius and Methicillin resistance staphylococcus aureus are the most common viral and bacterial zoonotic infections transmitted to humans by dogs. This review, focused on the mentioned infectious diseases by describing general information, signs and symptoms, transmission ways, prevention and treatment of the infection. As far as the infections are concerned, the increase of the knowledge and the awareness of dog owners and the general population regarding zoonotic infections could significantly mitigate zoonoses transmission and consequently their fatal complications.

Keywords: bacterial and zoonotic infections, viral infections, dogs, rabies, noroviruses

Introduction

Go to:

It is estimated that over 60% of the western families own a pet. The majority of these households keep a dog. Dogs have been kept as pets for over 14 centuries. Many studies have confirmed the precious roles of pets in the human life. Evidence has shown that owning a pet can increase the activity of pet owners and consequently reduced serum cholesterol, low triglyceride levels, and fewer cardiovascular events [1,2]. Also, some other studies demonstrated that pet owners suffer from depression and mental stress less and have a higher self esteem compared to others. Although dogs have several positive effects on the psychosocial and psychical health of their owners, many diseases among humans are attributed to them [3]. Children and immunocompromised individuals are especially at an increased risk of developing zoonoses infections. Several studies demonstrated that domestic dogs have a dramatic role in developing zoonoses disease and hospitalization [4,5].

Regarding domestic dogs, the increase in the population of stray and semi domestic dogs in urban areas has increased the risk of zoonoses diseases. About 5 million people throughout the world are annually bitten by dogs. Many parasitic and zoonotic pathogens are transmitted by dogs [6,7]. This review focused on the most important viral and bacterial zoonotic diseases, which can be transmitted by dogs.

Rabies

Rabies is a single strand RNA virus belonging to the Rhabdoviridae family. Rabies infection is an ancient disease with a high mortality rate in human and animal population. Based on the World Health Organization reports, annually between 30000 and 70000 deaths occurred throughout the world due to rabies infection [8]. Dogs are the major animal reservoirs for rabies infection. The majority of the infected patients in developing countries are infected by dog bites while, in developed countries, wild animals including raccoons, bats and foxes are the main cause for rabies transmission [9]. In a study in the United States, a rabies control program was conducted by using extensive vaccination in domestic dogs and reducing the rabies infection [8]. The incubation period for rabies varies between 4 days to several years depending on the location of the inoculating wound and the amount of induced viruses. Patients may present agitation, anxiety, confusion, hallucination, and hydrophobia. Post exposure prophylaxis with frequent doses of human rabies immunoglobulin (HRIG) within 14 days after the suspected dog bite can prevent the disease. Washing the wound with water and liquid soap can dramatically reduce the viral lead and consequently the probability of rabies infection [10].

Noroviruses

Noroviruses are a heterogeneous single strand RNA virus belonging to the Caliciviridae family. Noroviruses are the main cause of sporadic and epidemic gastroenteritis in humans [11]. This virus can affect humans of all ages. The virus can be found in the gastrointestinal tract and consequently in the feces or diarrhea of the infected dogs. It can be transmitted from contaminated food or water to humans and the infection can rapidly spread in the human population by fecal oral rate. Serum therapy should be considered for patients with acute gastroenteritis [12].

Pasteurella

Pasteurella species are Gram-negative coccobacilli, which were primarily found in animals. Pasteurella spp are normal flora of the upper respiratory tract of dogs and cats. Pasteurella infection can be transmitted to humans by direct and indirect contact such as dog or cat bites or licks and even cat scratches [6]. Several infectious diseases in humans are attributed to Pasteurella spp. The soft tissue infection is the most important infection transmitted by Pasteurella spp. However, meningitis, bone and joint infections and respiratory infection can be transmitted by Pasteurella spp [13]. In a prospective study in United States, the author demonstrated that Pasteurella spp. was the most frequent organism isolated from dog and cat bites [2]. Pasteurella infection can be treated by second and third generation cephalosporin, macrolides, fluoroquinolones, cotrimoxazole, and penicillin [14].

Salmonella

Salmonella species are anaerobic and motile gram-negative bacilli that colonize in the large intestine of a variety of mammals, especially in the distal part of the colon and the mesenteric lymph nodes of the canine. Humans can also get infected through the gastrointestinal tract [fecal transmission] and develop several infectious diseases such as gastroenteritis, enteric fever, bacteremia and osteomyelitis. Gastrointestinal diseases are the most prevalent clinical presentations of salmonella in human and dogs; however, the majority of infected animals or humans is asymptomatic and may shed the pathogen through feces for a period of 6 weeks and transmit the pathogen to other animals or individuals. In developing nations, Salmonella spp. is also more prevalent than in developed countries [15,16]. An antibiogram should be considered for patients infected with Salmonella spp. however, it could be treated by various families of antibiotics including fluoroquinolones, beta-lactams, and macrolides [17].

Brucella

Brucellosis is one of the most prevalent zoonoses, which imposes a heavy burden on the national health services. It is commonly transmitted to humans by consuming unpasteurized dairy products. Various

types of brucella spp. have been recognized; that resulted in human brucellosis such as B. melitensis, B. abortus and B. suis but, B. canis has been less known as an usual pathogen in brucellosis infection in humans [18,19]. Although B. canis is not responsible for the brucellosis infection in humans, the reported cases were more often seen among farmer populations who had a history of exposure to body fluids of dogs, which were infected with B. canis. The incubation period may last for one to four weeks up to several months [19]. The patients may be asymptomatic or may even present serious clinical symptoms especially fever, night sweats and low back pain in the endemic region that should be differentiated from tuberculosis and other malignancies [20]. Brucellosis should be treated in order to avoid complications and sequelae of the disease. Combination therapies, which are widely employed in the treatment of brucellosis, consisted of doxycycline plus streptomycin or rifampin for 6 weeks [21].

Yersinia enterocolitica

Y. enterocolitica is a gram-negative coccobacillus zoonotic pathogen that causes yersiniosis in human and animals. Several animals are main reservoirs for Y. enterocolitica including birds, pigs, deer, and cattle. The pathogen has been isolated from dog bite wound in some studies [22]. The patients may be asymptomatic in early stage and when the pathogen invades the mucosal surface of the intestine, watery or bloody diarrhea may be present. The pathogen can also involve the peyer's patches and represent the appendicitis symptoms [23,24]. Y. enterocolitica is mostly a self-limiting disease that does not need antibiotic therapy, however, patients with severe infection and immunocompromised patients should be treated with a combination of an aminoglycoside and doxycycline [24].

Campylobacter

Campylobacter spp. including campylobacter jejuni and campylobacter coli are gram-negative bacteria that usually result in campylobacter enteritis. This organism normally lives in the gastrointestinal tract of many animals. Direct contact with infected animals or their products is a leading cause of campylobacter transmission. Dogs and puppies are the major reservoirs for campylobacter. For example, in a study it was demonstrated that about 47% of the fecal specimens of dogs' campylobacter was isolated [25,26]. The incubation period in campylobacter enteritis varies from one to seven days. Most of the patients present fever, vomiting, diarrhea, and abdominal pain. Also, bloody diarrhea may be present in more than 50 percent of the infected patients. Convulsion and seizure may be observed in some patients [27]. This infection is usually self-limited and does not need antimicrobial therapy. Focus on correction of electrolyte imbalance and hydration should be considered. Antibiotic therapy with fluoroquinolones, macrolides, or aminoglycosides is indicated in patients with severe disease [28].

Capnocytophaga

Capnocytophaga canimorsus is a gram-negative bacterium, which is found in the normal flora of the oropharyngeal tract of dogs and cats. The pathogen is mostly transmitted to human by dogs bite and causes an overwhelming sepsis, particularly in elderly, immunocompromised or asplenic patients [25]. The pathogen can also lead to other fatal infections including meningitis, osteomyelitis, arthritis, lung abscess or empyema and endocarditis. In addition, thrombotic thrombocytopenic purpura and hemolytic uremic syndrome can be associated with capnocytophaga septicemia especially in immunocompromised patients [25,29]. The literature data have demonstrated that the mortality rate due to capnocytophaga septicemia is estimated to be of one third of the infected patients. Accordingly, early empirical therapy with third generation cephalosporins in patients who received a dog bite should be considered [30].

Bordetella bronchiseptica

Bordetella bronchiseptica is a gram-negative rod bacterium belonging to the genus Bordetella. The pathogen normally lives in the upper respiratory tract of the mammals such as dogs and cats and is

transmitted to humans by aerosol. B. bronchiseptica can lead to acute tracheobronchitis in dogs, which presents with harsh and kennel cough [31,32]. Human infection with B. bronchiseptica is very rare; however, the pathogen can also cause pneumonia and upper respiratory tract infection in dog owners [33]. Evidences demonstrated that this organism is resistant to macrolides and cephalosporins; however, in several studies, the organism was sensitive to fluoroquinolones and Trimethoprim/ sulfamethoxazole [34].

Coxiella burnetii

C. burnetii is an obligate intracellular gram-negative bacterium that causes Q fever in humans. The pathogen normally infects individuals via aerosol and direct contact with the body fluids of the infected animals. Although dogs are not the main reservoirs for C. burnetii, however, in a study it was demonstrated that C. burnetii was isolated from approximately 10 percent of farm dogs [35]. In addition, in another study by Buhariwalla and colleagues, it was reported that C. burnetii could be transmitted to human from an infected parturient dog. In addition, the patients developed the symptoms of Q fever including fever, chills, nausea, vomiting and productive cough. Opacity is a common finding in chest radiography, and, in physical examination, crackles may be heard during auscultation. The incubation period in this study was estimated to be between 8 and 12 days after the exposure to the infected animal. The patients with C. burnetii can be treated with fluoroquinolones or doxycycline successfully [36].

Leptospira

L. interrogans is an aerobic spirochete, which is the major cause of Leptospirosis in human. Leptospirosis is worldwide zoonoses that are mostly transmitted to human by environmental sources including contaminated soil, water, urine, or tissue of the infected animals. Rodents are the major reservoirs for Leptospirosis; however, domestic animals including dogs can play an important role in leptospirosis transmission in endemic regions [37]. Mucosal surfaces of the human body including eye, vagina, nose, mouth, or erosive lesions, which have a direct contact with the contaminated urine, are the main ways of Leptospirosis transmission. The incubation period for this infection is averagely of about 10 days (ranging from 2 to 26 days) [38,39]. Leptospirosis may present with a variety of symptoms from no symptom to fever, nonproductive cough, headache, musculoskeletal pain, diarrhea, nausea, vomiting, alveolar hemorrhage, and even meningitis [39]. Several antibiotics such as doxycycline, ceftriaxone, cefotaxime, penicillin, amoxicillin, and ampicilin have been successfully employed for the treatment of Leptospirosis [40].

Staphylococcus intermedius

S. intermedius is a gram-positive bacterium with a coagulase activity that normally lives in the anterior part of the nasal cavity of several animals such as dogs, pigeons, and horses. Some evidences demonstrated that this pathogen could also be isolated from the gingival of healthy dogs [41]. S. intermedius is not a common zoonotic pathogen in humans; however, several studies demonstrated that this bacterium is a potential pathogen associated with dog bite wounds and cellulitis can develop in inflicted humans [42,43]. This pathogen should be discriminated from staphylococcus aureus. Penicillin and amoxicillin-clavulanate are effective in the treatment of this infection [44].

Methicillin resistance staphylococcus aureus

Methicillin resistance staphylococcus aureus (MRSA) is a major cause of fatal infection in humans. Several investigations have reported that this pathogen has been isolated from some animals such as pigs, horses, cattle, cats and dogs. Of them, some believed that companion animals were the main reservoirs for the transmission of MRSA, being able to transmit the bacterium by direct contact with their owners. However, it seems that animal to human infection of MRSA is more seen in

immunocompromised patients. Nevertheless, some evidences showed that this bacterium could be transmitted to healthy humans who own an infected animal [45,46]. Traditional anti staphylococcal antibiotics are not more effective in the treatment of infections caused by MRSA. Accordingly, newer drugs including vancomycin, linezolid and daptomycin are widely used in the treatment of MRSA infections [47].

Conclusion

Go to:

Zoonoses are diseases that implicate both humans and animals and can be transmitted either by domestic pets or by wildlife animals. Many animals and their products can be reservoirs of zoonoses pathogens. Among them, dogs are responsible for the transmission of several zoonotic diseases to their owners. Thus, dog owners should be informed regarding the zoonotic diseases and their ways of transmission to reduce these infections in human population. Several prophylactic and therapeutic strategies have been introduced in order to decrease the zoonotic diseases. Dog owners are recommended to wash their hands after any direct contact with their dogs, their products, urine, or feces. Most of the viral and bacterial infections are transmitted from dogs to humans by dog bite; however, other infections caused by protozoa have a fecal oral transmission. Thus, food hygiene such as washing vegetables well and cooking meats adequately should be carefully done in order to eliminate the rate of zoonotic infections.

In addition, dogs should also be treated for diarrheal infections. Moreover, dog owners should feed their dogs with cooked meat to prevent campylobacter and salmonella infections. Raw meat and eggs should not be fed to dogs due to higher rate of infection susceptibility. Rabies vaccination should be considered for domestic dogs and the dog owners should also be aware of benefits of rabies vaccination before and after dogs bites. Many authors reported that increasing the knowledge of dog owners regarding dog associated zoonotic infections and prevention strategies can dramatically reduce the zoonotic infections in dog owners and their families.

References

Go to:

1. Katagiri S, Oliveira-Sequeira T. Prevalence of dog intestinal parasites and risk perception of zoonotic infection by dog owners in São Paulo State, Brazil. Zoonoses and Public Health. 2008;55(8-10):406–413. [PubMed]

2. Talan DA, Citron DM, Abrahamian FM, Moran GJ, Goldstein EJ. Bacteriologic analysis of infected dog and cat bites. New England Journal of Medicine. 1999;340(2):85–92. [PubMed]

3. Beth Tower R, Nokota M. Pet companionship and depression: results from a United States Internet sample. Anthrozoos: A Multidisciplinary Journal of The Interactions of People & Animals. 2006;19(1):50–64.

4. Vengust M, Anderson M, Rousseau J, Weese J. Methicillin-resistant staphylococcal colonization in clinically normal dogs and horses in the community. Letters in Applied Microbiology. 2006;43(6):602–606. [PubMed]

5. Moriello KA. Zoonotic skin diseases of dogs and cats. Animal Health Research Reviews. 2003;4(02):157–168. [PubMed]

6. Oehler RL, Velez AP, Mizrachi M, Lamarche J, Gompf S. Bite-related and septic syndromes caused by cats and dogs. The Lancet Infectious Diseases. 2009;9(7):439–447. [PubMed]

7. Ghasemzadeh I, Sadeghi P, Shahri RZ. A review of viruses related to prostatic cancer. Life Science Journal. 2014;11(8s)

8. Krebs JW, Mandel EJ, Swerdlow DL, Rupprecht CE. Rabies surveillance in the United States during 2003. Journal of the American Veterinary Medical Association. 2004;225(12):1837–1849. [PubMed]

9. Tang X, Luo M, Zhang S, Fooks AR, Hu R, Tu C. Pivotal role of dogs in rabies transmission, China. Emerg Infect Dis. 2005;11(12):1970–1972. [PMC free article] [PubMed]

10. Lucas C, Pino F, Baer G, Morales P, Cedillo V, Blanco M, et al. Rabies control in Mexico. Developments in Biologicals. 2007;131:167–175. [PubMed]

11. Summa M, von Bonsdorff C-H, Maunula L. Pet dogs—A transmission route for human noroviruses? Journal of Clinical Virology. 2012;53(3):244–247. [PubMed]

12. Wolf JM, Dawson L, Mountcastle SB, Owens BD. The incidence of scaphoid fracture in a military population. Injury. 2009;40(12):1316–1319. [PubMed]

13. Kristinsson G. Pasteurella multocida infections. Pediatrics in Review. 2007;28(12):472–473. [PubMed]

14. Lloret A, Egberink H, Addie D, Belák S, Boucraut-Baralon C, Frymus T, et al. Pasteurella Multocida Infection in Cats ABCD guidelines on prevention and management. Journal of Feline Medicine and Surgery. 2013;15(7):570–572. [PubMed]

15. Leonard F. Salmonella infection and carriage: the importance of dogs and their owners. Veterinary Record. 2014;174(4):92–93. [PubMed]

16. Cobb M, Stavisky J, Barrow P, Methner U. Salmonella infections in dogs and cats. Salmonella in Domestic Animals. 2013;2:318–336.

17. Leonard E, Pearl D, Finley R, Janecko N, Peregrine A, Reid-Smith R, et al. Evaluation of Pet-Related Management Factors and the Risk of Salmonella spp. Carriage in Pet Dogs from Volunteer Households in Ontario (2005–2006) Zoonoses and Public Health. 2011;58(2):140–149. [PubMed]

18. Seleem MN, Boyle SM, Sriranganathan N. Brucellosis: a re-emerging zoonosis. Veterinary Microbiology. 2010;140(3):392–398. [PubMed]

19. Lucero N, Corazza R, Almuzara M, Reynes E, Escobar G, Boeri E, et al. Human Brucella canis outbreak linked to infection in dogs. Epidemiology and Infection. 2010;138(2):280. [PubMed]

20. Hasanjani Roushan M, Mohrez M, Smailnejad Gangi S, Soleimani Amiri M, Hajiahmadi M. Epidemiological features and clinical manifestations in 469 adult patients with brucellosis in Babol, Northern Iran. Epidemiology and Infection. 2004;132(06):1109–1114. [PMC free article] [PubMed]

21. Pappas G, Akritidis N, Tsianos E. Effective treatments in the management of brucellosis. Expert Opinion on Pharmacotherapy. 2005;6(2):201–209. [PubMed]

22. Fredriksson-Ahomaa M. Yersinia enterocolitica and Yersinia pseudotuberculosis. Foodborne Diseases. Springer; 2007. pp. 79–113.

23. Abdel-Haq NM, Asmar BI, Abuhammour WM, Brown WJ. Yersinia enterocolitica infection in children. The Pediatric Infectious Disease Journal. 2000;19(10):954–958. [PubMed]

24. Casburn-Jones A, Farthing M. Management of infectious diarrhoea. Gut. 2004;53(2):296–305. [PMC free article] [PubMed]

25. Janda JM, Graves MH, Lindquist D, Probert WS. Diagnosing Capnocytophaga canimorsus infections. Emerging Infectious Diseases. 2006;12(2):340. [PMC free article] [PubMed]

26. Hermans D, Pasmans F, Messens W, Martel A, Van Immerseel F, Rasschaert G, et al. Poultry as a host for the zoonotic pathogen Campylobacter jejuni. Vector-Borne and Zoonotic Diseases. 2012;12 (2):89–98. [PubMed]

27. Gazaigne L, Legrand P, Renaud B, Bourra B, Taillandier E, Brun-Buisson C, et al. Campylobacter fetus bloodstream infection: risk factors and clinical features. European Journal of Clinical Microbiology & Infectious Diseases. 2008;27(3):185–189. [PubMed]

28. Ternhag A, Asikainen T, Giesecke J, Ekdahl K. A meta-analysis on the effects of antibiotic treatment on duration of symptoms caused by infection with Campylobacter species. Clinical Infectious Diseases. 2007;44(5):696–700. [PubMed]

29. Biedermann P, Deligne D. Meningitis due to Capnocytophaga canimorsus with misleading initial digestive symptom. Annales de Biologie Clinique. 2003 [PubMed]

30. Jolivet-Gougeon A, Sixou J-L, Tamanai-Shacoori Z, Bonnaure-Mallet M. Antimicrobial treatment of Capnocytophaga infections. International Journal of Antimicrobial Agents. 2007;29(4):367–373. [PubMed]

31. Woolfrey BF, Moody JA. Human infections associated with Bordetella bronchiseptica. Clinical Microbiology Reviews. 1991;4(3):243–255. [PMC free article] [PubMed]

32. Ner Z, A Ross L, Horn MV, Keens TG, MacLaughlin EF, Starnes VA, et al. Bordetella bronchiseptica infection in pediatric lung transplant recipients. Pediatric Transplantation. 2003;7(5):413 –417. [PubMed]

33. Hemsworth S, Pizer B. Pet ownership in immunocompromised children—a review of the literature and survey of existing guidelines. European Journal of Oncology Nursing. 2006;10(2):117–127. [PubMed]

34. Egberink H, Addie D, Belák S, Boucraut-Baralon C, Frymus T, Gruffydd-Jones T, et al. Bordetella bronchiseptica infection in cats. ABCD guidelines on prevention and management. Journal of Feline Medicine & Surgery. 2009;11(7):610–614. [PubMed]

35. Cosman F, Ruffing J, Zion M, Uhorchak J, Ralston S, Tendy S, et al. Determinants of stress fracture risk in United States Military Academy cadets. Bone. 2013;55(2):359–366. [PubMed]

36. Patel DS, Roth M, Kapil N. Stress fractures: diagnosis, treatment, and prevention. American Family Physician. 2011;83(1):39–46. [PubMed]

37. Moore GE, Guptill LF, Glickman NW, Caldanaro RJ, Aucoin D, Glickman LT. Canine leptospirosis, United States, 2002–2004. Emerging Infectious Diseases. 2006;12(3):501. [PMC free article] [PubMed]

38. Sehgal S. Epidemiological patterns of leptospirosis. Indian Journal of Medical Microbiology. 2006;24(4):310. [PubMed]

39. Organization WH. Human leptospirosis: guidance for diagnosis, surveillance and control. World Health Organization Malta. 2003

40. Kobayashi Y. Clinical observation and treatment of leptospirosis. Journal of Infection and Chemotherapy. 2001;7(2):59–68. [PubMed]

41. Hoekstra K, Paulton R. Clinical prevalence and antimicrobial susceptibility of Staphylococcus aureus and Staph. intermedius in dogs. Journal of Applied Microbiology. 2002;93(3):406–413. [PubMed]

42. Tanner MA, Everett CL, Youvan DC. Molecular phylogenetic evidence for noninvasive zoonotic transmission of Staphylococcus intermedius from a canine pet to a human. Journal of Clinical Microbiology. 2000;38(4):1628–1631. [PMC free article] [PubMed]

43. Talan DA, Staatz D, Staatz A, Overturf GD. Frequency of Staphylococcus intermedius as human nasopharyngeal flora. Journal of Clinical Microbiology. 1989;27(10):23–93. [PMC free article] [PubMed]

44. Barr JS, Elliston WA, Musnick H, Delorme TL, Hanelin J, Thibodeau AA. Fracture of the Carpal Navicular (Scaphoid) Bone An End-Result Study in Military Personnel. The Journal of Bone & Joint Surgery. 1953;35(3):609–625. [PubMed]

45. Armstrong III DW, Rue J-PH, Wilckens JH, Frassica FJ. Stress fracture injury in young military men and women. Bone. 2004;35(3):806–816. [PubMed]

46. Välimäki V-V, Alfthan H, Lehmuskallio E, Löyttyniemi E, Sahi T, Suominen H, et al. Risk factors for clinical stress fractures in male military recruits: a prospective cohort study. Bone. 2005;37(2):267–273. [PubMed]

47. Morgan M. Methicillin-resistant Staphylococcus aureus and animals: zoonosis or humanosis? Journal of Antimicrobial Chemotherapy. 2008;62(6):1181–1187. [PubMed]

Zoonotic parasitic infections contracted from dogs and cats: How frequent are they?

Your clients probably don't know that their lovable pets can transmit parasitic infections to them and their children, so it is up to you to educate them. Being aware of the gastrointestinal parasites with the most zoonotic potential will enable you to give clients the best advice for zoonosis prevention.

Mar 01, 2007
By Peter M. Schantz, VMD, PhD
From: DVM360.com [Veterinary Medicine]

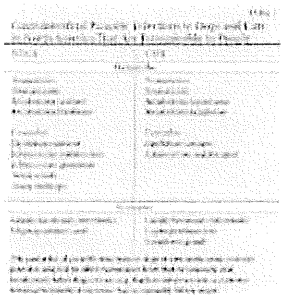
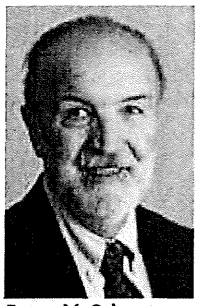


Table 1: Gastrointestinal Parasitic Infections in Dogs and Cats in North America That Are Transmissible to People

The popularity of dogs and cats as pets in the United States continues to increase. Recent surveys estimated that there were 73 million owned dogs and 90 million owned cats in the United States, and almost 60% of U.S. households owned a pet.[1] The highest rates of dog and cat ownership occur in households of families with young children. Dogs and cats are hosts to many intestinal parasites that may be transmitted to humans through direct contact with infected pets or exposure to environments contaminated with infected animals' feces (*Table 1*). Children are often at greatest risk of zoonotic infections because of their play habits and affection for pets.

Veterinarians in practice are on the front lines in preventing transmission of pet-associated zoonotic parasite infections because of their knowledge of the potential risks and through their contact with pet owners. Practicing veterinarians' services should include preventive treatments to eliminate parasites as well as advice to owners on minimizing the risk of zoonotic transmission. This article summarizes data on the modes of transmission of potentially zoonotic intestinal parasites of dogs and cats and the available data on the frequency at which they infect and cause disease in humans in the United States.*

HELMINTH INFECTIONS



Peter M. Schantz, VMD, PhD

Potentially zoonotic gastrointestinal parasites of dogs and cats include the maternally transmitted intestinal roundworms and hookworms whose infective stages may contaminate and persist in the peridomestic environment (*i.e.* in proximity to humans).

Ascarids

Infection of humans by *Toxocara canis* and *Toxocara cati*, the common roundworms of dogs and cats, respectively, cause larva migrans syndromes (visceral and ocular larva migrans and covert toxocariasis) in humans who accidentally ingest infective eggs from contaminated environments.[2] Toxocariasis ranks among the most common of all zoonotic infections; the results of numerous published surveys document seroprevalences in humans ranging from 1% to 20%, depending on the age, socioeconomic status, and pet ownership status of the tested populations.[3-5] Toxocaral larva migrans, or infection by the common ascarid worms of dogs and cats, is arguably the most common zoonotic infection associated with pets in the United States and other industrialized countries.[4] It has been estimated that every year in the United States this infection causes hundreds of cases of unilateral blindness and uncountable numbers of less permanent forms of illness in children.[3,4] The severity and type of disease in humans produced by *Toxocara* species infection depend on how many larvae are ingested, the frequency of reinfection, and other factors still poorly understood. Most human infections with *Toxocara* larvae are well-tolerated, even asymptomatic; however, a proportion of infected people develop larva migrans syndromes that may be systemic or confined to the eye. When a larva invades the eye, it almost always leaves the individual partially or totally blind in that eye. Based

on data obtained from the Centers for Disease Control and Prevention (CDC) serologic diagnostic reference service, an estimated minimum of 750 cases of ocular larva migrans are diagnosed by physicians every year in the United States.[4] Chart review of patients with a diagnosis of uveitis at the University of California Medical Center determined that ocular larva migrans accounted for 1% of cases of uveitis seen between 1977 and 1996.[5] All cases were associated with vision loss in the affected eye. The number of cases of toxocaral visceral larva migrans syndrome is much greater; however, estimates of these are quite imprecise.[4]
Epidemiologic investigations have consistently determined that the principal risk factor for infection was the presence of a household dog, particularly a pup, in a patient's household within six months of onset of illness.[2,3,6] When this condition is combined with pica, especially dirt eating, the statistical association becomes very strong.

Infections with the hookworms *Ancylostoma braziliense* and *Ancylostoma caninum* remain common in dogs and cats, with the highest prevalences in the southern United States, mainly in coastal areas from southern New Jersey to the Florida Keys and westward along the Gulf of Mexico to Texas. Infections in people are acquired from contact with moist or wet sand or loam soil containing filariform larvae of hookworms generated from the feces of dogs and cats, usually in unprotected sandboxes, on bathing beaches, and under houses where workers lie prone while repairing leaking water pipes. Larval invasion of skin in humans produces pruritic papules. In two or three days, these papules become serpiginous tunnels in the epidermis caused by inflammation resulting from intradermal migration of larvae (cutaneous larva migrans).[7] Without treatment, migration may continue for several weeks or months before the immune system kills the larvae. Zoonotic hookworm infection may also be acquired through ingestion of the larvae in soil or in tissues of paratenic hosts. Infection in humans acquired by these latter routes, especially *A. caninum*, may occasionally lead to enteric localizations of zoonotic hookworms, causing eosinophilic enteritis.[8] Although eosinophilic enteritis has been diagnosed with relative frequency in Australia where it was first noted, it has rarely been diagnosed in the United States.[9] The eosinophilic enteritis syndrome requires clinical experience and technical sophistication to diagnose and may occur more frequently than currently recognized and documented.

Tapeworms

Dipylidium caninum. Zoonotic tapeworm infections associated with dogs and cats include the flea tapeworm, *Dipylidium caninum*. Infection is acquired when a person, usually a young child, accidentally ingests a flea carrying the larval stage of the tapeworm. *Dipylidium caninum* infection can lead to diarrhea and pruritus in infected humans. This infection rarely causes serious symptoms; however, the stress associated with seeing tapeworm segments in a child's stool or diapers can be considerable.[10]
Echinococcus species. *Echinococcus* species of dogs may infect humans with larval stages that cause cystic or tumorous growths in the liver and other visceral organs. Cystic echinococcosis, or hydatid disease, is caused by infection with larval stage *Echinococcus granulosus*. Cases of cystic echinococcosis acquired in the northernmost regions of North America, especially Canada and Alaska, are caused by the northern sylvatic genotype maintained in cycles involving wolves, dogs, moose, caribou, and other cervids.[11] The practice of feeding the viscera of moose and caribou to working and pet dogs leads to infection in dogs and subsequent exposure to humans. Infection continues to be relatively commonly diagnosed in most Canadian provinces. A review of hospital records in Edmonton, Alberta, noted 42 cases diagnosed and treated between 1991 and 2001.[12]
Foci of local transmission involving a variety of domestic intermediate hosts have been described in various regions of the United States.[11] Distinct foci of *E. granulosus* transmission were noted in the 1970s in western states including California, Utah, New Mexico, and Arizona. Epidemiologic investigations revealed that transmission was associated with unique cultural practices involving home slaughter of sheep and the access of dogs to discarded viscera of these hosts. Human populations at risk in these settings were transhumant sheep ranchers, including Basque-Americans in California, Mormons in central Utah, and Navajo and Zuni Indians in New Mexico and Arizona. Active transmission appears to

have been eliminated in some of those foci; however, local hospital records indicate that an average of one to four cases continue to be diagnosed each year among Native American communities in Arizona and New Mexico.[11]

Echinococcus multilocularis, the cause of the alveolar form of human hydatid disease, is an emerging zoonotic parasite in the United States. The life cycle of *E. multilocularis* involves foxes and coyotes and their rodent prey in ecosystems generally separate from that of humans. However, there is ecologic overlap with humans because fox and coyote populations have increasingly encroached upon suburban and urban areas of many regions, and domestic dogs or cats may become infected when they eat infected wild rodents.[11] Infections in domestic pets increase the risk of human exposure. Humans may acquire infection when they accidentally ingest eggs by direct or indirect fecal-oral contamination from infected definitive hosts. Human alveolar echinococcosis in North America has been mainly confined to certain Eskimo populations in northern coastal Alaska in which annual diagnostic incidence rates during the 1970s and 1980s were among the highest ever reported for this infection (7 to 98 per 100,000 population).[13] A control intervention in endemic Alaskan villages initiated in 1990 involving education, improved housing, and preventive treatments of dogs has greatly reduced or eliminated transmission to humans.[14] No new cases have been diagnosed in humans since that time.[14]

This tapeworm also occurs in a large area of central North America, and its geographic range and prevalence may be increasing. Before 1964, there were no reports of *E. multilocularis* in North America south of the Arctic tundra zone, but, in that year, it was reported in foxes and rodents in North Dakota.[15] Subsequent surveys revealed that the cestode was enzootic in cycles involving red foxes, coyotes, and deer mice in North and South Dakota, Minnesota, Montana, Iowa, Wyoming, Nebraska, Wisconsin, and Illinois.[16,17] The most recent surveys have extended the range eastward to Indiana, Michigan, and Ohio.[18,19] Prevalence of infection in foxes and coyotes in the northern Great Plains (25% to 90%) is as high as in any region in the world. To date only two persons are known to have acquired their infections in the endemic region in central North America—a 54-year-old man from Manitoba, Canada, and a 60-year-old woman from Minnesota[20] —however, the potential exists for a more serious public health problem as domestic dogs and cats become involved in the life cycle.

***Taenia* species.** Coenurosis is an infection by larval forms of several related tapeworms of the genus *Taenia* (formerly designated *Multiceps*). The coenurus is a fluid-filled cyst that measures from a few millimeters to 2 cm or more in diameter. Dogs and other canids (wolves, coyotes, foxes) are the final hosts of *Taenia* tapeworms. *Taenia serialis*, the only coenurid-forming cestode currently present in North America, uses rodents or hares as intermediate hosts, and the coenuri are typically found in the intermuscular fascia and subcutaneous tissues. Humans become infected when they accidentally ingest tapeworm eggs in the feces of infected canids. The symptoms of coenurosis are due to the physical presence of the cyst and depend on the site of localization. In North America, fewer than 10 autochthonous (locally acquired) human cases have been documented; three involved the central nervous system or the eye, and the others involved intramuscular or subcutaneous localization.[21]

PROTOZOA INFECTIONS

Toxoplasma gondii

Toxoplasma gondii is a coccidian parasite widely dispersed in nature. Cats are the definitive hosts for this protozoan, which they acquire when they eat infected intermediate hosts (rodents and many other mammals) or ingest oocysts excreted in the stools of other infected cats. Infected cats are important in the epidemiology and public health importance of toxoplasmosis because they excrete and widely disperse the environmentally resistant oocysts.[22] Numerous herbivorous and omnivorous animals become infected when they ingest infective oocysts in soil or contaminated food.

Humans become infected by ingesting food and water contaminated with oocysts shed in the feces of infected cats, by eating undercooked meat from infected animals, or in utero (by congenital transmission from infected mothers). Rarely, humans become infected through blood transfusion or organ transplantation. Recent serosurveys of the U.S. population have documented antibodies (evidence of current or past infection) in about 23% of the U.S. population.[23] Ingestion of oocysts shed in the feces of

infected cats is believed to directly account for up to 50% of human cases in the United States. Clinical disease caused by toxoplasmosis is generally mild following primary infection of immunocompetent people. Self-limiting fever, malaise, and lymphadenopathy are the most common clinical abnormalities, and most infected people never realize when their first *T. gondii* infection occurred. However, acute infections acquired by pregnant women can be transmitted to the fetus and cause severe illness (*e.g.* mental retardation, blindness, epilepsy) and death. According to a 1999 report by the CDC, an estimated 400 to 4,000 cases of congenital toxoplasmosis occur each year in the United States.[24,25] Another permanent manifestation of toxoplasmosis is ocular disease, which is estimated to occur in up to 12,000 people per year in the United States. Toxoplasmosis can cause more severe or fatal illness in people who are immunosuppressed (people with human immunodeficiency virus [HIV], transplant recipients).

Giardia species

Giardia duodenalis (synonyms *Giardia lamblia, Giardia intestinalis*) is a protozoan parasite that infects the intestinal tract of many animal species including humans. Motile trophozoite stages occur in the intestines, and environmentally resistant cysts are passed in the feces of infected animals, which are immediately infective if ingested by other susceptible hosts. In all hosts, *G. duodenalis* can cause acute gastrointestinal signs as well as chronic disease, including chronic malabsorptive and allergic manifestations and childhood failure to thrive.[26] Transmission of infection occurs by fecal-oral routes either by direct contact or by ingestion of contaminated food or water. *Giardia* species infections are common in dogs and cats throughout North America; however, prevalences are often underestimated because the parasite detection methods commonly used in practice have low sensitivity.[27,28] *Giardia* species have long been considered zoonotic because morphologically similar organisms infect humans and a variety of mammals and birds.[29] However, evidence of giardiasis being directly transmitted from one host species to an immunocompetent host of another species is limited. Although variants of *Giardia* species in human, canine, and feline hosts lack differentiating morphologic characters, the application of molecular tools (*e.g.* PCR) has revealed genetic differences in isolates from different hosts such that it has become clear that the genotypes commonly infecting dogs and cats are not those commonly infecting humans.[29,30] Most confirmed infections in humans with *Giardia* species acquired from dogs or cats have been reported in individuals with recognized immunodeficiency disease (*e.g.* HIV infection).[29]

Cryptosporidium species

Protozoan parasites belonging to the genus *Cryptosporidium* are ubiquitous and among the most common nonbacterial causes of diarrhea in a wide range of vertebrates, including humans. *Cryptosporidium* species are transmitted via the fecal-oral route by environmentally resistant cysts that are shed in the feces, contaminating soil and water, and, thus, providing multiple routes into the food chain.[30] In an immunocompetent host, cryptosporidiosis of the intestinal tract may be asymptomatic or lead to self-limiting diarrhea, but in an immunocompromised host, it can be life-threatening.[31] *Cryptosporidium* species have been reported in numerous mammals and, like *Giardia* species, appear to have evolved with their respective hosts such that they do not readily cross-infect and develop in hosts of other species. The application of molecular tools has revealed that *Cryptosporidium* species are a phenotypically and genotypically heterogeneous assemblage of species and genotypes that are morphologically similar.[30,31] In humans, the most commonly detected species are the anthroponotic *Cryptosporidium hominis* and the zoonotic *Cryptosporidium parvum* (cattle). Both *Cryptosporidium canis* and *Cryptosporidium felis*, whose natural hosts are dogs and cats, respectively, have also been demonstrated in infected humans suffering diarrhea.[32] Young children and immunocompromised individuals are at greatest risk. Information regarding the role of pets in zoonotic transmission of *Cryptosporidium* species in immunocompetent humans is insufficient. While it is clear that most outbreaks and individual cases of cryptosporidiosis in humans are related to the contamination of water, food, or fomites with organisms of human or cattle origin, it is also clear that inter-species transmission from dogs or cats to humans can occur in certain situations, especially among very young children or immunodeficient individuals.[30]

CONCLUSION

Dogs and cats are infected with a number of helminths and protozoa that can infect and sometimes cause life-threatening illness in humans. Awareness of these infections and their zoonotic potential is essential for practicing veterinarians in order to diagnose and treat the infections in pets as well as to provide preventive advice to pet owners.

*None of the zoonotic parasitic infections acquired from dogs and cats are reportable diseases in the United States; consequently, no systematically collected data on the frequency of these zoonotic parasitic infections exist.

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TABLE 1

Gastrointestinal Parasitic Infections in Dogs and Cats in North America That Are Transmissible to People

DOGS	CATS
Helminths	
Nematodes	Nematodes
Toxocara canis	*Toxocara cati*
Ancylostoma caninum	*Ancylostoma tubaeforme*
Ancylostoma braziliense	*Ancylostoma braziliense*
Cestodes	Cestodes
Dipylidium caninum	*Dipylidium caninum*
Echinococcus multilocularis	*Echinococcus multilocularis*
Echinococcus granulosus	
Taenia serialis	
Taenia multiceps	
Protozoa	
Giardia duodenalis (intestinalis)	*Giardia duodenalis (intestinalis)*
Cryptosporidium canis	*Cryptosporidium felis*
	Toxoplasma gondii

This partial list of parasitic infections in dogs or cats omits some zoonotic parasites adapted to other mammalian hosts that occasionally and facultatively infect dogs or cats (*e.g. Baylisascaris procyonis*, a common intestinal helminth of raccoons that occasionally infects dogs).

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Parasit Vectors. 2017; 10: 43. PMCID: PMC5264337
Published online 2017 Jan 25. doi: 10.1186/s13071-017-1989-8

Abundance, zoonotic potential and risk factors of intestinal parasitism amongst dog and cat populations: The scenario of Crete, Greece

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Received 2016 Sep 4; Accepted 2017 Jan 18.

Abstract

Go to:

Background

The objectives of this study were to evaluate the prevalence and infection intensity of intestinal parasites in different dog and cat populations in Crete, Greece, estimate the zoonotic risk and identify risk factors.

Methods

Faecal samples from shelter, household and shepherd dogs and shelter and household cats were analyzed using sedimentation/flotation techniques. *Giardia* and *Cryptosporidium* were detected by a quantitative direct immunofluorescence assay (IFA). PCR and sequencing was performed to evaluate the zoonotic potential of *Giardia* and *Cryptosporidium* positive samples.

Results

Totals of 879 dog and 264 cat faecal samples were examined. In dogs, the overall prevalence was 25.2% (CI: 22.4–28.1) for *Giardia* spp.; 9.2% (CI: 7.3–11.1) for *Ancylostoma/Uncinaria* spp.; 7.6% (CI: 5.9–9.4) for *Toxocara* spp.; 5.9% (CI: 4.4–7.5) for *Cryptosporidium* spp.; 4.6% (CI: 3.2–5.9) for *Cystoisospora* spp.; 2.7% (CI: 1.7–3.8) for *Toxascaris leonina*; 1.7% (CI: 0.9–2.6) for *Capillaria* spp.;

0.8% (CI: 0.2–1.4) for taeniid eggs; 0.2% (CI: 0–0.5) for *Dipylidium caninum*; and 0.1% (CI: 0–0.3) for *Strongyloides stercoralis*. In cats, the prevalence was 20.5% (CI: 15.6–25.3) for *Giardia* spp.; 9.5% (CI: 5.9–13.0) for *Cystoisospora* spp.; 8.3% (CI: 5.0–11.7) for *Toxocara* spp.; 7.6% (CI: 4.4–10.8) for *Ancylostoma/Uncinaria* spp.; 6.8% (CI: 3.8–9.9) for *Cryptosporidium* spp.; 4.2% (CI: 1.8–6.6) for *Capillaria* spp.; 0.8% (CI: 0–1.8) for taeniid eggs; and 0.4% (CI: 0–1.1) for *Hammondia/Toxoplasma*. Concerning the risk factors evaluated, there was a negative association between age and *Giardia* infection and between age and *T. leonina* infection intensity for dogs. Sequencing results revealed the presence of mainly animal-specific *G. duodenalis* assemblages C and D in dogs and assemblages F, C and BIV-like in cats, with only a limited number of (co-)infections with assemblage A. As for *Cryptosporidium*, the dog-specific *C. canis* and the pig-specific *C. scrofarum* were detected in dogs and the cat-specific *C. felis* was detected in cats.

Conclusions

High levels of parasitism in both dogs and cats were recorded. *Giardia* was the most prevalent parasite in all dog and cat populations except for shepherd dogs. Genotyping results suggest a limited zoonotic risk of *Giardia* and *Cryptosporidium* infections from dogs and cats in Crete. Taeniid eggs were more prevalent in shepherd dogs suggesting access to carcasses and posing a threat for cystic echinococcosis transmission. Infection rates of *Toxocara* spp. in both dogs and cats show that companion animals could be a significant source of infection to humans.

Electronic supplementary material

The online version of this article (doi:10.1186/s13071-017-1989-8) contains supplementary material, which is available to authorized users.

Keywords: Intestinal parasites, Companion animals, Zoonotic, Molecular analyses

Background

Go to:

Intestinal parasite infections are still abundant in companion animals, despite all the highly efficient drug formulations available and the control measures taken by owners and veterinarians [1–8]. Moreover, parasites are responsible for some of the most important and well-recognized zoonoses transmitted from companion animals to man globally such as *Giardia* spp., *Cryptosporidium* spp., *Toxocara* spp., hookworms and *Echinococcus granulosus* [9–13].

Nowadays, changes due to climate alterations and social behaviour that affect humans' lives and consequently the lives of the animals which live close to them [14, 15], alter the interactions between humans and pathogens leading to (re)emergence of several diseases, including zoonotic ones [16, 17].

The distribution of zoonoses associated with companion animals is highly affected by animals' movements (between regions, countries and continents) which in fact are the means to relocate pathogens and vectors they harbour. The above is becoming more and more important since human travel continues to increase in parallel with the population and financial status increase, and when humans travel, they often take their companion animals, particularly dogs.

All the above is in fact unfolding the reasons why it is crucial to fill the gaps on the current distribution of these diseases in a constantly changing environment and to describe the risks associated with pet infection in order to assure their well-being and to prevent the free movement of zoonotic pathogens.

The aim of our study was to investigate the presence and infection intensity of intestinal parasites in dogs and cats, the risk factors (such as lifestyle, veterinary care, etc.) that influence those infections and their zoonotic potential. This was done by performing a cross-sectional epidemiological study within a defined animal/human community, i.e. the island of Crete, as a case scenario.

Methods

Go to:

Populations studied

Faecal samples were collected from different dog populations (shelter, household and shepherd) as well as shelter and household cats in Crete Island in Southern Greece (Fig. 1), from October 2011 to January 2015.

Fig. 1
Map of Crete demonstrating the locations of different sample points per animal population category. *Key*: triangles: shelter dogs/cats; rhombi: shepherd dogs; gray ellipses: household dogs/cats with the number of animals sampled

Crete is the largest and most densely populated island of Greece (623,000 residents recorded in 2011) with a population well distributed in urban and rural areas. The island is also a highly popular tourist destination (approximately 3.5 million international tourist passengers' arrivals in 2013) (Region of Crete: www.crete.gov.gr). Moreover in Crete, in addition to the high number of companion animals, there is a significant livestock and wildlife population (Hellenic Ministry of Rural Development and Food: http://minagric.gr).

Since data on the precise population of pets in the location were not available, the sample size was determined estimating the dog and cat population size as "infinite". The prevalence of intestinal parasitism in different dog and cat studies in Europe varies enormously depending on the sampled animal population and the diagnostic techniques that were used [3, 6, 18–24]. In this study, in order to calculate the sample size (with a precision of 5% and a 95% confidence interval) we selected to relate our "expected prevalence" values to recent reports of *Giardia* prevalence in Europe. Therefore, the targeted sample size was defined as follows: for household dogs up to 200 dogs (reported prevalence 10 –20%); for shelter dogs up to 400 dogs (reported prevalence 20–50%); for household cats 138 cats (reported prevalence < 10%) and for shelter cats 385 cats (reported prevalence 10–50%) [3, 5, 18, 19, 25–28]. For shepherd dogs there is little information available and given the difficulties in approaching and handling such dogs we aimed at collecting the maximum feasible number of samples. In order to achieve the most accurate coverage of the whole island, the animals enrolled in our study were allocated proportionally to the four different counties of the island according to the inhabitant's population density (Fig. 1).

Individual rectal faecal samples were randomly collected from dogs and cats of all ages with or without intestinal symptoms from 561 households, 11 shelters and 29 sheep and goat farms. After collection, the samples were immediately transported under vacuum [29] to the laboratory where they were stored at 4 °C and examined within 2 days. When a sample was found to be positive by coproscopic analysis for *Giardia* spp. or *Cryptosporidium* spp., it was stored at -20 °C until DNA extraction was performed and molecular genotyping followed.

For every animal/sample, a data-form was completed by interviewing the owner or in case of shelters the person who was responsible for the animals, providing information on age, sex, breed, living conditions (indoors or outdoors), presence of other animals, the presence or absence of diarrhoea (up to maximum 1 month before sampling), if the animal had travelled recently and the antiparasitic treatment plan followed (including time of last treatment). Faecal consistency was recorded for all faecal samples. The consistency of individual faecal samples was scored using the following scale: 1, formed; 2, soft; 3, diarrhoea, 4, haemorrhagic diarrhoea.

Parasitological techniques

The presence of worm eggs and protozoan oocysts was determined by applying two different methods, i.e. a sedimentation (acid/ether) and a sedimentation/flotation technique (using a saturated sugar salt solution as a flotation fluid with 1.28 specific gravity) [30]. For the detection of *Giardia* spp. and *Cryptosporidium* spp. (oo)cysts a quantitative direct immunofluorescence assay (IFA) based on the commercial MERIFLUOR *Cryptosporidium/Giardia* kit (Meridian Diagnostics Inc., Cincinnati, Ohio) was used [31, 32].

Molecular analyses

DNA was extracted from the positive *Giardia* spp. and *Cryptosporidium* spp. faecal samples using the QIAamp® Stool Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. For the amplification of the *Cryptosporidium* 18S ribosomal RNA gene (rDNA18S) and HSP70 gene, previously described PCR protocols were used [33, 34]. For the identification of *Giardia* DNA, the *Giardia* rRNA 18S gene (rDNA 18S) [35], the β-giardin gene [36], the triose phosphate isomerase (TPI) gene [37] and the glutamate dehydrogenase (GDH) gene [38] were used. Amplification products were visualised on 1.5% agarose gels with ethidium bromide. A positive (genomic DNA from a positive faecal sample) and negative (PCR water) control sample were included in each PCR reaction.

PCR products were purified and sequenced from both strands. PCR products were purified using the Qiaquick PCR purification kit (Qiagen) and fully sequenced using the Big Dye Terminator V3.1 Cycle sequencing Kit (Applied Biosystems, California, USA). Sequencing was performed by an external company (GATC Biotech) using the Big dye Terminator V3.1 Cycle sequencing Kit (Applies Biosystems) and the reactions were analyzed using a 3730xl DNA Analyzer (ThermoFisher Scientific). Sequences were assembled using Seqman 5.0 Software (Lasergene DNASTAR) and were aligned using the Basic Local Alignment Search Tool (BLAST) as well as compared with reference sequences using MegAlign (Lasergene DNASTAR) (Additional file 1). For multilocus genotyping Clustal X, 2.0.11 software was used and reference sequences were selected according to Caccio et al. [39].

Statistical analysis

Descriptive statistical analyses and multivariate methodologies were performed using the statistical language R [40] and the *pscl* package [41]. Two approaches were applied as follows.

Multivariate binary logistic models

The effect of the independent variables (age in months, gender, food, travel, neutering, living conditions, living with other animals, antiparasitic treatment, time between treatment and sampling date, diarrhoea during the last month, faecal score and type) on a sample being or not infected by a parasite was studied through the utilization of multivariate logistic models with forward LR selection.

Initially, a test of the full model against a constant only model was performed in order to assess whether there was a statistically significant effect of the examined independent predictors on the response variable through the utilization of the Omnibus Tests of Model Coefficients, which uses the Chi-square test to see if there is a significant difference between the log-likelihood (-2LL) of the baseline model (constant model) and the model with the predictors. In addition, the Hosmer & Lemeshow (H-L) test was performed to test whether the model provides a good fit to the data (Additional file 2: Table S1).

Multivariate zero-inflated models

The effect of the independent variables on the parasitic infection intensity (egg/(oo)cyst counts per gram) was studied through the utilization of a zero-inflated negative binomial model [42] due to the excess of zero counts and overdispersion of the data. In this analysis the group of shepherd dogs were not included due to the limited number of samples examined (Additional file 2: Table S2).

Results

Go to:

Dogs

A total of 879 faecal samples from dogs were investigated for the presence of intestinal parasites. Of these samples, 278 were derived from shelter dogs, 529 from household dogs and 72 from shepherd dogs (Table 1). In total, 38.3% of dogs were found harbouring at least one intestinal parasite. Precisely 25.5% were harbouring one parasite, 8.9% two and the rest 3–6 different species. The overall infection rate was 25.2% (CI: 22.4–28.1) for *Giardia* spp.; 9.2% (CI: 7.3–11.1) for *Ancylostoma/Uncinaria* spp.; 7.6% (CI: 5.9–9.4) for *Toxocara* spp.; 5.9% (CI: 4.4–7.5) for *Cryptosporidium* spp.; 4.6% (CI: 3.2–5.9) for *Cystoisospora* spp.; 2.7% (1.7–3.8) for *Toxascaris leonina*; 1.7% (CI: 0.9–2.6) for *Capillaria* spp.; 0.8% (CI: 0.2–1.4) for taeniid eggs, 0.2% (CI: 0–0.5) for *Dipylidium caninum*; and 0.1% (CI: 0–0.3) for *Strongyloides stercoralis*. The results for the different dog populations are shown in Table 1.

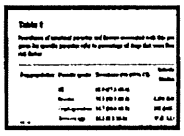

Table 1

Prevalence of intestinal parasites and factors associated with this prevalence in different dog populations. Percentages given for specific parasites refer to percentage of dogs that were found positive for an infection within a category of risk factor ...

Among the different canine populations studied, shelter dogs had the highest infection rates. In particular, 62.9% of the shelter dogs were infected with at least one species of endoparasite compared to 51.4% of the shepherd dogs and 23.8% of the household dogs. According to the multivariate binary logistic model analysis, the odds ratio (OR) of *Giardia* infection was higher in shelter dogs than household dogs (11.24 times higher) and shepherd dogs (15.63 times higher). However, based on the multivariate zero-inflated model, among *Giardia*-infected individuals, household dogs had generally higher cyst counts than shelter dogs (OR = 1.602). Regarding *Cryptosporidium*, and according to the multivariate zero-inflated model, the odds ratio in favour of zero *Cryptosporidium* OPG for household dogs was 8.248 times higher than that for shelter dogs, suggesting that household dogs were less prone to *Cryptosporidium* infection than shelter dogs. However, *Cryptosporidium*-positive household dogs shed more oocysts than infected shelter dogs (OR = 12.182). No statistically significant correlations between infection with the other parasites and their living conditions were detected in both models (Table 2).

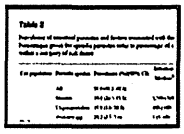

Table 2

Prevalence of intestinal parasites and factors associated with this prevalence in different cat populations. Percentages given for specific parasites refer to percentage of cats that were found positive for an infection within a category of risk factor ...

The mean age of the sampled dogs was approximately 3 years (39.5 months ± 41.8, SD). The majority of the dogs were adults (≥ 12 months, $n = 642$), while 229 of them were younger than 12 months and 8 were of unspecified age. There was a significant correlation between age and *Giardia* infection (Fig. 2) and between age and *T. leonina* infection intensity. According to the multivariate binary logistic model analysis, as age increased by one month, the odds of detecting *Giardia* cysts decreased by 1.9% = [(0.981–1) × 100] which is also confirmed by the multivariate zero-inflated model, according which the odds of absence of *Giardia* cysts are increased by one unit increase of age. Similarly, according to the multivariate zero-inflated model, as age increased by one month, the odds of detecting *T. leonina* eggs decreases by 7% = [(0.93–1) × 100]. Regarding the other parasites studied, their correlation with age was not statistically significant.

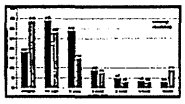

Fig. 2

Prevalence of *Giardia* spp. in different dog populations and different age groups

Of the dogs which had a history of recent diarrhoea, 43.1% were positive for at least one intestinal parasite. However, faecal consistency was not significantly associated with parasitic infection. The statistical analyses showed that signs of diarrhoea (based on faeces consistency) were significantly more often present in younger animals ($U = 100{,}667$, $P = < 0.001$). Moreover, there was a statistically significant association between the factors "recent record of diarrhoea" and "live with other animals", ($\chi^2_{(6, N = 1138)} = 29.495$, $P = < 0.001$).

On average, all of the dogs sampled received 2.1 anthelmintic treatments/year (range 0–6). The arithmetic mean of anthelmintic treatments/year was 2.3 for household dogs, 2.2 for shelter dogs and 0.5 for shepherd dogs. Information about anthelmintic treatments was not defined in 48 cases (5.5%). The frequency of antiparasitic treatment was also associated with diarrhoea and more specifically, the effect of the odds of one treatment per year increase resulted in a decrease by 0.828 times in the trace of "recent record of diarrhoea", implying diarrhoea to be caused by parasite infestation. However, the number of antiparasitic treatments/year received was not statistically associated with parasitic infection.

The risk analyses of all the other factors which were evaluated in this study, such as the gender of the animals, their living conditions (indoors/outdoors), the type of food, and recent travelling, showed no statistically significant correlation with parasitic infection. Since almost all shelter dogs had access to the external environment and the shepherd dogs were also living outside, the risk factor "living indoors/outdoors" was assessed only for household dogs. The risk factor "recent travelling" was also not analysed since only 4.2% of the dogs had been travelling during the last months before sampling, including within counties. The same applied for the "type of food" factor, since the majority of the dogs were eating industrial/cooked food and only 28 were fed with raw meat/offal, 64% of these being shepherd dogs.

Giardia spp. was the most prevalent parasite in all dogs (25.2%) and also in shelter (54.3%) and household (12.9%) dogs in particular. The range of the cysts being shed by the infected animals varied from 100 to 275,800 cysts per gram of faeces with 6,855 cysts shed on average. In the samples derived from shelter and household dogs, the dog-specific assemblages C and D were dominating, either alone ($n = 72$) or in mixed infections ($n = 15$). A limited number of dogs were infected with assemblage A ($n = 2$), assemblage AI ($n = 1$), assemblage AII ($n = 1$) or a mixture of A with C or D ($n = 5$) or BIV-like and C ($n = 1$) (Table 3). Regarding shepherd dogs, no positive PCR products were sequenced successfully. Multilocus genotyping was performed from one dog sample which was classified as sub-assemblage AI using 3 genetic loci (bg, TPIGEN and GDH). Alignment analysis of the isolate showed 100% homology when compared to reference sequences A5 for bg; A1 for TPIGEN and A1 for GDH [39], resulting in multilocus genotype MLGA1 [43].

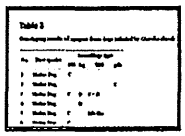

Table 3
Genotyping results of samples from dogs infected by *Giardia duodenalis* (at all different loci)

The PCR results for *Cryptosporidium* positive samples showed that the HSP70 gene amplified 23.6% of the samples, whereas the 18S rDNA gene amplified 5.6%. Sequencing revealed the presence of *Cryptosporidium canis* in 2 household dogs and *C. scrofarum* in a shelter dog.

Cats

In total, 264 faecal samples from cats were collected; 59 samples from shelters and 205 from owned cats. Unfortunately, it was not possible to reach the target of 385 shelter cats. Overall, 38.1% of the cats were harbouring at least one intestinal parasite. Precisely 26.4% were harbouring one parasite, 8.3% two and the rest 3–4 different species. The prevalence was 20.5% (CI: 15.6–25.3) for *Giardia* spp.; 9.5% (CI: 5.9–13.0) for *Cystoisospora* spp.; 8.3% (CI: 5.0–11.7) for *Toxocara* spp.; 7.6% (CI: 4.4–10.8) for *Ancylostoma/Uncinaria* spp.; 6.8% (CI: 3.8–9.9) for *Cryptosporidium* spp.; 4.2% (CI: 1.8–6.6) for *Capillaria* spp.; 0.8% (CI: 0.0–1.8) for taeniid eggs; and 0.4% (CI: 0–1.1) for *Hammondia/Toxoplasma*. The results among different feline populations are shown in Table 4.

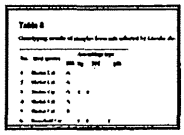

Table 4
Genotyping results of samples from cats infected by *Giardia duodenalis* (at all different loci)

The mean age of the sampled cats was 3.4 years (40.8 months ± 48.9, SD). The majority of the cats were adults (≥ 12 months, $n = 161$), while 97 of them were younger than 12 months and 6 were of unspecified age.

Among the different feline populations studied, shelter cats had the highest infection rates. Specifically, 55.9% of the shelter cats were infected with at least one species of intestinal parasite compared to 33.2% of the household cats. However, infection rates of the different parasites were not statistically different between different cat populations.

Of the cats which had a history of diarrhoea (30.9%), 32.9% were infected with at least one parasite. On average, all cats sampled received 2.3 anthelmintic treatments/year (range 0–6). The mean number of anthelmintic treatments/year was 1.9 for household cats and 2.7 for shelter cats. Information about

anthelmintic treatments was unknown in one case. Only 1.5% of the cats had been travelling during the last months including within counties. No significant associations were found between parasite infections and risk factors or between parasite infections and diarrhoea.

Giardia spp. was the most prevalent parasite (20.5%), both in shelter cats (39.0%) and household cats (15.6%). When targeting the 18S rRNA gene, assemblage A was identified in 10 cat samples. In 6 of these samples, no amplification was obtained with the other genes, while in 4 samples only assemblage F was detected in at least one of the other loci. Assemblage F was also found alone in 2 samples. Also, in two different cases, the typing revealed the presence of assemblage BIV-like ($n = 1$) or the dog specific assemblage C ($n = 1$) (Table 4).

Genotyping of *Cryptosporidium* positive samples showed the presence of the feline specific species *Cryptosporidium felis* ($n = 4$).

Discussion

Go to:

The infection rates of intestinal parasites detected in this study, revealed a high prevalence of parasitic infections (38.2%) and the presence of different species of endoparasites in both dogs and cats. These infection rates were equally distributed within animal species (38.3% for dogs and 38.1% for cats) involved in the study. With the exception of shepherd dogs, *Giardia* spp. was the most prevalent parasite detected in the dog and cat populations followed by significant prevalences of ascarids, hookworms and taeniid infections. These results are also reported in other studies which consider *Giardia* the most common enteric parasite of dogs and cats in developed countries [2, 3, 23, 28, 44–48]. In shepherd dogs, hookworms were the most prevalent parasite species detected.

Among the targets of this study was to investigate the potential effect of animal lifestyle to parasitism so animals living in households, shelters or farms were included. The results showed that more than half of the shelter dogs and cats were infected with at least one species of endoparasite, which was more or less expected, taking into consideration the less hygienic conditions that those animal are living in combined with a high population density that usually exists in shelters. A high level of parasitism has been previously reported in shelter dogs [3, 5, 22, 24] while in shelter cats the prevalence observed in other studies was lower [49, 50].

More than half of the shepherd dogs (51.4%) were positive for at least one species of intestinal parasite. The infection rate of intestinal parasites estimated in shepherd dogs in this study was higher than in a previous record from Greece (26.0%) [27]. Such differences are expected in cross-sectional studies especially given time and region differences. However, our results were in agreement with a study conducted in farm dogs in Portugal (57.4%) [51]. Shepherd/farm dogs often receive less veterinary care and preventive treatments. Compared to a general average of more than 2 anthelmintic treatments per year, shepherd dogs in our study received only 0.5 treatments per year.

Although the prevalence of intestinal parasites in household dogs was lower than in shelters, although not statistically significant for many species, the percentage of individuals infected was still noteworthy (23.8%). In similar studies conducted in Italy the prevalence of intestinal parasites in household dogs was even higher, reaching 57.0% of the animals [6, 23]. In our study there was no difference in the risk of infection between dogs living in an apartment with no access to a yard or a garden and dogs living in a house with access to outdoors. A reason for that could be that even dogs that are kept permanently indoors are regularly being walked by their owners in public places getting in close contact with other dogs (including stray ones) or their contaminated faeces. Similar results were recorded in the household

cats studied, but this was probably due to the fact that the majority of them also live partially outdoors. The parasitism reported in household cats in this study is in agreement with the infection rates reported in Austria, Belgium, the Netherlands, France, Hungary, Italy, Romania and Spain [6, 8, 21].

Although both household and shelter dogs received regular anthelmintic treatments (i.e. an average of 2.3 for household dogs and 2.2 for shelter dogs per year), this seemed not to control parasitism efficiently. This is in agreement with the general recommendation by ESCCAP for roundworms in which it is suggested that annual or twice yearly treatments do not have a significant impact on the prevalence of patent infections within a population, and therefore a treatment frequency of at least 4 times per year is recommended (Worm Control in Dogs and Cats - ESCCAP, www.esccap.org). Recent modeling indicated that the environmental *Toxocara* contamination by dogs can only be reduced significantly if compliance to the four times a year treatment advice is sufficiently high (90%) or if at least half of the dog owners consistently remove their dog's faeces [52]. In cats, the frequency of anthelmintic treatment differed between categories, with shelter cats being more frequently treated (i.e. an average of 1.9 for household cats and 2.7 for shelter cats per year). This could be explained by a misconception of the cat owners that indoor cats do not need preventive treatments [53].

Despite the high prevalence of parasitic infections, most animals were healthy with no obvious signs of suffering probably due to the low parasitic burden, as at least suggested by the low number of egg/(oo) cyst output recorded in most cases (even if usually there is not a clear correlation between numbers of eggs/(oo-)cysts and clinical signs). It was not statistically proven that recent records of diarrhoea were correlated to parasitism as also shown previously [54, 55] although there was evidence that anthelmintic treatment had a positive effect on reducing such records. A supporting argument for the absence of clinical disease could be that the majority of animals were adults at the time of sampling. Young animals are more sensitive to parasitism [56] but although in this study there was a tendency of older animals (> 2 year-old dogs and > 1 year-old cats) to be less infected, this was not statistically significant for most parasites. The only statistically proven facts were that the chance to get infected by *Giardia* spp. and the infection intensity of *T. leonina* was negatively correlated to age in dogs.

Given the high prevalence and the potential zoonotic importance, *Giardia* and *Cryptosporidium* positive samples were further investigated by PCR and sequencing of the positive PCR products. In dogs, the host-specific assemblages C and D dominated, which has been described before in various studies [23, 24, 36, 57–62]. Few dogs were (co)- infected with assemblage A, and the majority of these were identified as sub-assemblage AI. Sub-assemblage AI is frequently found in animals, while humans are most frequently infected with sub-assemblage AII [63, 64]. The sequence analysis in one *G. duodenalis* sample further revealed a multilocus genotype (MLG) which was previously described in calves in China [43]. Together, these results suggest that there is no significant risk for zoonotic transmission of *Giardia* infections from dogs in Crete.

In cats, the genotyping results seemed to indicate the dominance of the potentially zoonotic assemblage A in shelter animals and the co-infection of assemblages A and the feline specific assemblage F in household cats. However, the zoonotic assemblage A was identified only at the 18S rDNA locus, while only assemblage F was identified at the other loci. Since no distinction could be made between assemblages A and F in the amplified region of the conserved rRNA 18S gene, it cannot be excluded that (some of) the samples that were amplified with rDNA 18S gene were assemblage F instead of A. Therefore, no conclusion can be drawn on the zoonotic risk associated with *Giardia* infections in cats.

Regarding *Cryptosporidium*, the dog specific *C. canis* was identified in only two household dogs and the pig specific *C. scrofarum* in one shelter dog. *Cryptosporidium canis* has been also detected in household dogs in other studies [48, 65, 66] and isolated in humans, mainly children and immunocomprimised individuals in developing countries [67, 68], suggesting its potential public health impact. To our knowledge, this is the first case of *C. scrofarum* infection reported in a dog. Since keeping backyard pigs is quite a common practice in the area, it is possible that this dog ingested the oocysts before being transferred to the shelter. In such a scenario this could be a case of pseudoparasitism, given that this dog was 2.5 month-old and only recently introduced to the shelter. In cats, sequencing was not efficient; nevertheless, it revealed the presence of the feline-specific *C. felis*. Since our genotyping results revealed the presence of host-specific *Cryptosporidium* species in both dogs and cats which have been implicated in very few human infections and mainly in developing countries, we could suggest that the zoonotic potential of *Cryptosporidium* from dogs in the study area is low.

Apart from *Giardia* and *Cryptosporidium*, ascarids, hookworms and taeniids are also considered to be zoonotic [13, 69–72]. The two major ascarid species *T. canis* and *T. cati* (to a lesser extent) are responsible for human infections [13, 72]. In our study the prevalence of *Toxocara* spp. in dogs and cats was 7.6 and 8.3%, respectively. In dogs, we characterised all *Toxocara* eggs found as *Toxocara* spp. since those infections were only microscopically diagnosed and as previously suggested they could either belong to *T. canis* or *T. cati* since coprophagy is not unusual for dogs and the presence of *T. cati* eggs in dog faeces might in fact relate to pseudoparasitism [73, 74]. The infection rates found in the present study are similar to those reported in Europe which vary from 3.5 to 34.0% for *T. canis* in dogs from different epidemiological environments and from 7.2 to 76.0% for *T. cati* in cats [8, 10, 18, 52, 75–79]. The *Toxocara* infection was high, especially in shelter dogs and cats, as also reported before [24, 80, 81]. Although mainly *T. canis* is considered responsible for human toxocarosis [82], the role of *T. cati* in human toxocarosis should not be underestimated [82–84]. In Greece, toxocarosis in humans has not been studied extensively since published data are restricted only to some sporadic cases [85, 86] and one study regarding the seroprevalence of *T. canis* in children [87]. Our results combined to all European studies presented above strongly suggest that more information is needed.

Hookworm infection rates were 9.2% in dogs and 7.6% in cats. The highest infection rates of hookworms were identified in shepherd dogs (33.3%) similar to the study of Mateus et al. [51] in Portugal (31.0%). Since different hookworm species were not differentiated, the zoonotic risk associated with hookworm infections could not be determined.

The detection of taeniid eggs in shepherd dogs is worth mentioning. Unlike shelter and household dogs, shepherd dogs seem to be more prone to taeniid infection, which possibly is due to the frequent consumption of raw meat and carcasses [2, 88]. Echinococcosis is still endemic in Greece with a high prevalence reported in livestock [89–91]. However, there are no recent reports regarding the prevalence of taeniids in dogs. Taking into consideration our results in combination with the high prevalence of *E. granulosus* in livestock, which is transmitted through dogs, we could assume that shepherd dogs in Greece could be a reservoir for human infections.

Conclusions

Go to:

In conclusion, we have recorded high levels of (multi)parasitism in both dogs and cats in the study area. Most of the animals were harbouring different species of parasites sometimes in high numbers according to the egg/(oo)cyst counts. This is a proof that those parasites are greatly abundant within

animal populations regardless of lifestyle. Thus, the results of our study, stress the need for better anthelmintic control schemes in dogs and cats tailored to their individual needs in order to safeguard animal and public health.

Aknowledgements

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This work is a part of a PhD scholarship funded by the Greek State Scholarships Foundation. The authors will also like to thank all the veterinarians, the shelter owners, Dr Alexandros Stefanakis, Mr Anastasios Anastasiades and other colleagues in Crete who contributed in this study.

Funding

This work is a part of a PhD scholarship funded by the Greek State Scholarships Foundation.

Availability of data and materials

All data generated or analysed during this study are included in the article.

Authors' contributions

This study was conducted by DK as part of her PhD thesis. Also, DK participated in the data collection and analysis and developed the first draft of the manuscript. All other authors played a role in data collection and analysis, and interpretation of findings (EC, DA, PL, NV, SC and SS). All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval

The study was carried out in compliance with the national animal welfare regulations. Diagnostic veterinary procedures are not within the context of relevant EU legislation for animal experimentations (Directive 86/609/EC) and may be performed in order to diagnose animal diseases and improve animal welfare. Samples were collected by registered veterinarians who ensured owners consent and caused no suffering.

Additional files

Additional file 1:(3.5K, zip)

Examples of alignments analyzed (different target genes) in both dogs and cats. (ZIP 3 kb)

Additional file 2: Table S1.(18K, docx)

Binary logistic model for *Giardia* spp. infection rate in dogs. **Table S2.** Zero-inflation negative binomial model for parasite infection intensity in dog samples. (DOCX 17 kb)

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References

Go to:

1. Capelli G, Paoletti B, Iorio R, Frangipane di Regalbono A, Pietrobelli M, Bianciardi P, Giangaspero A. Prevalence of *Giardia* spp. in dogs and humans in nothern and central Italy. Parasitol Res. 2003;90:S154–S155. doi: 10.1007/s00436-003-0924-4. [PubMed] [Cross Ref]

2. Palmer CS, Thompson RCA, Traub RJ, Rees R, Robertson ID. National study of the intestinal parasites of dogs and cats in Australia. Vet Parasitol. 2008;151:181–190. doi: 10.1016/j.vetpar.2007.10.015. [PubMed] [Cross Ref]

3. Claerebout E, Casaert S, Dalemans AC, De Wilde N, Levecke B, Vercruysse J, Geurden T. *Giardia* and other intestinal parasites in different dog populations in Nothern Belgium. Vet Parasitol. 2009;161:41–46. doi: 10.1016/j.vetpar.2008.11.024. [PubMed] [Cross Ref]

4. Joffe D, Van Niekerk D, Gagne F, Gilleard J, Kutz S, Lobingier R. The prevalence of intestinal parasites in dogs and cats in Calgary, Alberta. Can Vet J. 2011;52:1323–1328. [PMC free article] [PubMed]

5. Ortuno A, Castella J. Intestinal parasites in shelter dogs and risk factors associated with the facility and its management. Isr J Vet Med. 2011;66:103–107.

6. Riggio F, Mannella R, Ariti G, Perrucci S. Intestinal and lung parasites in owned dogs and cats from Central Italy. Vet Parasitol. 2013;193:78–84. doi: 10.1016/j.vetpar.2012.11.026. [PubMed] [Cross Ref]

7. Itoh N, Kanai K, Kimura Y, Chikazawa S, Hori Y, Hoshi F. Prevalence of intestinal parasites in breeding kennel dogs in Japan. Parasitol Res. 2015;114(3):1221–4. doi: 10.1007/s00436-015-4322-5. [PubMed] [Cross Ref]

8. Nijsse R, Ploeger HW, Wagenaar JA, Mughini-Gras L. Prevalence and risk factors for patent *Toxocara* infections in cats and cat owners' attitude towards deworming. Parasitol Res. 2016;115 (12):4519–4525. doi: 10.1007/s00436-016-5242-8. [PMC free article] [PubMed] [Cross Ref]

9. Pereira A, Martins A, Brancal H, Vilhena H, Silva P, Pimenta P, Diz-Lopes D, Neves N, Coimbra M, Alves AC, Cardoso L, Maia C. Parasitic zoonoses associated with dogs and cats: a survey of Portuguese pet owners' awareness and deworming practices. Parasit Vectors. 2016;9:245. doi: 10.1186/s13071-016-1533-2. [PMC free article] [PubMed] [Cross Ref]

10. Lee ACY, Shantz PM, Kazacos KR, Montgomery SP, Bowman DD. Epidemiologic and zoonotic aspects of ascarid infection of dogs and cats. Trends Parasitol. 2010;26:155–161. doi: 10.1016/j.pt.2010.01.002. [PubMed] [Cross Ref]

11. Deplazes P, Van Knapen F, Schweiger A, Overgaauw PAM. Role of pet dogs and cats in the transmission of helminthic zoonoses in Europe, with a focus on echinococcosis and toxocarosis. Vet Parasitol. 2011;182:41–53. doi: 10.1016/j.vetpar.2011.07.014. [PubMed] [Cross Ref]

12. Chen J, Xu MJ, Zhou DH, Song HQ, Wang CR, Zhu XQ. Canine and feline parasitic zoonoses in China. Parasit Vectors. 2012;5:152. doi: 10.1186/1756-3305-5-152. [PMC free article] [PubMed] [Cross Ref]

13. Macpherson CNL. The epidemiology and public health importance of toxocariasis: a zoonosis of global importance. Int J Parasitol. 2013;43:999–1008. doi: 10.1016/j.ijpara.2013.07.004. [PubMed] [Cross Ref]

14. Sutherst RW. Global change and human vulnerability to vector-borne diseases. Clin Microbiol Rev. 2004;17:136–73. doi: 10.1128/CMR.17.1.136-173.2004. [PMC free article] [PubMed] [Cross Ref]

15. Tabachnick WJ. Challenges in predicting climate and environmental effects on vector-borne disease episystems in a changing world. J Exp Biol. 2010;213:946–54. doi: 10.1242/jeb.037564. [PubMed] [Cross Ref]

16. Jones KE, Patel NG, Levy MA, Storeygard A, Balk D, Gittleman JL, Daszak P. Global trends in emerging infectious diseases. Nature. 2008;451:990–994. doi: 10.1038/nature06536. [PubMed] [Cross Ref]

17. Otranto D, Eberhard ML. Zoonotic helminths affecting the human eye. Parasit Vectors. 2011;4:41. doi: 10.1186/1756-3305-4-41. [PMC free article] [PubMed] [Cross Ref]

18. Dubna S, Langrova I, Napravnik J, Jankovska I, Vadlejch J, Pekar S, Fechtner J. The prevalence of intestinal parasites in dogs from Prague, rural areas, and shelters of the Czech Republic. Vet Parasitol. 2007;145:120–128. doi: 10.1016/j.vetpar.2006.11.006. [PubMed] [Cross Ref]

19. Martinez-Moreno FJ, Hernandez S, Lopez-Cobos E, Becerra C, Acosta I, Martinez-Moreno A. Estimation of canine intestinal parasites in Cordoba (Spain) and their risk to public health. Vet Parasitol. 2007;143:7–13. doi: 10.1016/j.vetpar.2006.08.004. [PubMed] [Cross Ref]

20. Overgaauw PAM, van Zutphen L, Hoek D, Yaya FO, Roelfsema J, Pinelli E, et al. Zoonotic parasites in fecal samples and fur from dogs and cats in the Netherlands. Vet Parasitol. 2009;163:115–122. doi: 10.1016/j.vetpar.2009.03.044. [PubMed] [Cross Ref]

21. Beugnet F, Bourdeau P, Chalvet-Monfray K, Cozma V, Farkas R, Guillot J, et al. Parasites of domestic owned cats in Europe: co-infestations and risk factors. Parasit Vectors. 2014;7:291. doi: 10.1186/1756-3305-7-291. [PMC free article] [PubMed] [Cross Ref]

22. Ortuno A, Scorza V, Castella J, Lappin M. Prevalence of intestinal parasites in shelter and hunting dogs in Catalonia, Northeastern Spain. Vet J. 2014;199:465–467. doi: 10.1016/j.tvjl.2013.11.022. [PubMed] [Cross Ref]

23. Zanzani SA, Gazzonis AL, Scarpa P, Berrilli F, Manfredi MT. Intestinal parasites of owned dogs and cats from metropolitan and micropolitan areas: prevalence, zoonotic risks, and pet owner awareness

in nothern Italy. BioMed Res Int. 2014;2014:696508. doi:10.1155/2014/696508. [PMC free article] [PubMed]

24. Simonato G, Frangipane di Regalbono A, Cassini R, Traversa D, Beraldo P, Tessarin C, Pietrobelli M. Copromicroscopic and molecular investigations on intestinal parasites in kenneled dogs. Parasitol Res. 2015;114:1963–1970. doi: 10.1007/s00436-015-4385-3. [PubMed] [Cross Ref]

25. Hamnes IS, Gjerde BK, Robertson LJ. A longitudinal study on the occurrence of *Cryptosporidium* and *Giardia* in dogs during their first year of life. Acta Vet Scand. 2007;49:22. doi: 10.1186/1751-0147-49-22. [PMC free article] [PubMed] [Cross Ref]

26. Leonhard S, Pfister K, Beelitz P, Wielinga C, Thompson RCA. The molecular characterization of *Giardia* from dogs in southern Germany. Vet Parasitol. 2007;150:33–38. doi: 10.1016/j.vetpar.2007.08.034. [PubMed] [Cross Ref]

27. Papazahariadou M, Founta A, Papadopoulos E, Chliounakis S, Antoniadou-Sotiriadou K, Theodorides Y. Gastrointestinal parasites of shepherd and hunting dogs in the Serres Prefecture, Northern Greece. Vet Parasitol. 2007;148:170–173. doi: 10.1016/j.vetpar.2007.05.013. [PubMed] [Cross Ref]

28. Barutzki D, Schaper R. Results of parasitological examinations of faecal samples from cats and dogs in Germany between 2003 and 2010. Parasitol Res. 2011;109:S45–60. doi: 10.1007/s00436-011-2402-8. [PubMed] [Cross Ref]

29. Rinaldi L, Coles GC, Maurelli MP, Musella V, Cringoli G. Calibration and diagnostic accuracy of simple flotation, McMaster and Flotac for parasite egg counts in sheep. Vet Parasitol. 2011;177:345–352. doi: 10.1016/j.vetpar.2010.12.010. [PubMed] [Cross Ref]

30. MAFF. Manual of veterinary parasitology laboratory techniques. Ministry of Agriculture, Fisheries and Food. London: Her Majesty's Stationery Office; 1986.

31. Kostopoulou D, Casaert S, Tzanidakis N, van Doorn D, Demeler J, von Samson-Himmelstjerna G, et al. The occurrence and genetic characterization of *Cryptosporidium* and *Giardia* species in foals in Belgium, The Netherlands, Germany and Greece. Vet Parasitol. 2015;211(3–4):170–4. doi: 10.1016/j.vetpar.2015.04.018. [PubMed] [Cross Ref]

32. Geurden T, Berkvens D, Casaert S, Vercruysse J, Claerebout E. A Bayesian evaluation of three diagnostic assays for the detection of *Giardia duodenalis* in symptomatic and asymptomatic dogs. Vet Parasitol. 2008;157(1–2):14–20. doi: 10.1016/j.vetpar.2008.07.002. [PubMed] [Cross Ref]

33. Morgan UM, Monis PT, Xiao L, Limor J, Sulaiman I, Raidal S, et al. Molecular and phylogenetic characterization of *Cryptosporidium* from birds. Int J Parasitol. 2001;31:289–296. doi: 10.1016/S0020-7519(00)00164-8. [PubMed] [Cross Ref]

34. Xiao L, Singh A, Limor J, Graczyk TK, Gradus S, Lal AA. Molecular characterization of *Cryptosporidium* oocysts in samples of raw surface water and wastewater. Appl Environ Microbiol. 2001;6:1097–1101. doi: 10.1128/AEM.67.3.1097-1101.2001. [PMC free article] [PubMed] [Cross Ref]

35. Hopkins RM, Meloni BP, Groth DM, Wetherall JD, Reynoldson JA, Thompson RCA. Ribosomal RNA sequencing reveals differences between the genotypes of *Giardia* isolates recovered from humans and dogs living in the same locality. J Parasitol. 1997;83:44–51. doi: 10.2307/3284315. [PubMed] [Cross Ref]

36. Lalle M, Pozio E, Capelli G, Bruschi F, Crotti D, Caccio SM. Genetic heterogeneity at the beta-giardin locus among human and animal isolates of *Giardia duodenalis* and identification of potentially zoonotic subgenotypes. Int J Parasitol. 2005;35:207–213. doi: 10.1016/j.ijpara.2004.10.022. [PubMed] [Cross Ref]

37. Geurden T, Geldhof P, Levecke B, Martens C, Berkvens D, Casaert S, et al. Mixed *Giardia duodenalis* assemblage A and E infections in calves. Int J Parasitol. 2008;38:259–264. doi: 10.1016/j.ijpara.2007.07.016. [PubMed] [Cross Ref]

38. Read CM, Monis PT, Thompson RC. Discrimination of all genotypes of *Giardia duodenalis* at the glutamate dehydrogenase locus using PCR-RFLP. Infect Genet Evol. 2004;4:125–30. doi: 10.1016/j.meegid.2004.02.001. [PubMed] [Cross Ref]

39. Caccio SM, Beck R, Lalle M, Marinculic A, Pozio E. Multilocus genotyping of *Giardia duodenalis* reveals striking differences between assemblages A and B. Int J Parasitol. 2008;38:1523–31. doi: 10.1016/j.ijpara.2008.04.008. [PubMed] [Cross Ref]

40. R Core Team . R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2013.

41. Jackman S. pscl: Classes and methods for R developed in the political science computational laboratory, Stanford University. Department of Political Science, Stanford University, Stanford, California. R package version 0.95, URL http://CRAN.R-project.org/package=pscl. Accessed 25 Aug 2016.

42. Lambert D. Zero-inflated Poisson regression, with an application to defects in manufacturing. Technometrics. 1992;34:1–14. doi: 10.2307/1269547. [Cross Ref]

43. Wang XT, Wang RJ, Ren GJ, Yu ZQ, Zhang LX, Zhang SY, et al. Multilocus genotyping of *Giardia duodenalis* and *Enterocytozoon bieneusi* in dairy and native beef (Qinchuan) calves in Shaanxi province, northwestern China. Parasitol Res. 2016;115:1355–61. doi: 10.1007/s00436-016-4908-6. [PubMed] [Cross Ref]

44. Thompson RC, Palmer CS, O'Handley R. The public health and clinical significance of *Giardia* and *Cryptosporidium* in domestic animals. Vet J. 2008;177:18–25. doi: 10.1016/j.tvjl.2007.09.022. [PubMed] [Cross Ref]

45. Scaramozzino P, Di Cave D, Berrilli F, D'Orazi CD, Spaziani A, Mazzanti S, et al. A study of the prevalence and genotypes of *Giardia duodenalis* infecting kenneled dogs. Vet J. 2009;182:231–234. doi: 10.1016/j.tvjl.2008.07.003. [PubMed] [Cross Ref]

46. Ballweber LR, Xiao L, Bowman DD, Kahn G, Cama VA. Giardiasis in dogs and cats: update on epidemiology and public health significance. Trends Parasitol. 2010;26:180–189. doi: 10.1016/j.pt.2010.02.005. [PubMed] [Cross Ref]

47. Polak KC, Levy JK, Crawford PC, Leutenegger CM, Moriello KA. Infectious diseases in large-scale cat hoarding investigations. Vet J. 2014;201:189–195. doi: 10.1016/j.tvjl.2014.05.020. [PubMed] [Cross Ref]

48. Osman M, Bories J, El Safadi D, Poirel M, Gantois N, Benamrouz-Vanneste S, et al. Prevalence and genetic diversity of the intestinal parasites *Blastocystis* sp. and *Cryptosporidium* spp. in household

dogs in France and evaluation of zoonotic transmission risk. Vet Parasitol. 2015;214:167–170. doi: 10.1016/j.vetpar.2015.09.015. [PubMed] [Cross Ref]

49. Becker AC, Rohen M, Epe C, Schnieder T. Prevalence of endoparasites in stray and fostered dogs and cats in Northern Germany. Parasitol Res. 2012;111:849–857. doi: 10.1007/s00436-012-2909-7. [PubMed] [Cross Ref]

50. Ito Y, Itoh N, Kimura Y, Kanai K. Prevalence of intestinal parasites in breeding cattery cats in Japan. J Feline Med Surq. 2016;18:834–7. doi: 10.1177/1098612X15597023. [PubMed] [Cross Ref]

51. Mateus TL, Castro A, Ribeiro JN, Vieira-Pinto M. Multiple zoonotic parasites identified in dog feces collected in Ponte de Lima, Portugal - A potential threat to human health. Int J Environ Res Public Health. 2014;11:9050–9067. doi: 10.3390/ijerph110909050. [PMC free article] [PubMed] [Cross Ref]

52. Nijsse R, Mughini-Gras L, Wagenaar JA, Franssen F, Ploeger HW. Environmental contamination with *Toxocara* eggs: a quantitative approach to estimate the relative contributions of dogs, cats and foxes, and to assess the efficacy of advised interventions in dogs. Parasit Vectors. 2015;8:397. doi: 10.1186/s13071-015-1009-9. [PMC free article] [PubMed] [Cross Ref]

53. Matos M, Alho AM, Owen SP, Nunes T. Parasite control practices and public perception of parasitic diseases: a survey of dog and cat owners. Prev Vet Med. 2015;122:174–80. doi: 10.1016/j.prevetmed.2015.09.006. [PubMed] [Cross Ref]

54. Hill SL, Cheney JM, Taton-Allen GF, Reif JS, Bruns C, Lappin MR. Prevalence of enteric zoonotic organisms in cats. J Am Vet Med Assoc. 2000;216:687–692. doi: 10.2460/javma.2000.216.687. [PubMed] [Cross Ref]

55. Hackett T, Lappin MR. Prevalence of enteric pathogens in dogs of north-central Colorado. J Am Anim Hosp Assoc. 2003;39:52–56. doi: 10.5326/0390052. [PubMed] [Cross Ref]

56. Gates MC, Nolan TJ. Endoparasite prevalence and recurrence across different age groups of dogs and cats. Vet Parasitol. 2009;166(1–2):153–158. doi: 10.1016/j.vetpar.2009.07.041. [PMC free article] [PubMed] [Cross Ref]

57. Sulaiman IM, Fayer R, Bern C, Gilman RH, Trout JM, Schantz PM, et al. Triosephospate isomerase gene characterization and potential zoonotic transmission of *Giardia duodenalis*. Emerg Infect Dis. 2003;9:1444–1452. doi: 10.3201/eid0911.030084. [PMC free article] [PubMed] [Cross Ref]

58. Berilli F, Di Cave D, De Liberato C, Franco A, Scaramozzino P, Orecchia P. Genotype characterization of *Giardia duodenalis* isolates from domestic and farm animals by SSU-rRNA gene sequencing. Vet Parasitol. 2004;122:193–199. doi: 10.1016/j.vetpar.2004.04.008. [PubMed] [Cross Ref]

59. Barutzki D, Thompson RC, Wielinga C, Parka U, Schaper R. Observations on *Giardia* infection in dogs from veterinary clinics in Germany. Parasitol Res. 2007;101:153–156. doi: 10.1007/s00436-007-0623-7. [Cross Ref]

60. Souza SL, Gennari SM, Richtzenhain LJ, Pena HF, Funada MR, Cortez A, et al. Molecular identification of *Giardia duodenalis* isolates from humans, dogs, cats and cattle from the state of Sao Paulo, Brazil, by sequence analysis of fragments of glutamate dehydrogenase (gdh) coding gene. Vet Parasitol. 2007;149:258–264. doi: 10.1016/j.vetpar.2007.08.019. [PubMed] [Cross Ref]

61. Scorza AV, Ballweber LR, Tangtrongsup S, Panuska C, Lappin MR. Comparisons of mammalian *Giardia duodenalis* assemblages based on the β-giardin, glutamate dehydrogenase and triose phosphate isomerase genes. Vet Parasitol. 2012;189:182–188. doi: 10.1016/j.vetpar.2012.04.032. [PubMed] [Cross Ref]

62. Pallant L, Barutzki D, Schaper R, Thompson RCA. The epidemiology of infections with *Giardia* species and genotypes in well cared for dogs and cats in Germany. Parasit Vectors. 2015;8:2. doi: 10.1186/s13071-014-0615-2. [PMC free article] [PubMed] [Cross Ref]

63. Xiao L, Fayer R. Molecular characterisation of species and genotypes of *Cryptosporidium* and *Giardia* and assessment of zoonotic transmission. Int J Parasitol. 2008;38:1239–1255. doi: 10.1016/j.ijpara.2008.03.006. [PubMed] [Cross Ref]

64. Minetti C, Lamden K, Durband C, Cheesbrough J, Fox A, Wastling JM. Determination of *Giardia duodenalis* assemblages and multi-locus genotypes in patients with sporadic giardiasis from England. Parasit Vectors. 2015;8:44. doi: 10.1186/s13071-015-1059-z. [PMC free article] [PubMed] [Cross Ref]

65. Lucio-Forster A, Griffiths JK, Cama VA, Xiao L, Bowman DD. Minimal zoonotic risk of cryptosporidiosis from pet dogs and cats. Trends Parasitol. 2010;26:174–179. doi: 10.1016/j.pt.2010.01.004. [PubMed] [Cross Ref]

66. Ryan U, Fayer R, Xiao L. *Cryptosporidium* species in humans and animals: current understanding and research needs. Parasitology. 2014;11:1–19. doi: 10.1016/j.exppara.2014.04.016. [PubMed] [Cross Ref]

67. Xiao L, Cama VA, Cabrera L, Ortega Y, Pearson J, Gilman RH. Possible transmission of *Cryptosporidium canis* among children and a dog in a household. J Clin Microbiol. 2007;45:2014 –2016. doi: 10.1128/JCM.00503-07. [PMC free article] [PubMed] [Cross Ref]

68. Bowman DD, Lucio-Forster A. Cryptosporidiosis and giardiasis in dogs and cats: veterinary and public health importance. Exp Parasitol. 2010;124:121–7. doi: 10.1016/j.exppara.2009.01.003. [PubMed] [Cross Ref]

69. Dakkak A. Guidelines for diagnosis, surveillance and control of echinococcosis. Geneva Switzerland: Veterinary Public Health, World Health Organization; 1992.

70. Caccio S, Ryan U. Molecular epidemiology of giardiasis. Mol Biochem Parasitol. 2008;160:75–80. doi: 10.1016/j.molbiopara.2008.04.006. [PubMed] [Cross Ref]

71. Thompson RCA, Smith A. Zoonotic enteric protozoa. Vet Parasitol. 2011;182:70–78. doi: 10.1016/j.vetpar.2011.07.016. [PubMed] [Cross Ref]

72. Traversa D. Pet roundworms and hookworms: a continuing need for global worming. Parasit Vectors. 2012;5:91. doi: 10.1186/1756-3305-5-91. [PMC free article] [PubMed] [Cross Ref]

73. Fahrion AS, Schnyder M, Wichert B, Deplazes P. *Toxocara* eggs shed by dogs and cats and their molecular and morphometric species-specific identification: is the finding of *T. cati* eggs shed by dogs of epidemiological relevance? Vet Parasitol. 2011;177:186–9. doi: 10.1016/j.vetpar.2010.11.028. [PubMed] [Cross Ref]

74. Nijsse R, Mughini-Gras L, Wagenaar JA, Ploeger HW. Coprophagy in dogs interferes in the diagnosis of parasitic infections by faecal examination. Vet Parasitol. 2014;204(3–4):304–9. doi: 10.1016/j.vetpar.2014.05.019. [PubMed] [Cross Ref]

75. Parsons JC. Ascarid infections of cats and dogs. Vet Clin North Small Anim Pract. 1987;17:1307–39. doi: 10.1016/S0195-5616(87)50004-3. [PubMed] [Cross Ref]

76. Fok E, Szatmari V, Busak K, Rozqonyi F. Prevalence of intestinal parasites in dogs in some urban and rural areas of Hungary. Vet Q. 2001;23:96–8. doi: 10.1080/01652176.2001.9695091. [PubMed] [Cross Ref]

77. Habluetzel A, Traldi G, Ruggieri S, Attili AR, Scuppa P, Marchetti R, et al. An estimation of *Toxocara canis* prevalence in dogs, environmental egg contamination and risk of human infection in the Marche region of Italy. Vet Parasitol. 2003;113:243–52. doi: 10.1016/S0304-4017(03)00082-7. [PubMed] [Cross Ref]

78. Le Nobel WE, Robben SR, Döpfer D, Hendrikx WM, Boersema JH, Fransen F, Eysker M. Infections with endoparasites in dogs in Dutch animal shelters. Tijdschr Diergeneeskd. 2004;129:40–4. [PubMed]

79. Martínez-Carrasco C, Berriatua E, Garijo M, Martínez J, Alonso FD, de Ybáñez RR. Epidemiological study of non-systemic parasitism in dogs in southeast Mediterranean Spain assessed by coprological and post-mordem examination. Zoonoses Public Health. 2007;54:195–203. doi: 10.1111/j.1863-2378.2007.01047.x. [PubMed] [Cross Ref]

80. Haralambidis ST. Toxocarosis. In: Haralambidis ST, editor. Issues of parasitology that concern public health in Thessaloniki. Thessaloniki, Greece: University Studio Press; 1993. pp. 41–45.

81. Villeneuve A, Polley L, Jenkins E, Schurer J, Gilleard J, Kutz S, et al. Parasite prevalence in fecal samples from shelter dogs and cats across the Canadian provinces. Parasit Vectors. 2015;8:281. doi: 10.1186/s13071-015-0870-x. [PMC free article] [PubMed] [Cross Ref]

82. Fisher M. *Toxocara cati*: an underestimated zoonotic agent. Trends Parasitol. 2003;19:167–70. doi: 10.1016/S1471-4922(03)00027-8. [PubMed] [Cross Ref]

83. Overgaauw PA. Aspects of *Toxocara* epidemiology: human toxocarosis. Crit Rev Microbiol. 1997;23:215–31. doi: 10.3109/10408419709115137. [PubMed] [Cross Ref]

84. Smith H, Noordin R. Diagnostic limitations and future trends in the serodiagnosis of human toxocariasis. In: Holland CV, Smith HV, editors. *Toxocara*: the enigmatic parasite. Wallingford: CABI Publishing; 2006. pp. 89–112.

85. Xinou E, Lefkopoulos A, Gelagoti M, Drevelegas A, Diakou A, Milonas, Dimitriadis AS. CT and MR imaging findings in cerebral toxocaral disease. Am J Neuroradiol. 2003;24:714–8. [PubMed]

86. Haralambidou S, Vlachaki E, Ioannidou E, Milioni V, Haralambidis S, Klonizakis I. Pulmonary and myocardial manifestations due to *Toxocara canis* infection. Eur J Intern Med. 2005;16:601–2. doi: 10.1016/j.ejim.2005.04.008. [PubMed] [Cross Ref]

87. Theodoridis I, Frydas S, Papazahariadou M, Hatzistilianou M, Adamama-Moraitou KK, Di Gioacchino M, Felaco M. Toxocarosis as zoonosis. A review of literature and the prevalence of

Toxocara canis antibodies in 511 serum samples. Int J Immunopathol Pharmacol. 2001;14:17–23. doi: 10.1177/039463200101400104. [PubMed] [Cross Ref]

88. Jenkins DJ, Mckinlay A, Duolong H, Bradshaw H, Craig PS. Detection of *Echinococcus granulosus* coproantigens in faeces from naturally infected rural domestic dogs in south eastern Australia. Aust Vet J. 2006;84:12–16. doi: 10.1111/j.1751-0813.2006.tb13116.x. [PubMed] [Cross Ref]

89. Sotiraki S, Chaligiannis I. Cystic echinococcosis in Greece. Parasite. 2010;17:205–10. doi: 10.1051/parasite/2010173205. [PubMed] [Cross Ref]

90. Katzoura V, Diakou A, Kouam MK, Feidas H, Theodoropoulou H, Theodoropoulos G. Seroprevalence and risk factors associated with zoonotic parasitic infections in small ruminants in the Greek temperate environment. Parasitol Int. 2013;62:554–60. doi: 10.1016/j.parint.2013.08.010. [PubMed] [Cross Ref]

91. Chaligiannis I, Maillard S, Boubaker G, Spiliotis M, Saratsis A, Gottstein B, Sotiraki S. *Echinococcus granulosus* infection dynamics in livestock of Greece. Acta Trop. 2015;150:64–70. doi: 10.1016/j.actatropica.2015.06.021. [PubMed] [Cross Ref]

J. Comp. Path. 2016, Vol. 155, S54–S71

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Major Parasitic Zoonoses Associated with Dogs and Cats in Europe

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Summary

Some of the most important zoonotic infectious diseases are associated with parasites transmitted from companion animals to man. This review describes the main parasitic zoonoses in Europe related to dogs and cats, with particular emphasis on their current epidemiology. Toxoplasmosis, leishmaniosis, giardiosis, echinococcosis, dirofilariosis and toxocariosis are described from the animal, as well as from the human host perspectives, with an emphasis on parasite life cycle, transmission, pathogenicity, prevention and identification of knowledge gaps. In addition, priorities for research and intervention in order to decrease the risks and burden of these diseases are presented. Preventing zoonotic parasitic infections requires an integrated multidisciplinary 'One Health' approach involving collaboration between veterinary and medical scientists, policy makers and public health officials.

Keywords: companion animal; Europe; parasite; zoonotic disease

Contents

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0021-9975/$ - see front matter
http://dx.doi.org/10.1016/j.jcpa.2015.10.179

Introduction

Parasites are responsible for some of the most important and well recognized zoonotic infectious diseases transmitted from companion animals to man globally. The CALLISTO (**C**ompanion **A**nimal multisectoria**L** interprofessiona**L** and interdisciplinary **S**trategic **T**hink tank **O**n zoonoses) project, an EU Framework 7-funded project, was established to discuss and investigate infectious diseases transmitted between companion animals, man and food producing animals, aiming to focus on these diseases in Europe. Expert Advisory Group (EAG) V in the CALLISTO project discussed the most important parasitic zoonoses in Europe, describing their epidemiology and identifying priorities for research and intervention to decrease the burden of these infections. This review by the members of EAG V includes descriptions of the parasitic diseases considered as most relevant for CALLISTO, with further insights into their epidemiology, diagnosis and prevention, with identification of gaps in knowledge of these infections and recommendations for further research.

Toxoplasmosis

Aetiology

Toxoplasma gondii is a tissue cyst-forming coccidium (Protozoa, Apicomplexa) with a complex life cycle. The asexual phase of *T. gondii* development takes place in various tissues of herbivorous or omnivorous intermediate hosts and is linked to a sexual phase of development in the intestine of felids, the definitive hosts. There are three infectious stages in the life cycle of the parasite: tachyzoites, bradyzoites contained in tissue cysts and sporozoites contained in sporulated oocysts. The parasite can invade the gut, become systemic and localize in vital organs such as muscle and the nervous system. In most cases infection is subclinical, but devastating disease can occur (Cenci-Goga *et al.*, 2011). The virulence of *T. gondii* strains is highly variable and dependent on the genotype of the

parasite. Many atypical genotypes exist besides the 'commonest' genotypes (genotypes I, II and III) first described from Europe and the USA (Shwab *et al.*, 2014).

Hosts and Life Cycle

Felids are the definitive hosts for *T. gondii*, but all warm-blooded vertebrates including man may serve as intermediate hosts and potentially be infected by bradyzoites in meat, by sporulated oocysts or by intrauterine tachyzoites (Dabritz and Conrad, 2010; Elmore *et al.*, 2010). *T. gondii* has become adapted to exploit multiple routes of transmission through a sexual cycle in the definitive host and asexually, through carnivorous behaviour and by vertical transmission. These different routes may operate synergistically to enhance transmission, but they might also provide a vehicle for selection leading to partitioning of strains in the environment. Human infections are acquired from eating undercooked or raw meat, such as pork and lamb. However, the prevalence of *T. gondii* infection in human populations that do not consume meat or eat it well-cooked, suggests that the acquisition of infection from the environment, via oocysts in soil, water or on uncooked vegetables, may also play an important role in transmission. Only a small proportion (<0.1%) of infected people acquire infection congenitally (Lindsay and Dubey, 2011).

Epidemiology

Latent infections with *T. gondii* are common in domestic cats throughout the world. Antibodies to *T. gondii* may be detected in up to 74% of adult cats, depending on the type of feeding and whether cats are kept indoors or outdoors (Tenter *et al.*, 2000). After primary infection, cats spread *Toxoplasma* oocysts in their faeces within 3–10 days and shedding continues for approximately 7–21 days (median 8 days), with up to hundreds of millions of oocysts shed in the faeces of a single infected cat (Dubey, 2001). Afterwards, the direct risk for cat owners is limited.

T. gondii infects up to a third of the human population of the world. In Europe, European Commission (EC) Directive 2003/99 stipulates that member countries report human seroprevalence results every year or every other year, according to their epidemiological status (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2003:325:0031:0040:EN:PDF). Despite this directive, accurate information is incomplete and the EC has applied to the European Food Safety Authority (EFSA) for recommendations on surveillance and control methods for toxoplasmosis for man, animals and food.

Diagnosis of Infection in Man and Animals

A diagnosis of infection by *T. gondii* can be established by the isolation of the parasite from various tissues, detection of specific DNA by polymerase chain reaction (PCR) or by carrying out serological tests. Currently, routine diagnosis of toxoplasmosis relies mainly on the use of serological assays that are available for both man and animals such as the Sabin–Feldman dye test, indirect fluorescent antibody test (IFAT), enzyme-linked immunosorbent assay (ELISA) or various agglutination tests. Most clinical laboratories use an ELISA for the routine screening of specific immunoglobulin (Ig) G and IgM, while other techniques are mostly reserved for reference laboratories (Robert-Gangneux and Dardé, 2012).

Isolation of the parasite by mouse bioassay is a laborious and time-consuming technique, and represents the 'gold standard' for the detection of *T. gondii* in meat for human consumption (Villena *et al.*, 2012). It is still used for diagnosis in people with immunosuppression (Robert-Gangneux and Dardé, 2012).

Over the past two decades, PCR-based tests have been developed to detect parasite DNA in human and animal tissues. Nevertheless, this molecular diagnosis remains unsatisfactory due to a low sensitivity compared with the mouse bioassay, lack of standardization and a considerable diversity among DNA extraction methods, amplification systems and DNA primers (Sterkers *et al.*, 2010). In an attempt to increase the sensitivity of detection, a method based on sequence-specific magnetic capture of *T. gondii* DNA followed by DNA amplification has been developed (Opsteegh *et al.*, 2010).

Prevention of Infection in Man and Animals

Control measures should be aimed at the prevention of oocyst shedding in order to reduce infection of people with *T. gondii* (Tenter *et al.*, 2000). The risk for exposure to *T. gondii* parasites is greatest in cats that prey on wildlife and live outdoors or in farms. Kittens are very susceptible to infection and shed greater quantities of oocysts. Efforts to develop a *T. gondii* vaccine for cats should be renewed, which will lead to better protection of people (Robert-Gangneux and Dardé, 2012). Responsible cat ownership should also be encouraged. This includes measures such as collecting faeces in litter trays for ultimate disposal

in rubbish destined for landfills, which are designed to prevent waste materials leaking into groundwater. In addition, cat faeces should not be disposed of in toilets.

Human infection can be acquired either by ingestion of infected raw or undercooked meat or by ingestion of sporulated oocysts from the contaminated environment. As a consequence, it is highly recommended (especially for high-risk individuals, e.g. previously unexposed pregnant women) that meat is consumed only after thorough cooking or freezing and personal hygiene in handling meat is mandatory. The control of human toxoplasmosis also relies on the avoidance of direct or indirect exposure to cat faeces. Proper faecal handling, litter tray management, removal of faeces from public areas and yards and hand hygiene are critical. Litter trays should be thoroughly cleaned every day so that any potential oocysts do not have time to sporulate (i.e. in about 48 h) (Dubey *et al.*, 2011). People, particularly those vulnerable to infection, such as pregnant women and the immunosuppressed, should avoid this task. Similarly, drinking unfiltered surface water or accidental ingestion of soil must be avoided.

Gaps in Knowledge and Recommendations for Further Research

A major gap in knowledge is the relationship between seropositivity in the main livestock species and presence of *T. gondii* in meat. There is a straightforward relationship between the level of antibodies detected in serum and the likelihood of isolating a viable parasite in pigs and sheep, but this relationship appears not to be clear for horses and cattle (Opsteegh *et al.*, 2011) and needs further investigation.

Another gap resides in the identification of the different sources of infection in various human populations. While multicentre studies pointed out the consumption of undercooked lamb, beef or game, contact with soil and travel outside Europe and North America as strong risk factors for acquiring infection with *T. gondii*, little is known about the relative importance of transmissions via tissue cysts versus oocysts in a given human population (Cook *et al.*, 2000; Jones *et al.*, 2009). The discovery of a sporozoite-specific protein, which elicited differential antibody production in experimentally infected pigs and mice, may contribute to filling this gap in knowledge (Hill *et al.*, 2011).

Further studies need to be undertaken in the field of molecular biology for standardization of PCR methods to be applied both in man and animals, while improvements need to be made in the sensitivity of these techniques for detecting viable parasites. Concerning the definitive host, there is need for advancement in the field of vaccination, with the objective of significantly reducing oocyst excretion, since felids represent the major source of environmental contamination.

Leishmaniosis

Aetiology

Leishmaniosis (or leishmaniasis) is a complex of mammalian diseases caused by diphasic protozoans of the genus *Leishmania* (Kinetoplasta, Trypanosomatidae). The *Leishmania* species endemic in Europe is *Leishmania infantum* and its most common zymodeme is MON-1. However, other zymodemes are also found in Europe. In addition, it is important to highlight that because multilocus enzyme electrophoresis, the classical reference method for *Leishmania* typing (Rioux *et al.*, 1990), is laborious and expensive, molecular typing methods of *L. infantum* isolates have been developed such as multilocus microsatellite typing (Gouzelou *et al.*, 2013) or multilocus sequence analysis, PCR with restriction fragment length polymorphism (RFLP) and whole genome sequencing.

Hosts and Life Cycle

The leishmanioses affect man and domestic and wild animals worldwide. Most transmission cycles are zoonotic, involving reservoir hosts such as rodents, marsupials, edentates, monkeys, domestic dogs and wild canids. Only a few *Leishmania* species are strictly anthroponotic (i.e. transmitted directly from person to person via sand flies) (Quinnell and Courtenay, 2009). Dogs are the major reservoir for canine and human *L. infantum* infection, in an area that stretches from Portugal to China and across South, Central and parts of North America, with the exception of Oceania. In Europe, the domestic dog is the only reservoir host of major veterinary and human importance (Solano-Gallego *et al.*, 2009). Infection in cats (Martin-Sanchez *et al.*, 2007), wild canids (Sobrino *et al.*, 2008; Millan *et al.*, 2011) and horses (Fernandez-Bellon *et al.*, 2006) has also been reported in areas where disease is common in dogs, but the role of these species as reservoirs remains unclear.

Natural transmission of *L. infantum* between animals and from animals to man occurs usually by the bite of a phlebotomine sand fly species (Diptera, Psychodidae, Phlebotominae) of the genera *Phlebotomus* (Old World) and *Lutzomyia* (New World). Sand flies are the only arthropod vectors that are adapted for the transmission of *Leishmania* species. *Leishmania* completes its life cycle in the sand fly, which harbours the flagellated extracellular promastigote form and in a mammal where the intracellular amastigote form

develops. A female sand fly ingests *Leishmania* while blood feeding and then transmits the infective stages (metacyclic promastigotes) during a subsequent blood meal. The infective promastigotes inoculated by the sand fly are phagocytosed in the mammalian host by macrophages and other phagocytic cells, in which they transform to amastigotes.

Non-sand fly modes of transmission have also been described, but their role in the natural history and epidemiology of *L. infantum* infection remains unclear. Proven modes of non-sand fly transmission in dogs include infection through transfused blood products (Owens *et al.*, 2011) from blood donors that are carriers of infection (de Freitas *et al.*, 2006; Tabar *et al.*, 2008), vertical (Rosypal *et al.*, 2005; Pangrazio *et al.*, 2009; Boggiatto *et al.*, 2011) and venereal transmission (Silva *et al.*, 2009).

Epidemiology

Based on seroprevalence studies from Spain, France, Italy and Portugal, it has been estimated that 2.5 million dogs in these countries are infected with *L. infantum* and infection is spreading north in Europe, reaching the foothills of the Alps (Maroli *et al.*, 2008), Pyrenees (Chamaille *et al.*, 2010) and northwestern Spain (Amusategui *et al.*, 2004). The numbers of dogs travelling to southern Europe or imported as companion animals from areas where canine leishmaniosis is endemic have increased, as have the numbers of clinical cases reported in non-endemic countries such as the UK (Shaw *et al.*, 2009) and Germany (Menn *et al.*, 2010).

The seroprevalence in dogs in the Mediterranean basin ranges from 5% to 30% depending on the region (Solano-Gallego *et al.*, 2009). Surveys employing other detection methods to estimate the prevalence of *Leishmania* infection by amplification of *Leishmania* DNA from different tissues or by detection of specific anti-*Leishmania* cellular immunity have revealed even higher infection rates, approaching 70% in some foci. Most dogs in these areas appear to have chronic infection that may be lifelong, but only a small proportion of dogs develop severe disease (Baneth *et al.*, 2008).

In cats, serological and PCR surveys in southern Europe indicate that *Leishmania* infection is more widespread than clinical disease. Epidemiological studies have described rates ranging from 0.4% to 30% based on serological and molecular techniques (Martin-Sanchez *et al.*, 2007; Solano-Gallego *et al.*, 2007; Maia *et al.*, 2008; Millan *et al.*, 2011; Sherry *et al.*, 2011).

Human leishmaniosis, caused by several species of *Leishmania*, comprises a heterogeneous group of diseases. These include visceral leishmaniosis (VL), which involves internal organs and is fatal if untreated, and the cutaneous (CL) and mucocutaneous forms, which affect the skin or mucocutaneous junctions and may heal spontaneously, leaving disfiguring scars (Murray *et al.*, 2005). This group of infections is the third most important vector-borne disease after malaria and lymphatic filariosis. It is endemic in many tropical and subtropical regions of the Old and New World. Leishmaniosis is endemic in 88 countries, with more than 350 million people at risk. The estimated incidence is 2 million new cases per year: 0.5 million VL and 1.5 million CL cases (Desjeux, 2004).

There are only two transmission cycles with proven long-term endemicity in Europe: (1) visceral, cutaneous and mucocutaneous human leishmaniosis caused by *L. infantum* throughout the Mediterranean region and (2) anthroponotic cutaneous human leishmaniosis caused by *L. tropica*, which occurs sporadically in Greece. In Europe, about 1,000 people are estimated to be affected by clinical disease due to *L. infantum* annually (Dujardin *et al.*, 2008), although asymptomatic or subclinical infections are more frequent (Michel *et al.*, 2011). The high prevalence (2–40%) of asymptomatic human carriers of *L. infantum* in some areas of southern Europe suggests that this parasite is a latent public health threat. Asymptomatic infections are estimated to have a prevalence ratio of >100 asymptomatic:1 clinical case (Michel *et al.*, 2011).

Mediterranean VL primarily affects children as well as an increasing number of immunocompromised and immunosuppressed adult individuals, such as people who are positive for the human immunodeficiency virus (HIV) and people under immunosuppressive therapy. Mortality rates due to leishmaniosis in *Leishmania*–HIV co-infected patients can reach over 56% (Lopez-Velez *et al.*, 1998; Pasquau *et al.*, 2005). Therefore, risk factors for human infection include age, poor socioeconomic conditions, malnutrition and immunosuppressive conditions (Alvar *et al.*, 2006).

Diagnosis of Infection in Man and Animals

The most common techniques used for disease detection in man and animals include microscopical observation (i.e. cytology, biopsy or immunohistochemistry) and serological and molecular techniques (Solano-Gallego *et al.*, 2009; Elmahallawy *et al.*, 2014).

Prevention of Infection in Man and Animals

Control measures for man and dogs are available and include medical treatment, individual use of

sand fly repellents in dogs, canine vaccines and immunomodulating drugs (Otranto and Dantas-Torres, 2013; Wylie *et al.*, 2014a,b).

Treatment for people and dogs in Europe is different, thus limiting the likelihood of developing resistance. People are commonly treated with a short course of amphotericin B (Murray *et al.*, 2005), while moderately to severely sick dogs are usually treated with a combination of a 1-month course of meglumine antimoniate or miltefosine and a long-term course of allopurinol. Generally, treatment in dogs leads to a clinical cure and decreased parasite load. However, complete parasitological cure in the majority of dogs appears to be unlikely (Solano-Gallego *et al.*, 2009).

Gaps in Knowledge and Recommendations for Further Research

There are numerous gaps in knowledge regarding *Leishmania* infection. These include: (1) a better understanding of the immunopathogenesis of the disease in man and dogs and how clinical disease appears versus subclinical infection, (2) knowledge of the immune mechanisms that control infection and how to develop efficacious vaccines for man and dogs, (3) understanding the role of domestic or wild mammals other than the dog as reservoirs of *L. infantum* infection and (4) understanding the risk factors associated with human and animal infection in Europe.

Giardiosis

Aetiology

The genus *Giardia* (Diplomonadida, Hexamitidae) includes intestinal protozoan parasites that infect numerous hosts, ranging from mammals to amphibians and birds. Currently, six *Giardia* species are accepted: *Giardia agilis*, *Giardia ardeae*, *Giardia muris*, *Giardia microti* and *Giardia psittaci* infecting various species of animals, while *Giardia duodenalis* infects man and many other mammals. *Giardia* species differ significantly in host range, with *G. duodenalis* (syn. *Giardia lamblia* and *Giardia intestinalis*) having the broadest host range and greatest public health significance (Feng and Xiao, 2011).

Although *G. duodenalis* is found in man and other mammals, including pets and livestock, it is now considered a multispecies complex. Historically, allozyme analyses placed all isolates from man into two genetic assemblages (assemblages A and B). Multigenic sequence analyses confirmed this assemblage separation and identified additional lineages of *G. duodenalis* from animals including assemblages A and B in man and other animals, assemblages C and D from dogs, assemblage E from artiodactyls, assemblage F from cats and assemblage G from rodents (Caccio *et al.*, 2005; Thompson *et al.*, 2008; Tysnes *et al.*, 2014).

Hosts and Life Cycle

Giardia is a very common enteric protozoal parasite of domestic animals, including livestock, dogs, cats and wildlife. *G. duodenalis* causes giardiosis in man and in most mammals. The life cycle of *Giardia* is direct and the infective stage of the parasite, the cyst, is immediately infectious when released into the faeces. Cysts remain infectious for months in cool, damp areas and accumulate in the environment. When ingested by the host, cysts excyst in the duodenum, releasing the trophozoites. The latter undergo repeated mitotic division in the gut lumen and form environmentally resistant cysts. Cysts pass through the intestine in faeces and are spread by contaminated water, food and fomites and by direct physical contact (Feng and Xiao, 2011).

Epidemiology

It has been estimated that about 200 million people in Asia, Africa and Latin America have symptomatic infection with *Giardia* (Feng and Xiao, 2011). Once infected, *Giardia* causes a generally self-limited clinical illness characterized by diarrhoea, abdominal cramps, bloating, weight loss and malabsorption. However, asymptomatic giardiosis occurs frequently, especially in developing countries. In Germany, on average, 3,806 notified giardiosis cases (range 3,101–4,626) were reported between 2001 and 2007, which corresponded to an average incidence of 4.6 cases/100,000 population (Sagebiel *et al.*, 2009). Much higher incidence rates were reported for some other countries. In the Netherlands, there were 11,600 cases in 2004, corresponding to 69.9 cases/100,000 population (Vijgen *et al.*, 2007).

The relationship between human and animal *Giardia* infection is not clear. Although people share the same *G. duodenalis* assemblages with animals with which they have close contact, such as household dogs, it is not known how frequently infection is actually acquired from household animal contact or whether both people and pets acquire it from a common source, such as contaminated water. Undoubtedly, people also commonly infect each other.

Infection rates with *Giardia* in dogs were 24.8% in a large study in Europe (Epe *et al.*, 2010), 22.7% in Belgium (Claerebout *et al.*, 2009) and 21.0% in the UK (Upjohn *et al.*, 2010). Infection rates in cats were 20.3% in a multicountry study in Europe (Epe *et al.*, 2010). Giardiosis in animals is often subclinical,

but has been associated with the occurrence of diarrhoea and illness in puppies and kittens (Thompson, 2004).

Giardia infections are common in pigs, cattle, sheep, goats, elks and deer and other ruminants (Feng and Xiao, 2011). Although it is believed that infection with *Giardia* is associated with economic losses through the occurrence of diarrhoea, poor growth and even death in farm animals (Geurden *et al.*, 2005), only a few studies have been conducted to assess the effect of giardiosis on livestock production or growth rates. In bottle-fed specific-pathogen-free lambs infected experimentally with *Giardia* cysts, infection was associated with delay in reaching slaughter weight and decreased carcass weight (O'Handley and Olson, 2006).

Diagnosis of Infection in Man and Animals

Giardia infection can be diagnosed by stool examination to identify cyst and trophozite stages in direct fresh stool smears or by flotation for cysts. Rapid detection of *Giardia* antigen can be made using immunochromatographic kits, by immunofluorescence, ELISA or PCR in a suitably equipped parasitology laboratory (Feng and Xiao, 2011).

Prevention of Infection in Man and Animals

The prevention of giardiosis in man is closely associated with the provision of clean fresh water and adequate sewage systems. Boiling or filtering water from the environment before drinking it is essential and removal of infected faeces from infected animals or people followed by proper disinfection is necessary. Adherence to personal hygiene habits such as washing hands and cleaning fresh food is important in limiting infection.

Gaps in Knowledge and Recommendations for Further Research

Gaps in knowledge of giardiosis include the need to clarify if there are animal reservoirs for human giardiosis and to what extent, if at all, human giardiosis can be caused by contamination from an animal source. In that respect, it would also be important to find out whether animals may be infected by their owners and suffer from clinical giardiosis. A vaccine for giardiosis would be beneficial for people and also for domestic animals.

Echinococcosis

Aetiology

The genus *Echinococcus* includes several species and genotypes of zoonotic cestodes (tapeworms). The adult stages occur in the intestines of canids and felids without clinical relevance. The larval stages develop in tissues of various organs of a variety of mammalian intermediate hosts, including man, as aberrant hosts. Cystic echinococcosis (CE) is caused by species of the *Echinococcus granulosus* sensu lato (s. l.) complex. In Europe, *E. granulosus* sensu stricto (s. s.) ('sheep strain') and *Echinococcus canadensis* (*Echinococcus intermedius*, 'pig strain') are of major zoonotic significance (Table 1). The controversially discussed taxonomy and the molecular epidemiology of the *E. granulosus* complex has been reviewed recently (Romig *et al.*, 2015). Alveolar echinococcosis (AE) caused by *Echinococcus multilocularis* is one of the most pathogenic zoonoses in Europe and leads to death of people in 10–15 years if untreated (Eckert *et al.*, 2011).

Hosts and Life Cycle

E. granulosus s.s. is mainly transmitted within a dog–sheep cycle in pastoral regions (Table 1);

Table 1
***Echinococcus* spp. in Europe and their definitive and intermediate hosts**

Echinococcus species	*Echinococcus strains or E. granulosus s. l. genotypes (G)*	*Definitive hosts*	*Intermediate hosts*	*Zoonotic significance*
E. granulosus sensu stricto (s. s.)	Sheep strain (G1, 2, 3)	Dog (fox*)	Sheep, cattle†, pig and other herbivores†	+ + +
E. ortleppi	Cattle strain (G5)	Dog	Cattle	+
E. canadensis	Cervid strain (G8, 10)	Wolf (dog)	Cervids	+
E. canadensis, (proposed *E. intermedius*)	Pig strain (G7)	Dog (wolf)	Pig, other herbivores†	+ +
E. equinus	Horse strain (G4)	Dog	Equids	–
E. multilocularis	European strain	Fox, dog, raccoon dog, (cat*)	Arvicolids and other rodents	+ + +

Zoonotic significance is graded as: –, none; +, mild; + +, moderate; or + + +, marked.
*Mostly low worm numbers with very low egg production.
†Mostly with strongly reduced protoscolex formation in the cysts often resulting in infertile cysts.

however, other potential intermediate hosts can be involved. Interestingly, the development of protoscoleces in the cysts can be markedly reduced in cattle as compared with sheep. The *E. canadensis* (pig strain, G 7) cycle is characterized in the Baltic states and Poland by a small scale transmission pattern between farm dogs and pigs in family farms with the practice of traditional home slaughter (Bruzinskaite *et al.*, 2009), but possible wild or semi-wild animal cycles have been observed, including wolves in Portugal or wild boars in Corsica (Umhang *et al.*, 2014). *Echinococcus ortleppi* was prevalent in cattle all over central Europe, but has nearly disappeared without specific control programmes.

E. multilocularis is perpetuated in a wildlife cycle mainly by foxes as definitive hosts and small mammals as intermediate hosts. Definitive hosts with high reproductive potential of *E. multilocularis* are predominantly the red fox, the raccoon dog, the wolf and the domestic dog. After a prepatency of around 1 month, eggs are shed over a few months, but 95% of the total egg excretion occurs within the first month of patency (Kapel *et al.*, 2006). Wild felines and domestic cats have occasionally been found to harbour intestinal stages. Although cats are more likely to be infected with *E. multilocularis* than dogs, their zoonotic significance is estimated to be small, based on the low level of egg excretion. Dogs, on the other hand, may play a very important role in the transmission to man, but they probably do not contribute significantly to the contamination of rodent habitats as compared with foxes (Deplazes *et al.*, 2011; Hegglin and Deplazes, 2013).

Echinococcosis is not a food-borne zoonosis in the classical sense. Eggs are typically excreted fully developed and infectious (containing an oncosphere larva) by defecation in the environment. In addition, these eggs are highly resistant: *E. multilocularis* eggs survive in the environment for up to 8 months; however, they are sensitive to desiccation. Eggs can be dispersed from the deposition sites either by being washed away or carried by flies and other vectors (Eckert *et al.*, 2011). *Echinococcus* eggs may also adhere to tyres, shoes or animal paws, resulting in more widespread dispersal and contamination of the environment, including human dwellings.

Epidemiology

In Europe, the endemic area of *E. granulosus* s. s. covers southern and south-eastern Europe; *E. canadensis* G7 is prevalent in the Baltic countries, Poland and southwards to Romania. For *E. granulosus* s. l., most prevalence data are based on slaughterhouse investigations of intermediate hosts, while prevalence data concerning definitive hosts are scarce, especially for pet dogs. Prevalence rates of 0–31% are reported from farm and shepherd dogs in Italy and Spain and 14.2% from farm village dogs in Lithuania (Bruzinskaite *et al.*, 2009; Carmena and Cardona, 2013).

E. multilocularis occurs in the northern hemisphere, with large endemic areas in Europe including parts of the western continent (e.g. France, Benelux States) and all countries of central Europe including Northern Italy, Slovenia, Romania and the Baltic States. Furthermore, foci also exist in Denmark, Sweden and on Svalbard Island (Gottstein *et al.*, 2015) (Fig. 1).

Based on recently improved diagnostic strategies, several studies have investigated the prevalence of *E. multilocularis* in pet dog populations. Low prevalence rates of <0.5% were recorded in the privately owned dog populations in France, Germany, Switzerland and Denmark, but a higher prevalence (3–8%) was found in dogs with predatory habits and those able to roam more widely (Deplazes *et al.*, 2011). In Switzerland, 0.3% of randomly selected privately owned dogs were found to be infected with this tapeworm. Based on this prevalence, the individual probability of being infected at least once during 10 years can be estimated at 8.7%. Large population studies in Germany revealed that 0.13% of dogs in northern and 0.35% in southern Germany excreted *E. multilocularis* eggs in their faeces. Considering the total dog population in Germany (approximately 5.4×10^6 dogs), around 13,000 are estimated to be infected.

The prevalence of *E. multilocularis* in cat populations, as determined at necropsy examination, ranged between 0% and 5.5% in various endemic areas. Cat infections are characterized by low worm burdens and strongly reduced worm development, resulting in lower egg production compared with foxes or dogs. Therefore, the epidemiological role of the cat in spreading this infection is estimated to be low (Hegglin and Deplazes, 2013).

In the human population, CE is one of the five most frequently diagnosed zoonoses in the Mediterranean region and is re-emerging in South Eastern Europe (Jenkins *et al.*, 2005). Incidence rates for CE of 1.1–3.3/100,000 were recorded in Spain, up to 3.5 in Sardinia in Italy and 3.3 in Greece, Bulgaria and Romania (Torgerson *et al.*, 2011). Economic loss attributable to human CE was estimated for Spain at €133 million (Benner *et al.*, 2010).

Human AE is one of the most pathogenic helminthic zoonoses and causes a high burden of disease in Europe (Torgerson *et al.*, 2008). Recent studies support the hypothesis that the infection pressure caused

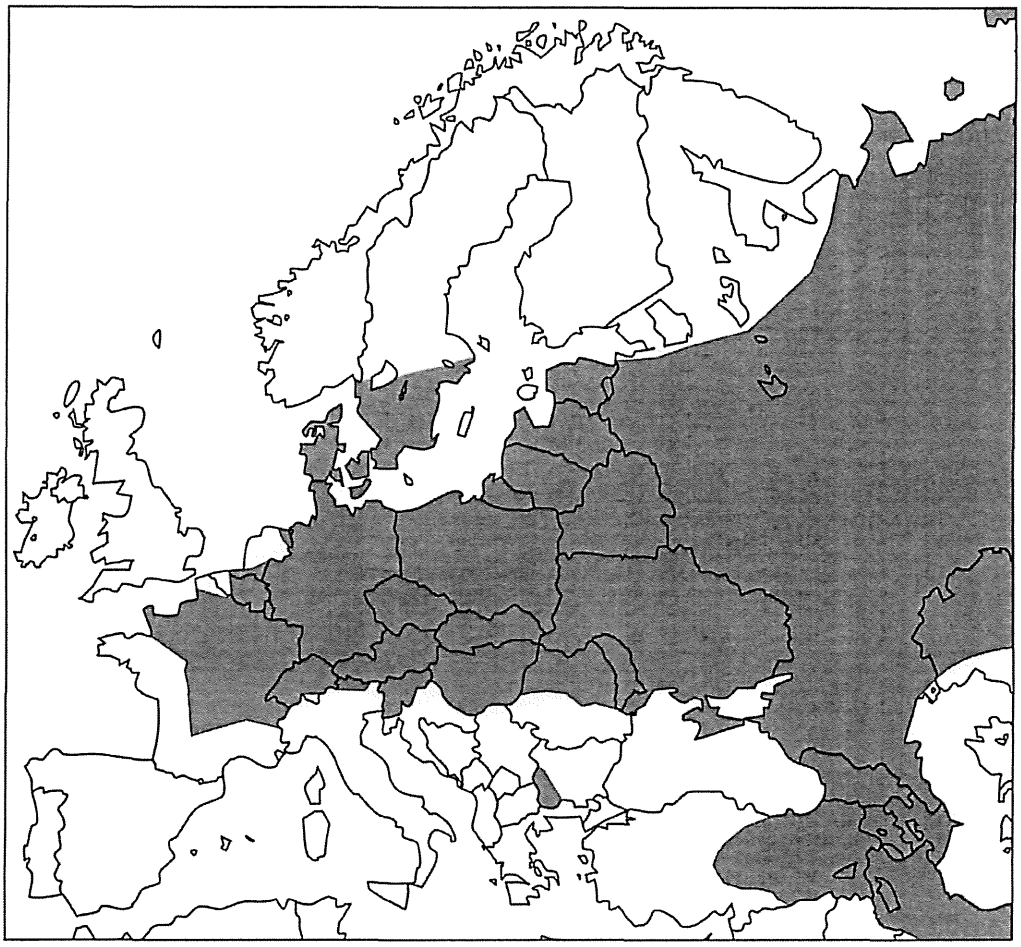

Fig. 1. Approximate distribution of *Echinococcus multilocularis* in Europe shown in dark orange colour (with permission from the Institute of Parasitology, University of Zurich, Switzerland).

by *E. multilocularis* eggs has increased across certain large European regions. In Switzerland, a representative endemic area for central Europe, the annual incidence rates of new human AE cases varied between 0.10 and 0.16/100,000 individuals over a 45-year period, suggesting a high degree of epidemiological stability. However, approximately 10–15 years (corresponding to the incubation time of AE) after a distinct increase in the fox populations (with *E. multilocularis* prevalences of 30–60%), a higher incidence rate of 0.25/100,000 was recorded (Deplazes *et al.*, 2011). Similar trends of increasing incidence have been observed in Austria, France and Lithuania. The overall incidence of AE is variable (0.03–0.26) in Central Europe, but estimated to be 200 new cases per year (Deplazes, personal communication).

Humans are exposed to eggs of *Echinococcus* spp. via different ways. The most important sources of infection are handling of definitive hosts and oral uptake of contaminated water, food or soil. Adherent eggs and even proglottids of *Echinococcus* have been observed on infected dogs in individual cases. Direct exposure to these eggs is influenced by occupation and behaviour, especially a close human–animal bond.

Domestic transmission of *E. granulosus* eggs from pet, stray and working dogs is particularly important in areas with inadequate educational standards and veterinary control. Risk factors for infection of intermediate and definitive animal hosts with *E. granulosus* s. l. have been recently reviewed (Otero-Abad and Torgerson, 2013; Craig *et al.*, 2015). Indeed, the number of owned dogs and the frequency of contact with dogs were identified as risk factors for human AE in studies from China (Craig *et al.*, 2015), while in a Spanish study, cohabitation with dogs and feeding of uncooked viscera were defined as risk factors for CE (Campos-Bueno *et al.*, 2000). As home

slaughter of sheep in parts of Southern Europe and of pigs in parts of Poland and the Baltic states is still widespread, local family dogs may be infected by feeding of infected offal.

Diagnosis of Infection in Animals

Intestinal infections with *E. granulosus* or *E. multilocularis* are typically subclinical in definitive hosts. The diagnosis of the infection in dogs or cats has been considerably improved in recent years by egg isolation methods, coproantigen ELISAs and PCR tests for *E. granulosus* s. l. and for *E. multilocularis* (Craig *et al.*, 2015; Conraths and Deplazes, 2015). These techniques can also be used for the examination of faecal samples collected in the environment.

Prevention of Infection in Man and Animals

Comprehensive control programmes have so far only been applied for CE, with varying degrees of success (Craig and Larrieu, 2006) including control of stray dogs, slaughter supervision and public education campaigns, routine anthelmintic treatment of dogs and vaccination of sheep. More detailed control options for CE have been reviewed by Lightowlers (2013) and Barnes *et al.* (2012).

A treatment schedule individually designed for pets based on infection risks (e.g. free roaming, uncontrolled access to rodents or offal) can improve treatment efficiency against cestodes. Uniform guidelines for the control and treatment of parasites in pet animals were developed and published by the European Scientific Council on Companion Animal Parasites (ESCCAP) in Europe (www.esccap.org). The current recommendation is to treat dogs with access to *Echinococcus* metacestodes monthly with praziquantel in order to reduce environmental contamination with eggs. However, even strict compliance of the pet owners will not reduce the environmental contamination with eggs of *E. granulosus* caused by stray dogs or of *E. multilocularis* caused by foxes. The growing fox populations in Central Europe, especially in urban areas, with a prevalence of *E. multilocularis* infection above 30% is causing a high infection pressure and maintaining the parasite cycle without the pet population. Therefore, a promising approach is to reduce the infection pressure by the delivery of anthelmintic baits for foxes (Hegglin and Deplazes, 2013).

To prevent the introduction of *E. multilocularis* into Great Britain, Ireland and as of yet non-endemic Scandinavian countries, where, due to the presence of suitable intermediate hosts, the establishment of the parasite would be possible, the Pet Travel Scheme prescribed strict deworming regime of all dogs entering these countries.

Gaps in Knowledge and Recommendations for Further Research

Recommendations for further research and actions against echinococcosis include: (1) establishment of a One Health concept for systematic, specific and standardized surveillance of AE and CE in man and of *Echinococcus* infection in animals, (2) definition of minimal standards and harmonized approaches for the monitoring of the epidemiological state of these infections in Europe and (3) further development of control strategies adapted to the local and socio-cultural epidemiological situation to prevent both AE and CE in man.

Vector-borne Helminths

Aetiology

Filaroids are roundworms that belong to the family Onchocercidae. Filaroid species are prevalent in Europe and some of them are of increasing concern due to the significant level of disease they cause in dogs and man (Genchi *et al.*, 2011; Otranto and Eberhard, 2011; Morchón *et al.*, 2012). The species *Dirofilaria immitis* and *Dirofilaria repens* (Spirurida, Onchocercidae) are the best known filaroids affecting dogs. They present different pathogenic potentials for man and animals; while *D. immitis* threatens dogs and cats, causing a severe and often fatal cardiocirculatory disease referred to as 'heartworm disease', *D. repens* induces a non-pathogenic subcutaneous infestation in dogs, but is a more prevalent zoonotic pathogen in man (Dantas-Torres and Otranto, 2013). Mosquitoes transmit these *Dirofilaria* species to dogs, cats and other wild carnivores. About 45% of the total human and pet population are exposed to the risk of vector-borne helminths (VBHs) in Europe (Petrić *et al.*, 2012). Although *Dirofilaria* spp. represent the most prevalent VBHs, other helminths of dogs and cats, such as the *Thelazia callipaeda* eyeworm (Spirurida, Thelaziidae), are emergent zoonotic agents in several European regions (Otranto *et al.*, 2013a). Finally, the recent finding of the zoonotic potential of a little known filaroid of dogs, *Onchocerca lupi* (Spirurida, Onchocercidae), rendered the puzzle of human VBH infections in Europe even more complicated.

Hosts and Life Cycle

Dirofilarioses are transmitted by bloodsucking mosquitoes, primarily to dogs, although cases of infection in man are reported increasingly (Otranto and Eberhard, 2011). Soon after mosquitoes inoculate

infective third-stage larvae (L3) to dogs and cats, developing larvae migrate to the definitive site of parasitism, the pulmonary arteries and right chambers of the heart for *D. immitis* and the subcutaneous tissues for *D. repens*. In these locations, following their development into adult worms (in 120–180 and 189–259 days for *D. immitis* and *D. repens*, respectively), females release microfilariae into the blood of the definitive host (Genchi *et al.*, 2009), which are thereafter ingested by mosquitoes during their blood intake. Microfilariae of *Dirofilaria* spp. develop in the intermediate mosquito vectors from embryos to infective L3 larvae in a variable period of time at a minimum threshold of 14°C and the requirement of a minimum of 130 days for larvae to reach infectivity (Genchi *et al.*, 2009).

T. callipaeda nematodes live in the orbital cavities and associated host tissues, causing ocular disease in carnivores and representing a potential public health concern due to the zoonotic impact. Adults live in the conjunctival sacs of animals under the nictitating membrane and the mature females release first-stage larvae (L1) into the lachrymal secretions, which are ingested subsequently by the zoophilic fruit fly *Phortica variegate* (Diptera, Drosophilidae), the known vector of this spirurid in Europe (Otranto *et al.*, 2005). In the intermediate host, L1s undergo development to L3s approximately 14–21 days after infestation (in laboratory conditions) and may also survive in overwintering flies for 6 months (Otranto *et al.*, 2004, 2005). Finally, mature L3s migrate through the arthropod coeloma to the labella to be then transmitted to a receptive host as soon as the drosophilid feeds on the lachrymal secretions (Otranto *et al.*, 2005).

Scant information is available on *O. lupi*, which localizes in nodular lesions under the sclera and perioocular tissues of dogs and cats or in the retrobulbar eye (Otranto *et al.*, 2013b). The biology of this filaroid in the definitive host is almost unknown and the vector of this infestation is not well characterized (Otranto *et al.*, 2012a).

Epidemiology

The interaction between helminths, vectors and animals is the consequence of a complex range of biological (e.g. vectorial capacity, biting rates) and environmental (e.g. climate, population movements and trade) factors, which ultimately affect the epidemiology of VBH infections. This picture is complicated further by the fact that new potential vectors are introduced into previously non-endemic areas, therefore increasing the risk for establishing new transmission cycles in populations of susceptible hosts. This was the case for the introduction of the invasive mosquito species *Stegomyia albopicta* (*Aedes albopictus*) into Italy (Romi and Majori, 2008), which most likely contributed to the spread of *D. immitis* from endemic areas of the Po river valley in northern Italy to southern Italy (Otranto *et al.*, 2009). However, several mosquito species of the genus *Anopheles*, *Aedimorphus*, *Armigeres*, *Ochlerotatus*, *Stegomyia*, *Culex*, *Coquillettidia* and *Mansonia* may act as intermediate hosts, although *Aedimorphus vexans* (*Aedes vexans*), *Culex pipiens pipiens* and *S. albopicta* are also implicated as the most important natural vectors of these worms in Europe. Since both *D. repens* and *D. immitis* grow under laboratory conditions in the same mosquito species with similar developmental times, these infections are often sympatric in animal populations (Genchi *et al.*, 2009). The relationship between the prevalence of *D. repens* in dogs and the occurrence of human cases of dirofilariosis, based on a review of the historical literature, was evident in some provinces of Sicily (Otranto *et al.*, 2011a). Indeed, while *D. immitis* is recognized as the main agent of human dirofilariosis in the Americas and was described in a few cases in Italy, Greece and Spain (Miliaras *et al.*, 2010; Morchón *et al.*, 2010; Avellis *et al.*, 2011), *D. repens* is the most prevalent species infesting people in Europe (Pampiglione *et al.*, 1995, 2009). Human cases of dirofilariosis are increasing in Europe, most likely paralleling the spreading of infection in dogs in central and north-eastern European countries including Poland, Switzerland, the Czech Republic, Hungary, Romania, Serbia and the Slovak Republic (Genchi *et al.*, 2014) (Fig. 2).

Over the last 20 years, *T. callipaeda* has been repeatedly reported to infest the eyes of domestic (dogs and cats) and wild carnivores (foxes, wolves, beech martens and wild cats). Countries considered as endemic for this worm in Europe include Italy, France, Switzerland, Spain and Portugal (Malacrida *et al.*, 2008; Miró *et al.*, 2011; Vieira *et al.*, 2012; Otranto *et al.*, 2013b). The same areas where the infection was recently diagnosed were predicted by a model published about 10 years before, which was based on the ecology and the seasonal occurrence of the drosophilid fly in a highly endemic area of southern Italy (Otranto *et al.*, 2006). Indeed, that model anticipated that large areas of Europe were likely to represent suitable habitats for *Phortica variegata* and, therefore, for the expansion of thelaziosis. Consequently, the first cases of human thelaziosis in Europe have been diagnosed in north-western Italy, south-eastern France (Otranto and Dutto, 2008) and Spain (Fuentes *et al.*, 2012).

O. lupi has been found to infect dogs in southern (Greece, Portugal) and Central Europe (Germany,

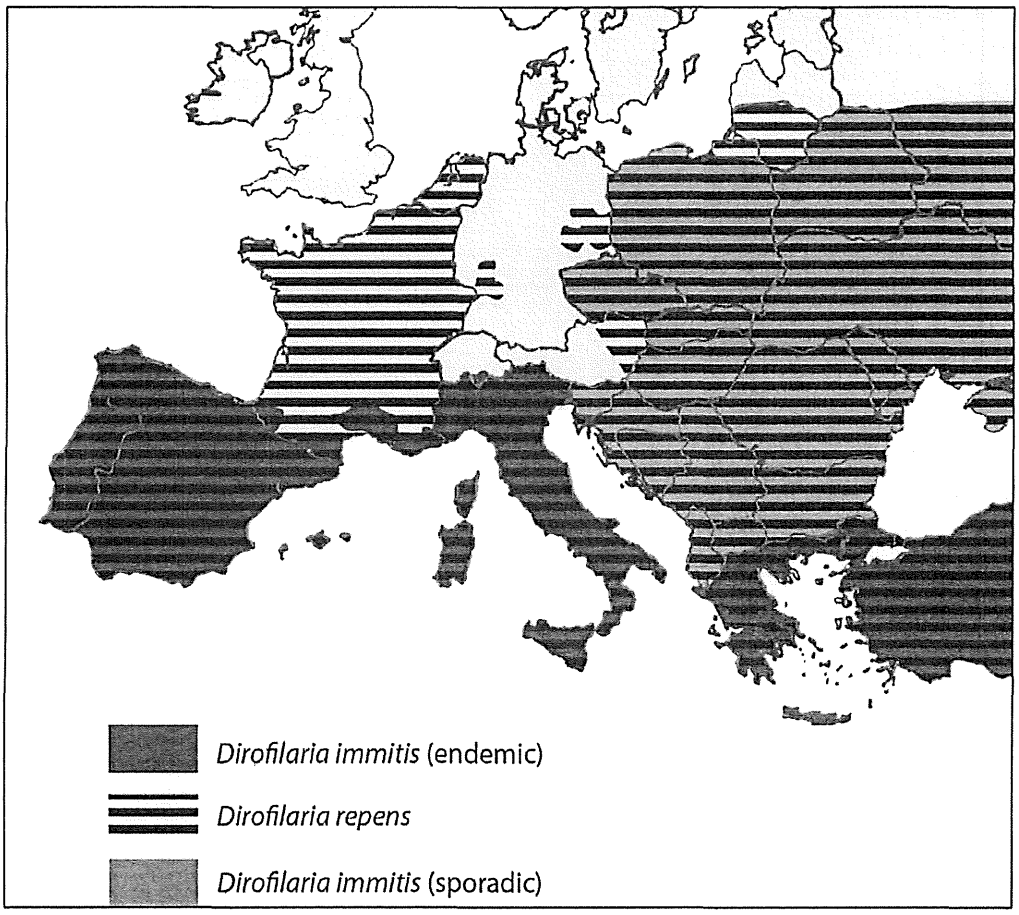

Fig. 2. Distributions of *Dirofilaria immitis* and *Dirofilaria repens* infections in Europe (with permission from the Institute of Parasitology, University of Zurich, Switzerland).

Hungary and Switzerland) (Széll *et al.*, 2001; Komnenou *et al.*, 2002; Hermosilla *et al.*, 2005; Faísca *et al.*, 2010; Otranto *et al.*, 2013a) and in the USA (Orihel *et al.*, 1991; Eberhard *et al.*, 2000; Zarfoss *et al.*, 2005) where it was recently found also in cats (Labelle *et al.*, 2011). Since the first report of human ocular infestation (Otranto *et al.*, 2011b), *O. lupi* has been recognized as a zoonotic agent in patients from Turkey (Otranto *et al.*, 2012b; Ilhan *et al.*, 2013), Tunisia (Otranto *et al.*, 2012b), Iran (Mowlavi *et al.*, 2013) and the USA (Eberhard *et al.*, 2013).

Diagnosis of Infection in Man and Animals

Diagnosis of VBH infections is achieved through detection of circulating microfilariae (e.g. *D. immitis* and *D. repens*) in the bloodstream of infected animals by microscopical techniques, with the Knott's method as the gold standard (McCall *et al.*, 2008). In contrast, dermal microfilariae of *O. lupi* can be detected in skin biopsy samples from the interscapular region and the head (Otranto *et al.*, 2013a). While the morphological discrimination of microfilariae might be challenging and lack in sensitivity, as other filaroids may infect dogs (e.g. *Acanthocheilonema reconditum*, *Acanthocheilonema dracunculoides*), an alternative method for diagnosing *D. immitis* infection in dogs is the use of commercial kits for the detection of antigens released into the blood by adult females. The acid phosphatase histochemical staining method can be useful for differentiating microfilariae of *D. immitis*, *D. repens* and *A. reconditum* based on species-typical staining patterns of their anatomical structures, although this method presents limitations for the identification of microfilariae and major disadvantages due to the short shelf-life of its reagents (Peribáñez *et al.*, 2001). Recent molecular-based assays have enabled identification of filaroids, irrespective of their life cycle stage (Latrofa *et al.*, 2012).

In man, *Dirofilaria* spp. localize predominantly in the subcutaneous tissues and lungs, but also in the

central nervous system, causing a range of clinical manifestations ranging from asymptomatic infection to fatal syndromes (Otranto and Eberhard, 2011). Diagnosis in human patients is usually only possible after surgery and extraction of the worm from the tissues for *Dirofilaria* spp. and *O. lupi* and often requires the assistance of a specialist with an appreciation of the microscopical features of helminth histology (Otranto and Eberhard, 2011). Molecular characterization of samples also assists in achieving a diagnosis from the tissue biopsies.

Prevention of Infection in Man and Animals

The prevention and the treatment of VBH infections in endemic areas is challenging, due to the many components involved in the epidemiology and biology of these infections in man and animals. In dogs, dirofilariosis can be prevented with a number of macrocyclic lactones administered in different formulations (e.g. tablets, chewable, spot on and injectable) with different protocols, from daily administration up to slow release products with effects lasting for 6 months, which kill *D. immitis* or *D. repens* larvae before they develop into adults. The injectable long-lasting formulation containing moxidectin is effective in controlling *D. immitis* and *D. repens* infestations for a period of 6 months after a single administration (Genchi *et al.*, 2002, 2010). Current guidelines on management of *D. immitis* infection in dogs formed by ESCCAP and by the American Heartworm Society suggest extending preventive treatment to 7–8 months or even year round. No data are available on the efficacy of macrocyclic lactones as chemoprophylactic agents against *O. lupi*, while preventing contact with the fly intermediate host of *T. callipaeda* by use of bed nets is currently the only strategy to prevent this infection.

Gaps in Knowledge and Recommendations for Further Research

While the scientific knowledge of the biology, epidemiology, control and treatment of *D. immitis* and *D. repens* has increased considerably over past decades, for other filaroids such as *O. lupi* there are still gaps in knowledge that impair a realistic appreciation of their impact in veterinary and human medicine. In addition, the reasons why human cases of VBH infections have increased in Europe are not fully known, but this most likely reflects the spread of arthropod vector species and lack of economic means for their control in the environment. Large epidemiological studies to estimate the occurrence of filaroid infections in animals, coupled with entomological surveillance programmes, are essential for providing information on the occurrence of these pathogens and to prevent the spread of filaroids into non-endemic areas, therefore limiting the outbreaks of zoonotic filariosis.

Toxocariosis

Aetiology

Toxocariosis is caused by *Toxocara canis* and *Toxocara cati* (syn. *Toxocara mystax*), which are ubiquitous, prolific nematodes with a complicated life cycle. Other ascarids that may potentially be of clinical importance in man include *Baylisascaris procyonis* of raccoons and *Ascaris suum* of pigs. In contrast to the other nematodes, the latter is expected to complete its migration and may reach patency in man (Nejsum *et al.*, 2012).

Hosts and Life Cycle

The definitive host of *T. canis* are canids, including dogs and foxes, while *T. cati* has cats and other felids as definitive hosts. Invertebrates (e.g. earthworms), rodents, foxes, birds and livestock (e.g. sheep, pigs and poultry) can serve as paratenic hosts (Taira *et al.*, 2004; Schnieder *et al.*, 2011). Dogs are infected with *T. canis* by ingestion of embryonated eggs or hypobiotic (arrested) L3 in paratenic hosts; even older immune dogs may acquire new patent infections if exposed to low numbers of eggs (Fahrion *et al.*, 2008). Pups are infected vertically, either prenatally in the last trimester of gestation or by larvae in milk from the bitch. Transplacental transmission accounts for many more infections than the lactational route (Burke and Roberson, 1985) and represents either recent infection of the pregnant bitch or reactivated hypobiotic larvae after somatic migration in the immune bitch (Schnieder *et al.*, 2011). Occasionally, bitches are reinfected by eating intestinal larvae (L4) from faeces of pups. *T. cati* is primarily transmitted to kittens by ingestion of larvae in milk following acute infection of the queen, while prenatal infection apparently does not take place (Coati *et al.*, 2004). The lack of reactivation indicates different characteristics of hypobiotic larvae in cats compared with dogs. Other infection routes in cats are intake of embryonated eggs from soil or larvae within paratenic hosts (e.g. rodents).

The life cycle is typically migratory: after ingestion of eggs in a fully susceptible host, hatched larvae migrate through the liver and lungs while moulting from L3 to L4, are coughed up through the trachea (L4 to L5) to finally develop into adults that reside in the small intestine of the definitive hosts. Eventually, eggs in large number (thousands per day) are voided in the faeces. In the immune host, the larvae do not perform tracheal migration, but re-enter the

circulation for somatic migration (i.e. L3 relocate to skeletal muscles, kidneys, mammary gland, CNS and other organs) (Schnieder *et al.*, 2011). For *T. canis*, the prepatent period thus varies with the route of infection; eggs can be found in puppies 2–3 weeks of age after prenatal infection, while prepatency is 4–5 weeks after ingestion of eggs followed by tracheal migration (Overgaauw, 1997). Eggs are usually excreted for 4 months. The prepatent period for *T. cati* is also variable, but is usually 6–8 weeks after ingestion of eggs. Patency lasts 4–6 months. Eggs undergo development outside the host for at least 2–4 weeks to reach the infective stage (L3), which remains inside the egg and shows extreme persistence in the environment for months to years, although it is generally sensitive to ultraviolet light, desiccation and high temperature.

Human infections are predominantly acquired from ingestion of embryonated eggs by geophagia in sandpits, parks or other places where cats, dogs or wildlife have defecated. *Toxocara* spp. eggs have been recovered worldwide from sand or soil in playgrounds and public parks (Overgaauw, 1997). Embryonated eggs have also been found in the hair coat of dogs, mainly puppies (Amaral *et al.*, 2010) and foxes, but the relative importance of this for human transmission remains unknown. Food-borne infections may also take place, for example by drinking water or eating vegetables contaminated with eggs and by eating raw liver or other viscera of paratenic hosts, including livestock, as experimentally demonstrated for pigs or chickens (Taira *et al.*, 2004). It is possible that food-borne infections may be relatively common in certain cultural settings, for instance in Japan where raw liver is eaten (Akao and Ohta, 2007), but the relative importance of this means of transmission in the European context is presently unknown.

Raccoons are the major definitive hosts of *B. procyonis*, but infection also reaches patency in dogs; the latter has been observed in many cases in the USA (Lee *et al.*, 2010), usually with low intensity infections. However, no data for dogs in Europe have been reported. A wide range of animals (>90 species of mammals and birds) may serve as intermediate hosts, as it is believed that the L2 stage is in the ingested infective egg and it develops to L3 in the intermediate host (Kazacos, 2001). In raccoons, there is no migration, while there is extensive somatic migration in the intermediate hosts. A proportion of larvae has propensity for migration in the CNS (neural larvae migrans, NLM). This is particularly harmful as development from L2 to L3 is accompanied by a four- to five-fold increase in length (up to 1,300–1,900 μm) and larvae do not readily encapsulate in eosinophilic granulomas as in other tissues, but continue migration for a prolonged period of time (Kazacos, 2001).

Epidemiology

The heaviest infections and highest morbidity are seen in pups and kittens. Heavy prenatal infections in pups may lead to severe disease with alternating diarrhoea and constipation, vomiting, typical 'pot belly', reduced growth with cachexia, poor hair coat and in some cases death (Schnieder *et al.*, 2011). Dogs older than 6 months are usually less severely or not affected. Clinical signs of *T. cati* infection in young cats are similar, but generally less severe; respiratory tract signs are also reported. The prevalence of *T. canis* in dogs, based on faecal examination, varies considerably in EU countries (1.4–30.5%) (Schnieder *et al.*, 2011) and depends on the composition of the host population, animal density (definitive and paratenic hosts), seasonality, region and methods employed. The prevalence of *T. cati* is generally higher due to the low level of resistance to reinfection in older cats, around 8–76% (Overgaauw, 1997), with large variation between domestic cats with or without access to the outdoors, stray cats or those in shelters. In foxes, *T. canis* has been reported with mean prevalence rates up to 49–87%, depending on age group (Saeed *et al.*, 2006; Morgan *et al.*, 2013). Similar infection levels of *B. procyonis* (39–80%) have been reported in raccoons in some areas of Germany (Bauer, 2011).

Seroprevalence of *Toxocara* spp. infections in man is around 3–19% in many European countries, varying by diagnostic methods, age profile (highest in young people) and cultural habits (Overgaauw and Knapen, 2013). A certain level of cross-reaction with other nematode infections cannot be ruled out; for example, *A. suum* from pigs may cause patent (or aborted) infections in man, particularly in young individuals (Nejsum *et al.*, 2012). Risk factors related to seropositivity include young age, playing in sandpits, dog ownership, poor sanitation, rural populations and low socioeconomic status, while the effect of gender is variable (Magnaval *et al.*, 2001; Rubinsky-Elefant *et al.*, 2010). The vast majority of human *Toxocara* spp. infections are asymptomatic. However, *T. canis* and, probably less commonly, *T. cati*, may cause clinical syndromes in man described as visceral larvae migrans (VLM), ocular larvae migrans (OLM), covert toxocariosis and more rarely NLM. VLM and OLM are most often observed in children (VLM at 1–5 years of age predominantly; OLM at 5–10 years), while the less well-defined covert toxocariosis is found in both children and adults (Smith *et al.*, 2009). The incidence in

the EU is largely unknown, but presumably very low (Smith *et al.*, 2009), and the relative contribution of the two species is unknown (Fisher, 2003; Rubinsky-Elefant *et al.*, 2010). Signs of VLM depend on the infective dose and are non-specific, including abdominal pain, fever, anorexia, respiratory signs, headache, skin lesions and occasionally neurological symptoms, accompanied by hepatomegaly and eosinophilia. OLM indicates the location of a *Toxocara* larva in an eye or optic nerve and is often painless, but leads to visual disturbances and unilateral blindness. It is increasingly seen also in adults (Akao and Ohta, 2007). Specific antibody levels in OLM are often low because the larvae evade the immune system or their number is low. There are some indications that *T. canis* and *T. cati* larvae have different tissue preferences during somatic migration in the same paratenic host or at least different time courses (Strube *et al.*, 2013). *T. cati* larvae predominantly locate in skeletal muscles while *T. canis* more rapidly migrate to the CNS in addition to the muscle, indicating perhaps a higher degree of neuroaffinity.

B. procyonis eggs are particularly abundant in latrine areas of raccoons and people contract infection mainly by geophagia (Bauer, 2013). As mentioned, *B. procyonis* causes severe OLM and NLM (acute eosinophilic meningoencephalitis) in intermediate hosts, including man. The NLM syndrome is often fatal or causes permanent neurological disease to the intermediate host, as observed in almost all reported human cases in the USA (Lee *et al.*, 2010). Only single cases in people have been reported from Europe (Bauer, 2013).

Diagnosis of Infection in Man and Animals

Patent infections in dogs and cats can be diagnosed by standard faecal flotation. A study combining PCR analysis and egg morphology showed that *T. cati* eggs are distinctly smaller than *T. canis* eggs, but also revealed that up to 30% of eggs found in dogs could be *T. cati* (Fahrion *et al.*, 2011). This is most likely due to coprophagia, as these species seem to be host specific. Ingestion of fox faeces by dogs may also lead to false-positive observations. *B. procyonis* eggs can easily be mistaken for *T. canis* eggs based on size; however, the latter have a regular pitted surface while *B. procyonis* eggs have a fine granular surface (Kazacos, 2001; Lee *et al.*, 2010). This may, however, be difficult to ascertain by routine microscopy and baylisascariosis needs in most cases to be confirmed by PCR on eggs.

Human toxocariosis is diagnosed by clinical manifestations, ophthalmology (OLM), clinical pathology, including eosinophilia, bioimaging (typically in CNS involvement) and serology. In cases of OLM and perhaps NLM, extirpation by biopsy and subsequent histopathology can be performed and parasite material can be speciated by PCR. Detection of IgG antibodies to *T. canis* excretory/secretory antigens (TES) by indirect ELISA, followed by western blot to limit cross-reactivity, is central to the diagnosis (Fillaux and Magnaval, 2013). However, antibody titres do not necessarily correlate with active versus non-active infection and false-positive outcomes exist (Smith *et al.*, 2009). These assays cross-react with *T. cati* and can be used for evaluating toxocariosis as such; none of the currently available tests are capable of discriminating between *T. canis* and *T. cati* infections in man or any other paratenic host.

Prevention of Infection in Man and Animals

A cornerstone in prevention is minimizing the environmental contamination with (infective) eggs by rigorous removal of faeces and by treatment of infected dogs and cats. Faeces should be removed and destroyed when dropped in public areas, streets, kennels and also in private gardens. Intestinal stages of *Toxocara* spp. are susceptible to the most commonly used anthelmintics, while hypobiotic stages in tissues are impossible to treat effectively, thus posing a problem of clearing breeding bitches of infection (Othman, 2012). Although some hypobiotic larvae may become susceptible to anthelmintics on reactivation, it is not advisable to treat pregnant animals to reduce perinatal transmission (Overgaauw and Knapen, 2013). Repeated application of anthelmintics is therefore recommended for puppies and kittens (and their mothers) during lactation and early life in order to avoid pathogenic infections and limit contamination (Fisher *et al.*, 1993). Older dogs and cats can either be treated on a routine basis or examined for eggs regularly followed by treatment of patent cases. Guidelines for the control and treatment of parasites in pet animals were developed and published by ESCCAP in Europe (www.esccap.org). Other preventive measures include avoiding transmission by feeding of raw liver or offal and coprophagy in dogs. The relative contribution of *T. canis* from foxes to environmental contamination is difficult to assess in an urban context and equally difficult to control. An attempt to quantify the contributions of dogs, cats and foxes in the Bristol area (UK) indicated that the main contributor was dogs, although obviously modified by the degree of removal of faeces and dog access to public spaces (Morgan *et al.*, 2013).

Prevention of human infections should be based on appropriate control of infections in pets, removal of

faeces from surroundings, limiting access of pets to children's play areas, good hygiene and lastly, education. The environmental efforts include fencing of playgrounds, covering of sandpits, regular application of new sand, exclusion of dogs from parks and recreational areas, provision of information (signs) and bags for faeces and management of stray animals. Furthermore, general good hand hygiene, rinsing of fresh produce from gardens and prevention of geophagia in children are essential.

Treatment of larvae migrans in people includes anti-inflammatory and anthelmintic treatments with moderate reduction in clinical symptoms (Strube *et al.*, 2013) and in the case of OLM, possible extirpation. Anthelmintics may have limited effect in OLM.

B. procyonis infections in dogs are treated with commonly available anthelmintics, such as benzimidazoles, macrocyclic lactones or tetrahydropyrimidines. Raccoon populations should be controlled as well as any animal considered infected. Latrines close to children's playgrounds should be cleaned by disposal of faeces and preferably by burning (or removal) of the upper soil layer (more info on http://www.cdc.gov). Raccoons kept as pets or in contact with the public should be treated regularly.

Gaps in Knowledge and Recommendations for Further Research

Gaps in knowledge that need to be addressed include: (1) evaluation of the importance of food-borne transmission, in comparison with other transmission routes; (2) standardization of case definitions for human infection throughout Europe, which will enable the gathering of good quality data on the incidence and prevalence of disease; (3) evaluation of burden of disease in man, including the potential impact of subclinical infections on human behaviour; (4) development of diagnostic methods to discriminate between *T. canis* and *T. cati* in paratenic hosts, including man. This will provide information on infection routes and assist in better targeting of control strategies; (5) quantifying the animal sources of *Toxocara* environmental contamination (dogs, foxes or cats); (6) development of rapid point-of-care diagnostic tools for *Toxocara* in pets (e.g. coproassays for antigen or DNA). At present, most infections will remain unnoticed by companion animal owners and veterinarians unless faecal evaluation is performed; and (7) development of specific rapid detection for *B. procyonis* infections in dogs, which is important as the eggs look like *Toxocara* eggs and at present, a subsequent PCR on isolated eggs is most often needed to verify the diagnosis.

Conclusions

Parasitic zoonoses constitute some of the most important and common infections threatening human populations in Europe as well as other continents. This review has presented the major diseases in this category associated with companion animals, describing the current status of infections in man and animals in an effort to highlight gaps in knowledge and potential interventions to prevent or limit their spread. Combating parasitic zoonoses requires an integrated multidisciplinary approach involving collaboration between veterinary and medical scientists and policy makers.

Acknowledgments

The authors thank the EU seventh framework CALLSITO programme for funding the meetings and discussions, which provided the basis for composing this manuscript, and for funding its publication.

Conflict of Interest Statement

The authors declare that they have no competing interests.

References

Akao N, Ohta N (2007) Toxocariasis in Japan. *Parasitology International*, **56**, 87–93.

Alvar J, Yactayo S, Bern C (2006) Leishmaniasis and poverty. *Trends in Parasitology*, **22**, 552–557.

Amaral HLC, Rassier GL, Pepe MS, Gallina T, Villela MM *et al.* (2010) Presence of *Toxocara canis* eggs on the hair of dogs: a risk factor for visceral larva migrans. *Veterinary Parasitology*, **174**, 115–118.

Amusategui I, Sainz A, Aguirre E, Tesouro MA (2004) Seroprevalence of *Leishmania infantum* in northwestern Spain, an area traditionally considered free of leishmaniasis. *Annals of the New York Academy of Sciences*, **1026**, 154–157.

Avellis FO, Kramer LH, Mora P, Bartolino A, Benedetti P *et al.* (2011) A case of human conjunctival dirofilariosis by *Dirofilaria immitis* in Italy. *Vector Borne Zoonotic Diseases*, **11**, 451–452.

Baneth G, Koutinas AF, Solano-Gallego L, Bourdeau P, Ferrer L (2008) Canine leishmaniosis – new concepts and insights on an expanding zoonosis: part one. *Trends in Parasitology*, **24**, 324–330.

Barnes TS, Deplazes P, Gottstein B, Jenkins DJ, Mathis A *et al.* (2012) Challenges for diagnosis and control of cystic hydatid disease. *Acta Tropica*, **123**, 1–7.

Bauer C (2011) Baylisascariose (*Baylisascaris procyonis*) – eine seltene parasitäre Zoonose in Europa. *Berliner und Münchener Tierärztliche Wochenschrift*, **124**, 465–472.

Bauer C (2013) Baylisascariosis – infections of animals and humans with 'unusual' roundworms. *Veterinary Parasitology*, **193**, 404–412.

Benner C, Carabin H, Sánchez-Serrano LP, Budke CM, Carmena D (2010) Analysis of the economic impact of cystic echinococcosis in Spain. *Bulletin of the World Health Organization*, **88**, 49–57, Erratum in: *Bulletin of the World Health Organization* (2010) **88**, 236.

Boggiatto PM, Gibson-Corley KN, Metz K, Gallup JM, Hostetter JM *et al.* (2011) Transplacental transmission of *Leishmania infantum* as a means for continued disease incidence in North America. *PLoS Neglected Tropical Diseases*, **5**, e1019.

Bruzinskaite R, Sarkunas M, Torgerson PR, Mathis A, Deplazes P (2009) Echinococcosis in pigs and intestinal infection with *Echinococcus* spp. in dogs in southwestern Lithuania. *Veterinary Parasitology*, **160**, 237–241.

Burke TM, Roberson EL (1985) Prenatal and lactational transmission of *Toxocara canis* and *Ancylostoma caninum*: experimental infection of the bitch before pregnancy. *International Journal for Parasitology*, **15**, 71–75.

Caccio SM, Thompson RC, McLauchlin J, Smith HV (2005) Unravelling *Cryptosporidium* and *Giardia* epidemiology. *Trends in Parasitology*, **21**, 430–437.

Campos-Bueno A, López-Abente G, Andrés-Cercadillo AM (2000) Risk factors for *Echinococcus granulosus* infection: a case-control study. *American Journal of Tropical Medicine and Hygiene*, **62**, 329–334.

Carmena D, Cardona G (2013) Canine echinococcosis: global epidemiology and genotypic diversity. *Acta Tropica*, **128**, 441–460.

Cenci-Goga BT, Rossitto PV, Sechi P, McCrindle CM, Cullor JS (2011) *Toxoplasma* in animals, food, and humans: an old parasite of new concern. *Foodborne Pathogens and Disease*, **8**, 751–762.

Chamaille L, Tran A, Meunier A, Bourdoiseau G, Ready P *et al.* (2010) Environmental risk mapping of canine leishmaniasis in France. *Parasites and Vectors*, **3**, 31.

Claerebout E, Casaert S, Dalemans AC, De Wilde N, Levecke B *et al.* (2009) *Giardia* and other intestinal parasites in different dog populations in Northern Belgium. *Veterinary Parasitology*, **161**, 41–46.

Coati N, Schnieder T, Epe C (2004) Vertical transmission of *Toxocara cati* Schrank 1788 (Anisakidae) in the cat. *Parasitology Research*, **92**, 142–146.

Conraths F, Deplazes P (2015) *Echinococcus multilocularis*: epidemiology, surveillance and state-of-the-art of diagnostics from a veterinary public health perspective. *Veterinary Parasitology*, **213**, 149–161. http://dx.doi.org/10.1016/j.vetpar.2015.07.027.

Cook AJ, Gilbert RE, Buffolano W, Zufferey J, Petersen E *et al.* (2000) Sources of *Toxoplasma* infection in pregnant women: European multicentre case-control study. European Research Network on Congenital Toxoplasmosis. *British Medical Journal*, **321**, 142–147.

Craig PS, Larrieu E (2006) Control of cystic echinococcosis/hydatidosis: 1863–2002. *Advances in Parasitology*, **61**, 443–508.

Craig P, Mastin A, van Kesteren F, Boufana B (2015) *Echinococcus granulosus*: epidemiology and state-of-the-art of diagnostics in animals. *Veterinary Parasitology*, **213**, 132–148. http://dx.doi.org/10.1016/j.vetpar.2015.07.028.

Dabritz HA, Conrad PA (2010) Cats and *Toxoplasma*: implications for public health. *Zoonoses and Public Health*, **57**, 34–52.

Dantas-Torres F, Otranto D (2013) Dirofilariosis in the Americas: a more virulent *Dirofilaria immitis*? *Parasites and Vectors*, **6**, 288.

de Freitas E, Mel MN, da Costa-Val AP, Michalick MS (2006) Transmission of *Leishmania infantum* via blood transfusion in dogs: potential for infection and importance of clinical factors. *Veterinary Parasitology*, **137**, 159–167.

Deplazes P, van Knapen F, Schweiger A, Overgaauw PAM (2011) Role of pet dogs and cats in the transmission of helminthic zoonoses in Europe, with a focus on echinococcosis and toxocarosis. *Veterinary Parasitology*, **182**, 41–53.

Desjeux P (2004) Leishmaniasis: current situation and new perspectives. *Comparative Immunology, Microbiology and Infectious Diseases*, **27**, 305–318.

Dubey JP (2001) Oocyst shedding by cats fed isolated bradyzoites and comparison of infectivity of bradyzoites of the VEG strain *Toxoplasma gondii* to cats and mice. *Journal of Parasitology*, **87**, 215–219.

Dubey JP, Ferreira LR, Martins J, Jones JL (2011) Sporulation and survival of *Toxoplasma gondii* oocysts in different types of commercial cat litter. *Journal of Parasitology*, **97**, 751–754.

Dujardin JC, Campino L, Canavate C, Dedet JP, Gradoni L *et al.* (2008) Spread of vector-borne diseases and neglect of leishmaniasis, Europe. *Emerging Infectious Diseases*, **14**, 1013–1018.

Eberhard ML, Ortega Y, Dial S, Schiller CA, Sears AW *et al.* (2000) Ocular *Onchocerca* infections in western United States. *Veterinary Parasitology*, **90**, 333–338.

Eberhard ML, Ostovar GA, Chundu K, Hobohm D, Feiz-Erfan I *et al.* (2013) Zoonotic *Onchocerca lupi* infection in a 22-month-old child in Arizona: first report in the United States and a review of the literature. *American Journal of Tropical Medicine and Hygiene*, **88**, 601–605.

Eckert J, Deplazes P, Kern P (2011) Alveolar echinococcosis (*Echinococcus multilocularis*) and neotropical forms of echinococcosis (*Echinococcus vogeli* and *Echinococcus oligarthrus*). In: *Zoonoses*, 2nd Edit., D Brown, S Palmer, PR Torgerson, EJL Soulsby, Eds., Oxford University Press, Oxford, pp. 669–699.

Elmahallawy EK, Sampedro Martinez A, Rodriguez-Granger J, Hoyos-Mallecot Y *et al.* (2014) Diagnosis of leishmaniasis. *Journal of Infection in Developing Countries*, **8**, 961–972.

Elmore SA, Jones JL, Conrad PA, Patton S, Lindsay DS *et al.* (2010) *Toxoplasma gondii*: epidemiology, feline clinical aspects, and prevention. *Trends in Parasitology*, **26**, 190–196.

Epe C, Rehkter G, Schnieder T, Lorentzen L, Kreienbrock L (2010) *Giardia* in symptomatic dogs and cats in Europe – results of a European study. *Veterinary Parasitology*, **173**, 32–38.

Fahrion AS, Schnyder M, Wichert B, Deplazes P (2011) *Toxocara* eggs shed by dogs and cats and their molecular

and morphometric species-specific identification: is the finding of *T. cati* eggs shed by dogs of epidemiological relevance? *Veterinary Parasitology*, **177**, 186–189.

Fahrion AS, Staebler S, Deplazes P (2008) Patent *Toxocara canis* infections in previously exposed and in helminth-free dogs after infection with low numbers of embryonated eggs. *Veterinary Parasitology*, **152**, 108–115.

Faísca P, Morales-Hojas R, Alves M, Gomes J, Botelho M *et al.* (2010) A case of canine ocular onchocercosis in Portugal. *Veterinary Ophthalmology*, **13**, 117–121.

Feng Y, Xiao L (2011) Zoonotic potential and molecular epidemiology of *Giardia* species and giardiasis. *Clinical Microbiology Reviews*, **24**, 110–140.

Fernandez-Bellon H, Solano-Gallego L, Bardagi M, Alberola J, Ramis A *et al.* (2006) Immune response to *Leishmania infantum* in healthy horses in Spain. *Veterinary Parasitology*, **135**, 181–185.

Fillaux J, Magnaval JF (2013) Laboratory diagnosis of human toxocariasis. *Veterinary Parasitology*, **193**, 327–336.

Fisher M (2003) *Toxocara cati*: an underestimated zoonotic agent. *Trends in Parasitology*, **19**, 167–170.

Fisher M, Jacobs DE, Hutchinson MJ, Abbott EM (1993) Efficacy of fenbendazole and piperazine against developing stages of *Toxocara* and *Toxascaris* in dogs. *Veterinary Record*, **132**, 473–475.

Fuentes I, Montes I, Saugar JM, Gárate T, Otranto D (2012) Thelaziosis, a zoonotic infection, Spain, 2011. *Emerging Infectious Diseases*, **18**, 2073–2075.

Genchi C, Bowman D, Drake J (2014) Canine heartworm disease (*Dirofilaria immitis*) in Western Europe: survey of veterinary awareness and perceptions. *Parasites and Vectors*, **7**, 206.

Genchi C, Kramer LH, Rivasi F (2011) Dirofilarial infections in Europe. *Vector Borne Zoonotic Diseases*, **11**, 1307–1317.

Genchi M, Pengo G, Genchi C (2010) Efficacy of moxidectin microsphere sustained release formulation for the prevention of subcutaneous filarial infections (*Dirofilaria repens*) in dogs. *Veterinary Parasitology*, **170**, 167–169.

Genchi C, Rinaldi L, Mortarino M, Genchi M, Cringoli G (2009) Climate and *Dirofilaria* infection in Europe. *Veterinary Parasitology*, **163**, 286–292.

Genchi C, Rossi L, Cardini G, Kramer LH, Venco L *et al.* (2002) Full season efficacy of moxidectin microsphere sustained release formulation for prevention of heartworm (*Dirofilaria immitis*) infection in dogs. *Veterinary Parasitology*, **110**, 85–91.

Geurden T, Claerebut E, Vercruysse J (2005) Protozoan infection causes diarrhea in calves. *Tijdschrift voor Diergeneeskunde*, **130**, 734–737.

Gottstein B, Stojkovic M, Vuitton DA, Millon L, Marcinkute A *et al.* (2015) Threat of alveolar echinococcosis to public health – a challenge for Europe. *Trends in Parasitology*, **31**, 407–412, pii: S1471–4922(15)00118-X.

Gouzelou E, Haralambous C, Antoniou M, Christodoulou V, Martinkovic F *et al.* (2013) Genetic diversity and structure in *Leishmania infantum* populations from southeastern Europe revealed by microsatellite analysis. *Parasites and Vectors*, **6**, 342.

Hegglin D, Deplazes P (2013) Control of *Echinococcus multilocularis*: strategies, feasibility and cost-benefit analyses. *International Journal for Parasitology*, **43**, 327–337.

Hermosilla C, Hetzel U, Bausch M, Grübl J, Bauer C (2005) First autochthonous case of canine ocular onchocercosis in Germany. *Veterinary Record*, **154**, 450–452.

Hill D, Coss C, Dubey JP, Wroblewski K, Sautter M *et al.* (2011) Identification of a sporozoite-specific antigen from *Toxoplasma gondii*. *Journal of Parasitology*, **97**, 328–337.

Ilhan HD, Yaman A, Morishima Y, Sugiyama H, Muto M *et al.* (2013) *Onchocerca lupi* infection in Turkey: a unique case of a rare human parasite. *Acta Parasitologica*, **58**, 384–388.

Jenkins DJ, Romig T, Thompson RCA (2005) Emergence/re-emergence of *Echinococcus* spp. – a global update. *International Journal for Parasitology*, **35**, 1205–1219.

Jones JL, Dargelas V, Roberts J, Press C, Remington JS *et al.* (2009) Risk factors for *Toxoplasma gondii* infection in the United States. *Clinical Infectious Diseases*, **49**, 878–884.

Kapel CMO, Torgerson PR, Thompson RCA, Deplazes P (2006) Reproductive potential of *Echinococcus multilocularis* in experimentally infected foxes, dogs, raccoon dogs and cats. *International Journal for Parasitology*, **36**, 79–86.

Kazacos KR (2001) *Baylisascaris procyonis* and related species. In: *Parasitic Diseases of Wild Animals*, 2nd Edit., WM Samuel, MJ Pybus, AA Kocan, Eds., Manson, London, pp. 301–341.

Komnenou A, Eberhard ML, Kaldrymidou E, Tsalie E, Dessiris A (2002) Subconjunctival filariasis due to *Onchocerca* sp. in dogs: report of 23 cases in Greece. *Veterinary Ophthalmology*, **5**, 119–126.

Labelle AL, Daniels JB, Dix M, Labelle P (2011) *Onchocerca lupi* causing ocular disease in two cats. *Veterinary Ophthalmology*, **14**, 105–110.

Latrofa MS, Dantas-Torres F, Annoscia G, Genchi M, Traversa D *et al.* (2012) A duplex real-time polymerase chain reaction assay for the detection of and differentiation between *Dirofilaria immitis* and *Dirofilaria repens* in dogs and mosquitoes. *Veterinary Parasitology*, **185**, 181–185.

Lee ACY, Schantz PM, Kazacos KR, Montgomery SP, Bowman DD (2010) Epidemiologic and zoonotic aspects of ascarid infections in dogs and cats. *Trends in Parasitology*, **26**, 155–161.

Lightowlers MW (2013) Cysticercosis and echinococcosis. *Current Topics in Microbiology and Immunology*, **365**, 315–335.

Lindsay DS, Dubey JP (2011) *Toxoplasma gondii*: the changing paradigm of congenital toxoplasmosis. *Parasitology*, **9**, 1–3.

Lopez-Velez R, Perez-Molina JA, Guerrero A, Baquero F, Villarrubia J *et al.* (1998) Clinicoepidemiologic characteristics, prognostic factors, and survival analysis of patients coinfected with human immunodeficiency virus and *Leishmania* in an area of Madrid, Spain. *American Journal for Tropical Medicine and Hygiene*, **58**, 436–443.

Magnaval JF, Glickman LT, Dorchies P, Morassin B (2001) Highlights of human toxocariasis. *Korean Journal of Parasitology*, **39**, 1–11.

Maia C, Nunes M, Campino L (2008) Importance of cats in zoonotic leishmaniasis in Portugal. *Vector Borne and Zoonotic Disease*, **8**, 555–559.

Malacrida F, Hegglin D, Bacciarini L, Otranto D, Nägeli F *et al.* (2008) Emergence of canine ocular Thelaziosis caused by *Thelazia callipaeda* in southern Switzerland. *Veterinary Parasitology*, **157**, 321–327.

Maroli M, Rossi L, Baldelli R, Capelli G, Ferroglio E *et al.* (2008) The northward spread of leishmaniasis in Italy: evidence from retrospective and ongoing studies on the canine reservoir and phlebotomine vectors. *Tropical Medicine International Health*, **13**, 256–264.

Martin-Sanchez J, Acedo C, Munoz-Perez M, Pesson B, Marchal O *et al.* (2007) Infection by *Leishmania infantum* in cats: epidemiological study in Spain. *Veterinary Parasitology*, **145**, 267–273.

McCall JW, Genchi C, Kramer LH, Guerrero J, Venco L (2008) Heartworm disease in animals and humans. *Advances in Parasitology*, **66**, 193–285.

Menn B, Lorentz S, Naucke TJ (2010) Imported and travelling dogs as carriers of canine vector-borne pathogens in Germany. *Parasites and Vectors*, **3**, 34.

Michel G, Pomares C, Ferrua B, Marty P (2011) Importance of worldwide asymptomatic carriers of *Leishmania infantum* (*L. chagasi*) in humans. *Acta Tropica*, **119**, 69–75.

Miliaras D, Meditskou S, Kelekis A, Papachristos I (2010) Human pulmonary dirofilariasis: one more case in Greece suggests that *Dirofilaria* is a rather common cause of coin lesions in the lungs in endemic areas of Europe. *International Journal of Immunopathology and Pharmacology*, **23**, 345–348.

Millan J, Zanet S, Gomis M, Trisciuoglio A, Negre N *et al.* (2011) An investigation into alternative reservoirs of canine leishmaniasis on the endemic island of Mallorca (Spain). *Transboundary and Emerging Diseases*, **58**, 352–357.

Miró G, Montoya A, Hernández L, Dado D, Vázquez MV *et al.* (2011) *Thelazia callipaeda* infection in dogs: a new parasite for Spain. *Parasites and Vectors*, **27**, 148.

Morchón R, Carretón E, González-Miguel J, Mellado-Hernández I (2012) Heartworm disease (*Dirofilaria immitis*) and their vectors in Europe – new distribution trends. *Frontiers in Physiology*, **3**, 196.

Morchón R, Moya I, González-Miguel J, Montoya MN, Simón F (2010) Zoonotic *Dirofilaria immitis* infections in a province of Northern Spain. *Epidemiology and Infection*, **138**, 380–383.

Morgan ER, Azam D, Pegler K (2013) Quantifying sources of environmental contamination with *Toxocara* spp. eggs. *Veterinary Parasitology*, **193**, 390–397.

Mowlavi G, Farzbod F, Kheirkhah A, Mobedi I, Bowman DD *et al.* (2013) Human ocular onchocerciasis caused by *Onchocerca lupi* (Spirurida, Onchocercidae) in Iran. *Journal of Helminthology*, **6**, 1–6.

Murray HW, Berman JD, Davies CR, Saravia NG (2005) Advances in leishmaniasis. *Lancet*, **366**, 1561–1577.

Nejsum P, Betson M, Bendall RP, Thamsborg SM, Stothard JR (2012) Assessing the zoonotic potential of *Ascaris suum* and *Trichuris suis*: looking to the future from an analysis of the past. *Journal of Helminthology*, **86**, 148–155.

O'Handley RM, Olson ME (2006) Giardiasis and cryptosporidiosis in ruminants. *Veterinary Clinics of North America: Food Animal Practice*, **22**, 623–643.

Opsteegh M, Langelaar M, Sprong H, den Hartog L, De Craeye S *et al.* (2010) Direct detection and genotyping of *Toxoplasma gondii* in meat samples using magnetic capture and PCR. *International Journal of Food Microbiology*, **139**, 193–201.

Opsteegh M, Teunis P, Züchner L, Koets A, Langelaar M *et al.* (2011) Low predictive value of seroprevalence of *Toxoplasma gondii* in cattle for detection of parasite DNA. *International Journal for Parasitology*, **41**, 343–354.

Orihel TC, Ash LR, Holshuh HJ, Santenelli S (1991) Onchocerciasis in a California dog. *American Journal of Tropical Medicine and Hygiene*, **44**, 513–517.

Otero-Abad B, Torgerson PR (2013) A systematic review of the epidemiology of echinococcosis in domestic and wild animals. *PLoS Neglected Tropical Diseases*, **7**, e2249.

Othman AA (2012) Therapeutic battle against larval toxocariasis: are we still far behind? *Acta Tropica*, **124**, 171–178.

Otranto D, Brianti E, Cantacessi C, Lia RP, Máca J (2006) The zoophilic fruit fly *Phortica variegata*: morphology, ecology and biological niche. *Medical and Veterinary Entomology*, **20**, 358–364.

Otranto D, Brianti E, Gaglio G, Dantas-Torres F, Azzaro S *et al.* (2011a) Human ocular infection with *Dirofilaria repens* (Railliet and Henry, 1911) in an area endemic for canine dirofilariasis. *American Journal of Tropical Medicine and Hygiene*, **84**, 1002–1004.

Otranto D, Capelli G, Genchi C (2009) Changing distribution patterns of canine vector borne disease in Italy: leishmaniosis *vs.* dirofilariosis. *Parasites and Vectors*, **26**(Suppl. 1), S2.

Otranto D, Dantas-Torres F (2013) The prevention of canine leishmaniasis and its impact on public health. *Trends in Parasitology*, **29**, 339–345.

Otranto D, Dantas-Torres F, Brianti E, Traversa D, Petrić D *et al.* (2013a) Vector-borne helminths of dogs and humans in Europe. *Parasites and Vectors*, **6**, 16.

Otranto D, Dantas-Torres F, Papadopoulos E, Petrić D, Ćupina AI *et al.* (2012a) Tracking the vector of *Onchocerca lupi* in a rural area of Greece. *Emerging Infectious Diseases*, **18**, 1196–1200.

Otranto D, Dantas-Torres F, Giannelli A, Latrofa MS, Papadopoulos E *et al.* (2013b) Zoonotic *Onchocerca lupi* infection in dogs, Greece and Portugal, 2011–2012. *Emerging Infectious Diseases*, **19**, 2000–2003.

Otranto D, Dantas-Torres F, Cebeci Z, Yeniad B, Buyukbabani N *et al.* (2012b) Human ocular filariasis: further evidence on the zoonotic role of *Onchocerca lupi*. *Parasites and Vectors*, **27**, 84.

Otranto D, Dutto M (2008) Human thelaziosis, Europe. *Emerging Infectious Diseases*, **14**, 647–649.

Otranto D, Eberhard ML (2011) Zoonotic helminths affecting the human eye. *Parasites and Vectors*, **23**, 41.

Otranto D, Lia RP, Buono V, Traversa D, Giangaspero A (2004) Biology of *Thelazia callipaeda* (Spirurida, Thelaziidae) eyeworms in naturally infected definitive hosts. *Parasitology*, **129**, 627–633.

Otranto D, Lia RP, Cantacessi C, Testini G, Troccoli A *et al.* (2005) Nematode biology and larval development of *Thelazia callipaeda* (Spirurida, Thelaziidae) in the drosophilid intermediate host in Europe and China. *Parasitology*, **131**, 847–855.

Otranto D, Sakru N, Testini G, Gürlü VP, Yakar K *et al.* (2011b) Case report: first evidence of human zoonotic infection by *Onchocerca lupi* (Spirurida, Onchocercidae). *American Journal of Tropical Medicine and Hygiene*, **84**, 55–58.

Overgaauw PAM (1997) Aspects of *Toxocara* epidemiology: toxocarosis in dogs and cats. *Critical Reviews in Microbiology*, **23**, 233–251.

Overgaauw PAM, Knapen F (2013) Veterinary and public health aspects of *Toxocara* spp. *Veterinary Parasitology*, **193**, 398–403.

Owens SD, Oakley DA, Marryott K, Hatchett W, Walton R *et al.* (2011) Transmission of visceral leishmaniasis through blood transfusions from infected English foxhounds to anemic dogs. *Journal of the American Veterinary Medical Association*, **219**, 1076–1083.

Pampiglione S, Canestri Trotti G, Rivasi F (1995) Human dirofilariasis due to *Dirofilaria* (*Nochtiella*) *repens* in Italy: a review of word literature. *Parasitologica*, **37**, 149–193.

Pampiglione S, Rivasi F, Gustinelli A (2009) Dirofilarial human cases in the Old World, attributed to *Dirofilaria immitis*: a critical analysis. *Histopathology*, **54**, 192–204.

Pangrazio KK, Costa EA, Amarilla SP, Cino AG, Silva TM *et al.* (2009) Tissue distribution of *Leishmania chagasi* and lesions in transplacentally infected fetuses from symptomatic and asymptomatic naturally infected bitches. *Veterinary Parasitology*, **165**, 327–331.

Pasquau F, Ena J, Sanchez R, Cuadrado JM, Amador C *et al.* (2005) Leishmaniasis as an opportunistic infection in HIV-infected patients: determinants of relapse and mortality in a collaborative study of 228 episodes in a Mediterreanean region. *European Journal of Clinical Microbiology and Infectious Diseases*, **24**, 411–418.

Peribáñez MA, Lucientes J, Arce S, Morales M, Castillo JA *et al.* (2001) Histochemical differentiation of *Dirofilaria immitis*, *Dirofilaria repens* and *Acanthocheilonema dracunculoides* microfilariae by staining with a commercial kit, Leucognost-SP. *Veterinary Parasitology*, **102**, 173–175.

Petrić D, Zgomba M, Bellini R, Becker N (2012) Surveillance of mosquito populations: a key element to understanding the spread of invasive vector species and vector-borne diseases in Europe. In: *Essays on Fundamental and Applied Environmental Topics*, D Mihailović, Ed., Nova Science Publishers, New York, pp. 192–224.

Quinnell RJ, Courtenay O (2009) Transmission, reservoir hosts and control of zoonotic visceral leishmaniasis. *Parasitology*, **136**, 1915–1934.

Rioux JA, Lanotte G, Serres E, Pratlong F, Bastien P *et al.* (1990) Taxonomy of *Leishmania*. Use of isoenzymes. Suggestions for a new classification. *Annales de Parasitologie Humaine et Comparee*, **65**, 111–125.

Robert-Gangneux F, Dardé ML (2012) Epidemiology of and diagnostic strategies for toxoplasmosis. *Clinical Microbiology Reviews*, **25**, 264–296.

Romi R, Majori G (2008) An overview of the lesson learned in almost 20 years of fight against the 'tiger' mosquito. *Parasitologica*, **50**, 117–119.

Romig T, Ebi D, Wassermann M (2015) Taxonomy and molecular epidemiology of *E. granulosus* sensu lato. *Veterinary Parasitology*, **213**, 76–84. http://dx.doi.org/10.1016/j.vetpar.2015.07.035.

Rosypal AC, Troy GC, Zajac AM, Frank G, Lindsay DS (2005) Transplacental transmission of a North American isolate of *Leishmania infantum* in an experimentally infected beagle. *Journal of Parasitology*, **91**, 970–972.

Rubinsky-Elefant G, Hirata CE, Yamamoto JH, Ferreira MU (2010) Human toxocariasis: diagnosis, worldwide seroprevalences and clinical expression of the systemic and ocular forms. *Annals of Tropical Medicine and Parasitology*, **104**, 3–23.

Saeed I, Maddox-Hyttel C, Monrad J, Kapel CMO (2006) Helminths of red foxes (*Vulpes vulpes*) in Denmark. *Veterinary Parasitology*, **139**, 168–179.

Sagebiel D, Weitzel T, Stark K, Leitmeyer K (2009) Giardiasis in kindergartens: prevalence study in Berlin, Germany, 2006. *Parasitology Research*, **105**, 681–687.

Schnieder T, Laabs E-M, Welz C (2011) Larval development of *Toxocara canis* in dogs. *Veterinary Parasitology*, **175**, 193–206.

Shaw SE, Langton DA, Hillman TJ (2009) Canine leishmaniosis in the United Kingdom: a zoonotic disease waiting for a vector? *Veterinary Parasitology*, **63**, 281–285.

Sherry K, Miro G, Trotta M, Miranda C, Montoya A *et al.* (2011) A serological and molecular study of *Leishmania infantum* infection in cats from the Island of Ibiza (Spain). *Vector Borne and Zoonotic Diseases*, **11**, 239–245.

Shwab EK, Zhu XQ, Majumdar D, Pena HF, Gennari SM *et al.* (2014) Geographical patterns of *Toxoplasma gondii* genetic diversity revealed by multilocus PCR-RFLP genotyping. *Parasitology*, **141**, 453–461.

Silva FL, Oliveira RG, Silva TM, Xavier MN, Nascimento EF *et al.* (2009) Venereal transmission of canine visceral leishmaniasis. *Veterinary Parasitology*, **160**, 55–59.

Smith H, Holland C, Taylor M, Magnaval J-F, Schantz P *et al.* (2009) How common is human toxocariasis? Towards standardizing our knowledge. *Trends in Parasitology*, **25**, 182–188.

Sobrino R, Ferroglio E, Oleaga A, Romano A, Millan J *et al.* (2008) Characterization of widespread canine leishmaniasis among wild carnivores from Spain. *Veterinary Parasitology*, **155**, 198–203.

Solano-Gallego L, Koutinas A, Miro G, Cardoso L, Pennisi MG *et al.* (2009) Directions for the diagnosis, clinical staging, treatment and prevention of canine leishmaniosis. *Veterinary Parasitology*, **165**, 1–18.

Solano-Gallego L, Rodriguez-Cortes A, Iniesta L, Quintana J, Pastor J *et al.* (2007) Cross-sectional serosurvey of feline leishmaniasis in ecoregions around the Northwestern Mediterranean. *American Journal of Tropical Medicine and Hygiene*, **76**, 676–680.

Sterkers Y, Varlet-Marie E, Marty P, Bastien P (2010) Diversity and evolution of methods and practices for the molecular diagnosis of congenital toxoplasmosis in France: a 4-year survey. *Clinical Microbiology and Infection*, **16**, 1594–1602.

Strube C, Heuer L, Janecek E (2013) *Toxocara* spp. infections in paratenic hosts. *Veterinary Parasitology*, **193**, 375–389.

Széll Z, Erdélyi I, Sréter T, Albert M, Varga I (2001) Canine ocular onchocercosis in Hungary. *Veterinary Parasitology*, **97**, 245–251.

Tabar MD, Roura X, Francino O, Altet L, Ruiz de Gopegui R (2008) Detection of *Leishmania infantum* by real-time PCR in a canine blood bank. *Journal of Small Animal Practice*, **49**, 325–328.

Taira K, Saeed I, Permin A, Kapel CMO (2004) Zoonotic risk of *Toxocara canis* through consumption of pig or poultry viscera. *Veterinary Parasitology*, **121**, 115–124.

Tenter AM, Heckeroth AR, Weiss LM (2000) *Toxoplasma gondii*: from animals to humans. *International Journal for Parasitology*, **30**, 1217–1258.

Thompson RC (2004) The zoonotic significance and molecular epidemiology of *Giardia* and giardiasis. *Veterinary Parasitology*, **126**, 15–35.

Thompson RC, Palmer CS, O'Handley R (2008) The public health and clinical significance of *Giardia* and *Cryptosporidium* in domestic animals. *Veterinary Journal*, **177**, 18–25.

Torgerson PR, MacPherson CNL, Vuitton DA (2011) Cystic echinococcosis. In: *Zoonoses*, 2nd Edit., D Brown, S Palmer, PR Torgerson, EJL Soulsby, Eds., Oxford University Press, Oxford, pp. 650–668.

Torgerson PR, Schweiger A, Deplazes P, Pohar M, Reichen J *et al.* (2008) Alveolar echinococcosis: from a deadly disease to a well-controlled infection. Relative survival and economic analysis in Switzerland over the last 35 years. *Journal of Hepatology*, **49**, 72–77.

Tysnes KR, Skancke E, Robertson LJ (2014) Subclinical *Giardia* in dogs: a veterinary conundrum relevant to human infection. *Trends in Parasitology*, **30**, 520–527.

Umhang G, Richomme C, Hormaz V, Boucher JM, Boue F (2014) Pigs and wild boar in Corsica harbor *Echinococcus canadensis* G6/7 at levels of concern for public health and local economy. *Acta Tropica*, **133**, 64–68.

Upjohn M, Cobb C, Monger J, Geurden T, Claerebout E *et al.* (2010) Prevalence, molecular typing and risk factor analysis for *Giardia duodenalis* infections in dogs in a central London rescue shelter. *Veterinary Parasitology*, **172**, 341–346.

Vieira L, Rodrigues FT, Costa A, Diz-Lopes D, Machado J *et al.* (2012) First report of canine ocular thelaziosis by *Thelazia callipaeda* in Portugal. *Parasites and Vectors*, **21**, 124.

Vijgen S, Mangen M, Kortbeek L, van Duijnhoven Y, Havelaar A (2007) Disease burden and related costs of cryptosporidiosis and giardiasis in the Netherlands. In: *RIVM Report 330081001/2007*, RIVM, Bilthoven.

Villena I, Durand B, Aubert D, Blaga R, Geers R *et al.* (2012) New strategy for the survey of *Toxoplasma gondii* in meat for human consumption. *Veterinary Parasitology*, **183**, 203–208.

Wylie CE, Carbonell-Antoñanzas M, Aiassa E, Dhollander S, Zagmutt FJ *et al.* (2014a) A systematic review of the efficacy of prophylactic control measures for naturally-occurring canine leishmaniosis, part I: Vaccinations. *Preventive Veterinary Medicine*, **117**, 7–18.

Wylie CE, Carbonell-Antoñanzas M, Aiassa E, Dhollander S, Zagmutt FJ *et al.* (2014b) Systematic review of the efficacy of prophylactic control measures for naturally occurring canine leishmaniosis. Part II: Topically applied insecticide treatments and prophylactic medications. *Preventive Veterinary Medicine*, **117**, 19–27.

Zarfoss MK, Dubielzig RR, Eberhard ML, Schmidt KS (2005) Canine ocular onchocerciasis in the United States: two new cases and a review of the literature. *Veterinary Ophthalmology*, **8**, 51–57.

[*Received, August 2nd, 2015*
Accepted, October 18th, 2015]

J. Comp. Path. 2016, Vol. 155, S27–S40

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Bacterial Zoonoses Transmitted by Household Pets: State-of-the-Art and Future Perspectives for Targeted Research and Policy Actions

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Summary

The close contact between household pets and people offers favourable conditions for bacterial transmission. In this article, the aetiology, prevalence, transmission, impact on human health and preventative measures are summarized for selected bacterial zoonoses transmissible by household pets. Six zoonoses representing distinct transmission routes were selected arbitrarily based on the available information on incidence and severity of pet-associated disease caused by zoonotic bacteria: bite infections and cat scratch disease (physical injuries), psittacosis (inhalation), leptospirosis (contact with urine), and campylobacteriosis and salmonellosis (faecal–oral ingestion). Antimicrobial resistance was also included due to the recent emergence of multidrug-resistant bacteria of zoonotic potential in dogs and cats. There is a general lack of data on pathogen prevalence in the relevant pet population and on the incidence of human infections attributable to pets. In order to address these gaps in knowledge, and to minimize the risk of human infection, actions at several levels are recommended, including: (1) coordinated surveillance of zoonotic pathogens and antimicrobial resistance in household pets, (2) studies to estimate the burden of human disease attributable to pets and to identify risk behaviours facilitating transmission, and (3) education of those in charge of pets, animal caretakers, veterinarians and human medical healthcare practitioners on the potential zoonotic risks associated with exposure to pets. Disease-specific recommendations include incentives to undertake research aimed at the development of new diagnostic tests, veterinary-specific antimicrobial products and vaccines, as well as initiatives to promote best practices in veterinary diagnostic laboratories and prudent antimicrobial usage.

Keywords: antimicrobial resistance; bacterial zoonoses; pet animal

Introduction

The number of pet animals kept within households is increasing and the range of animal species kept for this purpose has extended from traditional household pets such as dogs and cats to encompass rodents, rabbits, ferrets, birds, amphibians, reptiles and ornamental fish. It has been estimated that the population of dogs and cats alone exceeds 127 million in the EU countries (FEDIAF, 2012).

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0021-9975/S - see front matter
http://dx.doi.org/10.1016/j.jcpa.2015.03.004

Household pets, defined here as any animals kept within households by people for company, enjoyment, work or psychological support, can be colonized or infected with a wide variety of bacteria pathogenic to animals and people. Pet-associated bacterial zoonoses represent a relatively neglected area compared with food borne zoonoses. However, the close contact between household pets and people offers favourable conditions for transmission by direct contact (e.g. petting, licking or physical injuries) or indirectly through contamination of food and domestic environments. Indeed, frequent sharing of skin microbiota between people and their dogs has been shown, thus emphasizing the role of contact (Song *et al.*, 2013). Zoonoses are of special concern for people who are young, old, pregnant or immunocompromised, and therefore particularly susceptible to infections. Furthermore, young children may be more exposed to bacteria originating from household pets due to lower hygiene standards and closer physical contact with these animals and the household environment (e.g. floors and carpets).

This paper focuses on selected bacterial zoonoses (Table 1) representing distinct transmission routes, namely bite infections and cat scratch disease (CSD) (physical injuries), psittacosis (inhalation), leptospirosis (contact with urine or urine-contaminated environments) and campylobacteriosis and salmonellosis (faecal–oral ingestion). Selection of these diseases was based on a subjective assessment, considering the available information on the incidence and severity of pet-associated disease caused by zoonotic bacteria. Antimicrobial resistance is included among the selected zoonoses in view of the increasing evidence (Wieler *et al.*, 2011) that household pets are a source of infection of multidrug-resistant (MDR) bacteria of zoonotic potential such as meticillin-resistant *Staphylococcus aureus* (MRSA), meticillin-resistant *Staphylococcus pseudintermedius* (MRSP) and extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli*. Examples of other bacterial diseases reported in pets are shown in Table 2, but are not discussed further. The aim of the paper is to identify targeted research and policy actions to assess and reduce the risk of zoonotic transmission of bacteria from pets. For this purpose, the aetiology, prevalence, transmission, impact on human health and prevention of each selected zoonosis were reviewed.

Bite Infections

Dog and cat bites are frequent injuries among pet owners and those coming into more frequent contact with animals (e.g. veterinarians and animal-related workers and postal workers). Bites are one of the main sources of bacterial infections related to pet ownership.

Aetiology, Transmission and Prevalence

Bites from household pets may result in infections caused by a wide range of bacteria residing on the oral mucosa of the animal and on the skin of the bite victim. The most common bacteria transmitted by cat and dog bites are *Pasteurella multocida* and *Pasteurella canis*, respectively (Talan *et al.*, 1999; Oehler *et al.*, 2009; Patronek and Slavinski, 2009); however, the oral cavity of dogs and cats harbours a diverse microbiota and multiple potential zoonotic pathogens can be found in every animal (Sturgeon *et al.*, 2013, 2014). Dog bites are the most common and account for approximately 80% of all reported animal bites (Patronek and Slavinski, 2009), but cat bites are more likely to develop wound infection due to the puncture lesions caused by the cat's sharper teeth. It has been estimated that 20–80% of cat bite wounds become infected, while infection rates for dog bites are as low as 3–18% (Talan *et al.*, 1999). Bites by rodents can cause rat bite fever associated with *Streptobacillus moniliformis* or, less frequently, *Spirillum minus* (Gaastra *et al.*, 2009) as well as infections caused by a range of other opportunistic pathogens.

Impact on Human Health

Dog and cat bites comprise approximately 1% of accident and emergency department visits in both the USA and Europe (Oehler *et al.*, 2009). In the Netherlands, between 50,000 and 100,000 people are bitten by a pet animal each year (Gaastra and Lipman, 2010), corresponding to 0.3–0.6% of the total population. Factors such as the type of injury, injury location, quantity and type of bacteria, foreign material, wound care and patient health/immune status determine whether bite wounds become infected and the severity of infection (Patronek and Slavinski, 2009).

Capnocytophaga canimorsus is a rare cause of bite wound infection with around 200 cases reported worldwide (Macrea *et al.*, 2008); however, this is probably an underestimate of the impact of this serious infection. This agent can lead to severe bite infections with systemic manifestations such as septicaemia and meningitis. Disease almost always occurs in immunocompromised individuals (particularly individuals without a functional spleen) and alcoholics, and has a mortality rate of about 30% (Lion *et al.*, 1996).

Table 1
Bacterial zoonoses transmissible by household pets and described in this article

Human zoonotic disease	*Pathogen(s) involved*	*Main household pet reservoir*[*]	*Reasons for concern*
Bite wound infections	*Pasteurella multocida* *Pasteurella canis* *Capnocytophaga canimorsus* etc.	Dogs, cats (rodents)	Very high incidence. Risk of therapeutic failure due to inadequate antimicrobial prophylaxis.
Cat scratch disease	*Bartonella henselae* and other *Bartonella* spp.	Cats (dogs, rabbits)	Cats are a natural reservoir with high bacteraemia prevalence in some geographical regions. Lack of information on frequency of human infections (likely underreported). Can result in severe disease in man.
Leptospirosis	*Leptospira* spp.	Dogs, (rats)	Dogs are an important reservoir in some geographical regions. Emerging new serovars in dogs are not covered by vaccines. Epidemiology may be influenced by climate changes (e.g. floods in cities). Can result in severe disease in both man and animals. Highly contagious.
Multidrug-resistant infections[†]	MRSA MRSP ESBL-producing Enterobacteriaceae MDR *Acinetobacter baumanii*	Dogs, cats	Most of the bacteria have limited host barriers. Truly emerging problem, which is expected to increase. Both human and animal health problem.
Psittacosis	*Chlamydia psittaci*	Birds	Airborne and highly contagious. Lack of information on frequency of human infections (likely underreported). Can result in severe disease in both man and animals. Large outbreaks have occurred (e.g. bird fairs).
Salmonellosis	*Salmonella* spp.	Reptiles (birds, rodents, cats, dogs, fish)	Reptiles are a natural reservoir with very high prevalence. Reptiles have a proven relatively high contribution to human infections, especially in children.

[*]Potential reservoirs of low or unknown relevance are mentioned in parentheses.
[†]Only MDR bacteria that are relatively common in household pets and of known zoonotic potential are included here.

Table 2
Examples of bacterial zoonoses for which household pets have limited or unknown importance

Human zoonotic disease	*Pathogen(s) involved*	*Main household pet reservoir*[*]	*Reasons for concern*
Brucellosis	*Brucella canis* *Brucella suis* *Brucella melitensis* *Brucella abortus*	Dogs	Trade of dogs from endemic areas (e.g. Eastern Europe) may impose a risk. Both human and animal health problem.
Chlamydiosis	*Chlamydia felis*	Cats	High prevalence in cats. Affects cat health. Possibly underdiagnosed in man.
Clostridiosis	*Clostridium difficile*	Dogs	Both human and animal health problem. Common in human healthcare and emerging community pathogen. Same strains found in people and animals, with some evidence of interspecies transfer.
Mycobacterial infections	*Mycobacterium* spp.	Fish, cats, birds, (dogs)	High incidence of infection in homeless people and subsequent potential for exposure of pets who might then expose other people (*M. tuberculosis*). Potential problem in immunocompromised people (*M. avium* complex). May cause opportunistic infections in immunocompetent people (*M. marinum*).
Mycoplasma infections	*Mycoplasma haemofelis*	Cats	High prevalence in cats. Possibly underdiagnosed in man.
Tularaemia	*Francisella tularensis*	Cats, rabbits, (dogs)	Both human and animal health problem. Highly contagious.
Q fever	*Coxiella burnetii*	Dogs, cats	Airborne and highly contagious. May cause large outbreaks.

[*]Potential reservoirs of low or unknown relevance are mentioned in parentheses.

Prevention

Education plays a primary role in prevention of pet bites; owners should be advised by veterinarians on how to interact safely with pets and about the importance of proper socialisation of puppies to reduce aggressive behaviour in adult dogs. When bites occur, basic first aid measures are indicated to prevent infection. Regardless of wound size, antimicrobial prophylaxis is indicated: (1) when bite wounds are located at critical sites (e.g. face, joints and tendon sheaths) and (2) when the bite victim is immunocompromised (Gaastra and Lipman, 2010). Understanding the potential for bite infections and the appropriate medical response is important for attending physicians, to ensure that proper treatment is provided when required.

Specific Research/Policy Recommendations

Bite infection incidence estimates are based mostly on cases registered at hospitals, even though many cases are treated elsewhere. Large-scale multicentre studies are therefore needed to estimate the actual incidence of animal bite infections and their impact on human health (e.g. frequency of treatment failure, complications and economic burden). Monitoring of antimicrobial resistance in bite wound isolates and evaluation of treatment outcomes should form the basis for the development of evidence-based antimicrobial prophylaxis guidelines on bite wound management. Research involving pet interactions and bite avoidance programs, particularly those directed at children, is needed to help reduce the risk of bites.

Cat Scratch Disease

The high prevalence of *Bartonella henselae* in some geographical areas and certain cat populations (e.g. stray and shelter cats), combined with the fact that domestic cats represent one of the largest populations of household pets worldwide, implies a potentially high risk of humans acquiring CSD. The risk of misdiagnosis and the potential development of severe infections in immunocompromised patients are the main concerns regarding this disease.

Aetiology, Transmission and Prevalence

CSD is caused by members of the genus *Bartonella*, a diverse group of blood-borne, gram-negative bacteria. Domestic cats are the natural reservoir of *B. henselae*, which is the main agent of CSD. *Bartonella* bacteraemia is most frequent in stray cats, cats in shelters/catteries and young cats infested with fleas (Boulouis *et al.*, 2005; Chomel *et al.*, 2009; Breitschwerdt *et al.*, 2010). The prevalence of bacteraemia amongst pet cats is generally lower in countries with a cold climate (e.g. 0% in Norway)

than in warm, humid countries (e.g. 83% in Thailand) (Boulouis *et al.*, 2005; Juvet *et al.*, 2010; Ayllón *et al.*, 2012). *B. henselae* is generally carried by cats without clinical signs, although some cases of feline endocarditis and stomatitis have been described (Chomel and Kasten, 2010). The cat flea is a vector for transmission between cats, mainly by intradermal inoculation of flea faeces (Chomel *et al.*, 1996; Foil *et al.*, 1998). Zoonotic transmission from cats to people is likely acquired during scratching by contaminated flea faeces coming into contact with skin abrasions, or indirectly through exposure to faeces of fleas that have fed on infected cats. Transmission is less likely to occur through bites (Chomel *et al.*, 2009).

Impact on Human Health

CSD often results in mild, non-specific symptoms such as lymphadenopathy, fever or headaches, but severe clinical manifestations such as encephalitis, angiomatosis and endocarditis may also occur, especially in immunocompromised patients (Chomel *et al.*, 2006). The incidence of CSD has been estimated in few European countries. In France and the Netherlands, 7.6 and 11.9 cases occur per 100,000 inhabitants each year, respectively (Chomel *et al.*, 2004). This is comparable to the estimated 9.3 cases per 100,000 inhabitants in North America. It has also been estimated that *Bartonella* spp. account for around 3% of all cases of human endocarditis in Europe (Raoult *et al.*, 1996). These numbers are based on the number of reported cases and are likely to underestimate the actual incidence of CSD and *Bartonella* infections in people.

Prevention

There are two main aspects to control of CSD. One is reducing exposure in the feline reservoir, which is achieved mainly through flea control. Proper flea control can eliminate the vector and is likely to have a profound effect on the likelihood of transmission from cats to people. The other aspect involves prevention of human exposure, particularly through proper wound care and prevention of scratches and bites, as described in the previous section on bite infections.

Specific Research/Policy Recommendations

There is an overall need for a better understanding of the role of *Bartonella* spp. in human disease. International multicentre studies investigating the prevalence of seropositivity and the incidence of CSD in the general human population, as well as the relative frequency of different clinical pictures, are needed to establish a baseline for further research. Awareness of the disease might be heightened for healthcare practitioners, since CSD is currently severely underreported. Optimizing diagnosis of CSD is also a prerequisite for other initiatives, such as making the disease notifiable. We recommend that the disease becomes notifiable in risk groups (i.e. young, old, pregnant or immunocompromised people) in order to learn more about the consequences of severe infections in these patients.

Psittacosis

Pet birds, particularly those of the psittacine family (e.g. cockatoos, parrots, parakeets and lories), represent an extensive reservoir for *Chlamydia psittaci*, the causative agent of psittacosis or 'parrot fever'. While the incidence of psittacosis appears to be quite low, this infection can be life-threatening and control measures are complicated by potential misdiagnosis.

Aetiology, Transmission and Prevalence

C. psittaci is a gram-negative, obligate intracellular bacterium that may survive in the environment for months in its infectious form (i.e. elementary bodies). At least 465 avian species can be infected with this zoonotic agent (Kaleta and Taday, 2003). Among pet birds, *C. psittaci* is highly prevalent in psittacine birds (16–81%) and pigeons (12.5–95%) (Vanrompay *et al.*, 2007; Ling *et al.*, in press). Birds may present with respiratory distress or general signs of disease, but more often become persistent carriers without displaying clinical signs (Dickx *et al.*, 2010). This status is characterized by the presence of non-replicating aberrant bodies inside cells. On reactivation of replication, the birds shed *C. psittaci* from the respiratory and gastrointestinal tracts (Evans, 2011). Birds are mainly infected after inhalation of *C. psittaci*-containing aerosols or dust; however, avian infection also occurs by vertical transmission, ingestion and via blood-sucking parasites. People are mostly infected after inhalation of *C. psittaci*-infected aerosols or dust, after petting infected companion birds, after handling infected avian tissues or being exposed to *C. psittaci* in excretions (e.g. from cage bedding) (West, 2011).

Impact on Human Health

Populations most at risk include bird owners, pet shop employees, taxidermists and veterinarians. The occupational risk was evident during an outbreak where people in contact with infected birds developed psittacosis and the same *C. psittaci* genotype was detected in affected birds and people (Gaede *et al.*, 2008). The course of psittacosis may vary from asymptomatic to a flu-like syndrome and involvement of the

respiratory tract. Severe complications such as endocarditis, encephalitis or fetal death are rare. Between 2001 and 2007, fewer than 400 cases were reported annually in Europe with 0–2 fatalities per year (Beeckman and Vanrompay, 2009). However, these numbers are likely underestimated due to unrecognized cases.

Prevention

Currently no effective vaccine exists for avian chlamydiosis. Guidelines for control of psittacosis are available from the US National Association of State Public Health Veterinarians (NASPHV; http://www.nasphv.org). Key measures include good husbandry practices, such as regular cleaning of cages, avoiding spread of feathers, dust and litter between cages, and quarantine of diseased or newly purchased birds. Birds may be screened for antibodies specific for *Chlamydia* spp., for example if they have frequent public contact or prior to trade. People handling sick birds or cleaning their cages should wear protective clothing.

Specific Research/Policy Recommendations

Better diagnostic tools are needed to elucidate the role and frequency of *C. psittaci* in human disease. At present, serological tests are often used for diagnosis of *C. psittaci* infection in birds. However, serology cannot provide a definitive diagnosis due to the lack of specific antibody detection assays, the high 'background' in endemic bird populations, the long persistence of antibody titres in cured birds and the need for convalescent sera to detect seroconversion. There is a need to validate newly developed nucleic acid amplification techniques, such as polymerase chain reaction (PCR), for rapid detection of infected animals and for diagnosis of infection in human patients (Vanrompay, 2013). A vaccine targeting pet birds would have a huge impact on prevention of psittacosis.

Leptospirosis

Human exposure to *Leptospira* spp. has traditionally been associated with direct or indirect contact with wildlife. However, the re-emergence of *Leptospira* spp. in pet populations in some geographical areas, and the potential severity of this infection, are reasons for concern.

Aetiology, Transmission and Prevalence

Leptospirosis is caused by members of the genus *Leptospira*, a diverse group of gram-negative bacteria that can survive for long periods of time in warm, wet environments. Virtually any domestic animal species can be infected, and different serovars may be involved depending on the animal species. Serogroup distribution in dogs varies widely. The major serogroups to which dogs in Europe are exposed are Icterohaemorrhagiae, Grippotyphosa, Australis, Sejroe and Canicola (Ellis, 2010). Leptospirosis is considered a re-emerging disease in dogs and is endemic in many countries. Prevalence rates reported in different regions are difficult to compare because of methodological differences between studies. One common trend is that the highest seroprevalence rates (up to 84%) are observed in stray and kennelled dogs (Jittapalapong *et al.*, 2009). Risk factors for seropositivity or disease in dogs include exposure to wildlife, being a working, herding or hound dog, being >5 years of age and living in peri-urban or urban areas (Ward *et al.*, 2002; Alton *et al.*, 2009; Hennebelle *et al.*, 2014). However, the changing incidence has also been accompanied by anecdotal changes in at-risk populations and risk factors in some regions, with increases in disease concentrated on urban dogs, potentially as a consequence of changes in urban wildlife numbers and infection rates. While much less common, leptospirosis can occur in cats, particularly stray cats (Millán *et al.*, 2009). Important serovars of *Leptospira* can also occur in pet rats, although the prevalence is unknown (Gaudic *et al.*, 2008). Animals are often silent carriers of leptospires, but mild to severe infection may develop, most commonly in the urinary tract. Transmission occurs through ingestion or contact of leptospires with mucous membranes or broken skin (Levett, 2001). Most infections are acquired from urine-contaminated environmental sources, particularly water.

Impact on Human Health

The reported incidence of human infection in most countries is low, such as the 0.06/100,000 people incidence rate reported in Germany (Jansen *et al.*, 2005). Such figures likely represent a large underestimation of the actual incidence due to the problems of diagnosis associated with non-specific symptoms and the lack of diagnostic tests with high sensitivity. Most human infections are mild (e.g. rash, headache and lymphadenopathy) or asymptomatic, but severe cases of hepatic or renal failure (Weil's disease) are not infrequent, especially in risk groups (i.e. young, old, pregnant and immunocompromised). Despite the fact that people are often affected by the same serovars as dogs (Dupouey *et al.*, 2014), the overall contribution of these animals to the burden of human

leptospirosis is thought to be limited. Zoonotic transmission from dogs is poorly documented and largely involves anecdotal or poorly documented reports (Allard and Bedard, 2006; Vincent *et al.*, 2007). The risk is likely greatest for owners and veterinary personnel exposed to acutely ill animals and laboratory personnel exposed to blood, urine or tissue samples from patients. Pet rat owners may be the main risk group for pet-associated leptospirosis, since wild rats are the main reservoir for *Leptospira icterhaemorrhagiae*, the most human pathogenic *Leptospira* serovar (Gaudie *et al.*, 2008; Dupouey *et al.*, 2014).

Prevention

Vaccines for dogs generally protect against the serovars *L. canicola* and *L. icterohaemorrhagiae*, and some of them additionally provide protection against region-specific serovars. Suspected animal patients should be isolated and antimicrobial treatment should be initiated promptly. For all categories at risk, especially in endemic areas, general hygiene practices associated with handling of animals or contact with dog urine are critical considering the occurrence of healthy carriers and the vague nature of the clinical signs at early infection stages. Human risk groups (see above) should be particularly aware of the risks associated with handling of pet rats.

Specific Research/Policy Recommendations

Serology is commonly used for diagnosis of leptospirosis; however, most serological tests are suboptimal in clinical practice because of the time required (i.e. weeks to obtain convalescent titres), false-positive results due to vaccination and cross-reaction between different serotypes. Serology has therefore partially been replaced by DNA-based tests for detection of *Leptospira* spp. in dog urine or blood. Apart from facilitating diagnosis, and thereby proper infection control practices and early treatment, reliable DNA-based tests will facilitate future research to determine the prevalence of subclinical carriers and to evaluate the efficacy of different antimicrobial treatment strategies. Vaccines for dogs should be continuously validated for their efficacy, and new vaccines should be developed to ensure coverage of region-specific serovars.

Campylobacteriosis

Campylobacteriosis is predominantly a food-borne disease, but there is clear evidence of zoonotic transmission from pets. Current evidence suggests that transmission from household pets accounts for a minority of human cases.

Aetiology, Transmission and Prevalence

Dogs and cats are well-recognized carriers of *Campylobacter*, a gram-negative genus associated with human gastroenteritis. Carriage rates may reach figures up to 50% in healthy dogs and cats, with relatively higher rates in puppies and kittens and in stray and kennel populations (Baker *et al.*, 1999; Wieland *et al.*, 2005). *Campylobacter upsaliensis* is the most common species, followed by *C. jejuni*. Other household pets (e.g. rodents and reptiles) are potential carriers of *Campylobacter*, but prevalence data are sparse (Skirrow, 1994; Gilbert *et al.*, 2014). Pet animal carriers often do not manifest clinical signs of disease, although cases of diarrhoea in young animals <1 year of age, have been associated with the presence of *Campylobacter* (Burnens *et al.*, 1992). Transmission occurs by the faecal–oral route, either directly or indirectly via fomites such as contaminated food and water.

Impact on Human Health

Campylobacteriosis is a leading cause of gastroenteritis in industrialized countries (Humphrey *et al.*, 2007). The most common symptom is diarrhoea, which in 0.15% of cases develops into septicaemia. *C. jejuni* is the most common *Campylobacter* species isolated from human patients. Although food, in particular poultry, is the main source of infection, various epidemiological studies have also identified contact with pets as a risk factor for campylobacteriosis (Mughini Gras *et al.*, 2013). Stafford *et al.* (2008) estimated that approximately 3% of cases of human campylobacteriosis could be attributed to ownership of puppies and Buettner *et al.* (2010) estimated that 8% of cases of human campylobacteriosis might be due to contact with cats and dogs. Case-based studies have identified indistinguishable *C. jejuni* clones in human patients and their dogs (Wolfs *et al.*, 2001; Damborg *et al.*, 2004), but such studies are rarely able to infer the direction of transmission. *C. upsaliensis* is believed to play a minor role in human disease, but the frequency of infections caused by this species might be underestimated by some diagnostic laboratories, since it requires special growth media that are not used routinely.

Prevention

Prevention of campylobacteriosis relies on avoidance of direct or indirect exposure to animal faeces. As such, the main preventive measures include proper

handling of pet faeces and litter box management, removal of faeces from public areas, and hand hygiene after contact with pets and pet-contaminated items.

Specific Research/Policy Recommendations

Large-scale risk studies may identify human behaviours increasing the risk of *Campylobacter* spp. transmission from pets and further research would be necessary to assess the incidence of human infections with *C. upsaliensis*.

Salmonellosis

Reptiles are considered a reservoir of *Salmonella* spp. and constitute a significant source of human non-typhoidal salmonellosis. Reptile-acquired salmonellosis (RAS) often presents as a severe invasive disease, especially in young children. Since the role of other companion animals in transmission of *Salmonella* spp. to people is unclear and probably of less concern, this section will primarily focus on RAS.

Aetiology, Transmission and Prevalence

Salmonella is a gram-negative bacterium, which can survive for weeks to months in the environment, in particular in warm and moist places. Among household pets, reptiles belonging to all major extant orders (i.e. crocodilians, lizards, snakes and chelonians) constitute the most important reservoir. A wide variety of primarily non-host adapted *Salmonella* serovars are isolated from these animals. This includes several exotic serovars mostly related to reptiles (e.g. *S. poona*) and serovars that are well-established in people, but more often associated with transfer from food animals (e.g. *S. typhimurium*) (Pedersen *et al.*, 2009). *Salmonella* is generally considered a normal constituent of the reptilian intestinal microbiota, since cumulative prevalence studies often show rates approaching 100% (Hoelzer *et al.*, 2011). Clinical salmonellosis is rare in reptiles and is generally provoked by an underlying primary cause of disease, but might present as salpingitis or septicaemia (Pasmans *et al.*, 2008).

Data on the occurrence of *Salmonella* in other household pets are generally sparse. Prevalences ranging from 0 to 9% and 0 to 4% have been reported in dogs and cats, respectively (Marks *et al.*, 2011). However, much higher prevalences may be identified in stray or shelter cats/dogs as well as dogs fed raw food diets (Marks *et al.*, 2011). Dogs, cats and most other non-reptile household pet species are primarily infected subclinically, but infections ranging from mild (e.g. fever of unknown origin) to potential, fatal gastroenteritis and septicaemia can occur (Marks *et al.*, 2011). *Salmonella* is transmitted directly or indirectly by the faecal–oral route as described for *Campylobacter*.

Impact on Human Health

Most people infected with *Salmonella* spp. develop symptoms of gastroenteritis. Depending on the age or the immune status of the patient and the serovar involved, salmonellosis may evolve to septicaemia, abortion and even death. Children <5 years of age are particularly at risk of RAS, probably due to the combination of their higher susceptibility to infection, greater contact with pets and limited hygiene practices (Aiken *et al.*, 2010). In the 1970s, chelonians were the source of 11–22% of all registered cases of human salmonellosis (Lamm *et al.*, 1972; Cohen *et al.*, 1980). In 1975, the sale of small turtles was prohibited in the USA, which resulted in an estimated annual reduction of 100,000 *Salmonella* infections in children (Cohen *et al.*, 1980). A more recent case–control study estimated that 6% of all sporadic *Salmonella* infections in the USA can be attributed to reptiles or amphibians (Mermin *et al.*, 2004), while a case–case study estimated reptile exposure to account for 0.95% of *Salmonella* cases in the UK (Aiken *et al.*, 2010).

Other pet animal species appear to play a less important role in human salmonellosis, with only a few published cases of confirmed transfer from cats, dogs, rodents, a parakeet, amphibians, aquarium fish and non-traditional mammalian pets (e.g. hedgehogs) (Hoelzer *et al.*, 2011). One case–control study reported cat exposure, as well as reptile contact, to be a risk factor for childhood salmonellosis (Younus *et al.*, 2010).

Prevention

The ubiquitous presence of *Salmonella* in reptiles makes it difficult to eradicate this bacterium. Instead, focus should be on minimizing human exposure. A guideline published by the Association of Reptilian and Amphibian Veterinarians (http://www.arav.org/special-topics/) recommends that risk groups (i.e. young, old, pregnant and immunocompromised people) should avoid direct and indirect contact with reptiles, while other people in contact with reptiles must focus on good hygiene measures, particularly hand hygiene. Hygiene precautions should also be taken when handling feeder mice, which can be a reservoir for *Salmonella* (Harker *et al.*, 2011). Less stringent hygiene measures can probably be used to prevent transmission from other household pets. Feeding raw diets to carnivores should be limited as

they are more likely to have *Salmonella* compared with commercial dry diets (Hoelzer *et al.*, 2011).

Specific Research/Policy Recommendations

An apparent link between stress and *Salmonella* shedding (Verbrugghe *et al.*, 2012) suggests that husbandry practices could be optimized to reduce shedding. Further research is needed to evaluate whether this approach can be used to reduce human exposure to *Salmonella*, while improving animal welfare of reptiles kept in captivity. Minimizing exposure of dogs and cats to *Salmonella* spp. would require the creation of international pet food industry standards for raw pet food and raw animal-based pet treats, including the use of processing practices (e.g. high pressure pasteurization, irradiation) to reduce or eliminate contamination.

Antimicrobial Resistance

There is increasing concern about the rapid emergence and spread of MDR bacteria among household pets in recent years. Various genetic similarities have been observed between MDR isolates from human infections and from household pets. This implicates a zoonotic risk, which is further supported by recent studies indicating contact with pets as a risk factor for human infections with resistant bacteria, and by several case reports suggesting household transmission of resistant strains between pets and their owners.

Aetiology, Prevalence and Transmission

During the last decade, various MDR bacteria such as ESBL-producing *E. coli*, MRSA and MRSP have spread among dogs and cats on a worldwide basis (Guardabassi *et al.*, 2004; Wieler *et al.*, 2011; Ewers *et al.*, 2012). Multidrug resistance has also appeared in other bacterial pathogens encountered in small animal practice, including typical human nosocomial pathogens such as carbapenemase-producing *E. coli* and MDR *Klebsiella pneumoniae* and *Acinetobacter baumannii* (Müller *et al.*, 2014; Woodford *et al.*, 2014). All of these MDR bacteria can be hospital-acquired, and resistant to virtually all conventional antimicrobials licensed for animal use. Hospitalization and antimicrobial treatment, especially with broad-spectrum drugs such as cephalosporins and fluoroquinolones, are major risk factors associated with carriage and infection with MDR bacteria in animals (Weese and van Duijkeren, 2010). The prevalence of MDR bacteria in the pet population varies considerably between countries. The reason for this geographical variation is unclear, but it is likely related to local variations in patterns of antimicrobial use. Zoonotic transmission from infected or colonized pets to people can occur by direct contact or indirectly through environmental contamination of households, veterinary clinics and public spaces. It should be noted that human-to-pet transmission may also occur. The risk that pets acquire MRSA from people is particularly high, since the MRSA types found in dogs and cats often correspond to widespread clones in the local human population (Vincze *et al.*, 2014).

Impact on Human Health

Significant public health concerns exist because of the possible risk of animal-to-human transmission of resistant clones and/or resistance genes. Exposure to companion animals has been identified by two separate studies as a risk factor for ESBL carriage in people (Meyer *et al.*, 2012; Leistner *et al.*, 2013). Other evidence supporting a role for household pets in human ESBL infections include the occurrence of specific ESBL-producing *E. coli* clones (e.g. B2-O25b:H4-ST131 and CTX-M-15-ST648) and ESBL types (e.g. CTX-M-15 and CTX-M-1) in both people and pets (Ewers *et al.*, 2012). MRSA colonization (and perhaps infection) is a recognized occupational risk in veterinary staff and various studies have identified the same MRSA strains in people and pets sharing the same household (Weese, 2010). Although the most common MRSA clones infecting or colonizing pets (e.g. ST22) occurred in people a long time before their emergence in pets, and are likely to originate from man, pets may serve as infection sources for MRSA infection or (re)colonization of human patients (Loeffler and Lloyd, 2010). Considering that *S. pseudintermedius* has a canine origin and is not a commensal in people, the relatively high MRSP carriage rates (up to 8%) among owners of infected dogs and veterinary personnel provide indirect evidence of zoonotic transmission (Ishihara *et al.*, 2010; Walther *et al.*, 2012). MRSP infections have been reported in dog owners and their frequency may be underestimated due to diagnostic problems regarding identification of *S. pseudintermedius*, and consequently MRSP, in human clinical microbiology laboratories (Pottumarthy *et al.*, 2004). The occurrence of MDR bacteria in household pets has induced veterinary use of critically important antimicrobials (CIAs) authorized for human use only (e.g. carbapenems and glycopeptides) (Weese, 2006, 2008), which may further aggravate the problem. In addition to the risks of zoonotic transmission, untreatable MDR infections in household pets have negative emotional and social effects on the owners and their families (Bengtsson and Greko, 2014).

Prevention

Considering that hospitalization and antimicrobial treatment are the main risk factors for colonization and infection with MDR bacteria, hospital infection control and rational antimicrobial use are essential measures to prevent further spread of MDR bacteria in household pets and, ultimately, to reduce the risk of zoonotic transmission to people. Veterinarians play an important role in educating the owners of patients infected with MDR bacteria to follow best hygiene practices for prevention of zoonotic transmission. Both veterinarians and physicians should raise awareness about the risks of zoonotic infection, especially among risk groups (i.e. young, old, pregnant and immunocompromised people).

Specific Research/Policy Recommendations

Veterinary use of CIAs licensed for human use only must be reduced to an absolute minimum and regulated by legislation. Use of broad-spectrum antimicrobials licensed for veterinary use (e.g. cephalosporins and fluoroquinolones) should be controlled by implementation of antimicrobial stewardship programmes at both the national and the clinic level (Guardabassi and Prescott, 2015). Development of new narrow-spectrum, veterinary-specific antimicrobial products, including anti-infective biological agents such as phage and bacteriocins, is urgently needed for treatment of MDR infections in household pets.

Concluding Remarks

This paper summarizes the present knowledge of selected bacterial zoonoses transmissible by household pets, highlighting important research and policy actions needed to assess and reduce the zoonotic risks derived from exposure to pets. It is clear that the zoonotic risks attributable to household pets are difficult to quantify due to a multitude of knowledge gaps, mainly because most knowledge of the zoonoses transmissible by household pets relies on case reports. Large-scale case–control studies, including cases and matched, healthy controls, are needed to identify human–pet interactions that pose a risk for human disease. Population attributable fractions should be calculated to understand the relative contribution by household pets to zoonoses that may also be acquired from other sources.

For most bacterial zoonoses, there is a lack of baseline data on pathogen prevalence and antimicrobial susceptibility in the relevant pet population. Adequate surveillance of pet-associated zoonoses in

Table 3
Diagnostic challenges concerning the bacterial zoonoses in this article

Pathogen	*Diagnostic challenges*
Antimicrobial-resistant bacteria	Missing or insufficient veterinary-specific clinical breakpoints (bacterial species, animal host and infection-specific breakpoints).
	No global standards for antimicrobial susceptibility testing hampers surveillance and inter-laboratory comparison of data.
	Some carbapenemase-producing bacteria can be difficult to recognize by antibiograms.
Bartonella spp.	Slow growth and special growth requirements.
	Serology is suboptimal because of cross-reaction between species.
	Healthcare personnel often unaware of disease and symptoms often non-specific.
	Reliable PCR/antigen-based methods available mainly in specialized laboratories.
Campylobacter upsaliensis	Failure to grow on conventional agar media used for *Campylobacter* isolation in diagnostic laboratories.
	Some medical microbiologists unaware of the species.
Chlamydia psittaci	Only culturable in cell cultures.
	Serological tests suboptimal clinically because of: (1) the high background in endemic bird populations, (2) the long persistence of antibodies in cured birds, (3) cross-reaction to other bacterial species, and (4) need for convalescent sera to detect seroconversion.
	Healthcare personnel often unaware of disease and symptoms often non-specific.
	Current PCR/antigen-based methods need to be validated.
Leptospira spp.	Shedding only for limited period during disease.
	Very slow growth in conventional media (up to several months).
	Serological tests are suboptimal clinically due to: (1) the long time that is often required to obtain convalescent titres, (2) cross-reaction between different serotypes, (3) poor immune response elicited by especially host-adapted serovars.
	Symptoms often non-specific.
	Reliable PCR/antigen-based test available mainly in specialized laboratories.
Staphylococcus pseudintermedius	Can be misidentified as *S. aureus* by basic phenotypic tests. By MALDI-TOF the species is difficult to distinguish from other *S. intermedius* group (SIG) species.
	Some medical microbiologists unaware of the species.

large regions like Europe would require a centrally coordinated network collecting data from individual countries. Initially, mandatory reporting for selected zoonotic agents that are already reportable in people would be optimal to identify common geographical or temporal trends in people and pets. In the absence of such a network, data can be collected online by voluntary reporting from veterinary clinics and diagnostic laboratories. The Small Animal Veterinary Surveillance Network (SAVSNET, http://www.savsnet.co.uk) and the Worms and Germs Blog (http://www.wormsandgermsblog.com) are examples of successful online initiatives developed recently to collect and share information on infectious diseases in companion animals.

Proper diagnostic tests of high sensitivity and specificity provide an essential basis for any surveillance and research activities recommended in this paper. Various pitfalls regarding the methods used for diagnosis of the pet-associated zoonoses were identified and reviewed (Table 3). Research is needed to develop new rapid and reliable diagnostic tests, as well as to improve the performance of those currently available. Certification of diagnostic laboratories and definition of minimum quality standards are required to ensure best practices in veterinary diagnostic laboratories, including in-house diagnostic facilities located within veterinary clinics.

Finally, education is another key element for reducing the zoonotic risks associated with household pets. Certain zoonotic infections transmitted by household pets, such as CSD, psittacosis and MRSP infections, may be underdiagnosed by physicians. This is partly due to insufficient diagnostic tools, but also to the lack of awareness by primary healthcare practitioners about zoonoses transmitted by companion animals and difficulties of communication between veterinary and medical practitioners. The necessary space and attention should be given to companion animal zoonoses in medical and veterinary university curricula as well as in continuing education, for example by organizing joint courses and seminars for veterinarians and doctors. Education about the zoonotic risks associated with household pets should be extended to animal caretakers and pet owners, who often do not perceive pets as possible sources of infections, indirectly increasing exposure and infection risks.

Acknowledgments

The present work was supported by CALLISTO (Companion Animals multisectoriaL interprofessionaL Interdisciplinary Strategic Think tank On zoonoses), a project funded by the European Union's 7th Framework Programme for research, technological development and demonstration (project number 289316).

Conflict of Interest Statement

The authors declare no conflicts of interest.

References

Aiken AM, Lane C, Adak GK (2010) Risk of *Salmonella* infection with exposure to reptiles in England, 2004–2007. *Eurosurveillance*, **15**, 11–18.

Allard R, Bedard L (2006) *Explanatory Notes on Statistics for Reportable Disease and Other Infectious Diseases under Surveillance, Period 3, Year 2006 (Weeks 9–12) [26 February 2006 to 25 March 2006])*. Montreal Public Health Department, Montreal.

Alton GD, Berke O, Reid-Smith R, Ojkic D, Prescott JF (2009) Increase in seroprevalence of canine leptospirosis and its risk factors, Ontario 1998–2006. *Canadian Journal of Veterinary Research*, **73**, 167–175.

Ayllón T, Diniz PP, Breitschwerdt EB, Villaescusa A, Rodríguez-Franco F *et al.* (2012) Vector-borne diseases in client-owned and stray cats from Madrid, Spain. *Vector Borne Zoonotic Diseases*, **12**, 143–150.

Baker J, Barton MD, Lanser J (1999) *Campylobacter* species in cats and dogs in South Australia. *Australian Veterinary Journal*, **77**, 662–666.

Beeckman DSA, Vanrompay D (2009) Zoonotic *Chlamydophila psittaci* infections from a clinical perspective. *Clinical Microbiology and Infection*, **15**, 11–17.

Bengtsson B, Greko C (2014) Antibiotic resistance – consequences for animal health, welfare, and food production. *Uppsala Journal of Medical Sciences*, **119**, 96–102.

Boulouis HJ, Chang CC, Henn JB, Kasten RW, Chomel BB (2005) Factors associated with the rapid emergence of zoonotic *Bartonella* infections. *Veterinary Research*, **36**, 383–410.

Breitschwerdt EB, Maggi RG, Chomel BB, Lappin MR (2010) Bartonellosis: an emerging infectious disease of zoonotic importance to animals and human beings. *Journal of Veterinary Emergency and Critical Care*, **20**, 8–30.

Buettner S, Wieland B, Staerk KD, Regula G (2010) Risk attribution of *Campylobacter* infection by age group using exposure modelling. *Epidemiology and Infection*, **138**, 1748–1761.

Burnens AP, Angèloz-Wick B, Nicolet J (1992) Comparison of *Campylobacter* carriage rates in diarrheic and healthy pet animals. *Journal of Veterinary Medicine*, **39**, 175–180.

Chomel BB, Boulouis HJ, Breitschwerdt EB (2004) Cat scratch disease and other zoonotic *Bartonella* infections. *Journal of the American Veterinary Medical Association*, **224**, 1270–1279.

Chomel BB, Boulouis HJ, Breitschwerdt EB, Kasten RW, Vayssier-Taussat M *et al.* (2009) Ecological fitness and strategies of adaptation of *Bartonella* species to their hosts and vectors. *Veterinary Research*, **40**, 29.

Chomel BB, Boulouis HJ, Maruyama S, Breitschwerdt EB (2006) *Bartonella* spp. in pets and effect on human health. *Emerging Infectious Diseases*, **12**, 389–394.

Chomel BB, Kasten RW (2010) Bartonellosis, an increasingly recognized zoonosis. *Journal of Applied Microbiology*, **109**, 743–750.

Chomel BB, Kasten RW, Floyd-Hawkins K, Chi B, Yamamoto K *et al.* (1996) Experimental transmission of *Bartonella henselae* by the cat flea. *Journal of Clinical Microbiology*, **34**, 1952–1956.

Cohen ML, Potter M, Pollard R, Feldman RA (1980) Turtle-associated salmonellosis in the United States: effect of public health action, 1970 to 1976. *Journal of the American Medical Association*, **243**, 1247–1249.

Damborg P, Olsen KE, Møller Nielsen E, Guardabassi L (2004) Occurrence of *Campylobacter jejuni* in pets living with human patients infected with *C. jejuni*. *Journal of Clinical Microbiology*, **42**, 1363–1364.

Dickx V, Beeckman DS, Dossche L, Tavernier P, Vanrompay D (2010) *Chlamydophila psittaci* in homing and feral pigeons and zoonotic transmission. *Journal of Medical Microbiology*, **59**, 1348–1353.

Dupouey J, Faucher B, Edouard S, Richet H, Kodjo A (2014) Human leptospirosis: an emerging risk in Europe? *Comparative Immunology and Microbiology of Infectious Diseases*, **37**, 77–83.

Ellis WA (2010) Control of canine leptospirosis in Europe: time for a change? *Veterinary Record*, **167**, 602–605.

Evans EE (2011) Zoonotic diseases of common pet birds: psittacine, passerine, and columbiform species. *Veterinary Clinics of North America: Exotic Animal Practice*, **14**, 457–476.

Ewers C, Bethe A, Semmler T, Guenther S, Wieler LH (2012) Extended-spectrum β-lactamase-producing and AmpC-producing *Escherichia coli* from livestock and companion animals, and their putative impact on public health: a global perspective. *Clinical Microbiology and Infection*, **18**, 646–655.

FEDIAF (The European Pet Food Industry Federation). (2012) *The European Pet Food Industry Facts and Figures*. http://www.fediaf.org/facts-figures Last accessed 17 Mar 2015.

Foil L, Andress E, Freeland RL, Roy AF, Rutledge R *et al.* (1998) Experimental infection of domestic cats with *Bartonella henselae* by inoculation of *Ctenocephalides felis* (Siphonaptera: Pulicidae) feces. *Journal of Medical Entomology*, **35**, 625–628.

Gaastra W, Boot R, Ho HT, Lipman LJ (2009) Rat bite fever. *Veterinary Microbiology*, **133**, 211–228.

Gaastra W, Lipman LJ (2010) *Capnocytophaga canimorsus*. *Veterinary Microbiology*, **140**, 339–346.

Gaede W, Reckling KF, Dresenkamp B, Kenklies S, Schubert E *et al.* (2008) *Chlamydophila psittaci* infections in humans during an outbreak of psittacosis from poultry in Germany. *Zoonoses and Public Health*, **55**, 184–188.

Gaudie CM, Featherstone CA, Phillips WS, McNaught R, Rhodes PM *et al.* (2008) Human *Leptospira interrogans* serogroup icterohaemorrhagiae infection (Weil's disease) acquired from pet rats. *Veterinary Record*, **163**, 599–601.

Gilbert MJ, Kik M, Timmerman AJ, Severs TT, Kusters JG (2014) Occurrence, diversity, and host association of intestinal *Campylobacter*, *Arcobacter*, and *Helicobacter* in reptiles. *PLoS One*, **9**, e101599.

Guardabassi L, Prescott JF (2015) Antimicrobial stewardship in small animal veterinary practice: from theory to practice. *Veterinary Clinics of North America: Small Animal Practice*, **45**, 361–376.

Guardabassi L, Schwarz S, Lloyd DH (2004) Pet animals as reservoirs of antimicrobial-resistant bacteria. *Journal of Antimicrobial Chemotherapy*, **54**, 321–332.

Harker KS, Lane C, De Pinna E, Adak GK (2011) An outbreak of *Salmonella* Typhimurium DT191a associated with reptile feeder mice. *Epidemiology and Infection*, **139**, 1254–1261.

Hennebelle JH, Sykes JE, Foley J (2014) Risk factors associated with leptospirosis in dogs from northern California: 2001-2010. *Vector Borne Zoonotic Diseases*, **14**, 733–739.

Hoelzer K, Moreno Switt AI, Wiedmann M (2011) Animal contact as a source of human non-typhoidal salmonellosis. *Veterinary Research*, **42**, 34.

Humphrey T, O'Brien S, Madsen M (2007) Campylobacters as zoonotic pathogens: a food production perspective. *International Journal of Food Microbiology*, **117**, 237–257.

Ishihara K, Shimokubo N, Sakagami A, Ueno H, Muramatsu Y *et al.* (2010) Occurrence and molecular characteristics of methicillin-resistant *Staphylococcus aureus* and methicillin-resistant *Staphylococcus pseudintermedius* in an academic veterinary hospital. *Applied and Environmental Microbiology*, **76**, 5165–5174.

Jansen A, Schöneberg I, Frank C, Alpers K, Schneider T *et al.* (2005) Leptospirosis in Germany, 1962–2003. *Emerging Infectious Diseases*, **11**, 1048–1054.

Jittapalapong S, Sittisan P, Sakpuaram T, Kabeya H, Maruyama S *et al.* (2009) Coinfection of *Leptospira* spp. and *Toxoplasma gondii* among stray dogs in Bangkok, Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health*, **40**, 247–252.

Juvet F, Lappin MR, Brennan S, Mooney CT (2010) Prevalence of selected infectious agents in cats in Ireland. *Journal of Feline Medicine and Surgery*, **12**, 476–482.

Kaleta EF, Taday EM (2003) Avian host range of *Chlamydophila* spp. based on isolation, antigen detection and serology. *Avian Pathology*, **32**, 435–461.

Lamm SH, Taylor A, Gangarosa EJ, Anderson HW, Young W *et al.* (1972) Turtle-associated salmonellosis. An estimation of the magnitude of the problem in the United States. *American Journal of Epidemiology*, **95**, 511–517.

Leistner R, Meyer E, Gastmeier P, Pfeifer Y, Eller C *et al.* (2013) Risk factors associated with the community-acquired colonization of extended-spectrum beta-lactamase (ESBL) positive *Escherichia coli*. An exploratory case-control study. *PLoS One*, **8**, e74323.

Levett PN (2001) Leptospirosis. *Clinical Microbiology Reviews*, **14**, 296–326.

Ling Y, Chen H, Chen X, Yang X, Yang J *et al.* (2014) Epidemiology of *Chlamydia psittaci* infection in racing

pigeons and pigeon fanciers in Beijing, China. *Zoonoses and Public Health*. (in press).

Lion C, Escande F, Burdin JC (1996) *Capnocytophaga canimorsus* infections in human: review of the literature and cases report. *European Journal of Epidemiology*, **12**, 521–533.

Loeffler A, Lloyd DH (2010) Companion animals: a reservoir for methicillin-resistant *Staphylococcus aureus* in the community? *Epidemiology and Infection*, **138**, 595–605.

Macrea MM, McNamee M, Martin TJ (2008) Acute onset of fever, chills and lethargy in a 36-year-old woman. *Chest*, **133**, 1505–1507.

Marks SL, Rankin SC, Byrne BA, Weese JS (2011) Enteropathogenic bacteria in dogs and cats: diagnosis, epidemiology, treatment, and control. *Journal of Veterinary Internal Medicine*, **25**, 1195–1208.

Mermin J, Hutwagner L, Vugia D, Shallow S, Daily P *et al.* (2004) Reptiles, amphibians, and human *Salmonella* infection: a population-based, case-control study. *Clinical Infectious Diseases*, **38**, 253–261.

Meyer E, Gastmeier P, Kola A, Schwab F (2012) Pet animals and foreign travel are risk factors for colonisation with extended-spectrum β-lactamase-producing *Escherichia coli*. *Infection*, **40**, 685–687.

Millán J, Candela MG, López-Bao JV, Pereira M, Jiménez MA *et al.* (2009) Leptospirosis in wild and domestic carnivores in natural areas in Andalusia, Spain. *Vector Borne Zoonotic Diseases*, **9**, 549–554.

Mughini Gras L, Smid JH, Wagenaar JA, Koene MG, Havelaar AH *et al.* (2013) Increased risk for *Campylobacter jejuni* and *C. coli* infection of pet origin in dog owners and evidence for genetic association between strains causing infection in humans and their pets. *Epidemiology and Infection*, **141**, 2526–2535.

Müller S, Janßen T, Wieler LH (2014) Multidrug resistant *Acinetobacter baumannii* in veterinary medicine – emergence of an underestimated pathogen? *Berliner Münchener Tierärztliche Wochenschrift*, **127**, 435–446.

Oehler RL, Velez AP, Mizrachi M, Lamarche J, Gompf S (2009) Bite-related and septic syndromes caused by cats and dogs. *Lancet Infectious Diseases*, **9**, 439–447.

Pasmans F, Blahak S, Martel A, Pantchev N (2008) Introducing reptiles into a captive collection: the role of the veterinarian. *Veterinary Journal*, **175**, 53–68.

Patronek GJ, Slavinski SA (2009) Animal bites. *Journal of the American Veterinary Medical Association*, **234**, 336–345.

Pedersen K, Lassen-Nielsen AM, Nordentoft S, Hammer AS (2009) Serovars of *Salmonella* from captive reptiles. *Zoonoses and Public Health*, **56**, 238–242.

Pottumarthy S, Schapiro JM, Prentice JL, Houze YB, Swanzy SR *et al.* (2004) Clinical isolates of *Staphylococcus intermedius* masquerading as methicillin-resistant *Staphylococcus aureus*. *Journal of Clinical Microbiology*, **42**, 5881–5884.

Raoult D, Fournier PE, Drancourt M, Marrie TJ, Etienne J *et al.* (1996) Diagnosis of 22 new cases of *Bartonella* endocarditis. *Annals of Internal Medicine*, **125**, 646–652.

Skirrow MB (1994) Diseases due to *Campylobacter*, *Helicobacter* and related bacteria. *Journal of Comparative Pathology*, **111**, 113–149.

Song SJ, Lauber C, Costello EK, Lozupone CA, Humphrey G *et al.* (2013) Cohabiting family members share microbiota with one another and with their dogs. *eLife*, **2**, e00458.

Stafford RJ, Schluter PJ, Wilson AJ, Kirk MD, Hall G *et al.* (2008) Population-attributable risk estimates for risk factors associated with *Campylobacter* infection, Australia. *Emerging Infectious Diseases*, **14**, 895–901.

Sturgeon A, Pinder SL, Costa MC, Weese JS (2014) Characterization of the oral microbiota of healthy cats using next-generation sequencing. *Veterinary Journal*, **201**, 223–229.

Sturgeon A, Stull JW, Costa MC, Weese JS (2013) Metagenomic analysis of the canine oral cavity as revealed by high-throughput pyrosequencing of the 16S rRNA gene. *Veterinary Microbiology*, **162**, 891–898.

Talan DA, Citron DM, Abrahamian FM, Moran GJ, Goldstein EJ (1999) Bacteriologic analysis of infected dog and cat bites. Emergency Medicine Animal Bite Infection Study Group. *New England Journal of Medicine*, **340**, 85–92.

Vanrompay D (2013) Avian chlamydiosis. In: *Diseases of Poultry*, 13th Edit., D Swayne, Ed., Wiley-Blackwell, Hoboken, pp. 1055–1074.

Vanrompay D, Harkinezhad T, van de Walle M, Beeckman D, Van Droogenbroeck C *et al.* (2007) *Chlamydophila psittaci* transmission from pet birds to humans. *Emerging Infectious Diseases*, **13**, 1108–1110.

Verbrugghe E, Boyen F, Gaastra W, Bekhuis L, Leyman B *et al.* (2012) The complex interplay between stress and bacterial infections in animals. *Veterinary Microbiology*, **155**, 115–127.

Vincent C, Munger C, Labrecque O (2007) La leptospirose: cas de transmission d'un chien a un humain. *Reseau d'Alerte et d'Information Zoosanitaire (RAIZO) Bulletin Zoosanitaire*, **51**, 1–4.

Vincze S, Stamm I, Kopp PA, Hermes J, Adlhoch C *et al.* (2014) Alarming proportions of methicillin-resistant *Staphylococcus aureus* (MRSA) in wound samples from companion animals, Germany 2010–2012. *PLoS One*, **9**, e85656.

Walther B, Hermes J, Cuny C, Wieler LH, Vincze S *et al.* (2012) Sharing more than friendship – nasal colonization with coagulase-positive staphylococci (CPS) and co-habitation aspects of dogs and their owners. *PLoS One*, **7**, e35197.

Ward MP, Glickman LT, Guptill LE (2002) Prevalence of and risk factors for leptospirosis among dogs in the United States and Canada: 677 cases (1970–1998). *Journal of the American Veterinary Medical Association*, **220**, 53–58.

Weese JS (2006) Investigation of antimicrobial use and the impact of antimicrobial use guidelines in a small animal veterinary teaching hospital: 1995-2004. *Journal of the American Veterinary Medical Association*, **228**, 553–558.

Weese JS (2008) Issues regarding the use of vancomycin in companion animals. *Journal of the American Veterinary Medical Association*, **233**, 565–567.

Weese JS (2010) Methicillin-resistant *Staphylococcus aureus* in animals. *ILAR Journal*, **51**, 233–244.

Weese JS, van Duijkeren E (2010) Methicillin-resistant *Staphylococcus aureus* and *Staphylococcus pseudintermedius* in veterinary medicine. *Veterinary Microbiology*, **140**, 418–429.

West A (2011) A brief review of *Chlamydophila psittaci* in birds and humans. *Journal of Exotic Pet Medicine*, **20**, 18–20.

Wieland B, Regula G, Danuser J, Wittwer M, Burnens AP *et al.* (2005) *Campylobacter* spp. in dogs and cats in Switzerland: risk factor analysis and molecular characterization with AFLP. *Journal of Veterinary Medicine*, **52**, 183–189.

Wieler LH, Ewers C, Guenther S, Walther B, Lübke-Becker A (2011) Methicillin-resistant staphylococci (MRS) and extended-spectrum beta-lactamases (ESBL)-producing Enterobacteriaceae in companion animals: nosocomial infections as one reason for the rising prevalence of these potential zoonotic pathogens in clinical samples. *International Journal of Medical Microbiology*, **301**, 635–641.

Wolfs TF, Duim B, Geelen SP, Rigter A, Thomson-Carter F *et al.* (2001) Neonatal sepsis by *Campylobacter jejuni*: genetically proven transmission from a household puppy. *Clinical Infectious Diseases*, **32**, e97–e99.

Woodford N, Wareham DW, Guerra B, Teale C (2014) Carbapenemase-producing Enterobacteriaceae and non-Enterobacteriaceae from animals and the environment: an emerging public health risk of our own making? *Journal of Antimicrobial Chemotherapy*, **69**, 287–291.

Younus M, Wilkins M, Davies H, Rahbar MH, Funk J *et al.* (2010) Case-control study of disease determinants for nontyphoidal *Salmonella* infections among Michigan children. *BMC Research Notes*, **3**, 105.

[*Received, December 29th, 2014*
Accepted, March 29th, 2015]

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Principles of Epidemiology in Public Health Practice, Third Edition
An Introduction to Applied Epidemiology and Biostatistics

This is an online version of a printed textbook. It is not intended to be an online course.

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On This Page

Book originally published: October 2006
Book updated: November 2011

Introduction

This course [book] was developed by the Centers for Disease Control and Prevention (CDC) as a self-study course. Continuing Education for this course is no longer available.

Objectives

Students who successfully complete this course should be able to correctly:

Describe key features and applications of descriptive and analytic epidemiology.

Calculate and interpret ratios, proportions, incidence rates, mortality rates, prevalence, and years of potential life lost.
Calculate and interpret mean, median, mode, ranges, variance, standard deviation, and confidence interval.
Prepare and apply tables, graphs, and charts such as arithmetic-scale line, scatter diagram, pie chart, and box plot.
Describe the processes, uses, and evaluation of public health surveillance.
Describe the steps of an outbreak investigation.

Course Design

This course covers basic epidemiology principles, concepts, and procedures useful in the surveillance and investigation of health-related states or events. It is designed for federal, state, and local government health professionals and private sector health professionals who are responsible for disease surveillance or investigation. A basic understanding of the practices of public health and biostatistics is recommended.

Course Materials

The course materials consist of six lessons. Each lesson presents instructional text interspersed with relevant exercises that apply and test knowledge and skills gained.

Lesson One: Introduction to Epidemiology

Key features and applications of descriptive and analytic epidemiology

Section to Review

Self-Assessment Quiz
Answers to Self-Assessment Quiz

Lesson Two: Summarizing Data

Calculation and interpretation of mean, median, mode, ranges, variance, standard deviation, and confidence interval

Lesson Overview
Section 1: Organizing Data
Section 2: Types of Variables
Section 3: Frequency Distributions
Section 4: Properties of Frequency Distributions
Section 5: Methods for Summarizing Data
Section 6: Measures of Central Location
Section 7: Measures of Spread
Section 8: Choosing the Right Measure of Central Location and Spread
Summary and References
Exercise Answers
Self-Assessment Quiz
Answers to Self-Assessment Quiz

Lesson Three: Measures of Risk

Calculation and interpretation of ratios, proportions, incidence rates, mortality rates, prevalence, and years of potential life lost

Lesson Overview
Section 1: Frequency Measures
Section 2: Morbidity Frequency Measures
Section 3: Mortality Frequency Measures
Section 4: Natality (Birth) Measures
Section 5: Measures of Association
Section 6: Measures of Public Health Impact
Summary and References
Exercise Answers
Self-Assessment Quiz
Answers to Self-Assessment Quiz

Lesson Four: Displaying Public Health Data

Preparation and application of tables, graphs, and charts such as arithmetic-scale line, histograms, pie chart, and box plot

Lesson Five: Public Health Surveillance

Processes, uses, and evaluation of public health surveillance in the United States

Lesson Six: Investigating an Outbreak

Steps of an outbreak investigation

Glossary

A Glossary that defines the major terms used in the course is also provided at the end of Lesson Six.

Supplementary Materials

In addition to the course materials, students may want to use the following:

A calculator with square root and logarithmic functions for some of the exercises.
A copy of Heymann, DL, ed. Control of Communicable Diseases Manual, 18th edition, 2004, for reference. Available from the American Public Health Association (202) 777-2742.

General Instructions

Self-study courses are "self-paced." We recommend that a lesson be completed within two weeks. To get the most out of this course, establish a regular time and method of study. Research has shown that these factors greatly influence learning ability.

Each lesson in the course consists of reading, exercises, and a self-assessment quiz.

Reading Assignments

Complete the assigned reading before attempting to answer the self-assessment questions. Read thoroughly and re-read for understanding as necessary. A casual reading may result in missing useful information which supports main themes. Assignments are designed to cover one or two major subject areas. However, as you progress, it is often necessary to combine previous learning to accomplish new skills. A review of previous lessons may be necessary. Frequent visits to the Glossary may also be useful.

Exercises

Centers for Disease Control and Prevention
CDC 24/7: Saving Lives, Protecting People™

Lesson 1: Introduction to Epidemiology

Section 10: Chain of Infection

As described above, the traditional epidemiologic triad model holds that infectious diseases result from the interaction of agent, host, and environment. More specifically, transmission occurs when the agent leaves its **reservoir** or host through a **portal of exit**, is conveyed by some **mode of transmission**, and enters through an appropriate **portal of entry** to infect a **susceptible host.** This sequence is sometimes called the chain of infection.

Figure 1.19 Chain of Infection

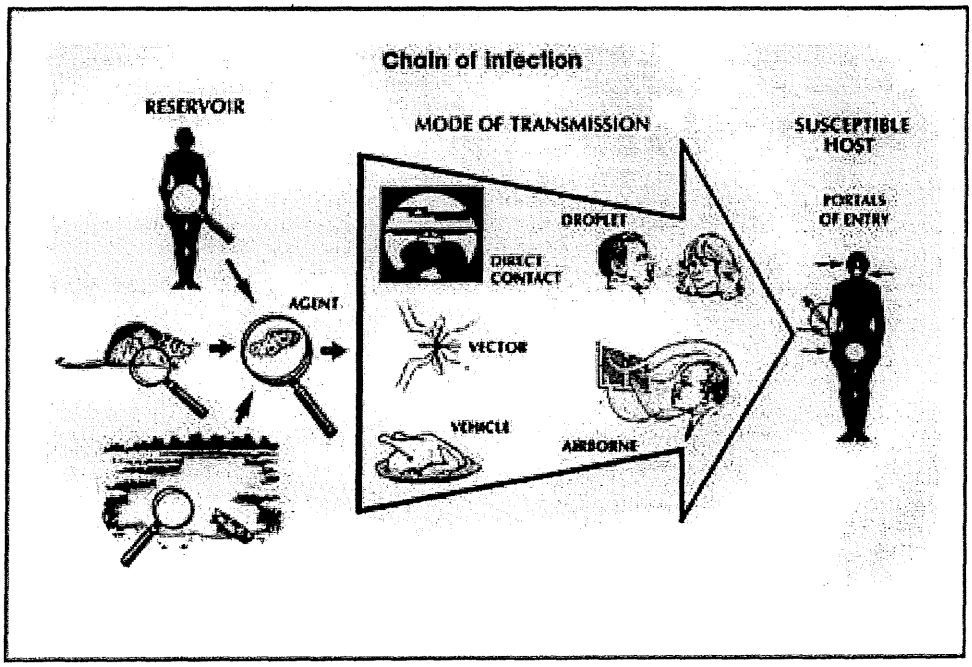

Image Description

Reservoir

The reservoir of an infectious agent is the habitat in which the agent normally lives, grows, and multiplies. Reservoirs include humans, animals, and the environment.

Source: Centers for Disease Control and Prevention. Principles of epidemiology, 2nd ed. Atlanta: U.S. Department of Health and Human Services;1992.

may or may not be the source from which an agent is transferred to a host. For example, the reservoir of *Clostridium botulinum* is soil, but the source of most botulism infections is improperly canned food containing *C. botulinum* spores.

Human reservoirs. Many common infectious diseases have human reservoirs. Diseases that are transmitted from person to person without intermediaries include the sexually transmitted diseases, measles, mumps, streptococcal infection, and many respiratory pathogens. Because humans were the only reservoir for the smallpox virus, naturally occurring smallpox was eradicated after the last human case was identified and isolated.8

Human reservoirs may or may not show the effects of illness. As noted earlier, a carrier is a person with inapparent infection who is capable of transmitting the pathogen to others. Asymptomatic or passive or healthy carriers are those who never experience symptoms despite being infected. Incubatory carriers are those who can transmit the agent during the incubation period before clinical illness begins. Convalescent carriers are those who have recovered from their illness but remain capable of transmitting to others. Chronic carriers are those who continue to harbor a pathogen such as hepatitis B virus or *Salmonella* Typhi, the causative agent of typhoid fever, for months or even years after their initial infection. One notorious carrier is Mary Mallon, or Typhoid Mary, who was an asymptomatic chronic carrier of *Salmonella* Typhi. As a cook in New York City and New Jersey in the early 1900s, she unintentionally infected dozens of people until

she was placed in isolation on an island in the East River, where she died 23 years later.(*45* (http://www.cdc.gov/OPHSS/CSELS/DSEPD/SS1978/Lesson1/Section10.htm

Carriers commonly transmit disease because they do not realize they are infected, and consequently take no special precautions to prevent transmission. Symptomatic persons who are aware of their illness, on the other hand, may be less likely to transmit infection because they are either too sick to be out and about, take precautions to reduce transmission, or receive treatment that limits the disease.

Animal reservoirs. Humans are also subject to diseases that have animal reservoirs. Many of these diseases are transmitted from animal to animal, with humans as incidental hosts. The term **zoonosis** refers to an infectious disease that is transmissible under natural conditions from vertebrate animals to humans. Long recognized zoonotic diseases include brucellosis (cows and pigs), anthrax (sheep), plague (rodents), trichinellosis/trichinosis (swine), tularemia (rabbits), and rabies (bats, raccoons, dogs, and other mammals). Zoonoses newly emergent in North America include West Nile encephalitis (birds), and monkeypox (prairie dogs). Many newly recognized infectious diseases in humans, including HIV/AIDS, Ebola infection and SARS, are thought to have emerged from animal hosts, although those hosts have not yet been identified.

Environmental reservoirs. Plants, soil, and water in the environment are also reservoirs for some infectious agents. Many fungal agents, such as those that cause histoplasmosis, live and multiply in the soil. Outbreaks of Legionnaires disease are often traced to water supplies in cooling towers and evaporative condensers, reservoirs for the causative organism *Legionella pneumophila.*

Portal of exit

Portal of exit is the path by which a pathogen leaves its host. The portal of exit usually corresponds to the site where the pathogen is localized. For example, influenza viruses and *Mycobacterium tuberculosis* exit the respiratory tract, schistosomes through urine, cholera vibrios in feces, *Sarcoptes scabiei* in scabies skin lesions, and enterovirus 70, a cause of hemorrhagic conjunctivitis, in conjunctival secretions. Some bloodborne agents can exit by crossing the placenta from mother to fetus (rubella, syphilis, toxoplasmosis), while others exit through cuts or needles in the skin (hepatitis B) or blood-sucking arthropods (malaria).

Modes of transmission

An infectious agent may be transmitted from its natural reservoir to a susceptible host in different ways. There are different classifications for modes of transmission. Here is one classification:

- Direct
  - Direct contact
  - Droplet spread
- Indirect
  - Airborne
  - Vehicleborne
  - Vectorborne (mechanical or biologic)

In direct transmission, an infectious agent is transferred from a reservoir to a susceptible host by direct contact or droplet spread.

Direct contact occurs through skin-to-skin contact, kissing, and sexual intercourse. Direct contact also refers to contact with soil or vegetation harboring infectious organisms. Thus, infectious mononucleosis ("kissing disease") and gonorrhea are spread from person to person by direct contact. Hookworm is spread by direct contact with contaminated soil.

Droplet spread refers to spray with relatively large, short-range aerosols produced by sneezing, coughing, or even talking. Droplet spread is classified as direct because transmission is by direct spray over a few feet, before the droplets fall to the ground. Pertussis and meningococcal infection are examples of diseases transmitted from an infectious patient to a susceptible host by droplet spread.

Indirect transmission refers to the transfer of an infectious agent from a reservoir to a host by suspended air particles, inanimate objects (vehicles), or animate intermediaries (vectors).

> **Airborne transmission** occurs when infectious agents are carried by dust or droplet nuclei suspended in air. Airborne dust includes material that has settled on surfaces and become resuspended by air currents as well as infectious particles blown from the soil by the wind. Droplet nuclei are dried residue of less than 5 microns in size. In contrast to droplets that fall to the ground within a few feet, droplet nuclei may remain suspended in the air for long periods of time and may be blown over great distances. Measles, for example, has occurred in children who came into a physician's office after a child with measles had left, because the measles virus remained suspended in the air.(*46* (http://www.cdc.gov/OPHSS/CSELS/DSEPD/SS1978/Lesson1/Section1

Vehicles that may indirectly transmit an infectious agent include food, water, biologic products (blood), and fomites (inanimate objects such as handkerchiefs, bedding, or surgical scalpels). A vehicle may passively carry a pathogen — as food or water may carry hepatitis A virus. Alternatively, the vehicle may provide an environment in which the agent grows, multiplies, or produces toxin — as improperly canned foods provide an environment that supports production of botulinum toxin by *Clostridium botulinum*.

Vectors such as mosquitoes, fleas, and ticks may carry an infectious agent through purely mechanical means or may support growth or changes in the agent. Examples of mechanical transmission are flies carrying *Shigella* on their appendages and fleas carrying *Yersinia pestis*, the causative agent of plague, in their gut. In contrast, in biologic transmission, the causative agent of malaria or guinea worm disease undergoes maturation in an intermediate host before it can be transmitted to humans (Figure 1.20).

Portal of entry

The portal of entry refers to the manner in which a pathogen enters a susceptible host. The portal of entry must provide access to tissues in which the pathogen can multiply or a toxin can act. Often, infectious agents use the same portal to enter a new host that they used to exit the source host. For example, influenza virus exits the respiratory tract of the source host and enters the respiratory tract of the new host. In contrast, many pathogens that cause gastroenteritis follow a so-called "fecal-oral" route because they exit the source host in feces, are carried on inadequately washed hands to a

vehicle such as food, water, or utensil, and enter a new host through the mouth. Other portals of entry include the skin (hookworm), mucous membranes (syphilis), and blood (hepatitis B, human immunodeficiency virus).

Figure 1.20 Complex Life Cycle of *Dracunculus medinensis* (Guinea worm)

Host

The final link in the chain of infection is a susceptible host.

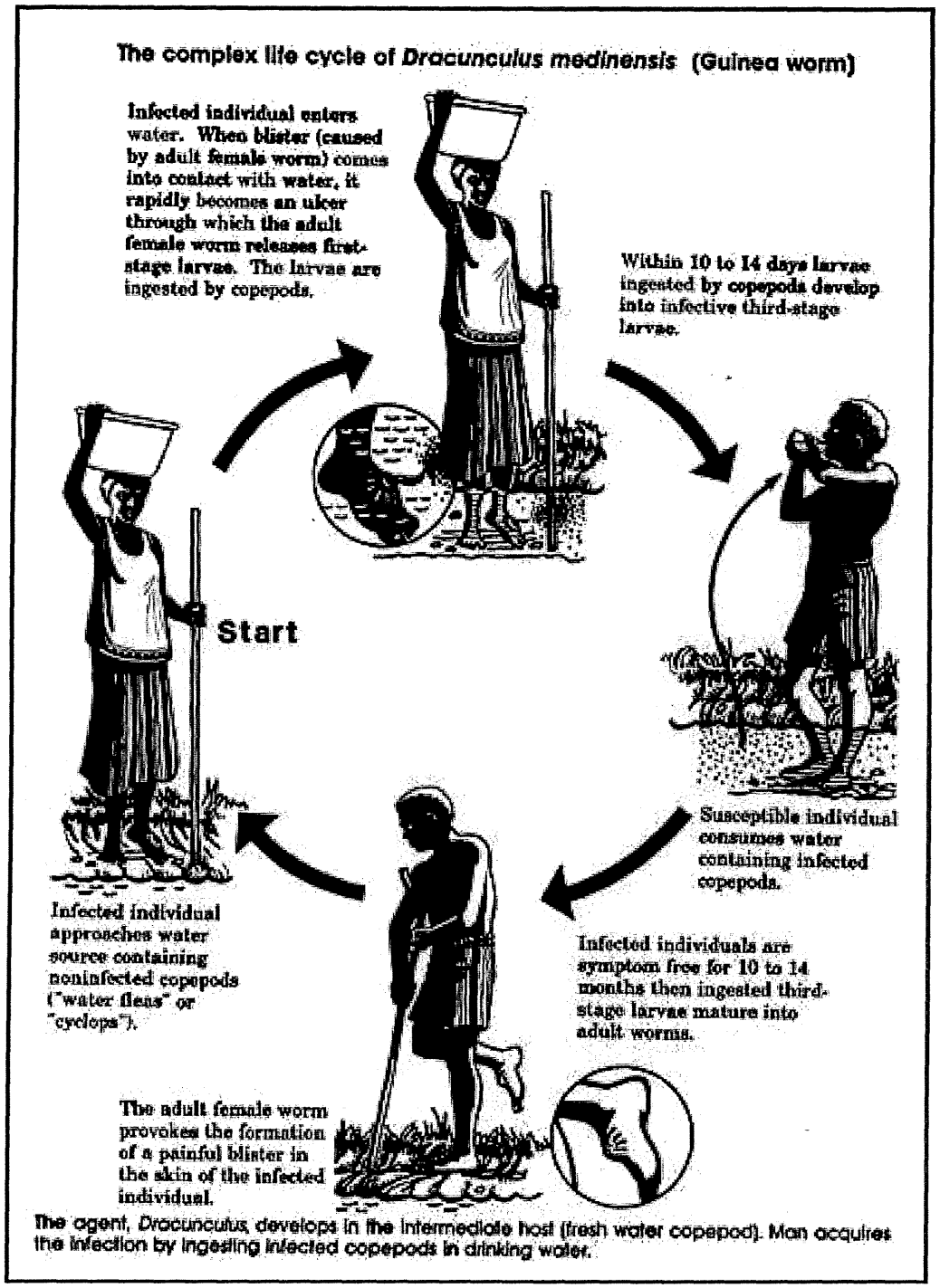

Image Description (http://www.cdc.gov/OPHSS/CSELS/DSEPD/SS1978/Lesson1/Section10.htm

Source: Centers for Disease Control and Prevention. Principles of epidemiology, 2nd ed. Atlanta: U.S. Department of Health and Human Services;1992.

Susceptibility of a host depends on genetic or constitutional factors, specific immunity, and nonspecific factors that affect an individual's ability to resist infection or to limit pathogenicity. An individual's genetic makeup may either increase or decrease susceptibility. For example, persons with sickle cell trait seem to be at least partially protected from a particular type of malaria. Specific immunity refers to protective antibodies that are directed against a specific agent. Such antibodies may develop in response to infection, vaccine, or toxoid (toxin that has been deactivated but retains its capacity to stimulate production of toxin antibodies) or may be acquired by transplacental transfer from mother to fetus or by injection of antitoxin or immune globulin. Nonspecific factors that defend against infection include the skin, mucous membranes, gastric acidity, cilia in the respiratory tract, the cough reflex, and nonspecific immune response. Factors that may increase susceptibility to infection by disrupting host defenses include malnutrition, alcoholism, and disease or therapy that impairs the nonspecific immune response.

Implications for public health

Knowledge of the portals of exit and entry and modes of transmission provides a basis for determining appropriate control measures. In general, control measures are usually directed against the segment in the infection chain that is most susceptible to intervention, unless practical issues dictate otherwise.

Interventions are directed at:

- Controlling or eliminating agent at source of transmission
- Protecting portals of entry
- Increasing host's defenses

For some diseases, the most appropriate intervention may be directed at controlling or eliminating the agent at its source. A patient sick with a communicable disease may be treated with antibiotics to eliminate the infection. An asymptomatic but infected person may be treated both to clear the infection and to reduce the risk of transmission to others. In the community, soil may be decontaminated or covered to prevent escape of the agent.

Some interventions are directed at the mode of transmission. Interruption of direct transmission may be accomplished by isolation of someone with infection, or counseling persons to avoid the specific type of contact associated with transmission. Vehicleborne transmission may be interrupted by elimination or decontamination of the vehicle. To prevent fecal-oral transmission, efforts often focus on rearranging the environment to reduce the risk of contamination in the future and on changing behaviors, such as promoting handwashing. For airborne diseases, strategies may be directed at modifying ventilation or air pressure, and filtering or treating the air. To interrupt vectorborne transmission, measures may be directed toward controlling the vector population, such as spraying to reduce the mosquito population.

Some strategies that protect portals of entry are simple and effective. For example, bed nets are used to protect sleeping persons from being bitten by mosquitoes that may transmit malaria. A dentist's mask and gloves are intended to protect the dentist from a patient's blood, secretions, and droplets, as well to protect the patient from the dentist. Wearing of long pants and sleeves and use of insect repellent are recommended to reduce the risk of Lyme disease and West Nile virus infection, which are transmitted by the bite of ticks and mosquitoes, respectively.

Some interventions aim to increase a host's defenses. Vaccinations promote development of specific antibodies that protect against infection. On the other hand, prophylactic use of antimalarial drugs, recommended for visitors to malaria-endemic areas, does not prevent exposure through mosquito bites, but does prevent infection from taking root.

Finally, some interventions attempt to prevent a pathogen from encountering a susceptible host. The concept of **herd immunity** suggests that if a high enough proportion of individuals in a population are resistant to an agent, then those few who are susceptible will be protected by the resistant majority, since the pathogen will be unlikely to "find" those few susceptible individuals. The degree of herd immunity necessary to prevent or interrupt an outbreak varies by disease. In theory, herd immunity means that not everyone in a community needs to be resistant (immune) to prevent disease spread and occurrence of an outbreak. In practice, herd immunity has not prevented outbreaks of measles and rubella in populations with immunization levels as high as 85% to 90%. One problem is that, in highly immunized populations, the relatively few susceptible persons are often clustered in subgroups defined by socioeconomic or cultural factors. If the pathogen is introduced into one of these subgroups, an outbreak may occur.

Exercise 1.9

Information about dengue fever is provided on the following pages. After studying this information, outline the chain of infection by identifying the reservoir(s), portal(s) of exit, mode(s) of transmission, portal(s) of entry, and factors in host susceptibility.

Reservoirs:

Portals of exit:

Modes of transmission:

Portals of entry:

Factors in host susceptibility:

Check your answer.

Dengue Fact Sheet

What is dengue?

Dengue is an acute infectious disease that comes in two forms: dengue and dengue hemorrhagic fever. The principal symptoms of dengue are high fever, severe headache, backache, joint pains, nausea and vomiting, eye pain, and rash. Generally, younger children have a milder illness than older children and adults.

Dengue hemorrhagic fever is a more severe form of dengue. It is characterized by a fever that lasts from 2 to 7 days, with general signs and symptoms that could occur with many other illnesses (e.g., nausea, vomiting, abdominal pain, and headache). This stage is followed by hemorrhagic manifestations, tendency to bruise easily or other types of skin hemorrhages, bleeding nose or gums, and possibly internal bleeding. The smallest blood vessels (capillaries) become excessively permeable ("leaky"), allowing the fluid component to escape from the blood vessels. This may lead to failure of the circulatory system and

shock, followed by death, if circulatory failure is not corrected. Although the average case-fatality rate is about 5%, with good medical management, mortality can be less than 1%.

What causes dengue?

Dengue and dengue hemorrhagic fever are caused by any one of four closely related flaviviruses, designated DEN-1, DEN-2, DEN-3, or DEN-4.

How is dengue diagnosed?

Diagnosis of dengue infection requires laboratory confirmation, either by isolating the virus from serum within 5 days after onset of symptoms, or by detecting convalescent-phase specific antibodies obtained at least 6 days after onset of symptoms.

What is the treatment for dengue or dengue hemorrhagic fever?

There is no specific medication for treatment of a dengue infection. Persons who think they have dengue should use analgesics (pain relievers) with acetaminophen and avoid those containing aspirin. They should also rest, drink plenty of fluids, and consult a physician. Persons with dengue hemorrhagic fever can be effectively treated by fluid replacement therapy if an early clinical diagnosis is made, but hospitalization is often required.

How common is dengue and where is it found?

Dengue is endemic in many tropical countries in Asia and Latin America, most countries in Africa, and much of the Caribbean, including Puerto Rico. Cases have occurred sporadically in Texas. Epidemics occur periodically. Globally, an estimated 50 to 100 million

cases of dengue and several hundred thousand cases of dengue hemorrhagic fever occur each year, depending on epidemic activity. Between 100 and 200 suspected cases are introduced into the United States each year by travelers.

How is dengue transmitted?

Dengue is transmitted to people by the bite of an Aedes mosquito that is infected with a dengue virus. The mosquito becomes infected with dengue virus when it bites a person who has dengue or DHF and after about a week can transmit the virus while biting a healthy person. Monkeys may serve as a reservoir in some parts of Asia and Africa. Dengue cannot be spread directly from person to person.

Who has an increased risk of being exposed to dengue?

Susceptibility to dengue is universal. Residents of or visitors to tropical urban areas and other areas where dengue is endemic are at highest risk of becoming infected. While a person who survives a bout of dengue caused by one serotype develops lifelong immunity to that serotype, there is no cross-protection against the three other serotypes.

What can be done to reduce the risk of acquiring dengue?

There is no vaccine for preventing dengue. The best preventive measure for residents living in areas infested with *Aedes aegypti* is to eliminate the places where the mosquito lays her eggs, primarily artificial containers that hold water.

Items that collect rainwater or are used to store water (for example, plastic containers, 55-gallon drums, buckets, or used automobile tires) should be covered or properly discarded. Pet and animal watering

containers and vases with fresh flowers should be emptied and scoured at least once a week. This will eliminate the mosquito eggs and larvae and reduce the number of mosquitoes present in these areas.

For travelers to areas with dengue, as well as people living in areas with dengue, the risk of being bitten by mosquitoes indoors is reduced by utilization of air conditioning or windows and doors that are screened. Proper application of mosquito repellents containing 20% to 30% DEET as the active ingredient on exposed skin and clothing decreases the risk of being bitten by mosquitoes. The risk of dengue infection for international travelers appears to be small, unless an epidemic is in progress.

Can epidemics of dengue hemorrhagic fever be prevented?

The emphasis for dengue prevention is on sustainable, community-based, integrated mosquito control, with limited reliance on insecticides (chemical larvicides and adulticides). Preventing epidemic disease requires a coordinated community effort to increase awareness about dengue/DHF, how to recognize it, and how to control the mosquito that transmits it. Residents are responsible for keeping their yards and patios free of sites where mosquitoes can be produced.

Source: Centers for Disease Control and Prevention [Internet]. Dengue Fever. [updated 2005 Aug 22]. Available from http://www.cdc.gov/ncidod/dvbid/dengue/index.htm.

References (This Section)

15. Leavitt JW. Typhoid Mary: captive to the public's health. Boston: Beacon Press; 1996.

16. Remington PL, Hall WN, Davis IH, Herald A, Gunn RA. Airborne transmission of measles in a physician's office. JAMA 1985;253:1575-7.

Previous Page Next Page: Epidemic Disease Occurrence
Lesson 1 Overview

Image Description

Figure 1.19

Description: The chain of infection has 3 main parts. A reservoir such as a human and an agent such as an amoeba. The mode of transmission can include direct contact, droplets, a vector such as a mosquito, a vehicle such as food, or the airborne route. The susceptible host has multiple portals of entry such as the mouth or a syringe. Return to text. (http://www.cdc.gov/OPHSS/CSELS/DSEPD/SS1978/Lesson1/Section10.h

Figure 1.20

Description: The agent *Dracunculus medinensis*, develops in the intermediate host (fresh water copepod). Man acquires the infection by ingesting infected copepods in drinking water.

An infected individual enters the water. When a blister (caused by adult female worm) comes into contact with water, it rapidly becomes an ulcer through which the adult female worm releases first-stage larvae. The larvae are ingested by copepods.

Within 10 to 14 days larvae ingested by the copepods develop into infective third stage larvae. The susceptible individual consumes water containing infected copepods. Infected individuals are symptom free for 10 to 14 months then ingested third-stage larvae mature into adult worms.

The adult female worm provokes the formation of a painful blister in the skin of the infected individual. The infected individual approaches water source containing noninfected copepods ("water fleas" or "Cyclops"). Then the cycle starts over. Return to text.
(http://www.cdc.gov/OPHSS/CSELS/DSEPD/SS1978/Lesson1/Section10.h

Section 11: Epidemic Disease Occurrence
Summary, References, and Websites
Exercise Answers
Self-Assessment Quiz
Answers to Self-Assessment Quiz

Page last reviewed: May 18, 2012
Page last updated: May 18, 2012
Content source: Centers for Disease Control and Prevention (/index.htm)
Office of Public Health Scientific Services (/ophss/index.html)
Center for Surveillance, Epidemiology, and Laboratory Services
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Whitehouse Station, NJ
2006

growth and prevents administration of topical drugs. Patients with UTIs without systemic signs or symptoms can often be treated with levofloxacin 500 mg po once/day or ciprofloxacin 400 mg po bid.

Systemic infection: Parenteral therapy is required, generally with an aminoglycoside plus an antipseudomonal β-lactam, antipseudomonal cephalosporin (eg, cefepime, cefoperazone), or meropenem.

Right-sided endocarditis can be treated with antibiotics, but usually the infected valve must be removed to cure an infection involving the mitral, aortic, or prosthetic valve.

In neutropenic patients with marginal renal function, nonaminoglycoside combinations, such as double β-lactams or a β-lactam plus a fluoroquinolone, are also satisfactory.

P. aeruginosa resistance may occur among patients treated with ceftazidime, ciprofloxacin, gentamicin, or imipenem.

SALMONELLA INFECTIONS

The 2200 known serotypes of *Salmonella* may be grouped into:

- Those highly adapted to human hosts, including *S. typhi* and *S. paratyphi* types A, B (*S. schottmülleri*), and C (*S. hirschfeldii*), which are pathogenic only in humans and commonly cause enteric (typhoid) fever
- Those adapted to nonhuman hosts or causing disease almost exclusively in animals, although 2 strains within this group, *S. dublin* and *S. choleraesuis*, also cause disease in humans
- Those unadapted to specific hosts. This group, designated *S. enteritidis*, includes > 2000 serotypes that cause gastroenteritis and accounts for 85% of all *Salmonella* infections in the US.

TYPHOID FEVER

Typhoid fever is a systemic disease caused by *Salmonella typhi*. Symptoms are high fever, prostration, abdominal pain, and a rose-colored rash. Diagnosis is clinical and confirmed by culture. Treatment is with ceftriaxone or ciprofloxacin.

Etiology and Pathophysiology

About 400 to 500 cases of typhoid fever are reported annually in the US. Typhoid bacilli are shed in stool of asymptomatic carriers or in the stool or urine of those with active disease. Inadequate hygiene after defecation may spread *S. typhi* to community food or water supplies. In endemic areas where sanitary measures are generally inadequate, *S. typhi* is transmitted more frequently by water than by food. In developed countries, transmission is chiefly by food that has been contaminated during preparation by healthy carriers. Flies may spread the organism from feces to food. Occasional transmission by direct contact (fecal-oral route) may occur in children during play and in adults during sexual practices. Rarely, hospital personnel who have not taken adequate enteric precautions have acquired the disease when changing soiled bedclothes.

The organism enters the body via the GI tract and gains access to the bloodstream via the lymphatic channels. Ulceration, hemorrhage, and intestinal perforation may occur in severe cases.

About 3% of untreated patients, referred to as chronic enteric carriers, harbor organisms in their gallbladder and shed them in stool for > 1 yr. Some carriers have no history of clinical illness. Most of the estimated 2000 carriers in the US are elderly women with chronic biliary disease. Obstructive uropathy related to schistosomiasis may predispose certain typhoid patients to developing a urinary carrier state. Epidemiologic data indicate that typhoid carriers are more likely than the general population to acquire hepatobiliary cancer.

Symptoms and Signs

The incubation period (usually 8 to 14 days) is inversely related to the number of organisms ingested. Onset is usually gradual, with fever, headache, arthralgia, pharyngitis, constipation, anorexia, and abdominal pain and tenderness. Less common symptoms include dysuria, nonproductive cough, and epistaxis.

Untreated, the temperature rises in steps over 2 to 3 days, remains elevated (usually 39.4 to 40° C) for another 10 to 14 days, begins to fall gradually at the end of the 3rd wk, and reaches normal levels during the 4th wk. Prolonged fever is often accompanied by relative bradycardia and prostration. CNS symptoms such as delirium, stupor, or coma occur in severe cases. In about 10% of patients, discrete pink, blanching lesions (rose spots) appear in crops on the chest and abdomen during the 2nd wk and resolve in 2 to 5 days. Splenomegaly, leukopenia, anemia, liver function abnormalities, proteinuria, and a mild

consumption coagulopathy are common. Acute cholecystitis and hepatitis may occur.

Late in the disease, when intestinal lesions are most prominent, florid diarrhea may occur, and the stool may contain blood (20% occult, 10% gross). In about 2% of patients, severe bleeding occurs during the 3rd wk, with a mortality rate of about 25%. An acute abdomen and leukocytosis during the 3rd wk may suggest intestinal perforation, usually involving the distal ileum, which occurs in 1 to 2% of patients. Pneumonia may develop during the 2nd or 3rd wk and is usually due to secondary pneumococcal infection, although *S. typhi* can also cause pulmonary infiltrates. Bacteremia occasionally leads to focal infections such as osteomyelitis, endocarditis, meningitis, soft-tissue abscesses, glomerulitis, or GU tract involvement. Atypical presentations such as pneumonitis, fever only, or symptoms consistent with UTI may delay diagnosis. Convalescence may last several months.

In 8 to 10% of untreated patients, symptoms and signs similar to the initial clinical syndrome recur about 2 wk after defervescence. For unclear reasons, antibiotic therapy during the initial illness increases the incidence of febrile relapse to 15 to 20%. If antibiotics are restarted at the time of relapse, the fever abates rapidly, unlike the slow defervescence that occurs during the primary illness. Occasionally, a 2nd relapse occurs.

Diagnosis

Other infections causing similar presentation include other *Salmonella* infections, the major rickettsioses, leptospirosis, disseminated TB, malaria, brucellosis, tularemia, infectious hepatitis, psittacosis, *Yersinia enterocolitica* infection, and lymphoma. Early in its clinical course, typhoid fever may resemble influenza, viral URI, or UTI.

Cultures of blood, stool, and urine should be obtained. Blood cultures are usually positive only during the first 2 wk of illness, but stool cultures are usually positive during the 3rd to 5th wk. If these cultures are negative and typhoid fever is strongly suspected, culture from a bone marrow biopsy specimen may reveal the organism.

Typhoid bacilli contain antigens (O and H) that stimulate the host to form corresponding antibodies. A 4-fold rise in O and H antibody titers in paired specimens obtained 2 wk apart suggests *S. typhi* infection. However, this test is only moderately (70%) sensitive and lacks specificity; many nontyphoidal *Salmonella* strains cross-react, and liver cirrhosis causes false positives.

Prognosis and Treatment

Without antibiotics, the mortality rate is about 12%. With prompt therapy, the mortality rate is < 1%. Most deaths occur in malnourished people, infants, and the elderly. Stupor, coma, or shock reflects severe disease and a poor prognosis. Complications occur mainly in patients who are untreated or in whom treatment is delayed.

Preferred antibiotics include ceftriaxone 1 g/kg IM or IV bid (25 to 37.5 mg/kg in children) for 7 to 10 days and various fluoroquinolones (eg, ciprofloxacin 500 mg po bid for 10 to 14 days, levofloxacin 500 mg po or IV once/day for 14 days, gatifloxacin 400 mg po or IV once/day for 14 days, moxifloxacin 400 mg po or IV once/day for 14 days). Chloramphenicol 500 mg po or IV q 6 h is still widely used, but resistance is increasing. Fluoroquinolones may be used in children. Alternative therapies, depending on in vitro sensitivity, include amoxicillin 25 mg/kg po qid, trimethoprim-sulfamethoxazole (TMP-SMX) 320/1600 bid or 10 mg/kg bid (of the TMP component), and azithromycin 1 g po on day one, then 500 mg once/day for 6 days.

Corticosteroids may be added to antibiotics to treat severe toxicity. Defervescence and clinical improvement usually follow. Prednisone 20 to 40 mg once/day po (or equivalent) for the first 3 days of treatment usually suffices. Higher doses of corticosteroids (dexamethasone 3 mg/kg IV initially, followed by 1 mg/kg q 6 h for 48 h total) are used in patients with marked delirium, coma, or shock.

Nutrition should be maintained with frequent feedings. Patients are generally kept on bed rest while febrile. Salicylates, which may cause hypothermia and hypotension, as well as laxatives and enemas, should be avoided. Diarrhea may be minimized with a clear liquid diet; parenteral nutrition may be needed temporarily. Fluid and electrolyte therapy and blood replacement may be needed.

Intestinal perforation and associated peritonitis call for surgical intervention and broader gram-negative and anti–*Bacteroides fragilis* coverage.

Relapses are treated the same as the initial illness, although duration of antibiotic therapy seldom needs to be > 5 days.

Patients must be reported to the local health department and prohibited from handling food until proven free of the organism.

Typhoid bacilli may be isolated for as long as 3 to 6 mo after the acute illness in people who do not become carriers. Thereafter, 3 stool cultures at weekly intervals must be negative to exclude a carrier state.

Carriers with normal biliary tracts should be given antibiotics. The cure rate is about 60% with amoxicillin 2 g po tid for 4 wk. In some carriers with gallbladder disease, eradication has been achieved with TMP-SMX and rifampin. In other cases, cholecystectomy with 1 to 2 days of preoperative and 2 to 3 days of postoperative antibiotics is effective.

Prevention

Drinking water should be purified, sewage should be disposed of effectively, milk should be pasteurized, chronic carriers should avoid handling food, and adequate patient isolation precautions should be implemented. Special attention to enteric precautions is important. Travelers in endemic areas should avoid ingesting raw leafy vegetables, other foods stored or served at room temperature, and untreated water. Unless water is known to be safe, it should be boiled or chlorinated before drinking.

A live attenuated oral typhoid vaccine is available (Ty21a strain) and is about 70% effective. It is administered every other day for a total of 4 doses. Because the vaccine contains living *S. typhi* organisms, it is contraindicated in patients who are immunosuppressed. In the US, the Ty21a vaccine is not used in children < 6 yr. An alternative is the single-dose, IM Vi polysaccharide vaccine, which is 64 to 72% effective and is well tolerated.

NONTYPHOIDAL *SALMONELLA* INFECTIONS

Nontyphoidal salmonellae, mainly *Salmonella enteritidis*, primarily produce gastroenteritis, bacteremia, and focal infection. Symptoms may be diarrhea, high fever with prostration, or those of focal infection. Diagnosis is by cultures of blood, stool, or site specimens. Treatment, when indicated, is with trimethoprim-sulfamethoxazole or ciprofloxacin, with surgery for abscesses, vascular lesions, and bone and joint infections.

Most nontyphoidal *Salmonella* infections are caused by *S. enteritidis*. These infections are common and remain a significant public health problem in the US. Many serotypes of *S. enteritidis* have been given names and are referred to informally as if they were separate species even though they are not. The most common *Salmonella* serotypes in the US include *S. typhimurium, S. heidelberg, S. newport, S. infantis, S. agona, S. montevideo*, and *S. saint-paul*.

Human disease occurs by direct and indirect contact with numerous species of infected animals, the foodstuffs derived from them, and their excreta. Infected meat, poultry, raw milk, eggs, and egg products are common sources of *Salmonella*. Other reported sources include infected pet turtles and reptiles, carmine red dye, and contaminated marijuana.

Subtotal gastrectomy, achlorhydria (or ingestion of antacids), sickle cell anemia, splenectomy, louse-borne relapsing fever, malaria, bartonellosis, cirrhosis, leukemia, lymphoma, and HIV infection are all risk factors for *Salmonella* infection.

Each *Salmonella* serotype can produce any or all of the clinical syndromes described below, although given serotypes tend to produce specific syndromes. Enteric fever, for instance, is caused by *S. paratyphi* types A, B, and C.

An asymptomatic carrier state may also occur. However, carriers do not appear to play a major role in large outbreaks of nontyphoidal gastroenteritis. Persistent shedding of organisms in the stool for ≥ 1 yr occurs in only 0.2 to 0.6% of patients with nontyphoidal *Salmonella* infections.

Symptoms and Signs

Salmonella infection may present as gastroenteritis, enteric fever, a bacteremic syndrome, or focal disease.

Gastroenteritis usually starts 12 to 48 h after ingestion of organisms, with nausea and cramping abdominal pain followed by diarrhea, fever, and sometimes vomiting. Usually the stool is watery but may be a pastelike semisolid. Rarely, mucus or blood is present. The disease is usually mild, lasting 1 to 4 days. Occasionally, a more severe, protracted illness occurs.

Enteric fever in a less severe form than typhoid is characterized by fever, prostration, and septicemia.

Bacteremia is relatively uncommon in patients with gastroenteritis. However, *S. choleraesuis, S. typhimurium*, and *S. heidelberg*, among others, can cause a sustained and fre-

quently lethal bacteremic syndrome lasting ≥ 1 wk, with prolonged fever, headache, malaise, and chills but rarely diarrhea. Patients may have recurrent episodes of bacteremia or other invasive infections (eg, septic arthritis) due to *Salmonella*. Multiple *Salmonella* infections in a patient without other risk factors should prompt HIV testing.

Focal *Salmonella* infection can occur with or without sustained bacteremia, producing pain in or referred from the involved organ—the GI tract (liver, gallbladder, and appendix), endothelial surfaces (atherosclerotic plaques, ileofemoral or aortic aneurysms, heart valves), pericardium, meninges, lungs, joints, bones, GU tract, or soft tissues. Preexisting solid tumors will occasionally be seeded and develop abscesses that may, in turn, become a source of *Salmonella* bacteremia. *S. choleraesuis* and *S. typhimurium* are the most common causes of focal infection.

Diagnosis, Treatment, and Prevention

Diagnosis is by isolating the organism from stool or another infected site. In bacteremic and focal forms, blood cultures are positive, but stool cultures are generally negative. In stool specimens stained with methylene blue, WBCs are often seen, indicating inflammatory colitis.

Gastroenteritis is treated symptomatically with oral or IV fluids (see p. 136). Antibiotics do not hasten resolution, may prolong excretion of the organism, and are unwarranted in uncomplicated cases. However, in elderly nursing home residents, infants, and patients with HIV infection, increased mortality dictates treatment with antibiotics. Antibiotic resistance is more common with nontyphoidal *Salmonella* than with *S. typhi*. Trimethoprim-sulfamethoxazole (TMP-SMX) 5 mg/kg (of the TMP component) po q 12 h for children and ciprofloxacin 500 mg po q 12 h for adults are acceptable regimens. Nonimmunocompromised patients should be treated for 3 to 5 days; patients with AIDS may require prolonged suppression to prevent relapses. Systemic or focal disease should be treated with antibiotic doses as outlined above for typhoid fever. Sustained bacteremia is generally treated for 4 to 6 wk. Abscesses should be drained surgically. At least 4 wk of antibiotic therapy should follow surgery. Infected aneurysms and heart valves and bone or joint infections usually require surgical intervention and prolonged courses of antibiotics. The prognosis is usually good, unless severe underlying disease is present.

Asymptomatic carriage is usually self-limited, and antibiotic treatment is rarely required. In unusual cases (eg, in food handlers or health care workers), eradication may be attempted with ciprofloxacin 500 mg po q 12 h for 1 mo. Follow-up stool cultures should be obtained in the weeks after drug administration to document elimination of *Salmonella*.

Preventing contamination of foodstuffs by infected animals and humans is paramount. Preventive measures for travelers discussed under Typhoid Fever on p. 1472 also apply to most other enteric infections. Case reporting is essential.

SHIGELLOSIS

(Bacillary Dysentery)

Shigellosis is an acute infection of the intestine caused by *Shigella* sp. Symptoms include fever, nausea, vomiting, and diarrhea that is usually bloody. Diagnosis is clinical and confirmed by stool culture. Treatment is supportive, mostly with rehydration; antibiotics (eg, ampicillin or trimethoprim-sulfamethoxazole) are optional.

Etiology and Pathophysiology

The genus *Shigella* is distributed worldwide and is the typical cause of inflammatory dysentery, responsible for 5 to 10% of diarrheal illness in many areas. *Shigella* is divided into 4 major subgroups: A, B, C, and D, which are subdivided into serologically determined types. *S. flexneri* and *S. sonnei* are found more widely than *S. boydii* and the particularly virulent *S. dysenteriae*. *S. sonnei* is the most common isolate in the US.

The source of infection is the feces of infected people or convalescent carriers. Direct spread is by the fecal-oral route. Indirect spread is by contaminated food and fomites. Flies serve as vectors. Epidemics occur most frequently in overcrowded populations with inadequate sanitation. Shigellosis is particularly common in younger children living in endemic areas. Adults usually have less severe disease.

Convalescents and subclinical carriers may be significant sources of infection, but true long-term carriers are rare. Infection imparts little or no immunity.

Shigella organisms penetrate the mucosa of the lower intestine, causing mucus secretion, hyperemia, leukocytic infiltration, edema, and

often superficial mucosal ulcerations. *Shigella dysenteriae* type 1 (not present in the US) produces Shiga toxin, which causes marked watery diarrhea and sometimes hemolytic-uremic syndrome.

Symptoms and Signs

The incubation period is 1 to 4 days. The most common presentation, watery diarrhea, is indistinguishable from other bacterial, viral, and protozoan infections that induce secretory activity of intestinal epithelial cells.

In adults, initial symptoms may be episodes of gripping abdominal pain, urgency to defecate, and passage of formed feces that temporarily relieves the pain. These episodes recur with increasing severity and frequency. Diarrhea becomes marked, with soft or liquid stools containing mucus, pus, and often blood. Rectal prolapse and consequent fecal incontinence may result from severe tenesmus. However, adults may present without fever, with nonbloody and nonmucoid diarrhea, and with little or no tenesmus. The disease usually resolves spontaneously in adults—mild cases in 4 to 8 days, severe cases in 3 to 6 wk. Significant dehydration and electrolyte loss with circulatory collapse and death occur mainly in debilitated adults and infants < 2 yr.

Rarely, shigellosis starts suddenly with rice-water or serous (occasionally bloody) stools. The patient may vomit and rapidly become dehydrated. Infection may present with delirium, seizures, and coma, but little or no diarrhea. Death may occur in 12 to 24 h.

In young children, onset is sudden, with fever, irritability or drowsiness, anorexia, nausea or vomiting, diarrhea, abdominal pain and distention, and tenesmus. Within 3 days, blood, pus, and mucus appear in the stools. The number of stools may increase to ≥ 20/day, and weight loss and dehydration become severe. If untreated, a child may die in the first 12 days. If the child survives, acute symptoms subside by the 2nd wk.

Secondary bacterial infections may occur, especially in debilitated and dehydrated patients. Severe mucosal ulcerations may cause significant acute blood loss. Other complications are uncommon but include toxic neuritis, arthritis, myocarditis, and, rarely, intestinal perforation. The hemolytic-uremic syndrome may complicate shigellosis in children. Infection does not become chronic and is not an etiologic factor in ulcerative colitis. However, patients with the HLA-B27 genotype more commonly develop reactive arthritis after shigellosis (and other enteritides).

Diagnosis

Diagnosis is facilitated by a high index of suspicion during outbreaks and in endemic areas and by the presence of fecal leukocytes on smears stained with methylene blue or Wright's stain. Stool cultures are diagnostic and should be obtained. In patients with symptoms of dysentery (bloody and mucoid stools), the differential diagnosis should include invasive *Escherichia coli, Salmonella, Yersinia, Campylobacter*, amebiasis, and viral diarrheas.

The mucosal surface, as seen through a proctoscope, is diffusely erythematous with numerous small ulcers. Although the WBC count is often reduced at onset, it averages 13,000/μL. Hemoconcentration is common, as is diarrhea-induced metabolic acidosis.

Treatment and Prevention

Fluid loss is treated symptomatically with oral or IV fluids (see p. 564). Antibiotics can reduce the symptoms and shedding of *Shigella* but are not necessary for mild illness in healthy adults. However, children, the elderly, debilitated patients, and those with severe disease generally should be treated. For adults, a fluoroquinolone, such as ciprofloxacin 500 mg po for 3 to 5 days, or trimethoprim-sulfamethoxazole (TMP-SMX) one double-strength tablet q 12 h is the treatment of choice. For children, the treatment is TMP-SMX at a dosage of 4 mg/kg po q 12 h of the TMP component. Many *Shigella* isolates are likely to be resistant to ampicillin and tetracycline.

Hands should be washed thoroughly before handling food, and soiled garments and bedclothes should be immersed in covered buckets of soap and water until they can be boiled. Proper isolation techniques (especially stool isolation) should be used with patients and carriers. A live oral vaccine is being developed, and field trials in endemic areas hold promise. Immunity is, however, generally type specific.

TULAREMIA

(Rabbit or Deer Fly Fever)

Tularemia is a febrile disease caused by *Francisella tularensis* that resembles typhoid fever. Symptoms are a primary local ulcerative lesion, regional lymphadenopathy, profound systemic symptoms, and occa-

develop into hermaphroditic adult worms. Worms may also develop in the brain, liver, lymph nodes, skin, and spinal cord. Adult flukes may persist for 20 to 25 yr.

Symptoms, Signs, and Diagnosis

Most damage is to the lungs, but other organs may be involved. About 25 to 45% of all extrapulmonary infections affect the CNS. Manifestations of pulmonary infection develop slowly and include chronic cough, chest pain, hemoptysis, and dyspnea. The clinical picture resembles, and is often confused with, TB. Cerebral infections present as space-occupying lesions, often within a year after the onset of pulmonary disease. Seizures, aphasia, paresis, and visual disturbances are common. Migratory allergic skin lesions similar to those of cutaneous larva migrans are common in infections with *P. skriabini* but also occur with other species.

Diagnosis is by identifying the characteristic large operculated eggs in sputum or stool. Occasionally, eggs may be found in pleural or peritoneal fluid. Eggs may be difficult to find because they are released intermittently and in small numbers. Concentration techniques increase sensitivity. X-rays provide ancillary information but are not diagnostic; chest x-rays may show a diffuse infiltrate, nodules and annular opacities, cavitations, lung abscesses, pleural effusion, and pneumothorax. Serologic tests may assist in diagnosis of light or extrapulmonary infections.

Treatment and Prevention

Praziquantel 25 mg/kg po tid for 2 days cures 80 to 100% of pulmonary infections and is the drug of choice. Bithionol 30 to 50 mg/kg po every other day for 10 to 15 doses is an alternative but has more adverse effects. Praziquantel is used to treat extrapulmonary infections, but multiple courses may be required. Surgery may be needed to excise skin lesions or, rarely, brain cysts.

The best prevention is to avoid eating raw or undercooked freshwater crabs and crayfish from endemic waters.

SCHISTOSOMIASIS

(Bilharziasis)

Schistosomiasis is infection with blood flukes of the genus *Schistosoma*, which are acquired transcutaneously by swimming or wading in contaminated waters. The organisms infect the vasculature of the GI or GU system. Acute symptoms are dermatitis, followed several weeks later by fever, chills, nausea, abdominal pain, diarrhea, malaise, and myalgia. Chronic symptoms vary with species but include bloody diarrhea and hematuria. Diagnosis is by identifying eggs in stool, urine, or biopsy specimens. Serologic tests are sensitive and specific. Treatment is with praziquantel.

Etiology and Pathophysiology

Schistosomiasis is by far the most important trematode infection. *Schistosoma* is the only trematode that invades through the skin; all other trematodes infect only via ingestion. About 200 million people are infected worldwide. The risk of infection is spreading as new dams are built in endemic areas.

There are 5 species of schistosomes, all with similar life cycles involving freshwater snails. *S. haematobium*, which causes urinary tract disease, is widely distributed over the African continent with smaller foci in the Middle East and India. The other *Schistosoma* sp cause intestinal disease. *S. mansoni* is widespread in Africa and is the only species in the Western Hemisphere, endemic in Brazil, Surinam, Venezuela, and on some Caribbean islands. *S. japonicum* is present only in Asia, mainly in China and the Philippines. *S. mekongi* is in Laos and Cambodia; *S. intercalatum* is in Central Africa. The disease may be imported in travelers and immigrants from endemic areas, but transmission does not occur within the US and Canada.

Adult worms live and copulate within the veins of the mesentery or bladder, depending on the species. Some eggs penetrate the intestinal or bladder mucosa and are passed in stool or urine; other eggs remain within the host organ or are transported through the portal system to the liver, and occasionally to other sites (eg, lungs, CNS, spinal cord). Excreted eggs hatch in freshwater, liberating miracidia that enter snails. After multiplication, thousands of free-swimming cercariae are released. These penetrate human skin within a few minutes after exposure and transform into schistosomulae, which travel through the bloodstream to the lungs, where they mature in about 6 wk. Subsequently they migrate to their ultimate home in the intestinal veins or the venous plexus of the GU tract. Eggs appear in stool or urine 1 to 3 mo after cercarial penetration. Estimates of the adult worm life span range from 3 to 37 yr.

Symptoms and Signs

Schistosome dermatitis is a pruritic papular rash where the cercariae penetrate the skin (see also Dermatitis Caused by Avian and Animal Schistosomes, below) in previously sensitized people.

Acute schistosomiasis (Katayama fever) occurs with onset of egg laying, typically 2 to 4 wk after heavy exposure. Symptoms include fever, chills, nausea, abdominal pain, malaise, myalgia, urticarial rashes, and marked eosinophilia, resembling serum sickness. Manifestations are more common and usually more severe in visitors than in residents of endemic areas and typically last for several weeks.

Chronic schistosomiasis results mostly from host responses to eggs retained in tissues. Early on, intestinal mucosal ulcerations caused by *S. mansoni* or *S. japonicum* may bleed and produce bloody diarrhea. As lesions progress, focal fibrosis, strictures, fistulas, and papillomatous growths may develop. With *S. haematobium*, ulcerations in the bladder wall may cause dysuria, hematuria, and urinary frequency. Over time, chronic cystitis develops. Strictures may lead to hydroureter and hydronephrosis. Papillomatous masses in the bladder are common, and squamous cell carcinoma may develop. Blood loss from both GI and GU tracts frequently results in anemia.

Secondary bacterial infection of the GU tract and persistent *Salmonella* septicemia associated with *S. mansoni* are also common. Several species, notably *S. haematobium*, can cause genital disease in both men and women, resulting in numerous symptoms including infertility.

Granulomatous reactions to eggs of *S. mansoni* and *S. japonicum* in the liver usually do not compromise liver function but may produce fibrosis and cirrhosis, which can lead to portal hypertension and subsequent hematemesis from esophageal varices. Eggs in the lungs may produce granulomas and focal obliterative arteritis, which may cause pulmonary hypertension and cor pulmonale. Eggs lodged in the spinal cord can cause transverse myelitis, and those in the CNS can cause seizures.

Diagnosis

Eggs are sought in the stool (*S. japonicum, S. mansoni, S. mekongi, S. intercalatum*) or urine (*S. haematobium* and occasionally *S. japonicum*). Repeated examinations using concentration techniques may be necessary. Geography is a primary determinant of species, so a history of exposure should be communicated to the laboratory. If the clinical picture suggests schistosomiasis but no eggs are found on repeated examination of urine or feces, intestinal or bladder mucosa can be biopsied for eggs.

Serologic tests are highly sensitive and specific for infection but do not provide information on worm burdens, clinical status, or prognosis.

Treatment and Prevention

Single-day oral treatment with praziquantel (20 mg/kg bid for *S. haematobium, S. mansoni*, and *S. intercalatum*; 20 mg/kg tid for *S. japonicum* and *S. mekongi*) is recommended. However, treatment does not affect developing schistosomulae and thus may not abort an early infection. Adverse effects are generally mild and include abdominal pain, diarrhea, headache, and dizziness. Therapeutic failures have been reported, but it is difficult to determine whether they are due to reinfection or drug-resistant strains. Oxamniquine (not available in the US) is effective only against *S. mansoni*. African strains are more resistant to this drug than South American strains and require larger doses (30 mg/kg po once/day for 1 or 2 days vs 15 mg/kg once). Oxamniquine-resistant cases have been observed.

Patients should be examined for living eggs 3 and 6 mo after treatment. Retreatment is indicated if egg excretion has not decreased markedly. In the future, antigen detection tests may supplant quantitative egg counts as tools to monitor response to chemotherapy.

Scrupulously avoiding contact with contaminated water prevents infection. The sanitary disposal of urine and feces reduces the likelihood of infection. Adult residents of endemic areas are more resistant to reinfection than children, suggesting the possibility of acquired immunity. Vaccine development is under way.

Dermatitis Caused by Avian and Animal Schistosomes

(Cercarial Dermatitis; Swimmers' Itch; Clam Diggers' Itch)

Cercarial dermatitis is a skin condition that develops when *Schistosoma* sp that cannot develop in humans penetrate the skin.

Cercariae of *Schistosoma* sp that infect birds and mammals other than humans can penetrate the skin. Although the organisms

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Open Defecation to End by 2025, Vows UN Chief, Marking World Toilet Day

By Thalif Deen Reprint | | Print | Send by email |En español

UNITED NATIONS, Nov 19 2015 (IPS) - The state of the world's toilets reveals the good, the bad and the ugly – but not necessarily in that order.

As the UN commemorated its annual World Toilet Day on November 19, a new study says, contrary to popular belief, not everyone in the rich nations of the developed world has access to a toilet.

The study, released by the UK based WaterAid, points out that Canada, UK, Ireland and Sweden are among nations with measurable numbers still without safe, private household toilets.

Russia has the lowest percentage of household toilets of all developed nations, while India, the world's second-most populous country, holds the record for the most people waiting for sanitation (774 million) and the most people per square kilometre (173) practising open defecation.

The report highlights the plight of more than 2.3 billion people in the world (out of a total population of over 7.3 billion) who do not have access to a safe, private toilet.

Of these, nearly 1.0 billion have no choice but to defecate in the open – in fields, at roadsides or in bushes.

The result is a polluted environment in which diseases spread fast. An estimated 314,000 children under five die each year of diarrhoeal illness which could be prevented with safe water, good sanitation and good hygiene.

Still, the tiny South Pacific island of Tokelau has made the most progress on delivering sanitation, holding number one position since 1990, followed by Vietnam, Nepal and Pakistan.

Nigeria has seen a dramatic slide in the number of people with access to toilets since 1990 despite considerable economic development.

The world's youngest country, South Sudan, has the worst household access to sanitation in the world, followed closely by Niger, Togo and Madagascar, according to the study.

WaterAid's Chief Executive Barbara Frost says just two months ago, all UN member states promised to deliver access to safe, private toilets to everyone everywhere by 2030.

"Our analysis shows just how many nations in the world are failing to give sanitation the political prioritisation and financing required. We also know that swift progress is possible, from the impressive advances in sanitation achieved in nations like Nepal and Vietnam."

No matter where you are in the world, everyone has a right to a safe, private place to relieve themselves, and to live healthy and productive lives without the threat of illness from poor sanitation and hygiene.

"On this World Toilet Day, it's time for the world to make good on their promises and understand that while we all love toilet humour, the state of the world's sanitation is no joke," said Frost.

The UN children's agency UNICEF says lack of sanitation, and particularly open defecation, contributes to the incidence of diarrhoea and to the spread of intestinal parasites, which in turn cause malnutrition.

"We need to bring concrete and innovative solutions to the problem of where people go to the toilet, otherwise we are failing millions of our poorest and most vulnerable children," said Sanjay Wijesekera, head of UNICEF's global water, sanitation and hygiene programmes.

"The proven link with malnutrition is one more thread that reinforces how interconnected our responses to sanitation have to be if we are to succeed."

In a report released Wednesday, the 21-member UN Advisory Board on Water and Sanitation (UNSGAB), calls for the mainstreaming of sanitation.

The focus should widen beyond the home – because toilets are needed in schools, clinics, workplaces, markets and other public places.

"Prioritize sanitation as preventive medicine and break the vicious cycle of disease and malnutrition, especially affecting women and children."

And "get serious about scaling up innovative technologies along the sanitation chain and unleash another sanitation revolution, as key economic and medical enabler in the run-up to 2030, and make a business case for sanitation by realizing the resource potential of human waste."

Additionally, it says, "de-taboo the topic of menstrual hygiene management, which deserves to be addressed as a priority by the UN and governments."

In its report, WaterAid is calling on world leaders to fund, implement and account for progress towards the new UN Global Goals on sustainable development.

Goal 6 – water, sanitation and hygiene for all – is fundamental to ending hunger and ensuring healthy lives, education and gender equality and must be a priority.

"Improving the state of the world's toilets with political prioritisation and long-term increases in financing for water, sanitation and hygiene, by both national governments and donor countries like the UK."

Secretary-General Ban Ki-moon said the recently adopted 2030 Agenda for Sustainable Development recognizes the central role sanitation plays in sustainable development.

"The integrated nature of the new agenda means that we need to better understand the connections between the building blocks of development."

In that spirit, he said, this year's observance of World Toilet Day focuses on the vicious cycle connecting poor sanitation and malnutrition. He said poor sanitation and hygiene are at the heart of disease and malnutrition.

Each year, too many children under the age of five have their lives cut short or altered forever as a result of poor sanitation: more than 800,000 children worldwide — or one every two minutes– die from diarrhea, and almost half of all deaths of children under five are due to undernutrition.

A quarter of all children under five are stunted, and countless other children, as well as adults, are falling seriously ill, often suffering long-term, even lifelong, health and developmental consequences.

Parents and guardians carry the cost of these consequences. Women in particular women bear the direct brunt, he noted.

"Despite the compelling moral and economic case for action on sanitation, progress is too little and too slow," Ban complained.

By many accounts, sanitation is the most-missed target of the Millennium Development Goals.

"This is why the Call to Action on Sanitation was launched in 2013, and why we aim to end open defecation by 2025," he added.

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Journal of Helminthology (2015) 89, 740–747
doi:10.1017/S0022149X14000716

Stray animal and human defecation as sources of soil-transmitted helminth eggs in playgrounds of Peninsular Malaysia

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(Received 15 July 2014; Accepted 30 August 2014; First Published Online 2 October 2014)

Abstract

Soil contaminated with helminth eggs and protozoan cysts is a potential source of infection and poses a threat to the public, especially to young children frequenting playgrounds. The present study determines the levels of infection of helminth eggs in soil samples from urban and suburban playgrounds in five states in Peninsular Malaysia and identifies one source of contamination via faecal screening from stray animals. Three hundred soil samples from 60 playgrounds in five states in Peninsular Malaysia were screened using the centrifugal flotation technique to identify and determine egg/cyst counts per gram (EPG) for each parasite. All playgrounds, especially those in Penang, were found to be contaminated with eggs from four nematode genera, with *Toxocara* eggs (95.7%) the highest, followed by *Ascaris* (93.3%), *Ancylostoma* (88.3%) and *Trichuris* (77.0%). In addition, faeces from animal shelters were found to contain both helminth eggs and protozoan cysts, with overall infection rates being 54% and 57% for feline and canine samples, respectively. The most frequently occurring parasite in feline samples was *Toxocara cati* (37%; EPG, 42.47 ± 156.08), while in dog faeces it was *Ancylostoma* sp. (54%; EPG, 197.16 ± 383.28). Infection levels also tended to be influenced by season, type of park/playground and the texture of soil/faeces. The occurrence of *Toxocara*, *Ancylostoma* and *Trichuris* eggs in soil samples highlights the risk of transmission to the human population, especially children, while the presence of *Ascaris* eggs suggests a human source of contamination and raises the issue of hygiene standards and public health risks at sites under investigation.

Introduction

Soil-transmitted helminths (STH) are listed as one of the world's neglected parasites in tropical regions (Molyneux *et al.*, 2005). Soil contaminated with helminth eggs is a potential source of infection that poses a threat to the public, especially young children, to *Toxocara*, *Ascaris*, *Ancylostoma* and *Trichuris* eggs. Children are at risk when playing in sandpits in playgrounds and parks contaminated with infective eggs or larvae of parasites (Glickman & Schantz, 1981; Duwel, 1984) and mainly acquire infection after ingestion of eggs embedded under unwashed fingernails.

Many worldwide reports have highlighted the importance of STH to children, especially in developing countries, where reduced physical activity, impaired learning ability and poor growth have been reported (Stephenson *et al.*, 1990; Nokes *et al.*, 1992; Adams *et al.*, 1994; Koroma *et al.*, 1996). However, most studies have focused on soil contaminated with *Toxocara* eggs, especially in industrialized countries, with prevalences

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ranging from 1.2% in Brazil (Chieffi & Muller, 1976), 2.7% in Argentina (Sommerfelt *et al.*, 1992), 15.5% in Iraq (Mahdi & Ali, 1993), 20.6% in Kansas (Dada & Lindquist, 1979), 24% in urban areas in Italy (Habluetzel *et al.*, 2003) and up to 67.7% in Kobe, Japan (Zibaei & Uga, 2008) and 97.5% in Greece (Himonas *et al.*, 1992). Only a small number of studies has highlighted soil contamination with other helminths, including eggs of *Ascaris*, *Ancylostoma* and *Trichuris* (Ajala & Asaolu, 1995; Blaszkowska *et al.*, 2011).

To date, there are only a few studies on helminth contamination of public playgrounds in Malaysia, although Loh & Israf (1998) reported high prevalences of over 50% of *Toxocara* in soil from public playgrounds in Serdang and Petaling Jaya. In addition, Noor Azian *et al.* (2008) reported on contamination of 182 soil samples examined from urban (Setapak, Kuala Lumpur) and rural residential parks (Kuala Lipis, Pahang); 12.1% were contaminated with *Toxocara* eggs, followed by *Ascaris* (7.4%), hookworm (4.9%) and *Trichuris* (1.6%).

Helminth and protozoan infections are also common in Malaysian schoolchildren. Rajeswari *et al.* (1994) reported that faecal samples from 456 schoolchildren in Gombak, Malaysia showed an overall prevalence of 62.9%, mainly comprising *Trichuris trichiura* (47.1%), *Giardia intestinalis* (14.7%), *Entamoeba coli* (11.4%), *Entamoeba histolytica* (9.9%) and *Ascaris lumbricoides* (7.9%). Bundy *et al.* (1988) found that 66% of 1574 children living in a slum area of Kuala Lumpur, Malaysia, were infected with *T. trichiura*, 49.6% with *A. lumbricoides* and 5.3% with hookworm. Moreover, Rahman (1998) showed that intestinal infections in schoolchildren from an urban area in Penang were dominated by *Trichuris* (100%), *Ascaris* (37.9%) and hookworm (18.7%).

Therefore the present study was undertaken to provide a comprehensive update on soil contaminated primarily with helminths in public playgrounds in Peninsular Malaysia. The determination of sources of contamination and confounding factors such as season, together with differences in soil types in both urban and suburban areas, are also considered.

Materials and methods

Study sites

Soil samples were collected from playgrounds in urban and suburban areas from five states in Peninsular Malaysia (table 1). These included Kuala Lumpur, the capital city of Malaysia, together with Petaling Jaya, Klang and Shah Alam districts in Selangor representing the west, the town of Kuantan in the state of Pahang representing the east, Georgetown (Penang) representing the north, and Malacca city representing the south state of Peninsular Malaysia (table 1). Kuala Lumpur is the largest city, with a population of 1.6 million, and is an enclave within the state of Selangor, in the central west coast Peninsular Malaysia. Petaling Jaya, Klang and Shah Alam districts are located in the state of Selangor, neighbouring Kuala Lumpur and comprising mostly residential and some industrial areas. The coastal city of Malacca, located approximately 130 km south of Kuala Lumpur, has a population of 788,706. Georgetown is the capital of Penang Island and located on the north-west coast of Peninsular Malaysia on the Straits of Malacca. Finally Kuantan, which is another coastal city in the state capital of Pahang, is situated along the east coast of Peninsular Malaysia, near the mouth of the Kuantan River and faces the South China Sea.

Malaysia features a tropical rainforest climate which is hot and humid throughout the year, along with abundant rainfall. Generally, the wet season occurs from April to May and October to December, with the dry season occurring between January to March and June to September. Temperatures remain constant, with maximum temperatures ranging between 31 and 37.2°C

Table 1. The location and number of sampling sites/samples from five geographical regions in Peninsular Malaysia from August 2009 to July 2010; mean temperature range from 25.5°C to 28.6°C.

Region	Cities/District	No. of playgrounds	No. of samples	Coordinates
West	Federal territory of Kuala Lumpur			
	Kuala Lumpur City	12	60	3°8′51″N 101°41′36″E
	Keramat	3	15	3°10′32″N 101°44′21″E
	Selangor			
	Petaling Jaya	5	25	3°05′N 101°39′E
	Shah Alam	5	25	3°5′00″N 101°32′00″E
	Klang	5	25	3°02′N 101°27′E
East	Pahang			
	Kuantan City	5	25	3°49′00″N 103°20′00″E
	Indera Mahkota	4	20	3°49′19″N 103°18′17″E
	Teluk Chempedak	1	5	3°48′48″N 103°21′5″E
North	Penang Island			
	Georgetown City	5	25	5°24′37″N 100°18′57″E
	USM Campus	1	5	5°21′26″N 100°17′58″E
	Jelutong	2	10	5°23′15″N 100°18′57″E
	Butterworth	2	10	5°25′10″N 100°19′46″E
South	Malacca			
	Malacca City	4	20	2°11′20″N 102°23′4″E
	Batu Berendam	4	20	2°15′27″N 102°15′13″E
	Ayer Keroh	2	10	2°16′2″N 102°17′54″E

(88–99.0°F) and minimum temperatures between 17.7°C (63.9°F) and 23.5°C (72–74°F), as detailed in Meteorology Malaysia (2010). The survey was conducted during both wet and dry seasons, with the mean temperature ranging between 25.5 and 28.6°C, and rainfall between >0.1 mm and 32.4 mm.

Soil and faecal sampling

Between August 2009 and July 2010, a total of 300 soil samples was collected and examined from sandpits of 60 playgrounds, including public parks and residential playgrounds, from five localities in Peninsular Malaysia (table 1). All playgrounds surveyed were unfenced or semi-fenced, allowing access by animals and the public at all times. Using a small shovel, five soil samples, each weighing approximately 300 g, were taken at a depth of 0–5 cm from an area of 1.0 m^2 selected randomly per 5 m^2 of each sandpit and surrounding play areas. Samples were taken from the sites without grass to avoid intensified drainage on grassy soil.

Playgrounds were classified according to location, either in open public parks or residential areas, and the condition of the soil texture was noted as being either silty (0.02–0.10 mm) or sandy (0.11–2.0 mm). All samples were kept in air-tight plastic containers at room temperature, labelled with the name of playground, number of sample and date of collection, and immediately transported to the laboratory for analysis. Results from this study were presented as infection rates rather than prevalence as the experimental design was not ideal for obtaining a reliable estimate of the prevalence of STH in soil samples in Malaysia. In addition, from January 2011 to February 2012, faecal samples from 100 stray cats and 100 stray dogs were screened from public and residential areas within the vicinity of Kuala Lumpur and from animal shelters, in association with the Society for the Prevention of Cruelty to Animals (SPCA) and the Animal Pound, Vector Control Unit of Kuala Lumpur City Hall (DBKL). Up to 10–20 g of faecal samples were collected using a wooden spatula and classified according to their texture (hard, smooth or runny). The date of collection was recorded and samples were placed in a stool container and kept at 4°C until examination.

Detection of eggs/cysts in soil and faeces

Samples of 1 g in weight were air-dried at room temperature and thoroughly ground in a pestle and mortar with a small amount of distilled water. The suspension was washed and sieved through a 2-mm mesh sieve to remove debris, and placed in a 15-ml centrifuge tube for centrifugation at 1500 *g* for 2 min. The supernatant was discarded, and the sediment re-suspended in 15 ml of saturated sodium chloride solution (SG1.25) using a Pasteur pipette and thoroughly mixed until all particles were evenly distributed. These procedures were replicated three times for each sample.

Helminth eggs and protozoan cysts were recovered using the modified McMaster flotation technique (Dunn & Keymer, 1986), which uses a counting chamber in a known volume of sample suspension (0.15 ml). For every sample, the number of eggs present within the grid chamber was counted and their genus determined microscopically. The mean number of eggs/cysts per gram (EPG) was calculated when both the weights of soil or faeces and the volume of flotation fluid used were known. Samples of the suspension were drawn off with a Pasteur pipette and added to the chambers of the McMaster slide (0.15 ml). Eggs/cysts present within the grid were counted and identified using the McMaster slide under low magnification (×10) and five replicates were counted.

Data analysis

The overall infection rate and EPG for each parasite species were calculated and analysed using the software Quantitative Parasitology 3.0 (Reiczigel & Rózsa, 2001) with 95% confidence intervals (Margolis *et al.*, 1982). The infection rates were compared using Fisher's Exact Test proposed by Rozsa *et al.* (2000) and the frequency distribution of eggs/cysts in soil samples was tested using a reformulated method of measuring the *k* parameter (Pal & Lewis, 2004). Helminth egg burdens were analysed using the SPSS version 16.0 (SPSS Inc., Chicago, Illinois, USA), together with GLIM (generalized linear models) and a Poisson regression model (Wilson & Grenfell, 1997) using two-way interactions between selected independent variables. These were transformed into two levels to confirm any significant differences, given as $P < 0.01$ unless otherwise stated, between variables such as wet and dry seasons, soil textures comprising sand and silt, and public and residential playgrounds.

Results

Soil screening

All 60 playground samples from the five states in Peninsular Malaysia were mainly contaminated with helminth eggs, comprising four nematode genera. Soil contamination with *Toxocara* was the highest (95.7%), followed by *Ascaris* (93.3%), *Ancylostoma* (88.3%) and *Trichuris* (77%). *Toxocara* also showed the highest EPG with a mean of 251.51 ± 220.51, followed by *Ascaris* (116.64 ± 149.02), *Ancylostoma* (105.25 ± 101.82) and, finally, *Trichuris* (56.45 ± 50.18) (table 2).

Relative to sites, playgrounds in Penang (northern coast) and Selangor (western coast) were the most contaminated, with all four nematode genera exhibiting 100% infection rates. Similar infection rates were shown for *Toxocara* and *Ancylostoma* in Malacca, with slightly lower values of 98% being recorded for *Ascaris* and *Trichuris* (table 2). Playground soils in Kuantan were slightly less contaminated with *Toxocara* (90%), *Ascaris* (76%), *Ancylostoma* (76%) and *Trichuris* (52%) and, similarly, in Kuala Lumpur, with *Toxocara* (89.3%), *Ascaris* (90.7%), *Ancylostoma* (70.7%) and *Trichuris* (40%). All ten playgrounds in Penang also exibited high EPG for all nematodes, especially the playground in Popus Lane Park (5°25′10″N 100°19′46″E) with egg counts for *Toxocara* as high as 856.4 ± 1.054, and Kota Lama Esplanade Park with counts of 388.40 ± 1.21 for *Ancylostoma* and 189.20 ± 0.24 for *Trichuris*.

Table 2. The infection rate (%) and mean number of eggs per gram (EPG ± SD) of four nematode genera in soil samples from playgrounds in five sites from Peninsular Malaysia, August 2009 to July 2010.

Site	No. of playgrounds	No. of samples	*Toxocara*		*Ascaris*		*Ancylostoma*		*Trichuris*	
			(%)	EPG ± SD	(%)	EPG ± SD	(%)	EPG ± SD	(%)	EPG ± SD
Penang Island	10	50	100.0	546.12 ± 245.57	100.0	405.62 ± 156.98	100.0	261.72 ± 98.54	100.0	116.04 ± 55.04
Kuala Lumpur	15	75	89.3	143.41 ± 157.98	90.7	48.59 ± 46.65	70.7	66.11 ± 95.74	40.0	28.51 ± 49.09
Selangor	15	75	100.0	309.88 ± 164.92	100.0	64.76 ± 27.72	100.0	102.25 ± 57.38	100.0	62.48 ± 20.76
Malacca	10	50	100.0	218.96 ± 85.47	98.0	90.72 ± 38.59	100.0	84.38 ± 30.22	98.0	72.18 ± 26.14
Kuantan	10	50	90.0	64.04 ± 41.51	76.0	33.46 ± 27.53	76.0	32.84 ± 26.19	52.0	13.98 ± 18.25

All four nematode genera comprising *Toxocara* sp., *Ascaris* sp., *Trichuris* sp. and *Ancylostoma* sp. showed infection rates above 70%. The Poisson regression model, using two-way interactions, showed significant effects for soil contamination in all nematode genera relative to season, soil texture and, especially, the type of park contaminated (table 3). With reference to seasonality, soil contamination was highest during the wet compared with the dry season for all four nematodes ($P < 0.001$). However, across the four genera, the two-way interaction was highly significant for soil texture with type of park only (table 3).

Faecal screening

More than half of the stray cat and dog populations were infected with parasites. Of the feline faecal samples screened, 54% were positive for two nematodes, one cestode and one protozoan species. The highest contamination in feline samples comprised eggs of *Toxocara* (37%; EPG, 42.47 ± 156.08) followed by the protozoan *Isospora* (35%; EPG, 65.83 ± 191.75), *Ancylostoma* (29%; EPG, 38.64 ± 273.70) and the cestode *Spirometra* (22%; EPG, 21.09 ± 81.63) (table 4). Up to 57% of canine samples were infected with four nematodes and one protozoan species. This included a high infection rate for *Ancylostoma* (54%; EPG, 197.16 ± 383.28) followed by *Toxocara* (25%; EPG, 42.51 ± 198.29), *Isospora* (25%; EPG, 43.52 ± 196.07), *Trichuris* (16%; EPG, 20.80 ± 108.96) and *Toxascaris leonina* (7%; EPG, 29.06 ± 281.94). In both canine and feline hosts, the frequency distribution of eggs within the faeces was found to be overdispersed and fitted a negative binomial distribution with k values ranging from 0.041–0.078 in feline and 0.009–0.121 in canine samples. With regard to stool texture and consistency there was little association between parasitic infections in both types of faeces, although in feline samples the protozoan *Isospora* tended to occur in runny stools ($P < 0.05$) whereas *Ancylostoma* was more frequent in runny stools of canines ($P < 0.01$).

Discussion

Soil samples from playgrounds in five states from Peninsular Malaysia were found to be highly contaminated with helminth eggs and occasionally protozoan cysts. Four genera of nematodes dominated the infections, with *Toxocara* being the most frequent (95.7%), which was similar to the results of previous studies undertaken by Himonas *et al.* (1992), Correa *et al.* (1995), Uga *et al.* (2000) and Alonso *et al.* (2001), but higher than prevalences of 54.5% and 12.1%, respectively, recorded by Loh & Israf (1998) and Noor Azian *et al.* (2008).

It was also noted that high levels of contamination were observed, with egg counts as high as 800 EPG in one study site in Penang. Higher infection rate of zoonotic nematodes, especially with *Toxocara* eggs, were likely to be linked with playgrounds being exposed to stray animals scavenging and defecating in residential areas. Such high incidences, especially of *Toxocara*, *Ancylostoma* and *Trichuris* eggs in the soil, were due to the open access of playgrounds, with no fencing to protect these locations from stray animals. These animals were free to roam and

Table 3. Variation in the infection rate of parasitic eggs in soil samples relative to season (wet and dry), soil texture (sand and silt) and type of park (public and residential), using a Poisson regression model. EC, expected log count for one unit of increase in each parameter; ER, expected infection rate of nematode eggs and level of significant differences given as $P < 0.01$, except for *, $P < 0.05$; and **, $P < 0.10$.

	Nematode genus							
	Toxocara		*Ascaris*		*Trichuris*		*Ancylostoma*	
Principle interactions	EC	ER	EC	ER	EC	ER	EC	ER
Season	1.029	2.799	1.463	4.319	0.637	1.890	1.218	3.380
Soil texture	−0.212	0.809**	−0.350	0.705	−0.318	0.727*	−0.353	0.703
Type of park	−1.202	0.301	−1.170	0.310	−2.032	0.131	−1.140	0.319
Soil texture × type of park	1.063	2.894	1.059	2.883	1.860	6.423	1.297	3.658

defecate repeatedly, thus contaminating the soil with eggs that can survive for many years (Zibaei *et al.*, 2010). On the other hand, the presence of *Ascaris* eggs was possibly due to human defecation. Similar findings were also found by Horiuchi *et al.* (2013) in the Philippines, where helminth egg counts in soil were as high as 410 (*A. lumbricoides*), 134 (*Toxocara* spp.) and 134 (*Trichuris* spp.). These authors concluded that contamination of soil was mainly due to stray animal and human defecation.

These circumstances, combined with optimum temperatures and high levels of humidity and moisture in the soil, particularly during the wet season in Malaysia, would undoubtedly enhance the survival and viability of ascarid and trichurid eggs and larval stages of hookworms such as *Ancylostoma*.

Higher infection rates of other nematode species were also observed in the playgrounds under investigation, including *Ascaris* (93.3%), *Ancylostoma* (88.3%) and *Trichuris* (77%), and these findings were significantly higher than those reported by Noor Azian *et al.* (2008) in Malaysia, Ajala & Asaolu (1995) in Nigeria, Mandarino-Pereira *et al.* (2010) in Brazil and Blaszkowska *et al.* (2011) in Poland. Playgrounds in the two states of Penang and Selangor were found to be more highly contaminated than other sites, with all nematode genera being present with high numbers of helminth EPG, especially in Penang.

It may seem premature to conclude the role of strays in contaminating playgrounds based on only one sampling site in Kuala Lumpur. However, Mohd Zain *et al.* (2013) confirmed the presence of *Toxocara cati*, *Toxocara malaysiensis*, *Ancyclostoma ceylanicum* and *Ancyclostoma brasiliensis* in stray cat populations from four states in Malaysia, while Mahdy *et al.* (2012) recorded *A. ceylanicum* and *Ancyclostoma caninum* in stray dogs. This further confirms the role of strays as a source of environmental contamination, particularly for *Toxocara* and *Ancylostoma*, but molecular approaches are required to identify eggs of other helminth species.

Although several authors have reported that dogs can mechanically transmit human parasites such as *Ascaris* (Traub *et al.*, 2002, 2005; Shalaby *et al.*, 2010), the present study found no evidence of *Ascaris* eggs despite screening up to 100 dog faecal samples. The relatively high incidence of *Ascaris* was more likely to originate from human defecation, because potential animal sources of infection, such as pigs, were strictly confined to farms on the outskirts of urban areas as a result of Islamic prohibitions. Therefore, pigs are unlikely to inhabit playgrounds in residential sites. Previous studies in Malaysia have shown that children are infected with *Ascaris lumbricoides*, *Trichuris trichiura* and *A. ceylanicum* (Bundy *et al.*, 1988; Rajeswari *et al.*, 1994; Rahman, 1998; Ngui *et al.*, 2012) and therefore molecular characterization

Table 4. The infection rate (%) and mean number of eggs /cysts (EPG ± SD) in each of 100 stray feline and canine faecal samples from animal shelters in Kuala Lumpur, January 2011 to February 2012; CI, 95% confidence intervals.

Parasite species/genus	(%)	CI	EPG ± SD	Range
Feline samples				
Toxocara cati	37.0	0.28–0.47	42.47 ± 156.08	6–1187
Ancylostoma	29.0	0.21–0.30	38.64 ± 273.70	10–2733
Isospora	35.0	0.26–0.45	65.83 ± 191.75	6–1140
Spirometra	22.0	0.15–0.31	21.09 ± 81.63	10–690
Canine samples				
Toxocara canis	25.0	0.17–0.34	42.51 ± 198.29	7–1920
Ancylostoma	54.0	0.44–0.63	197.16 ± 383.28	7–2267
Trichuris vulpis	16.0	0.10–0.24	20.80 ± 108.96	6–1020
Toxascaris leonina	7.0	0.03–0.14	29.06 ± 281.94	7–2820
Isospora	25.0	0.17–0.34	43.52 ± 196.07	7–1913

is also necessary to confirm whether or not playgrounds contaminated with *Ascaris* are of human or animal origin.

In the case of zoonotic helminths, the arrival of the wet season in playgrounds significantly increased the number of eggs of all four nematode genera compared with the dry season. Stojcevic *et al.* (2010) also reported an increase in the number of helminth eggs during the rainy season, as embryonation of eggs increases in tropical temperatures with high soil humidity. This result is similar to the results of studies by Uga & Kataoka (1995), Rai *et al.* (2000) and Nurdian (2004), where high levels of rainfall contributed to higher diversities and prevalence of parasite species. In addition, helminth eggs such as those of *Toxocara* and *Ascaris* possess thick external layers, which provide protection from environmental factors (Mizgajska, 1997).

The effects of two factors, the type of soil and size of playgrounds, also played a significant role in determining the number of helminth eggs recovered from soil samples. Ayaji & Duhlinska (1998), Duwel (1984) and Omudu *et al.* (2003) observed that *Toxocara* eggs were found more readily in soil rich with sand compared with other soil types, and also that particle size was important.

High levels of contamination with eggs of *Toxocara*, *Ascaris* and *Trichuris* occurred in smaller residential playgrounds compared with public parks and these results are similar to those of Mizgajska (2001) and Dubna *et al.* (2007). The open access of smaller residential playgrounds allowed stray animals, pets and the public to defecate repeatedly and indiscriminately in confined spaces, thereby increasing the density of eggs in the soil. On the other hand, a significant reduction in contamination with eggs was found in the soil of public playgrounds as these tend to be protected with fencing. Nevertheless, the presence of eggs of *Toxocara*, *Ancylostoma* and *Trichuris* in playground soil and stool samples suggests that stray animal populations play an important role in contaminating sandpits in both public and residential areas. Dogs, in particular, exhibit behavioural patterns by selecting previously used defecation sites (Rubel & Wisnivesky, 2005).

Canine faeces in the present study revealed a high infection rate of *Ancylostoma* (54%) followed by *Toxocara canis* (25%), *Isospora* (25%), *Trichuris* (16%) and *T. leonina* (7%), and these results were significantly higher than those reported by Noor Azian *et al.* (2008) in Malaysia for *T. canis* (12.1%), *Ancylostoma* (4.9%) and *Trichuris* (1.6%). Subsequent reports by Mahdy *et al.* (2012) in Malaysia showed that both hookworm species *A. caninum* and *A. ceylanicum* were found in urban stray dogs, with *A. ceylanicum* being the more prevalent species (76.2%), which was comparable with *Ancylostoma* (88%) recorded by Tanwar & Kachawha (2007) in India. Kutdang *et al.* (2010) also recorded a high infection rate of hookworms, with up to 50% infected in a dog population in Nigeria, together with 38.2% and 31.8% infected with *T. canis* and *Trichuris vulpes*, respectively. In the present investigation on feline faeces, the other *Toxocara* species (*T. cati/T. malaysiensis)* was also most frequently recovered (37%), followed by *Isospora* (35%), *Ancylostoma* (29%) and *Spirometra* (22%). On the other hand, Jittapalapong *et al.* (2007) reported much lower infections of *Toxocara* spp. (3.5%) and *Anyclostoma* spp. (9.9%) in Thailand.

Apart from host parameters, extrinsic factors such as faecal texture may also be linked with infectivity, as in the present study the number of infective stages of *Ancylostoma* ($P < 0.01$) and *Isospora* ($P < 0.05$) was higher in runny compared with soft/hard stools, but whether the viability of eggs or cysts is related to texture of stools requires further investigation.

Dubinsky *et al.* (1995) reported that the main source of food for most stray cats and dogs included small mammals such as rodents, which can act as paratenic hosts for helminths, thus increasing levels of infection in these feline and canine definitive hosts. The large numbers of parasite eggs recovered in this study clearly highlight the potential health risk to children, who may acquire infection when playing in the sandpits of playgrounds located in various sites in Peninsular Malaysia.

The present study has revealed high numbers of helminth eggs contaminating soil in playgrounds surveyed in Peninsular Malaysia, and such high levels of environmental contamination, especially with *Toxocara*, were mainly due to defecation by stray animals (and also domestic pets), or the public in the case of *Ascaris*. The origins of some helminth genera (*Trichuris, Ancylostoma*) were not determined, but high prevalences of *Toxocara* and *Ascaris* clearly confirm that both animals and humans are important sources of contamination. However, molecular approaches are now required to identify helminth species from soil samples, notably the identification and host origin of *Ascaris* since dog faeces were free from infection.

Factors contributing to soil contamination with helminth eggs include the type of playground, soil texture and season. In addition, the presence of helminth eggs/larvae in the stools of dogs and cats can be influenced by faecal texture. Nevertheless, highly contaminated soil in playgrounds in urban areas does highlight the need for substantially improving the management of stray animals and enhancing hygiene practices in Malaysia. Municipalities nationwide must be responsible for the control of stray animals and should include awareness programmes; improve playground designs for children, to exclude strays and the public from defecating in public and residential parks; and promote better hygiene practices within the community.

Acknowledgements

Special thanks are extended to the staff of the Society for the Prevention of Cruelty to Animals (SPCA), Vector Control Unit of Kuala Lumpur City Hall (DBKL), Setapak and staff members from Institute of Biological Science, University of Malaya, for their support and assistance.

Financial support

This research was fully supported by the Fundamental Research Grant Scheme (FP005/2008C) from the Ministry of Education and Postgraduate Research Fund (PV098/2011A) from the University of Malaya, Kuala Lumpur, Malaysia.

Conflict of interest

None.

Ethical standards

The study approach was approved by the University of Malaya Ethical Committee, reference number ISB/31/01/2013/SNMZ (R).

References

Adams, E.J., Stephenson, L.S., Latham, M.C. & Kinoti, S.N. (1994) Physical activity and growth of Kenyan school children with hookworm, *Trichuris trichiura* and *Ascaris lumbricoides* infections are improved after treatment with Albendazole. *Journal of Nutrition* **124**, 1199–1206.

Ajala, M.O. & Asaolu, S.O. (1995) Efficiency of the salt floatation technique in the recovery of *Ascaris lumbricoides* eggs from the soil. *Journal of Helminthology* **69**, 1–5.

Alonso, J.M., Stein, M., Chamorro, M.C. & Bojanich, M.V. (2001) Contamination of soils with eggs of *Toxocara* in a subtropical city in Argentina. *Journal of Helminthology* **75**, 165–168.

Ayaji, O.O. & Duhlinska, D.D. (1998) Distribution of *Toxocara* eggs in Jos and Dutse-Kura, Plateau State, Nigeria. *Science Forum* **1**, 31–37.

Blaszkowska, J., Kurnatowski, P. & Damiecka, P. (2011) Contamination of the soil by eggs of geohelminths in rural areas of Lodz district, Poland. *Helminthologia* **48**, 67–76.

Bundy, D.A.P., Kan, S.P. & Rose, R. (1988) Age related prevalence, intensity, and frequency distribution of gastrointestinal helminth infection in urban slum school children from Kuala Lumpur, Malaysia. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **82**, 289–294.

Chieffi, P.P. & Muller, E.E. (1976) Prevalencia de parasitismo por *Toxocara canis* em caese presenca de ovos de *Toxocara* sp. no solo de localidades publicas da zona urbana do município de Londrina, estado do Parana, Brasil. *Revista de Saude Publica* **10**, 367–372.

Correa, G.L.B., Michelon, E. & Lagaggio, V.R.A. (1995) Contaminação do solo por ovos, larvas de helmintos e oocistos de protozoários, em praças públicas de Santa Maria e sua importância em saúde pública. *Revista Brasileira de Parasitologia Veterinaria* **4**, 137.

Dada, B.J.O. & Lindquist, W.D. (1979) Prevalence of *Toxocara* spp. eggs in some public grounds and highway rest areas in Kansas. *Journal of Helminthology* **53**, 145–146.

Dubinsky, P., Havasivoa-Reiterova, K. & Petko, B. (1995) Role of small mammals in the epidemiology of toxocariasis. *Journal of Parasitology* **110**, 187–193.

Dubna, S., Langrova, I., Jankovska, I., Vadlejch, J., Pekar, S., Napravnik, J. & Fechtner, J. (2007) Contamination of soil with *Toxocara* eggs in urban (Prague) and rural areas in the Czech Republic. *Veterinary Parasitology* **144**, 81–86.

Dunn, A. & Keymer, A. (1986) Factors affecting the reliability of the McMaster technique. *Journal of Helminthology* **60**, 260–262.

Duwel, D. (1984) The prevalence of *Toxocara* eggs in the sand in children's playgrounds in Frankfurt. *Annals of Tropical Medicine and Parasitology* **78**, 633–636.

Glickman, L.T. & Schantz, P.M. (1981) Epidemiology and pathogenesis of zoonotic toxocariosis. *Epidemiologic Reviews* **3**, 230–250.

Habluetzel, A., Traldi, G., Ruggieri, S., Scuppa, P., Marchetti, R., Menghini, G. & Esposito, F. (2003) An estimation of *Toxocara canis* prevalence in dogs, environmental egg contamination and risk of human infection in the Marche region of Italy. *Veterinary Parasitology* **133**, 243–252.

Himonas, C., Antoniadou-Sotiriadou, K. & Frydas, S. (1992) Research survey on the prevalence of *Toxocara* ova in the soil of public parks in Thessaloniki. *Helliniki-Tatriki* **58**, 333–339.

Horiuchi, S., Paller, V.G. & Uga, S. (2013) Soil contamination by parasite eggs in rural village in the Philippines. *Tropical Biomedicine* **30**, 495–503.

Jittapalapong, S., Inparnkaew, T., Pinyopanuwat, N., Kengradomkij, C., Sangvaranond, A. & Wongnakphet (2007) Gastrointestinal parasites of stray cats in Bangkok Metropolitan Areas, Thailand. *Kasetsart Journal* **41**, 69–73.

Koroma, M.M., Williams, A.M., De La Haye, R.R. & Hodges, M. (1996) Effects of albendazole on growth of primary school children and the prevalence and intensity of soil-transmitted helminths in Sierra Leone. *Journal of Tropical Pediatrics* **42**, 371–372.

Kutdang, E.T., Bukbuk, D.N. & Ajayi, J.A. (2010) The prevalence of intestinal helminths of dogs (*Canis familaris*) in Jos, Plateau State, Nigeria. *Researcher* **2**, 51–56.

Loh, A.G. & Israf, D.A. (1998) Tests on the centrifugal floatation technique and its use in estimating the prevalence of *Toxocara* in soil samples from urban and suburban areas of Malaysia. *Journal of Helminthology* **72**, 39–42.

Mahdi, N.K. & Ali, H.A. (1993) *Toxocara* eggs in the soil of public places and schools in Basrah, Iraq. *Annals of Tropical Medicine and Parasitology* **87**, 201–205.

Mahdy, A.K., Yvonne, A.L., Ngui, R., Siti Fatimah, M.R., Choy, S.H., Yap, N.J., Al-Mekhlafi, H.M., Ibrahim, J. & Surin, J. (2012) Prevalence and zoonotic potential of canine hookworms in Malaysia. *Parasites and Vectors* **5**, 88.

Mandarino-Pereira, A., de Souza, F.S., Lopes, C.W. & Pereira, M.J. (2010) Prevalence of parasites in soil and dog feces according to diagnostic tests. *Veterinary Parasitology* **170**, 176–181.

Margolis, L., Esch, G.W., Holmes, J.C., Kuris, A.M. & Schad, G.A. (1982) The use of ecological terms in parasitology (report of an Ad Hoc committee of the American Society of Parasitologists). *Journal of Parasitology* **68**, 131–133.

Mizgajska, H. (1997) The role of some environmental factors in the contamination of soil with *Toxocara* spp. and other geohelminth eggs. *International Journal of Parasitology* **46**, 67–72.

Mizgajska, H. (2001) Eggs of *Toxocara* spp. in the environment and their public health implications. *Journal of Helminthology* **75**, 147–151.

Mohd Zain, S.N., Norhidayu, S., Pal, P. & Lewis, J.W. (2013) Macroparasite communities in stray cat populations from urban cities in Peninsular Malaysia. *Veterinary Parasitology* **196**, 469–477.

Molyneux, D.H., Hotez, P.J. & Fenwick, A. (2005) 'Rapid impact interventions': how a health policy of integrated control of Africa's neglected tropical diseases could benefit the poor. *PLoS Medicine* **10**, 1371.

Ngui, R., Lim, Y.A.L., Traub, R., Mahmud, R. & Mistam, M.S. (2012) Epidemiological and genetic data supporting the transmission of *Ancylostoma ceylanicum* among human and domestic animals. *PLoS Neglected Tropical Diseases* **6**, 1522.

Nokes, C., Grantham-McGregor, S.M., Sawyer, A.W., Cooper, E.S. & Bundy, D.A.P. (1992) Parasitic helminth infection and cognitive function in school children. *Proceedings of the Royal Society of London* **247**, 77–81.

Noor Azian, M.Y., Sakhone, L., Hakim, S.L., Yusri, M.Y., Nurulsyamzawaty, Y., Zuhaizam, A.H., Rodi, I.M. & Maslawaty, M.N. (2008) Detection of helminth infections in dogs and soil contamination in rural and urban areas. *The Southeast Asian Journal of Tropical Medicine and Public Health* **39**, 205–212.

Nurdian, Y. (2004) Soil contamination by parasite eggs in two urban villages of Jember. *Jurnal Ilmu Dasar* **5**, 50–54.

Omudu, E.A., Amuta, E.U., Unoqur, L.B. & Okoye, L.A. (2003) Prevalence of *Toxocara canis* ova in dog faeces and soil samples collected from public parks in Makurdi, Nigeria. *Nigerian Journal of Parasitology* **24**, 137–142.

Pal, P. & Lewis, J.W. (2004) Parasite aggregations in host populations using reformulated negative binomial model. *Journal of Helminthology* **78**, 57–61.

Rahman, W.A. (1998) Helminthic infections of urban and rural schoolchildren in Penang Island, Malaysia: implications for control. *Southeast Asian Journal of Tropical Medicine and Public Health* **29**, 596–598.

Rai, S.K., Uga, S., Ono, K., Rai, G. & Matsumura, T. (2000) Contamination of soil with helminth parasite eggs in Nepal. *Southeast Asian Journal of Tropical Medicine and Public Health* **31**, 388–393.

Rajeswari, B., Sinniah, B. & Hasnah, H. (1994) Socioeconomic factors associated with intestinal parasites among children living in Gombak, Malaysia. *Asia Pacific Journal of Public Health* **7**, 21–25.

Reiczigel, J. & Rózsa, L. (2001) Quantitative Parasitology 2.0. Budapest, distributed by the authors. Available at http://bio.univet.hu (accessed 10 December 2012).

Rozsa, L., Reiczigel, J. & Majoros, G. (2000) Quantifying parasites in samples of hosts. *Journal of Parasitology* **86**, 228–232.

Rubel, D. & Wisnivesky, C. (2005) Magnitude and distribution of canine fecal contamination and helminth eggs in two areas of different urban structure, Greater Buenos Aires, Argentina. *Veterinary Parasitology* **133**, 339–347.

Shalaby, H., Abdel-Shafy, S. & Derbala, A. (2010) The role of dogs in transmission of *Ascaris lumbricoides* for humans. *Parasitology Research* **106**, 1021–1026.

Sommerfelt, I., Degregorio, O., Barrera, M. & Gallo, G. (1992) Presence of *Toxocara* eggs in public parks of the city of Buenos Aires, Argentina, 1989–1990. *Revue de Medecine Veterinaire Buenos Aires* **73**, 70–74.

Stephenson, L.S., Latham, M.C., Kinoti, S.N., Kurz, K.M. & Brigham, H. (1990) Improvements in physical fitness of Kenyan schoolboys infected with hookworm, *Trichuris trichiura* and *Ascaris lumbricoides* following a single dose of albendazole. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **84**, 277–282.

Stojcevic, D., Susic, V. & Lucinger, S. (2010) Contamination of soil and sand parasite elements as a risk factor for human health in public parks and playgrounds in Pula, Croatia. *Veterinarski Arhiv* **80**, 733–742.

Tanwar, R.K. & Kachawha, S. (2007) Prevalence of worm infestations in stray dogs in and around Jodhpur. *Journal of Veterinary Parasitology* **21**, 171–172.

Traub, R.J., Robertson, I.D., Irwin, P., Mencke, N. & Thompson, R.C. (2002) The role of dogs in transmission of gastrointestinal parasites in a remote tea-growing community in Northeastern India. *American Journal of Tropical Medicine and Hygiene* **67**, 539–545.

Traub, R.J., Robertson, I.D., Irwin, P.J., Mencke, N. & Thompson, R.C. (2005) Canine gastrointestinal parasite zoonoses in India. *Trends in Parasitology* **21**, 42–48.

Uga, S. & Kataoka, N. (1995) Measures to control *Toxocara* egg contamination in sandpits of public parks. *American Journal of Tropical Medicine and Hygiene* **52**, 21–24.

Uga, S., Matsuo, J., Kimura, D., Rai, S.K., Koshino, Y. & Igarashi, K. (2000) Differentiation of *Toxocara canis* and *T. cati* eggs by light and scanning electron microscopy. *Veterinary Parasitology* **92**, 287–294.

Wilson, K. & Grenfell, B.T. (1997) Generalized linear modeling for parasitologists. *Parasitology Today* **13**, 33–38.

Zibaei, M. & Uga, S. (2008) Contamination by *Toxocara* spp. eggs in sandpits in Kobe, Japan. *Journal of Environment Contamination Technology* **26**, 32–37.

Zibaei, M., Abdollahpour, F., Birjandi, M. & Firoozeh, F. (2010) Soil contamination with *Toxocara* spp. eggs in the public parks from three areas of Khorram Abad, Iran. *Nepal Medical College Journal* **12**, 63–65.

REVIEW ARTICLE

Human Zoonotic Infections Transmitted by Dogs and Cats

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Dogs and cats are the 2 most common household pets. However, they may be a direct or indirect source of human infections. This article aims to familiarize physicians with some common and uncommon bacterial, rickettsial, parasitic, and fungal zoonotic infections of dogs and cats. Animal bites with or without infection continue to be a common problem. Treatment of infected animal bites must include early débridement and concern for organisms from the mouth flora of the animal. The diagnosis and treatment of cat-scratch disease have become easier since *Bartonella henselae* has been established as the main causal agent. Less common bacterial and rickettsial zoonotic infections are included to increase the reader's awareness. Parasitic infections, such as creeping eruptions, visceral larva migrans, cryptosporidiosis, and toxoplasmosis, are diseases associated with contact with dogs and cats. Pets can also be the source of dermatophyte infections. An increase in awareness that some of these diseases may be associated with animals could provide a better plan for the prevention and treatment of common and uncommon zoonotic infections.

Arch Intern Med. 1997;157:1933-1943

There are more than 110 million pet dogs and cats in the United States. These pets are found in approximately 70% of households and have been directly or indirectly associated with the transmission of at least 30 infectious agents to humans.[1,2] Pet owners often are not aware of and most health care workers are not trained to recognize these zoonotic diseases. Fortunately, despite close contact with these pets, the number of clinical zoonotic illnesses in humans is relatively low. This article covers the important clinical aspects of bacterial, rickettsial, parasitic, and fungal diseases acquired directly or indirectly from dogs and cats. This review is not intended to be comprehensive and inclusive, but emphasizes how the infection is acquired, as well as diagnosis and current recommended treatment. For detailed information about specific diseases, the reader is referred to standard textbooks of infectious diseases or parasitology.

Table 1 lists the associated clinical manifestations of diseases caused by infectious agents that may be directly or indirectly transmitted by dogs and cats to humans. The clinical manifestations are divided into the following categories: cutaneous, respiratory, gastrointestinal, neurologic, systemic, and other manifestations. **Table 2** lists the diagnostic methods and suggested treatment for bacterial infections transmitted by dogs and cats.

BACTERIAL INFECTIONS

Dog and Cat Bite Infections

Animal bites represent 1% of all emergency department visits. Between 70% and 90% of these visits are caused by dog bites, which have been estimated at more than 1 million occurrences per year.[2,3] Cats account for only 3% to 15% of animal bites, but these bites are more likely to become infected. The risk of infection after a bite is estimated to be 5% to 15%.[4] Infection following a bite is frequently caused by a mixture of microorganisms.[3] The prominent microorganisms include *Staphylococcus* spe-

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This article is also available on our Web site: www.ama-assn.org/internal.

Table 1. Clinical Manifestations of Zoonotic Infections Transmitted by Dogs and Cats

Agent	Manifestation: Skin	Respiratory	Gastrointestinal
Bacteria			
Bartonella henselae	Papule at the inoculation site, bacillary angiomatosis, and erythema nodosum	Pneumonia	Peliosis hepatis and splenomegaly
Borrelia burgdorferi	Erythema migrans	...	...
Brucella canis	...	Pneumonia	Hepatitis
Capnocytophaga canimorsus	Infected bite wound	...	...
Campylobacter jejuni	...	...	Diarrhea
Chlamydia psittaci	...	Pneumonia	...
Francisella tularensis	...	Pneumonia	...
Helicobacter heilmannii	...	...	Gastritis
Leptospira interrogans serovar-canicola	Petechiae	...	Hepatitis
Pasteurella multocida	Infected bite wound	Pneumonia	...
Salmonella enterica	...	...	Diarrhea
Streptococcus pyogenes	Cellulitis	Pharyngitis	...
Yersinia enterocolitica and *Yersinia pseudotuberculosis*	Erythema nodosum	Exudative pharyngitis	Diarrhea
Yersinia pestis	Pustule, eschar, ecthyma, gangrenosum, and bubo	Pneumonic plague	...
Rickettsia			
Coxiella burnetii	...	Pneumonia	Hepatitis
Ehrlichia spp	Rash	...	...
Rickettsia rickettsii	Petechiae and purpura	Pulmonary infiltrate	Jaundice, hepatomegaly, and splenomegaly
Parasites			
Ancylostoma spp	Creeping eruption	...	...
Cheyletiella yasguri and *Cheyletiella blakei*	Dermatitis and pruritis	...	...
Cryptosporidium parvum	...	...	Diarrhea
Ctenocephalides canis and *Ctenocephalides felis*	Flea bite dermatitis	...	...
Dipylidium caninum	Pruritus ani	...	Indigestion
Dirofilaria immitis	...	Pulmonary infiltrates and "coin lesion"	...
Echinococcus granulosus	...	Pulmonary cyst	Hepatic cyst
Giardia lamblia	...	...	Diarrhea
Isospora belli	...	...	Diarrhea
Sarcoptes scabiei	Dermatitis and pruritus	...	...
Toxocara canis and *Toxocara cati*	Rash	Pulmonary infiltrates	Hepatomegaly
Toxoplasma gondii	...	Pulmonary infiltrate (IC)	Hepatomegaly and splenomegaly
Fungi			
Microsporum canis	Ringworm	...	...
Sporothrix schenckii	Lymphocutaneous lesions	Pulmonary infiltrates	...

*Ellipses indicate no major clinical manifestations apply; IC, manifestations in immunocompromised patients.

cies, *Streptococcus* species, *Corynebacterium* species, *Pasteurella multocida* (isolated in 50%-75% of healthy cats), *Capnocytophaga canimorsus* (formerly called DF-2), and anaerobes. Anaerobes are isolated in approximately 41% of animal bite wounds, and always in mixed cultures.[5]

Patients with a bite wound should be examined thoroughly for nerve, tendon, joint, bone, and vascular injury. Aerobic and anaerobic cultures of the infected site should be taken. Immunization against rabies and tetanus should be considered. The decision to hospitalize should be based on the severity of disease and the presence of underlying illness.

Antimicrobial prophylaxis for a patient with a low infection risk and fresh bite wound is controversial. Antibiotic prophylaxis is recommended for a patient with a puncture wound or a wound that is considered a high risk for infection (eg, wounds located on the head, face, hand, wrist, foot, ankle, other major joints, bones, tendons, nerves, and vascular structures). These wounds should be promptly surgically débrided and irrigated. The an-

Neurologic	Systemic	Other
Meningitis and encephalitis		Regional lymphadenopathy, thrombocytopenia, osteomyelitis, arthralgia, and parotid gland swelling
Meningitis, encephalitis, and neuropathy		Joint and heart symptoms
Meningitis and encephalitis	Nonspecific fever	Bone and joint symptoms
Meningitis	Septicemia and endocarditis	
	Endocarditis	
		Regional lymphadenopathy and eye symptoms
Meningitis and encephalitis	Shock	Renal failure, myositis, uveitis, and thrombocytopenia
	Sepsis	
	Sepsis	
	Septicemic form in impaired hosts; endocarditis	Appendicitislike symptoms (mesenteric lymphadenitis) and reactive arthritis
Meningitis	Septicemia	Regional lymphadenopathy
Meningitis and encephalitis	Endocarditis	Osteomyelitis
Meningitis	Nonspecific fever	Anemia, leukopenia, and thrombocytopenia
Meningitis and encephalitis	Shock	Lymphadenopathy
		Bone, eye, and brain symptoms
Brain mass		Visceral larva migrans and liver, lung, and eye symptoms
Brain mass (IC)	Mononucleosis syndrome	Lymphadenopathy and retinochoroiditis

timicrobial agent selected should be indicated for mixed bacterial infections. A combination product of amoxicillin and clavulanate potassium should provide adequate coverage.

Treatment of an infected bite wound should include aggressive surgical débridement and timely appropriate antimicrobial therapy with agents active against the commonly encountered mixed flora. In addition to the gram-positive cocci and anaerobes, *P multocida* is frequently isolated from infected bite wounds. Therapy with amoxicillin-clavulanate, ampicillin, cefuroxime, a combination product of trimethoprim and sulfamethoxazole, tetracycline, or ciprofloxacin is effective. Drugs commonly used for skin and skin structure infections, such as dicloxacillin, cephalexin, cefadroxil, cefaclor, erythromycin, and clindamycin, are not recommended.[6] Another microbe commonly found as part of the oral flora of dogs is *C canimorsus*. This organism is consistently susceptible to most antimicrobial drugs except aztreonam, trimethoprim-sulfamethoxazole, and aminoglycosides.[7] β-Lactam and β-lactamase inhibitor combinations, such as amoxicillin and clavulanate, ampicillin and sulbactam, ticarcillin disodium and clavulanate, and piperacillin sodium and tazobactam, appear to cover the spectrum of bacteria commonly associated with bite wound infections.

Cat-Scratch Disease

Cat-scratch disease is caused by *Bartonella henselae* (formerly named *Rochalimaea henselae*). This organism also causes bacillary angiomatosis and bacillary peliosis hepatis. Domestic cats serve as a major persistent reservoir for *B henselae*. Cats with chronic *Bartonella* bacteremia are contagious through their saliva. The fleas removed from these cats may contain the infectious agent and bites from these fleas can transmit cat-scratch disease. In the United States, up to 50% of cats tested have antibodies against this organism.[8]

Approximately 1 week following exposure to *B henselae*, a primary papule appears at the site of inoculation. Tender regional lymphadenopathy develops 2 weeks later. In most cases, the disease is self-limited and complete resolution occurs within 2 to 6 months. Systemic disease and other organ involvement may occur,[9] and prolonged and relapsing illnesses have been observed.[10] Patients with human immunodeficiency virus infection may have skin lesions reminiscent of pyogenic granuloma, histiocytoid hemangioma, and epithelial hemangioma.

A history of exposure to cats and lymph node biopsy findings of microabscesses or granuloma should increase the index of suspicion for infection with *B henselae*. A definitive diagnosis can be made via microscopy using Warthin-Starry silver stain,[11] with serologic tests using

Table 2. Diagnosis and Suggested Therapy for Infections Transmitted by Dogs and Cats*

Agent	Diagnostic Methods	Suggested Therapy†	Prevention
Bartonella henselae	Tissue biopsy and special staining; culture; serologic testing (not species specific)	Observation, needle aspiration, or surgical removal; recommended antimicrobial agents: doxycycline, minocycline, and erythromycin; other agents: gentamicin sulfate, rifampin, ciprofloxacin, and T-S (controlled study needed)	Antimicrobial treatment of the pet cat and control of flea infestation
Borrelia burgdorferi	Clinical picture and serologic testing	Amoxicillin and other β-lactam antibiotics; doxycycline and erythromycin	Avoid letting pet cats roam in tick-infested areas
Brucella canis	Serologic testing (ask for *B canis* antibody); blood culture	Doxycycline plus rifampin; T-S may be used in children and pregnant patients	Avoid birth products of infected animals and eradicate infection from the source animal
Capnocytophaga canimorsus	Culture of bite wound	Most antimicrobial agents used for bite infections are effective, except aztreonam	Cleanse and débride wound early
Campylobacter jejuni	Stool culture	Macrolides and quinolones	Hygienic measures taken while caring for dogs or puppies with diarrhea
Chlamydia psittaci	Sputum culture and serologic testing	Tetracycline and macrolides	Avoid contact with infected animals, particularly birds and cats
Francisella tularensis	Serologic testing and culture	Streptomycin	Avoid contact with animals suspected to be infected
Leptospira interrogans serovar-canicola	Serologic testing for agglutination; dark-field microscopic examination of body fluids (reliable when performed by experienced individuals)	High-dose penicillin or tetracycline must be given early in the septicemia phase	Prevent exposure to infected materials
Pasteurella multocida	Culture of wound	Susceptible to penicillin, cephalosporins, T-S, tetracycline, and quinolones; resistant to clindamycin, erthromycin, and vancomycin	Cleanse and débride wound as soon as possible
Salmonella enterica	Stool culture	Therapy is usually not necessary; for those with systemic disease, T-S or quinolones may be given	Hygienic measures
Yersinia enterocolitica and Yersinia pseudotuberculosis	Culture of stool sample and specimens from suspected sites	Gentamicin sulfate, chloramphenicol, T-S, doxycycline, and ciprofloxacin	Avoid contact with infected animals
Yersinia pestis	Culture of blood and specimens from suspected sites	Streptomycin is recommended; chloramphenicol and tetracycline have been found to be effective	Avoid exposure to infected fleas and patients with pneumonic plague
Coxiella burnetii	Serologic testing	Doxycycline and fluoroquinolone, singly or in combination	Avoid contact with infected material; aerosolization of the organism will result in respiratory infection
Ehrlichia spp	Peripheral blood smear, immunocytologic testing, immunohistologic testing, PCR, and serologic testing	Doxycycline	Not well established
Rickettsia rickettsii	Serologic testing	Doxycycline	Carefully examining body after entering a tick-infested area

** Information compiled from previously published articles.[2,7,8,15,17,20-22,24,26,36,38,40]*
† T-S indicates trimethoprim-sulfamethoxazole combination product; PCR, polymerase chain reaction.

an indirect fluorescent antibody (it does not distinguish between *B quintana* and *B henselae*),[12] and with a bacterial culture. A blood culture using lysis centrifugation appears to have the best yield.[8]

Lymph node inflammation is usually self-limited and does not require drainage. Enlarged lymph nodes may be aspirated or excised to obtain tissue to rule out other causes and to relieve discomfort. Antimicrobial agents should be administered to patients with more serious illness, to those with complications, and to those who are immunocompromised. In vitro susceptibility studies[13] have shown that erythromycin, doxycycline, rifampin, and sparfloxacin are most active; however, only aminoglycosides are bactericidal in cat-scratch disease. Susceptibility testing has not been shown to correlate with clinical outcomes. The response of *B henselae* to antimicrobial therapy has not been evaluated prospectively. Erythromycin, doxy-

cycline, and minocycline are the recommended first-line agents for cat-scratch disease. Gentamicin sulfate, rifampin, ciprofloxacin, trimethoprim-sulfamethoxazole, and certain third-generation cephalosporins may also be clinically effective.[8]

Brucellosis

Brucellosis is a disease of domestic farm animals caused by *Brucella abortus*, *B melitensis*, and *Brucella suis*. Rural dogs may serve as a reservoir of human and livestock infections. Although *Brucella* infection in dogs and cats is usually benign with a subclinical and self-limited course, transient bacteremia with lymphadenopathy, epididymitis, orchitis, and abortion have been observed.

Brucella canis was identified in 1966 as associated with outbreaks of abortions in beagle dogs.[14] Human infections caused by canine species are not common. Similar to that of other *Brucella* species, the infection may present with nonspecific clinical manifestations and laboratory findings.[15,16] A diagnosis of brucellosis can be made using serologic tests and a blood culture. A request for *B canis* antibodies should be specified when *B canis* infection is suspected. The *Brucella* antigen used in most clinical laboratories does not cross-react with antibodies to *B canis*.[15] Treatment with doxycycline, 200 mg/d, plus rifampin, 600 to 900 mg/d, orally for at least 6 weeks has been found effective. For children younger than 8 years or pregnant women, treatment with trimethoprim-sulfamethoxazole may be used in place of doxycycline.

Campylobacteriosis

Infection with *Campylobacter* ranks among the most common causes of human bacterial diarrheas. Most human infections are acquired by the ingestion of water, milk, or food contaminated by domestic or farm animals. Pets are an infrequent source of this illness. Up to 11% of asymptomatic dogs and 28% of dogs with diarrhea have been found to excrete this organism transiently. *Campylobacter* infection also has been transmitted by sick puppies.[17,18] Fever and diarrhea lasting for about a week are the most common clinical manifestations. Diagnosis of campylobacteriosis can be made using a stool culture. Symptomatic treatment is recommended for most patients and antimicrobial therapy is recommended for more seriously ill patients. Most strains of *Campylobacter* are susceptible to the macrolides and fluoroquinolones.

Leptospirosis

Leptospirosis, a common zoonotic infection transmitted by livestock, pet animals, and wildlife, is caused by the spirochete *Leptospira interrogans*. Rats and dogs are important in the transmission of this disease. Infected animals may be asymptomatic or have an illness characterized by fever, jaundice, conjunctivitis, and hemoglobinuria.[19] The organism survives in the distal renal tubules of dogs and the urine may be contagious for life. Human infection occurs following exposure of a mucous membrane or abraded skin to the urine or tissue of an infected animal either directly or through contaminated water or soil. The clinical illness has a septicemic phase and an immune phase. The symptoms of the septicemic phase are nonspecific and difficult to differentiate from other acute febrile illnesses. Fever, rash, meningeal signs, myositis, uveitis, and leptospiruria occur in the immune phase. The diagnosis of leptospirosis is made clinically and by a 4-fold increase in results of agglutination tests, positive culture results, or dark-field microscopic examination of body fluids, including blood, urine, and other body fluids.[20] Treatment with high-dose penicillin or doxycycline should begin early to be effective.

Tularemia

Human illness caused by *Francisella tularensis* occurs among individuals exposed to deerflies or infected animals or their ticks. Cats become infected after feeding on infected animals. The disease is similar in humans. Cats can transmit the disease through their contaminated teeth and claws, while dogs can indirectly transmit the disease through tick vectors. Manifestations of the disease are dependent on the point of entry. For example, in the oculoglandular form, the site of inoculation is the conjunctiva; in the ulceroglandular form, an extremity; in the oropharyngeal and typhoidal forms, by ingestion of contaminated food or water; in pneumonic disease, by inhalation; and in the glandular form, primary site is unknown. Inhalation and typhoidal forms have the highest mortality rates and are most commonly found in laboratory workers. Diagnosis of tularemia is based on clinical suspicion and results of cultures and serologic tests. The drug of choice for tularemia is streptomycin sulfate, taken at a dosage of 7.5 to 10 mg/kg every 12 hours for 7 to 14 days.[21]

Salmonellosis

Based on molecular analysis, the genus *Salmonella* has only 1 species, *Salmonella enteritidis*, and has more than 2000 different variants or subspecies. Between 10% and 27% of dogs have been infected with *Salmonella*, usually with serotypes similar to those affecting humans. Animal infection may be subclinical but may present with fever, diarrhea, or spontaneous abortion. Dogs and cats are rarely the source of human infections with *Salmonella*. Appropriate samples, such as feces, should be sent for culture when *Salmonella* is suspected. Most patients with *S enteritidis* require no therapy. If the patient has systemic manifestations, the choice of antibiotic therapy should be based on local laboratory information and susceptibility testing. In the United States, treatment with trimethoprim-sulfamethoxazole and fluoroquinolones is usually effective.[22]

Plague (*Yersinia pestis*)

Yersinia pestis infection is most commonly transmitted to humans, cats, and dogs by infected flea bites. Cats acquire plague also by eating infected rodents. Cats are susceptible to a severe and often fatal infection but dogs usually have a brief and self-

limited illness. Pneumonia in humans has been reported following exposure to infected cats.[23]

There are 5 forms of the disease, including bubonic (suppurative lymphadenopathy), cutaneous, pneumonic, meningitis, and septicemic. The bubonic plague is most common and all forms are accompanied in humans by fever, headache, and weakness.[24]

In a patient presenting with regional lymphadenopathy, a careful history of exposure to an infected animal, infected flea bite, or a patient with pneumonic plague should be obtained. A laboratory diagnosis of plague is made by aspiration of the lymph node for a culture. In a patient suspected of having the pneumonic or septicemic form, blood cultures should also be performed. The antimicrobial agent of choice is streptomycin. Chloramphenicol and tetracycline have also been found to be effective against *Y pestis*.

Yersinia enterocolitica and *Yersinia pseudotuberculosis*

The natural reservoirs of *Y enterocolitica* and *Y pseudotuberculosis* include cats and dogs. These infections are most commonly foodborne but human infections have been traced to contact with infected household dogs and cats.[24,25] The most common clinical manifestations are fever, diarrhea, and abdominal pain. Other symptoms include reactive arthritis, erythema nodosum, and exudative pharyngitis. The septicemic form is more common in patients with impaired immunity, such as those with diabetes, iron overload disease, cirrhosis, and malignancy.[24] Cultures of stool samples and specimens from other infected sites will yield the organism, and serologic tests are available. Gentamicin, chloramphenicol, trimethoprim-sulfamethoxazole, doxycycline, and ciprofloxacin have been used for *Y enterocolitica* infection with good results.

Chlamydia psittaci

It is well known that *Chlamydia psittaci* is a disease transmitted by birds to humans. A recent discovery of the association of this agent with cats should alert clinicians of the probable transmission from these pets.[26,27]

Suspected Zoonotic Bacterial Diseases

At least 3 other bacteria have been suspected to be transmitted to humans from dogs and cats, including animal *Helicobacter* species, *Streptococcus*, and *Anaerobiospirillum* species.

Large, spiral-shaped bacteria, which are morphologically distinct from *Helicobacter pylori*, have been found in human and animal stomachs. Two morphological types have been isolated from the stomachs of cats and dogs: *Helicobacter felis* and *Helicobacter heilmannii* (formerly called *Gastrospirillum hominis*).[28] Similar to *H pylori* infection in humans, infection with these organisms is lifelong. Since no environmental source of these agents has been documented, it has been postulated that human infection is zoonotically acquired. Suspected cases of transmission of *H heilmannii* and *H felis* from dogs and cats to humans have been reported.[29-31]

Anaerobiospirillum have been associated with cases of human diarrhea. Human disease presents with 3 to 7 days of diarrhea, fever, abdominal pain, and vomiting. Cats are the suspected reservoir and the vector in human disease,[32] although this relationship has not been proven. The role of pet dogs acting as reservoirs for recurrent streptococcal infection has been considered but not proven.[1,33]

RICKETTSIAL INFECTIONS

Q Fever

Q fever is a disease transmitted by the inhalation of *Coxiella burnetii* endospores from contaminated soil, exposure to ticks, and parturient cats.[34,35] The disease has a worldwide distribution and has as its reservoir common livestock animals, such as cattle, sheep, and goats. Unlike other rickettsial illnesses, a rash is rarely found. Q fever is frequently a self-limited febrile illness lasting for 2 to 14 days. Other presentations include pneumonia, endocarditis, hepatitis, osteomyelitis, and neurologic manifestations.

Diagnosis of Q fever can be confirmed by results of serologic tests or a culture.[36] Isolation of the organism can only be performed in a limited number of laboratories. For the treatment of acute Q fever, doxycycline, 100 mg twice per day for 15 to 21 days, is recommended, and for meningoencephalitis, a fluoroquinolone compound is preferred because of higher cerebrospinal fluid levels of the drug.[37]

Ehrlichiosis

Two varieties of human ehrlichial infection have been described: human monocytic ehrlichiosis, caused by *Ehrlichia chaffeensis*, and human granulocytic ehrlichiosis, caused by a species that is closely related or identical to *Ehrlichia phagocytophila* and *Ehrlichia equi*.[38] *Ehrlichia chaffeensis* has been isolated from the Lone Star tick (*Amblyomma americanum*) and the dog tick (*Dermacentor variabilis*). Dogs, horses, deer, and other mammals have been suspected as the hosts. However, a definite association with domestic dogs has not been shown. In the granulocytic variety, the definitive tick vector has not been identified, but the deer tick (*Ixodes scapularis*) and *D variabilis* have been suspected. The epidemiologic patterns of human monocytic ehrlichiosis and human granulocytic ehrlichiosis are not well established. Both diseases have been encountered in upper midwestern and eastern states and frequently in areas where Lyme disease and babesiosis are prevalent. The clinical and laboratory manifestations of the 2 infections are similar to one another. Prominent findings include fever, headache, myalgia, anemia, leukopenia, thrombocytopenia, and mildly elevated transaminase levels. Elderly patients are more prone to severe illness and death. Results of a peripheral blood smear may show the characteristic morula in the cytoplasm of mononuclear or polymorphonuclear leukocytes, but this method is rather insensitive. A serologic diagnosis of human monocytic ehrlichiosis is based on a 4-fold increase in *E chaffeensis* antibody ti-

ter or a single high titer (≥128). The diagnosis is confirmed by results of an antibody titer or polymerase chain reaction (available from the Centers for Disease Control and Prevention, Atlanta, Ga). Treatment with doxycycline, 100 mg twice daily or 3 mg/kg per day in 2 doses divided for 5 to 7 days or 3 days after defervescence, has been recommended based on clinical experience.[38]

Rickettsia rickettsii

Rocky Mountain spotted fever is an endemic tick-borne disease caused by *Rickettsia rickettsii*. This microbe is transmitted by the wood tick (*Dermacentor andersoni*) or the dog tick (*D variabilis*). Both humans and dogs can be infected; however, when a dog becomes infected, rickettsemia is not sufficient for the animal to become a reservoir for transmission. Hence, dogs act as sentinels to the presence of this disease.[39] Human disease has not been associated with transmission from dogs. Rocky Mountain spotted fever has been reported in most of the continental United States, but the highest incidence appears to be in the southern Atlantic and south central states.[40] Diagnosis of Rocky Mountain spotted fever is by serologic means. The organism is susceptible to tetracycline, chloramphenicol, rifampin, and some fluoroquinolones, such as ciprofloxacin. Treatment with doxycycline, 100 mg twice daily for 7 days or 2 days after defervescence, is recommended.

PARASITIC INFECTIONS

Larva migrans occurs when nematode larvae enter a host that does not support maturation of the parasites to their adult stage. When the larvae migrate at the level of the skin, the condition is called *cutaneous larva migrans*; when deeper organs are involved, the term *visceral larva migrans* is applied.

Cutaneous Larva Migrans

Two species of dog hookworms are frequently associated with cutaneous larva migrans, also known as creeping eruptions. *Ancylostoma caninum* is found in dogs and *Ancylostoma braziliense* infests both dogs and cats. This disease is more common in the southeastern United States. Lesions typically develop following exposure of bare feet to a playground or beach that has been contaminated with feces containing dog hookworm ova. The ova develop into infective larvae in the soil, and these larvae attach to and penetrate the skin of the host. Their migration in the epidermis results in the clinical manifestation of creeping eruption that is characterized by red, itching papules within a few hours after skin penetration. These larvae migrate a few millimeters per day and produce the characteristic serpiginous, elevated, reddened, pruritic skin eruptions that can be observed approximately 2 weeks after exposure.[2] Visceral migration is rare and may cause abdominal symptoms when it occurs.[41] The clinical appearance of the skin is diagnostic; therefore, further studies, such as biopsies and serologic tests, are superfluous. The disease is self-limited; however, many patients frequently request treatment to relieve discomfort. Freezing the lesion using carbon dioxide snow or ethyl chloride spray, although helpful at times, is no longer recommended. Topical application of a 10% suspension of thiabendazole overlaid with 0.1% dexamethasone cream for 3 days can ease symptoms. In addition, thiabendazole, 25 mg/kg orally twice per day for 2 to 3 days, or mebendazole, 100 mg 3 times daily for 7 days, may be tried.

Visceral Larva Migrans

The syndrome of visceral larva migrans is found worldwide. In the United States, this syndrome is mostly found in toddlers. *Toxocara canis*, a dog roundworm, and less commonly, *Toxocara cati*, a cat roundworm, are the ascarids most commonly responsible for human disease. This disease is acquired by ingestion of ova from materials contaminated with dog or cat feces. Ova from these ascarids are found in the yards of many homes and public parks.[42,43] Although most human infection is subclinical, a serologic survey[2] showed that up to one third of all individuals had a past experience with this parasite. Symptoms are generally nonspecific and visceral organs, such as the lung, liver, brain, eyes, and myocardium (rarely), are targets.[2] The finding of eosinophilia with liver, lung, eye, or brain involvement in a child is suggestive of visceral larva migrans. Hypereosinophilia may be the only clue, but it is absent in 27% of patients with high antibody titer.[44] The definitive diagnosis is finding the larva in tissue. Serologic tests using enzyme-linked immunosorbent assay titers have been reported to be 91% sensitive and 86% specific.[43] Since the disease is self-limited, watchful waiting is advised. For more severe cases, treatment with corticosteroids and antiparasitic agents should be tried. Thiabendazole, 25 mg/kg twice daily, plus corticosteroids in severe cases can be administered until symptoms abate. Albendazole has also been found to be effective.[46,47] Treatment with mebendazole and diethylcarbamazine citrate can also cause disease regression. Appropriate antibacterial agents should be given when a secondary bacterial infection is suspected.

Cryptosporidiosis

Cryptosporidium parvum is a coccidia found worldwide that causes diarrhea in animals and humans. Mammals, including humans, rodents, and companion animals such as puppies and kittens, are known hosts. The parasite is acquired by the ingestion of fecally contaminated material, such as from the water supply, swimming pool water, food, and fomites, and from sexual activities that favor fecal-oral inoculation. In 1993, *Cryptosporidium* was responsible for a massive waterborne diarrheal outbreak that affected more than 400 000 individuals in Milwaukee, Wis.[48] The source was a contaminated public water supply. The oocysts are difficult to destroy, even with chlorination. The most common clinical presentation of cryptosporidiosis in both immunocompetent and immunocompromised hosts is profuse and watery diarrhea. In immunocompetent patients, the incubation period is approximately 3 weeks and the dis-

cal, and even physical welfare.[2] The responsibility of pet ownership does not end with the health of the pets alone. The welfare and health of the owners, the neighborhood, and the community must be considered. Therefore, the responsibilities for the pets begin with proper immunization, disease prevention, hygiene, and treatment of diseases. With ownership comes the responsibility for the pet's well-being and the health of the owners and neighbors. With the recent increase of rabies in the United States and the low vaccination rate of cats, pet owners should understand the importance of rabies vaccination. Other types of immunization, such as leptospiral vaccination, should be considered depending on local situations. Many regions have a high rate of dirofilarial infections in dogs. Dogs should be given chemoprophylaxis to protect against heartworm disease. Pets should be provided with a hygienic environment to reduce the chance of skin diseases and ectoparasitic as well as other parasitic infestations. Pets that are allowed to have outdoor activities are susceptible to parasitic infestation, including ectoparasites. Appropriate preventive measures should include regular inspection for ticks and fleas, as well as regular checkups by veterinarians for possible deworming.

The responsibilities of pet owners to themselves and the community are just as important. Pets should not be permitted to defecate on beaches or playgrounds. Animal feces in private yards and on public grounds should be removed to avoid dissemination of parasitic diseases to other animals, as well as to humans. For the same reasons, pet feces should not be used as fertilizer. Small children should not be left unattended with pets. Children should be taught to avoid unfamiliar dogs and not to disturb or startle a feeding or sleeping dog. Hands should be washed after every animal contact. Pets with diarrhea may be a source for human infection and special attention to hygiene should be stressed. Medical attention should be sought for any animal bite, no matter how insignificant.

Accepted for publication May 29, 1997.

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REFERENCES

1. Goldstein EJC. Household pets and human infections. *Infect Dis Clin North Am.* 1991;5:117-130.
2. Elliot DL, Tolle SW, Goldberg L, Miller JB. Pet-associated illness. *N Engl J Med.* 1985;313:985-995.
3. Jones RS, Lorber B. With man's best friend. In: Schlossberg D, ed. *Infections of Leisure.* New York, NY: Springer-Verlag NY Inc; 1994:181-203.
4. Weber DJ, Hansen AR. Infections resulting from animal bites. *Infect Dis Clin North Am.* 1991;5:663-680.
5. Goldstein EJC. Bites. In: Mandell GL, Bennett JE, Dolin R, eds. *Principles and Practice of Infectious Diseases.* New York, NY: Churchill Livingstone Inc; 1995:2765-2769.
6. Boyce JM. *Pasteurella* species. In: Mandell GL, Bennett JE, Dolin R, eds. *Principles and Practice of Infectious Diseases.* New York, NY: Churchill Livingstone Inc; 1995:2068-2070.
7. Gill VJ. *Capnocytophaga.* In: Mandell GL, Bennett JE, Dolin R, eds. *Principles and Practice of Infectious Diseases.* New York, NY: Churchill Livingstone Inc; 1995:2103-2106.
8. Koehler JE. *Bartonella* infections. In: Aronoff SC, ed. *Advances in Pediatric Infectious Diseases.* St Louis, Mo: Mosby–Year Book Inc; 1996;11:1-27.
9. Goldstein EJC, Greene EG. Around cats. In: Schlossberg D, ed. *Infections of Leisure.* New York, NY: Springer-Verlag NY Inc; 1994:229-242.
10. Lucey D, Dolan MJ, Moss W, et al. Relapsing illness due to *Rochalimaea henselae* in immunocompetent hosts: implication for therapy and new epidemiological associations. *Clin Infect Dis.* 1992;14:683-688.
11. Margileth AM. Cat scratch disease. In: Aronoff SC, ed. *Advances in Pediatric Infectious Diseases.* St Louis, Mo: Mosby–Year Book Inc; 1993;8:1-21.
12. Zangwill KM, Hamilton DH, Perkins BA, et al. Cat scratch disease in Connecticut: epidemiology, risk factors, and evaluation of a new diagnostic test. *N Engl J Med.* 1993;329:8-13.
13. Maurin M, Gasquet S, Ducco C, Raoult D. MICs of 28 antibiotic compounds for 14 *Bartonella* (formerly *Rochalimaea*) isolates. *Antimicrob Agents Chemother.* 1995;39:2387-2391.
14. Young EJ. *Brucella* species. In: Mandell GL, Bennett JE, Dolin R, eds. *Principles and Practice of Infectious Diseases.* New York, NY: Churchill Livingstone Inc; 1995:2053-2060.
15. Polt SS, Dismukes WE, Flint A, Shaefer J. Human brucellosis caused by *Brucella canis*: clinical features and immune response. *Ann Intern Med.* 1982;97:717-719.
16. Rumley RL, Chapman SW. *Brucella canis*: an infectious cause of prolonged fever of undetermined origin. *South Med J.* 1986;79:626-628.
17. Blaser MJ, Cravens J, Powers J, Wang WL. *Campylobacter* enteritis associated with canine infection. *Lancet.* 1978;2:979-981.
18. Salfield NJ, Pugh EJ. *Campylobacter* enteritis in young children living in households with puppies. *BMJ.* 1987;294:21-22.
19. Feigin RD, Lobes LAJ, Anderson D, Pickering L. Human leptospirosis from immunized dogs. *Ann Intern Med.* 1973;79:777-785.
20. Farrar WE. *Leptospira* species (leptospirosis). In: Mandell GL, Bennett JE, Dolin R, eds. *Principles and Practice of Infectious Diseases.* New York, NY: Churchill Livingstone Inc; 1995:2137-2141.
21. Penn RL. *Francisella tularensis* (tularemia). In: Mandell GL, Bennett JE, Dolin R, eds. *Principles and Practice of Infectious Diseases.* New York, NY: Churchill Livingstone Inc; 1995:2060-2069.
22. Miller SI, Hohmann EI, Pegues DA. Salmonella (including *Salmonella typhi*). In: Mandell GL, Bennett JE, Dolin R, eds. *Principles and Practice of Infectious Diseases.* New York, NY: Churchill Livingstone Inc; 1995:2013-2033.
23. Doll JM, Zeitz PS, Ettestad P, Bucholtz AL, Davis T, Gage K. Cat-transmitted fatal pneumonic plague in a person who traveled from Colorado to Arizona. *Am J Trop Med Hyg.* 1994;51:109-114.
24. Butler T. *Yersinia* species (including plague). In: Mandell GL, Bennett JE, Dolin R, eds. *Principles and Practice of Infectious Diseases.* New York, NY: Churchill Livingstone Inc; 1995:2070-2078.
25. Fukushima H, Gomyoda M, Ishikura S, et al. Cat-contaminated environmental substances lead to *Yersinia pseudotuberculosis* infection in children. *J Clin Microbiol.* 1989;27:2706-2709.
26. Peeling RW, Brunham RC. Chlamydia as pathogens: new species and new issues. *Emerg Infect Dis.* 1996;2:307-319.
27. Regan RJ, Dathan JRE, Treharne JD. Infective endocarditis with glomerulonephritis associated with cat chlamydia (*C psittaci*) infection. *Br Heart J.* 1979;42:349-352.
28. Otto G, Hazell SH, Fox JG, et al. Animal and public health implications of gastric colonization of cats by *Helicobacter*-like organisms. *J Clin Microbiol.* 1994;32:1043-1049.
29. Yang H, Dixon MF, Li X, Xu Z, Zhou D, Blum AL. Acute gastritis associated with infection of large spiral-shaped bacteria. *Am J Gastroenterol.* 1995;90:307-309.
30. Wegmann W, Aschwanden M, Schaub N, Aenishanslin W, Gyr K. Gastritis associated with *Gastrospirillum hominis*: a zoonosis? *Schweiz Med Wochenschr.* 1991;121:245-254.
31. Lavelle JP, Landas S, Mitros FA, Conklin JL. Acute gastritis associated with spiral organisms from cats. *Dig Dis Sci.* 1994;39:744-750.
32. Malnick H, Williams K, Phil-Eboise J, Levy AS. Description of a medium for isolating *Anaerobiospirillum* spp, a possible cause of zoonotic disease, from diarrheal feces and blood of humans and use of the medium in a survey of human, canine and feces. *J Clin Microbiol.* 1990;28:1380-1384.
33. Wilson KS, Maroney SA, Gander RM. The family pet as an unlikely source of group A beta-hemolytic streptococcal infection in humans. *Pediatr Infect Dis J.* 1995;14:372-375.
34. Pinsky RL, Fishbein DB, Greene CR, Genshelmer KF. An outbreak of cat-associated Q fever in the United States. *J Infect Dis.* 1991;164:202-204.
35. Langley JM, Marrie TJ, Covert A, Waag DM, Williams JC. Poker players' pneumonia: an urban outbreak of Q fever following exposure to a parturient cat. *N Engl J Med.* 1988;319:354-356.
36. Marrie TJ. *Coxiella burnetii* (Q fever). In: Mandell GL, Bennett JE, Dolin R, eds. *Principles and Practice of Infectious Diseases.* New York, NY: Churchill Livingstone Inc; 1995:1727-1735.
37. Raoult D. Treatment of Q fever. *Antimicrob Agents Chemother.* 1993;37:1733-1736.
38. Dumler JS, Bakken JS. Ehrlichial diseases of hu-

mans: emerging tick-borne infections. *Clin Infect Dis.* 1995;20:1102-1110.
39. Comer KM. Rocky Mountain spotted fever. *Vet Clin North Am Small Anim Pract.* 1991;21:27-44.
40. Walker DH, Raoult D. *Rickettsia rickettsii* and other spotted fever group rickettsiae (Rocky Mountain spotted fever and other spotted fevers). In: Mandell GL, Bennett JE, Dolin R, eds. *Principles and Practice of Infectious Diseases.* New York, NY: Churchill Livingstone Inc; 1995:1721-1727.
41. Croese J, Loukas A, Opdebeeck J, Fairley S, Prociv P. Human enteric infection with canine hookworms. *Ann Intern Med.* 1994;120:369-374.
42. Ludlam KE, Platt TR. The relationship of park maintenance and accessibility to dogs to the presence of *Toxocara* ova in soil. *Am J Public Health.* 1989;79:633-634.
43. Childs JE. The prevalence of *Toxocara* species ova in backyards and gardens of Baltimore, Maryland. *Am J Public Health.* 1985;75:1092-1094.
44. Taylor MRH, Keane CT, O'Connor P, Mulvihill E, Holland C. The expanded spectrum of toxocaral disease. *Lancet.* 1988;1:892-894.
45. Jacquier P, Gottstein B, Stingelin Y, Eckert J. Immunodiagnosis of toxocarosis in humans: evaluation of a new enzyme-linked immunosorbent assay kit. *J Clin Microbiol.* 1991;29:1831-1835.
46. Kollaritsch H, Jeschko E, Wiedermann G. Albendazole is highly effective against cutaneous larva migrans not against *Giardia* infection: result of an open pilot trial in travellers returning from the tropics. *Trans R Soc Trop Med Hyg.* 1993;87:689.
47. Davies HD, Sakuls P, Keystone JS. Creeping eruption: a review of clinical presentation and management of 60 cases presenting to a tropical disease unit. *Arch Dermatol.* 1993;129:588-591.
48. MacKenzie WR, Hoxie NJ, Proctor ME. A massive outbreak in Milwaukee of *Cryptosporidium* infection transmitted through the public water supply. *N Engl J Med.* 1994;331:162-167.
49. Current WL, Garcia LS. Cryptosporidiosis. *Clin Microbiol Rev.* 1991;4:325-358.
50. Markell EK, Voge M, John DT. *Medical Parasitology.* 7th ed. Philadelphia, Pa: WB Saunders Co; 1994:226-260.
51. Neva FA, Brown HW. *Basic Clinical Parasitology.* 6th ed. East Norwalk, Conn: Appleton & Lange; 1994.
52. Neafie RC, Marty AM. Unusual infections in humans. *Clin Microbiol Rev.* 1993;6:34-56.
53. Wijesundera MD. The use of praziquantel in human infection with *Dipylidium. Trans R Soc Trop Med Hyg.* 1989;83:383.
54. Jones WE. Niclosamide as a treatment for *Hymenolepis diminuta* and *Dipylidium caninum* infection in man. *Am J Trop Med Hyg.* 1979;28:300-302.
55. Glickman LT, Magnaval J-F. Zoonotic roundworm infections. *Infect Dis Clin North Am.* 1993;7:717-732.
56. Nicholson CP, Allen MD, Trastek VF, Tazelaar HD, Pairolero PC. *Dirofilaria immitis*: a rare, increasing cause of pulmonary nodules. *Mayo Clin Proc.* 1992;67:646-650.
57. Watson J, Wetzel WJ, Burkhalter J. Human disease caused by dog heartworm. *J Miss State Med Assoc.* 1991;32:399-401.
58. Remington JS, McLeod R. Toxoplasmosis. In: Gorbach SL, Bartlett JG, Blacklow NR, eds. *Infectious Diseases.* Philadelphia, Pa: WB Saunders Co; 1992:1328-1342.
59. Hill DR. *Giardia lamblia.* In: Mandell GL, Bennett JE, Dolin R, eds. *Principles and Practice of Infectious Diseases.* New York, NY: Churchill Livingstone Inc; 1995:2487-2493.
60. Katoh T, Maruyama R, Nishioka K, Sano T. Tinea corporis due to *Microsporum canis* from an asymptomatic dog. *J Dermatol.* 1991;18:356-359.
61. Moriello KA, Deboer DJ. Fungal flora of the haircoat of cats with and without dermatophytosis. *J Med Vet Mycol.* 1991;29:285-292.
62. Reed KD, Moore FM, Geiger GE, Stemper ME. Zoonotic transmission of sporotrichosis: case report and review. *Clin Infect Dis.* 1993;16:384-387.

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J Clin Diagn Res. 2016 Jul; 10(7): IL01–IL02. PMCID: PMC5020240
Published online 2016 Jul 1. doi: 10.7860/JCDR/2016/20723.8098

Open Defecation in India: A Major Health Hazard and Hurdle in Infection Control

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Received 2016 Apr 14; Revisions requested 2016 Apr 19; Accepted 2016 Apr 22.

Keywords: Clean India mission, Field defecation, Sanitation

Sir,

"Cleanliness is next to Godliness", a proverbial adage that traces its inception to ancient Indian times, is the epitome of irony in the current Indian health situation. The lost Indus Valley Civilization, with modern cities like Harappa and Mohenjodaro, was once the gold standard of sanitation infrastructure. Its extensive and efficient sewage system was not only an exemplary gem, but also a gift of knowledge to entire mankind. However history resides in books and has little relevance to the current situation.

Though over the last 50 years, the general health of Indians has improved and the life expectancy has increased, myriad health and sanitation problems still stare one in the face. The biggest one, open defecation, is the mother of all infection and morbidity. The WHO declared the year 2008 as International Year of Sanitation. It was here that the term 'Open Defecation' was widely publicized. Community Led Total Sanitation (CLTS) programs helped spread the term all around the globe.

It is a matter of national concern as India has the most number of people practicing open defecation in the world, around 600 million [1], and is followed by Indonesia, Pakistan, Nigeria and Ethiopia. Still these countries come nowhere close to the staggering number contributed by India.

Most of it occurs in villages with a prevalence of 65% [2]. In urban settings the prevalence is close to 16%. The problem has thick deep roots with a multi-factorial origin. Unavailability of proper toilets or toilets with dimly lit, broken or clogged latrines is common. However, the biggest problem is the mindset of people in both rural and urban settings. Children grow watching parents and grandparents practice open defecation. Most farmers believe that waking up early and defecating in the field, not only adds natural fertilizer to the soil, but also rejuvenates the bowel and the mind.

Open defecation is a major cause of fatal diarrhea. Everyday about 2000 children aged less than five succumb to diarrhea and every 40 seconds a life is lost [3]. It is depressing that all this needless suffering is actually preventable. In densely populated countries like India, the health impact is

magnified many fold [4]. There is evidence to suggest that water sanitation and hygiene practices are associated with child linear growth [5]. Children have a tendency to put common things in their mouth. In rural settings where open defecation is prevalent, large amounts of fecal pathogens via human and animal feces, are ingested by children. This creates a massive reservoir of bacteria, parasites and viruses that keep spreading gastrointestinal infection. An eventual result is growth stunting and malnutrition.

Though the health challenges seem to compound with time, the health budget allocation by the Government of India is getting smaller every year. This year also it is quite meager, only about 1% of the Gross Domestic Product. This may put financial constraints on dealing with sanitation linked diseases.

In 2013, the United Nations brought world attention to "Open Defecation" for the first time and World Toilet Day was celebrated. The UN has vowed to eliminate open defecation from the globe before 2025. In India, the largest contributor of open defecation to the world, widespread concern about abysmal sanitation practices is being slowly raised as well. An incident in Badaun district, where two teenage sisters who ventured out to defecate due to lack of a private toilet, were raped and murdered, stimulated the masses into anger and dissent. Investigating authorities admitted that 95% of all rapes in India occur when girls go out alone in secluded places, to urinate or defecate. Delinquent men have been known to cluster at such locations, awaiting helpless victims. Similar situation exists in many African countries like Kenya, Zimbabwe and Somalia. Thus, a direct correlation between crime and open defecation seems to exist.

The Government can employ a carrot and stick policy; incentives for building and using toilets and strict penalty if found openly defecating. A multipronged attack at the problem is essential and the Indian media has to play a key role in disseminating information. A vital driving force remains the political will. The incredulous rural population in India is the largest vote bank that determines fate of governments periodically. Lofty promises to eradicate open defecation and establish exemplary sanitation infrastructure have been made on several occasions before. But the problem has only gott worse. Health programs have failed mostly due to a waning momentum and lack of a true belief in the objective.

In order to reach maximum people, the government should explore collaborations with public and private sector organizations that function at the primary level to bring about behavioural change. Health schemes and demerits of open defecation should be advertised clearly on national media channels. Technological options, like self sustaining personal toilets should be explored. Leading think tanks should be encouraged to come up with ingenious and novel ways to curb the problem.

Wary of the burgeoning health threat, newly elected Prime Minister Narendra Modi, launched the nationwide 'Clean India Mission', literally with a broom in hand. The government's promise of "Toilets First, Temples Later" is the call of the hour, in a country where religion is a synonym of fanaticism and where health and hygiene often take a back seat. The movement plans to galvanize 3 million government workers and students to salvage the health of the country. The goal is to create a clean India before Mahatma Gandhi's 150th birthday in 2019. Nearly 800 million toilets with extensive sewage systems are to be built. The campaign aims to construct 17 million toilets a year to achieve its tall ambition. The World Bank estimates takes about 6-8 months to convince a village to adopt toilets and give up open defecation. In the CLTS concept, a village can be declared 'Open Defecation Free", if all its denizens use toilets regularly. Thus an intervention at the psyche of the people is required. Support from WHO, UNICEF and financial aid from private organizations, seems to show that India is

finally making the right noises at the world health stage. Wealth without health is of little consequence. Though India is emerging as an economic power, the overall health of the nation will be a useful barometer to gauge its true developmental progress. Putting an end to the menace of open defecation, requires a mass effort with a personal vendetta. Shying away complaining is no more an option. The National 'Clean India Movement", with a deadline of 2019, requires all citizens to get their hands dirty in order to clean the country. Exactly how this concerted effort pans out, remains to be seen.

Notes

Go to:

Financial or Other Competing Interests

None.

References

Go to:

[1] JMP (2014) Progress on drinking water and sanitation, 2014 Update. WHO/UNICEF Joint Monitoring Programme for Water Supply and Sanitation (JMP), ISBN 978924 1507240, page 19.

[2] JMP (2014) Progress on drinking water and sanitation, 2014 Update. WHO/ UNICEF Joint Monitoring Programme for Water Supply and Sanitation (JMP), ISBN 978 92 4 150724 0, Annex 3: Country, area or territory estimates on sanitation and drinking water.

[3] WHO Diarrhoeal Disease. WHO Int Retrieved 2014-03-10.

[4] Vyas S, et al. (2014). Population density and the effect of sanitation on early-life health, slide 19 (presentation at UNC conference in Oct. 2014) Research Institute for Compassionate Economics, project SQUAT (Sanitation Quality, Use, Access, and Trends): Evidence based sanitation advocacy for India (r.i.c.e.), USA.

[5] Ngure FM, Reid BM, Humphrey JH, et al. Water, sanitation, and hygiene (WASH), environmental enteropathy, nutrition, and early child development: making the links. Ann N Y Acad Sci. 2014;1308:118–28. [PubMed]