Review

Toxoplasmosis – a waterborne zoonosis

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Abstract

Humans become infected with *Toxoplasma gondii* mainly by ingesting uncooked meat containing viable tissue cysts or by ingesting food or water contaminated with oocysts from the feces of infected cats. Circumstantial evidence suggests that oocyst-induced infections in humans are clinically more severe than tissue cyst-acquired infections. Until recently, water-borne transmission of *T. gondii* was considered uncommon but a large human outbreak linked to contamination of a municipal water reservoir in Canada by wild felids and the widespread infection by marine mammals in the USA provide reasons to question this view. The present paper reviews information on the biology of oocyst-induced infections of *T. gondii* in humans and animals and examines possible importance of transmission by water.
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Contents

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1. Introduction

Infection with the protozoan *Toxoplasma gondii* is one of the most common parasitic infections of man and other warm-blooded animals (Dubey and Beattie, 1988). It has been found worldwide in nearly one-third of the human population (Dubey and Beattie, 1988; Tenter et al., 2000). In most adults it does not cause serious illness, blindness and mental retardation can result in congenitally infected children and severe disease in those with depressed immunity. Toxoplasmosis, until recently, was not considered a water-borne zoonosis. A major outbreak of toxoplasmosis in humans in Canada in 1994 (Bowie et al., 1997) was associated with *T. gondii* in municipal waters. Recently, *T. gondii* has been reported in many marine mammals, suggesting the possibility that the contamination of seawater with *T. gondii* may be more common than realized (Dubey et al., 2003b). This review focuses on water-borne aspects of *T. gondii*.

2. Life cycle

T. gondii is a coccidian parasite using felids as the definitive hosts, and warm-blooded animals as intermediate hosts (Frenkel et al., 1970; Dubey et al., 1970b). It is among the most common of parasites of animals and *T. gondii* is the only known species. Coccidia in general have complex life cycles. Although most are host-specific, and only transmitted by a fecal-oral cycle, *T. gondii* can also be transmitted transplacentally, and by carnivorism.

There are three infectious stages of *T. gondii* for all hosts: tachyzoites (individually and in groups), bradyzoites (in tissue cysts), and sporozoites (in oocysts) (Fig. 1).

The tachyzoite is often crescent-shaped and 2 μm × 6 μm in size. It enters the host cell by active penetration of the cell membrane and becomes surrounded by a parasitophorous vacuole that protects it from host defense mechanisms. The tachyzoite multiplies asexually by repeated binary divisions until the host cell ruptures.

After an unknown numbers of divisions, *T. gondii* tachyzoites give rise to another stage called a tissue cyst. Tissue cysts grow and remain intracellular. They vary in size from 5 to
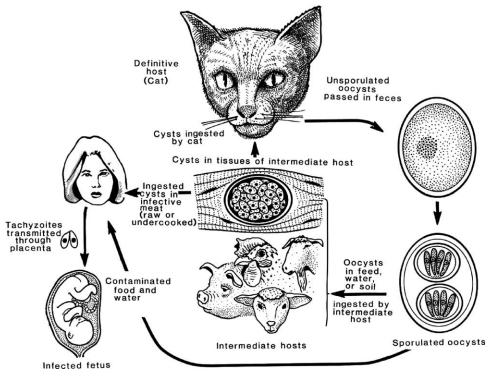
Fig. 1. Life cycle of *T. gondii*.

70 μm and contain a few to several hundred bradyzoites (Dubey et al., 1998a). Although tissue cysts may develop in visceral organs, including lungs, liver, and kidneys, they are more prevalent in muscular and neural tissues, including the brain, eye, skeletal, and cardiac muscle. Intact tissue cysts are probably harmless and can persist for the life of the host (Dubey et al., 1998a).

The tissue cyst wall is elastic, thin (<0.5 μm), and may enclose hundreds of crescent-shaped slender bradyzoites each measuring 7 μm × 1.5 μm. Bradyzoites differ only slightly from tachyzoites in having a nucleus situated toward the posterior end whereas the nucleus in tachyzoites is more central. As well, bradyzoites are more slender than are tachyzoites and less susceptible to destruction by proteolytic enzymes.

Upon ingestion by cats, the wall of the tissue cyst is digested by the proteolytic enzymes in the stomach and small intestine and bradyzoites are released. Some penetrate the lamina propria of the intestine and multiple as tachyzoites. Within a few hours, *T. gondii* may disseminate to extra-intestinal tissues. Other bradyzoites penetrate epithelial cells of the small intestine and initiate development of numerous generations of asexual (types A–E schizonts) (Dubey and Frenkel, 1972). The organisms (merozoites) released from schizonts form male and female gametes. The male gamete has two flagella

and it swims to and enters the female gamete. After the female gamete is fertilized by the male gamete, oocyst wall formation begins around the fertilized gamete. When oocysts are mature, they are discharged into the intestinal lumen by the rupture of intestinal epithelial cells. *T. gondii* persists in intestinal and extra-intestinal tissue of cats for at least several months, and possibly for the life of the cat.

Oocysts of *T. gondii* are formed only in cats, including both domestic and wild felids. Cats shed oocysts after ingesting tachyzoites, bradyzoites, or sporozoites (Frenkel et al., 1970; Dubey and Frenkel, 1972, 1976; Freyre et al., 1989; Dubey, 1996a, 2002a). However, less than 50% of cats shed oocysts after ingesting tachyzoites or oocysts whereas nearly all shed oocysts after ingesting tissue cysts (Dubey and Frenkel, 1976).

Oocysts in freshly passed feces are unsporulated (non-infective) and subspherical to spherical in shape and 10 μm $\times$ 12 μm in diameter. Sporulation occurs outside the cat and within 1–5 days, depending upon aeration and temperature. Sporulated oocysts contain two ellipsoidal sporocysts. Each sporocyst contains four sporozoites. The sporozoites are 2 μm $\times$ 6–8 μm in size (Dubey et al., 1998a).

Hosts, including felids can acquire *T. gondii* by ingesting either tissues of infected animals or food or drink contaminated with sporulated oocysts, or by transplacental transmission. After ingestion, bradyzoites released from tissue cysts or sporozoites from oocysts penetrate intestinal tissues, transform to tachyzoites, multiply locally, and are disseminated in the body via blood or lymph. After a few multiplication cycles, tachyzoites give rise to bradyzoites in a variety of tissues. *T. gondii* infection during pregnancy can lead to infection of the fetus. Congenital toxoplasmosis in humans, sheep, and goats can kill the fetus.

The life cycle of oocyst-transmitted infection has been studied in mice (Dubey et al., 1997a; Speer and Dubey, 1998). After ingestion of sporulated oocysts, sporozoites excyst, penetrate enterocytes and goblet cells of the intestinal epithelium, and are carried to the lamina propria via an unknown mechanism. Some sporozoites can be found circulating in peripheral blood as early as 4 h after ingestion. However, most remain in the lamina propria where they multiply in a variety of cells including vascular endothelium, fibroblasts, mononuclear cells and segmented leukocytes, but not in erythrocytes. Edema, necrosis of the lamina propria, and sloughing of the intestinal mucosa can produce severe enteritis. Infection can eventually spread to all other organs.

3. Host–parasite relationship

T. gondii can multiply in most mammalian cells. How it is destroyed by immune cells is not completely known. All extracellular forms of the parasite are directly affected by antibodies but intracellular forms are not. Cellular factors, including lymphocytes and lymphokines, are thought to be more important than humoral factors in the immune mediated destruction of *T. gondii*.

Immunity does not eliminate an established infection. *T. gondii* tissue cysts persist several years after acute infection. The ultimate fate of tissue cysts is not fully known. Some may rupture during the life of the host and the released bradyzoites be destroyed by

the host's immune responses. However, in immunosuppressed individuals, infection can be reactivated by dissemination of bradyzoites and conversion to tachyzoites.

Pathogenicity of *T. gondii* is determined by many factors including the susceptibility of the host species, virulence of the parasitic strain and the stage. Oocyst-induced infections are the most severe clinically in intermediate hosts, and this is not dose-dependent.

T. gondii has also adapted to an oocyst-oral cycle in herbivores (intermediate hosts) and tissue cyst-oral cycle in carnivores, especially in the cat. *T. gondii* oocysts are less infective and less pathogenic for the cat than for rats and mice (Dubey, 1996a,b). For example, one live oocyst is orally infective to mice and pigs (Dubey et al., 1996) whereas 100 or more oocysts may be required to establish infection in a cat (Dubey, 1996a). The reverse may be true for bradyzoites. By mouth, bradyzoites are less infective to mice than cats (Dubey, 2001). Cats can shed millions of oocysts after ingesting as few as one bradyzoite whereas 100 bradyzoites may not be infective to mice by the oral route (Dubey, 2001). Although *T. gondii* can be transmitted orally by ingesting tissue cysts, epidemiologic evidence indicates that cats are essential in perpetuation of the life cycle as *T. gondii* infection is rare or absent in areas devoid of cats (Wallace, 1969; Munday, 1972; Dubey et al., 1997b).

4. Epidemiology

T. gondii oocysts are shed by domestic cats and other felines resulting in widespread contamination of the environment (Jewell et al., 1972; Dubey and Beattie, 1988; Lukešová and Literák, 1998; Ocholi et al., 1989; Dubey and Odening, 2001). Domestic cats are probably the major source of contamination as they are common and produce large numbers of oocysts (Dubey and Frenkel, 1972; Dubey, 2001). Sporulated oocysts survive for long periods under moderate environmental conditions. For example, they can survive in moist soil for months to years (Dubey and Beattie, 1988). Oocysts in soil can be spread mechanically by flies, cockroaches, dung beetles, and earthworms. Oocysts are known to survive on fruits and vegetables for long periods (Kniel et al., 2002). Humans may acquire toxoplasmosis by petting dogs that have rolled over in infected cat feces (Frenkel et al., 1995; Lindsay et al., 1997).

While only a few cats may be shedding *T. gondii* oocysts at any given time (Table 1) the enormous numbers produced and their resistance to destruction (Tables 2 and 3) assure widespread contamination. Latently infected cats can shed oocysts after challenge infection (Dubey, 1976, 1995; Ruiz and Frenkel, 1980b). Congenitally infected kittens can also excrete oocysts. (Dubey and Johnstone, 1982; Dubey and Carpenter, 1993b). Infection rates in cats are largely determined by the rate of infection in the local avian and rodent populations, which serve as a food source. For example, *T. gondii* oocysts were found in 23.2% of 237 cats in Costa Rica where infection in local rodents and birds was high (Ruiz and Frenkel, 1980a). For epidemiologic surveys (Table 4), seroprevalence data for cats are more useful than results of fecal examination because cats with antibodies have probably already shed oocysts and are an indicator of environmental contamination (Dubey and Frenkel, 1972).

Table 1
Select reports of *T. gondii* oocysts[a] in feces of naturally infected cats

Country	No. of fecal samples	No. of positive	Prevalence (%)	Ref.
Australia	74	1	1.3	Jakob-Hoff and Dunsmore (1983)
Brazil	185	1	0.5	Nery-Guimarães and Lage (1973)
Costa Rica	237	55	23.2	Ruiz and Frenkel (1980b)
Egypt	213	88	41.3	Rifaat et al. (1976)
Germany	502	5	1.0	Janitschke and Kuhn (1972)
Hungary	200	2	1.0	Varga (1982)
Italy	250	1	0.4	Pampiglione et al. (1973)
Japan	446	4	0.8	Ito et al. (1974)
Spain	169	1	0.6	Aparicio Garrido et al. (1972)
Uruguay	138	1	0.7	Freyre et al. (1981)
USA	1604	12	0.7	Wallace (1971, 1973)
	1000	7	0.7	Dubey et al. (1977)
	274	5	1.8	Dubey et al. (1995)

[a] Demonstrated by mouse bioassay.

5. Clinical toxoplasmosis in animals

Severe disease is caused by *T. gondii* in many species of animals and has been reviewed by Dubey and Beattie (1988) and Tenter et al. (2000). This includes embryonic death and resorption, fetal death and mummification, abortion, stillbirth and neonatal death in goats and sheep. Outbreaks of toxoplasmosis in pigs cause higher mortality in young pigs than in adult pigs. Sporadic and widespread outbreaks of toxoplasmosis occur in rabbits, mink, birds and other domesticated and wild animals. Toxoplasmosis causes severe, often fatal disease in Australasian marsupials, New World monkeys, Pallas cats, and canaries (*Serinus canarius*) (Dubey and Beattie, 1988; Dubey and Odening, 2001; Dubey, 2002b; Kenny et al., 2002).

Among companion animals, fatal toxoplasmosis may occur in dogs that are immunosuppressed following infection with concurrent distemper virus (Dubey et al., 1989). Although cats of any age can die of toxoplasmosis, kittens and those with depressed immunity are the most likely (Dubey and Carpenter, 1993a,b).

6. Toxoplasmosis in humans

T. gondii infection is widespread in humans and prevalence varies with geography and increase in age. In the USA and the UK it is estimated that 16–40% of people become infected whereas in Central and South America and continental Europe infection estimates reach 50–80% (Dubey and Beattie, 1988; Tenter et al., 2000; Jones et al., 2001b). Infections in healthy adults are usually asymptomatic; however, severe disease can occur in immunocompomised individuals and newborns.

Congenital infection may occur following maternal infection during pregnancy. The severity of the disease may depend upon the stage of pregnancy at the time of infection. A wide spectrum of clinical disease occurs in congenitally infected children (Remington

Table 2
Effect of disinfectants on *T. gondii* oocysts

Reagent	Concentration (%)	Duration of treatment	Killed	Ref.
Formalin	10	48 h	No	Ito et al. (1975)
Sulfuric acid + dichromate	63/7	30 min	No	Dubey et al. (1970a)
	63/7	24 h	Yes	
Ethanol + acetic acid	95/5	1 h	No	Dubey et al. (1970a)
	95/5	24 h	Yes	
Ammonium hydroxide	5.0	10 min	No	Dubey et al. (1970a)
	5.0	30 min	Yes	Dubey et al. (1970a)
Sodium hyporchlorite (Purex)	6.0	24 h	No	Dubey et al. (1970a)
Sodium lauryl sulfate	0.1	24 h	No	Dubey et al. (1970a)
Cetyl trimethyl ammonium	0.1	24 h	No	Dubey et al. (1970a)
Tween 80	0.1	24 h	No	Dubey et al. (1970a)
Ammonia[a], liquid	5.5	1 h	No	Frenkel and Dubey (1972)
	5.5	3 h	Yes	Frenkel and Dubey (1972)
Tincture of Iodine	2.0	10 min	No	Frenkel and Dubey (1972)
	2.0	3 h	Yes	Frenkel and Dubey (1972)
	7.0	10 min	Yes	Frenkel and Dubey (1972)
Aldesol[b]	33	24 h	No	Kuticic and Wikerhauser (1993)
Tincture of Hibisept[c]		24 h	No	Kuticic and Wikerhauser (1993)
Izosan-G[d]	0.02	24 h	No	Kuticic and Wikerhauser (1993)
Drying at relative humidity	19	11 days	Yes	Frenkel and Dubey (1972)
	0	2 days	No	Frenkel and Dubey (1972)
	60–68%	7 weeks	No	Kuticic and Wikerhauser (1994)
Lomasept	1	1 h	No	Ito et al. (1975)
		3 h	Yes	Ito et al. (1975)
Neo Kurehasol	5	24 h	No	Ito et al. (1975)
Paracetic acid	5	48 h	Yes	Ito et al. (1975)

[a] Undiluted household ammonia.
[b] Aldesol, a solution for disinfection, contains 5 g benzalchoniumchloride, 6 g glutaraldehyde, and 8 g gloxal in 100 g of solution.
[c] Hibisept tincture contains 0.5 g chorhexidine gluconate in 70% ethanol in 100 ml of tincture.
[d] Izosan-G granulate contains 99 g of sodium dichloroizicyanurate-dihydrate in 100 g granulate.

et al., 2001; Jones et al., 2001a). Mild disease may consist of slightly diminished vision only whereas severely diseased children may have the full tetrad of signs including retinochoroiditis, hydrocephalus, convulsions, and intracerebral calcification. Of these, hydrocephalus is the least common but most dramatic lesion of toxoplasmosis. This condition is unique to congenitally acquired toxoplasmosis in humans and has not been reported in other animals.

The socioeconomic impact of toxoplasmosis in terms of human suffering and long term care of children with mental retardation and blindness are enormous (Roberts et al., 1994). Testing of all pregnant women for *T. gondii* infection is compulsory in France and Austria, and the cost benefits of such mass screening are being debated in many countries (Remington et al., 2001). Toxoplasmosis is considered as a leading cause of food related illness in humans in the United States (Mead et al., 1999).

Postnatally acquired toxoplasmosis in humans can be localized or generalized. Lymphadenitis is the most frequently observed clinical form of toxoplasmosis in humans.

Table 3
Effect of environmental influences on *T. gondii* oocysts

Temperature (°C)	Medium	Duration of treatment	Killed	Ref.
Indoors				
−21	Water	28 days	No	Frenkel and Dubey (1973)
4	2% H_2SO_4	578 days	No	Dubey (1977)
4	Water	1620 days	No	Dubey (1998)
10	Water	200+ days	No	Dubey (1998)
15	Water	200+ days	No	Dubey (1998)
20–22	Water	548 days	No	Hutchison (1967)
30	Water	107 days	No	Dubey (1998)
35	Water	32 days	No	Dubey (1998)
40	Water	28 days	Yes	Dubey (1998)
40	Water	24 h	No	Dubey (1998)
45	Water	3 days	Yes	Dubey (1998)
50	Water	60 min	No	Dubey et al. (1970a)
52	Water	5 min	No	Dubey (1998)
55	Water	1 min	No	Dubey (1998)
		2 min	Yes	Dubey (1998)
60	Water	1 min	Yes	Dubey (1998)
Outdoors				
Texas				
−6.5 to 37.5	Native feces	334–410 days	No	Yilmaz and Hopkins (1972)
Kansas				
−20 to 35	Native feces	548 days	No	Frenkel et al. (1975)
Costa Rica				
15–30	Native feces	56–357 days	No	Frenkel et al. (1975)

Lymphadenopathy may be associated with fever, malaise, fatigue, muscle pain, sore throat and headache. Although the condition may be benign, its diagnosis is vital in pregnant women because of the risk to the fetus. Encephalitis is the most clinically important manifestation of toxoplasmosis in immunosuppressed patients. Toxoplasmosis is a major cause of death among patients with AIDS.

Table 4
Seroprevalence of *T. gondii* antibodies in domestic cats from the United States

Location	Type of cats	No. of cats	% Seropositive	Serologic test	Cut-off titer	Ref.
Ohio	Urban	1000	39	IFAT[a]	1:16	Claus et al. (1977)
Ohio	Rural	275	48	MAT[b]	1:25	Dubey et al. (2002b)
Iowa	Rural	74	42	MAT	1:32	Smith et al. (1992)
Illinois	Rural	391	68	MAT	1:25	Dubey et al. (1995)
Rhode Island	Urban	200	42	MAT	1:25	DeFeo et al. (2002)
Iowa	Rural	157	58	DT[c]	1:4	Dubey (1973)
Kansas	Rural	510	16	DT	1:4	Dubey (1973)

[a] Indirect fluorescent antibody test.
[b] Modified agglutination test.
[c] Dye test.

7. Oocyst-transmitted toxoplasmosis

There are currently no tests to identify the source of infection in an individual, i.e. either oocysts or meat. Evidence is based exclusively on epidemiological surveys. In certain areas of Brazil, approximately 60% of 6–8-year-old children have antibodies to *T. gondii* linked to the ingestion of oocysts in a heavily contaminated environment with *T. gondii* oocysts (Bahia-Oliveira et al., 2003).

An outbreak of toxoplasmosis in humans of a western Canadian city was linked epidemiologically to oocyst contamination of a municipal water supply (Bowie et al., 1997; Burnett et al., 1998). Between 2894 and 7718 persons were considered to have acquired *T. gondii* infection. Among these, 100 cases of acute toxoplasmosis were reported in patients 6–83 years old. Of these 100 patients, 37 were women identified through a routine perinatal screening program. The most striking clinical feature of this outbreak was the occurrence of ocular toxoplasmosis in 20 patients, seven of whom were 75–83 years old. Eight patients had generalized toxoplasmosis with symptoms of night sweats, fever, chills and headaches. Fifty-one persons had enlarged lymph nodes. All had unusually high levels of IgG, IgM and IgE antibodies, which had not previously been described in groups of patients with acute toxoplasmosis retinitis. There were 12 additional congenitally infected children born to women who had acquired *T. gondii* during pregnancy. Six of these had retinal lesions, three of which were bilateral. Although oocysts were not identified in the municipal reservoir, runoff from soil contaminated with feces of infected domestic cats or cougars was considered the likely source (Isaac-Renton et al., 1998; Aramini et al., 1998, 1999).

Similar but smaller oocyst-associated outbreaks of toxoplasmosis have been reported previously including infection of 31 young army recruits on a jungle exercise in Panama (Benenson et al., 1982) and 35 individuals who had used a riding stable in Atlanta, Georgia (Teutsch et al., 1979). The Panamanian outbreak was linked to drinking water from a local pond although the water had been treated with iodine tablets. In the Panamanian outbreak, 90% had fever, 77% had headaches and lymphadenopathy, 68% had myalgia, 55% had abdominal pain and stiff neck and 26% had eye pain.

The Atlanta outbreak was linked to nasopharyngeal ingestion of oocysts aerosolized in the riding stable where *T. gondii* infected cats were present (Teutsch et al., 1979; Dubey et al., 1981). The symptoms were similar to those in the Panamanian outbreak. Whether severity of these outbreaks in Panama, USA, and Canada was due to the numbers of oocysts ingested, the virulence of the parasite or the susceptibility of persons is not known.

Numerous reports exist of *T. gondii* infections in marine mammals including sea otters, dolphins, seals, whales (Dubey et al., 2003b) and toxoplasmosis has been considered a cause of death in sea otters (Miller et al., 2002a,b) yet how marine mammals become infected is unknown. Ingesting oocysts directly from seawater or ingesting tissues of animals that have ingested oocysts are most likely. Felids are the only known hosts that can excrete environmentally resistant oocysts. As stated earlier, individual cats can excrete millions of oocysts, and there are >100 million cats in the United States. The prevalence of *T. gondii* in feral cats is very high (Dubey, 1973; Dubey et al., 1995, 2002b). Miller et al. (2002b) presented evidence that coastal fresh water surface runoff presented a risk of infection to sea otters, so it is possible that *T. gondii* oocysts could be washed into the sea

via runoff contaminated by cat excrement. Grey seals developed patent *T. gondii* infection after they were fed oocysts (Gajadhar et al., 2004). *T. gondii* oocysts are extremely resistant to environmental influences (Tables 2 and 3) and oocysts can sporulate and survive in seawater for months (Lindsay et al., 2003). Even unsporulated oocysts can remain viable at 4 °C for months (Lindsay et al., 2002).

The role of marine invertebrates in the life cycle of *T. gondii* is unknown. Although *T. gondii* does not parasitize cold-blooded animals, mollusks are filter feeders of water and may thus concentrate oocysts from the water. Experimentally, *T. gondii* oocysts were removed by oysters from water tanks seeded with oocysts (Lindsay et al., 2001b). Viable *T. gondii* was recovered from tissues of oysters for at least 21 day postexposure with oocysts using bioassay in mice (Lindsay et al., 2004). Similarly, (Arkush et al., 2003) found viable *T. gondii* in tissues of mussels (*Mytilus galloprovincialis*) 3 days postexposure.

T. gondii infection of dolphins is even more intriguing because they drink little or no water and their nutritional requirements are derived from fish, squid, or other cold-blooded sea animals they consume. Finding *T. gondii* antibodies in 100% of the wild Atlantic bottlenose dolphins from the USA raises concerns about the specificity of low antibody titers and needs confirmation by mouse bioassay (Dubey et al., 2003b).

8. Detection of *T.* gondii oocysts in the environment

Oocysts are detected in the feces of infected cats by concentration methods (e.g. flotation in high density sucrose solution) because too few may be present to be detected by direct smear (Ruiz and Frenkel, 1980b). In one study, oocysts were detected microscopically in only 12.7% and by mouse assay in additional 87.3% of naturally infected cats (Ruiz and Frenkel, 1980b). For definitive identification, *T. gondii* oocysts should be sporulated and then bioassayed in mice to distinguish them from other related coccidians. At any given time only 1% of cats will be shedding oocysts (Dubey and Beattie, 1988). Therefore, for epidemiological surveys, detection of *T. gondii* oocysts in cat feces is impractical and less informative. Determining serological prevalence is a better measure of *T. gondii* infection, assuming that seropositive cats have already shed *T. gondii* oocysts (Dubey and Frenkel, 1972). In an epidemiological survey for *T. gondii* on pig farms, oocysts were detected in only 5 of 274 (1.8%) samples of cat feces, 2 of 491 (0.4%) samples of feed and 1 of 79 (1.3%) samples of soil yet 267 of 391 (68.3%) cats had antibodies to *T. gondii* (Dubey et al., 1995). Serologic surveys indicate that approximately 50% of cats surveyed in the United States have antibodies to *T. gondii* (Table 4) and most of these cats probably ceased shedding oocysts.

Although *T. gondii* oocysts have been isolated from soil, there is no simple reliable method for large-scale epidemiological use. Bioassay of soil samples by feeding to pigs and chickens may be more sensitive than direct determination of oocysts in soil since pigs can be infected by feeding as few as one oocyst (Dubey et al., 1996). In a study of feral chickens, *T. gondii* was isolated from 54% of 50 chickens by bioassay in mice (Ruiz and Frenkel, 1980a). Because feral chickens on small farms feed from the ground, finding *T. gondii* in chickens may be a good indicator of infection in the environment. Recently, serological surveys and isolation of viable *T. gondii* in free ranging chickens

were used to asses environmental contamination with oocysts in rural area of São Paulo, Rio de Janeiro, Brazil with high endemicity of toxoplasmosis in humans (Silva et al., 2002; Dubey et al., 2002a, 2003a). Epidemiologic evidence indicated that drinking water contaminated with oocysts was the primary source of infection (Bahia-Oliveira et al., 2003).

Detection of *T. gondii* oocysts in water is more difficult than that of other coccidian oocysts and there are no standardized methods to do it. Attempts to recover *T. gondii* oocysts from water samples in the British Columbia outbreak were unsuccessful (Isaac-Renton et al., 1998). Dumètre and Dardé (2003) and Dubey et al. (2002a) suggested several methods that may be used to detect *T. gondii* oocysts in the water based on experiences with detection of the related coccidians, *Cryptosporidium* and *Giardia* species. These include, concentration of oocysts by centrifugation, filtration through small pore filters, elution of oocysts from filters, immunomagnetic separation and fluorescence-activated cell sorting (Dumètre and Dardé, 2003). *T. gondii* oocysts cannot be distinguished by direct microscopic examination because the oocysts of at least four other coccidians, *Hammondia hammondi*, *H. heydorni*, *Neospora caninum*, and *Besnoitia* species are morphologically similar. *T. gondii* oocysts have a specific pattern of autofluorescence that may be useful in identification of these coccidians (Lindquist et al., 2003). At present there are no commercial reagents available to detect *T. gondii* oocysts in the environment. Detection of *T. gondii* oocysts in municipal water systems is more difficult than the detection of cryptosporidial oocysts because relatively few *T. gondii* oocysts are likely to be present. *T. gondii* oocysts are likely to survive flocculation methods used to remove some of the impurities from the water (Kourenti et al., 2003) and it is noteworthy that the Panamanian outbreak of toxoplasmosis was associated with consumption of pond water that had been treated with salt tablets.

Methods to extract DNA from *T. gondii* oocysts have not been standardized. Arkush et al. (2003) reported on detection of DNA in tissues of mussels that were experimentally contaminated with *T. gondii* oocysts. They found *T. gondii* DNA up to 18 days postexposure of mussels but viable oocysts were detected only for 3 days exposure (Arkush et al., 2003). The molecular detection of DNA does not distinguish viable from non-viable oocysts and thus its utility will be limited. Villena et al. (2004) found *T. gondii* DNA in 10 of 125 environmental water samples but none were positive by bioassay in mice.

9. Prevention and control

At present there are no specific recommendations that will prevent transmission of *T. gondii* by drinking water because the level of contamination is unknown and because it can be transmitted by several modes. Therefore general recommendations are stated. To prevent infection of human beings by *T. gondii*, people handling meat should wash their hands thoroughly with soap and water before going to other tasks (Dubey and Beattie, 1988; Lopez et al., 2000). All cutting boards, sink tops, knives, and other materials contacting uncooked meat should also be washed with soap and water. Washing is effective because the stages of *T. gondii* in meat are killed by contact with soap and water

(Dubey and Beattie, 1988). *T. gondii* in meat are killed by exposure to extreme cold or heat. Tissue cysts in meat are killed by heating to an internal temperature of 67 °C (Dubey et al., 1990) or by cooling to −13 °C (Dubey, 1974; Kotula et al., 1991). *T. gondii* in tissue cysts or oocysts is killed by exposure to 0.5 krad of gamma irradiation (Dubey and Thayer, 1994, Dubey et al., 1998b). Meat of any animal should be cooked to a minimum of 67 °C before consumption, and tasting meat while cooking or while seasoning should be avoided.

Pregnant women should avoid contact with cats, cat litter, soil, and raw meat. Pet cats should be fed only dry, canned, or cooked food and the cat litter box should be emptied daily. Gloves should be worn while gardening and vegetables should be washed thoroughly before eating because they may have been contaminated with cat feces. People should avoid drinking unfiltered water from lakes, ponds, and rivers. Access to water reservoirs by cats should be prevented.

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