UPDATE

Toxoplasma gondii: transmission, diagnosis and prevention

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Toxoplasmosis, caused by the protozoan parasite *Toxoplasma gondii*, is one of the most common parasitic infections of man and other warm-blooded animals. It has been found world-wide from Alaska to Australia. Nearly one-third of humanity has been exposed to this parasite. In most adults it does not cause serious illness, but it can cause blindness and mental retardation in congenitally infected children and devastating disease in immunocompromised individuals.

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Toxoplasma gondii infection is widespread in humans, although its prevalence varies widely from place to place. In the United States and the United Kingdom, it is estimated that 16–40% of the population are infected, whereas in Central and South America and continental Europe, estimates of infection range from 50 to 80% [1]. Most infections in humans are asymptomatic but at times the parasite can produce devastating disease. Infection may be congenitally or postnatally acquired. Congenital infection occurs only when a woman becomes infected during pregnancy. Congenital infections acquired during the first trimester are more severe than those acquired in the second and third trimester [2,3]. While the mother rarely has symptoms of infection, she does have a temporary parasitemia. Focal lesions develop in the placenta and the fetus may become infected. At first there is generalized infection in the fetus. Later, infection is cleared from the visceral tissues and may localize in the central nervous system. A wide spectrum of clinical diseases occurs in congenitally infected children [2]. Mild disease may consist of slightly diminished vision, whereas severely diseased children may have the full tetrad of signs: retinochoroiditis, hydrocephalus, convulsions and intracerebral calcification. Of these, hydrocephalus is the least common, but most dramatic, lesion of toxoplasmosis. By far the most common sequel of congenital toxoplasmosis is ocular disease [2,3].

The socio-economic impact of toxoplasmosis in human suffering and the cost of care of sick children, especially those with mental retardation and blindness, are enormous [4,5]. The testing of all pregnant women for *T. gondii* infection is routine in some European countries, including France and Austria. The cost-benefit of such mass screening is being debated in many other countries [3].

Postnatally acquired infection may be localized or generalized. Humans become infected by ingesting tissue cysts in undercooked or uncooked meat or by ingesting food and water contaminated with oocysts from infected cat faeces. Oocyst-transmitted infections may be more severe than tissue cyst-induced infections [1,6–10]. Enlarged lymph nodes are the most frequently observed clinical form of toxoplasmosis in humans (Table 1). Lymphadenopathy may be associated with fever, 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. In an outbreak in British Columbia, of 100 people who were diagnosed with acute infection, 51 had lymphadenopathy and 20 had retinitis [7,8].

Encephalitis is the most important manifestation of toxoplasmosis in immunosuppressed

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Table 1 Frequency of symptoms in people with postnatally acquired toxoplasmosis

Symptoms	Patients with symptoms (%)	
	Atlanta outbreak[a] (35 patients)	Panama outbreak[b] (35 patients)
Fever	94	90
Lymphadenopathy	88	77
Headache	88	77
Myalgia	63	68
Stiff neck	57	55
Anorexia	57	NR[c]
Sore throat	46	NR
Arthralgia	26	29
Rash	23	0
Confusion	20	NR
Earache	17	NR
Nausea	17	36
Eye pain	14	26
Abdominal pain	11	55

[a]From Teutsch et al. [9].
[b]From Benenson et al. [6].
[c]Not reported.

patients as it causes the most severe damage to the patient [1]. Infection may occur in any organ. Patients may have headache, disorientation, drowsiness, hemiparesis, reflex changes and convulsions, and many become comatose. Encephalitis caused by *T. gondii* is now recognized with great frequency in patients treated with immunosuppressive agents.

Toxoplasmosis ranks high on the list of diseases which lead to death in patients with acquired immunodeficiency syndrome (AIDS); approximately 10% of AIDS patients in the USA and up to 30% in Europe are estimated to die from toxoplasmosis [11] Although in AIDS patients any organ may be involved, including the testis, dermis and the spinal cord, infection of the brain is most frequently reported. Most AIDS patients suffering from toxoplasmosis have bilateral, severe and persistent headache which responds poorly to analgesics. As the disease progresses, the headache may give way to a condition characterized by confusion, lethargy, ataxia and coma. The predominant lesion in the brain is necrosis, especially of the thalamus [12].

Contamination of the environment by oocysts is widespread as oocysts are shed by domestic cats and other members of the Felidae [1,13] (Figure 1). Domestic cats are probably the major source of contamination since oocyst formation is greatest in domestic cats. Cats may excrete millions of oocysts after ingesting only one bradyzoite or one tissue cyst, and many tissue cysts may be present in one infected mouse [14,15]. Generally, only about 1% of cats in a population are found to be shedding oocysts at any given time. Oocysts are shed for

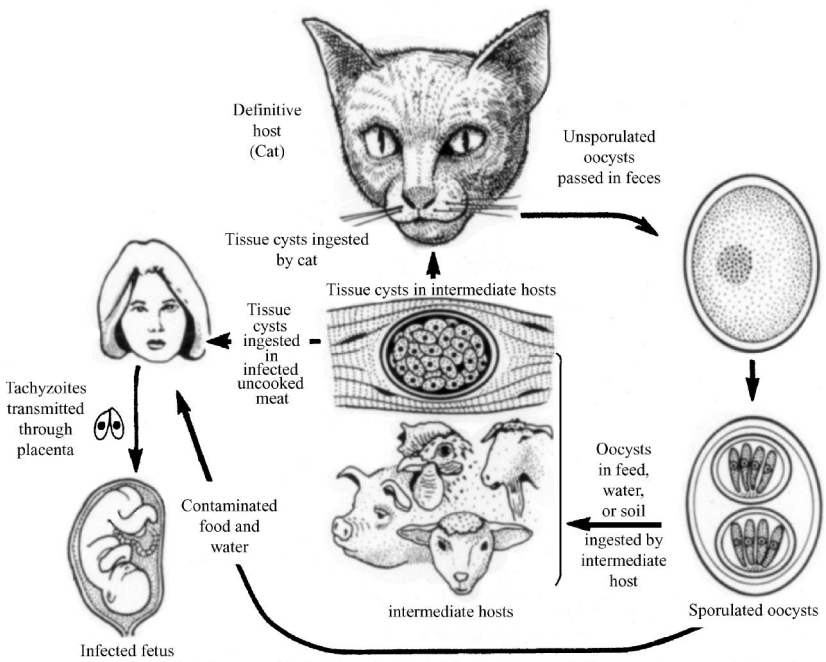

Figure 1 Life cycle of *Toxoplasma gondii*.

only a short period (1–2 weeks) in the life of the cat [1], however, the enormous numbers shed assure widespread contamination of the environment. Under experimental conditions, infected cats can shed oocysts after reinoculation with tissue cysts [16]. It is not known whether repeated shedding of oocysts occurs in nature, but this would greatly facilitate oocyst spread. Sporulated oocysts survive for long periods under most ordinary environmental conditions. They can survive in moist soil, for example, for months and even years [1]. Oocysts in soil do not always stay there, as invertebrates such as flies, cockroaches, dung beetles and earthworms can mechanically spread these oocysts and even carry them onto food. Congenital infection can occur in cats, and congenitally infected kittens can excrete oocysts, providing another source of oocysts for contamination. Infection rates in cats reflect the rate of infection in local avian and rodent populations because cats are thought to become infected by eating these animals. The more oocysts there are in the environment, the more likely it is that prey animals will become infected, and this results in increased infection rates in cats. Oocysts can be detected by examination of cat feces, though for epidemiological surveys, detection of *T. gondii* oocysts in cat feces is not practical. Concentration methods (e.g. flotation in high-density sucrose solution) are often used because the number of *T. gondii* oocysts in cat feces may be too few to be detected by direct smear. For definitive identification, *T. gondii* oocysts should be sporulated and then bioassayed in mice to distinguish them from other related coccidians [1]. Determining serological prevalence is a better measure of exposure of cats to *T. gondii* infection than detection of oocysts. It is a fair assumption that cats that are seropositive have already shed *T. gondii* oocysts.

Currently, there are no tests which can discriminate between oocyst ingestion and tissue cyst ingestion as the infection route. Available evidence for the oocyst infection route is based upon epidemiological surveys. For example, in certain areas of Brazil, approximately 60% of 6–8-year-old children have antibodies to *T. gondii* linked to the ingestion of oocysts from an environment heavily contaminated with *T. gondii* oocysts [17]. Infections in aquatic mammals indicate contamination and survival of oocysts in sea water [18]. The largest outbreak of clinical toxoplasmosis in humans was epidemiologically linked to drinking water from a municipal water reservoir in British Columbia, Canada [19]. This water reservoir was thought to be contaminated with *T. gondii* oocysts excreted by cougars (*Felis concolor*) [20,21].

Although *T. gondii* has been isolated from soil, there is no simple method for oocyst isolation from soil that is useful on an epidemiological scale. Although attempts to recover *T. gondii* oocysts from water samples in the British Columbia outbreak were unsuccessful, methods to detect oocysts were reported [19]. At present, there are no commercial reagents available to detect *T. gondii* oocysts in the environment.

Infection in humans often results from ingestion of tissue cysts contained in undercooked meat [1,22–24]. *T. gondii* infection is common in many animals used for food, including sheep, pigs and rabbits. Infection in cattle, horses and water buffaloes is less prevalent than infection in sheep or pigs. *Toxoplasma gondii* may survive in food animals for years in tissue cysts.

Virtually all edible portions of an animal can harbor viable *T. gondii*. In one study, viable *T. gondii* was isolated from 17% of 1000 adult pigs (sows) from a slaughter plant in Iowa [25]. *T. gondii* infection is also prevalent in game animals. Among wild game, *T. gondii* infection is most prevalent in black bears and in white-tailed deer. Approximately 80% of black bears are infected in the USA [26], and about 60% of raccoons have antibodies to *T. gondii* [26,27]. Because raccoons and bears scavenge for their food, infection in these animals is a good indicator of the prevalence of *T. gondii* in the environment.

The number of *T. gondii* tissue cysts in meat from food animals is very low. It is estimated that as few as one tissue cyst may be present in 100 g of meat. Therefore, without using a concentration method, it is not practical to detect this low level of *T. gondii* infection. Therefore, digestion of meat samples in trypsin or pepsin is used to concentrate *T. gondii* in meat [28]. Digestion in trypsin or pepsin ruptures the *T. gondii* tissue cyst wall, releasing hundreds of bradyzoites. The bradyzoites survive in the digests for several hours. Even in the digested samples, only a few *T. gondii* tissue cysts are present and their identification by direct microscopic examination is not practical. Therefore, the digested material is bioassayed in mice [28]. The mice inoculated with digested material have to be kept for 6–8 weeks before *T. gondii* infection can be detected reliably, and therefore this procedure is not

practical for mass scale samples. The detection of *T. gondii* DNA in meat samples by polymerase chain reaction (PCR) has been reported [29], but there are no data on the specificity and sensitivity of this method to detect *T. gondii* in meat samples. A highly sensitive method using a real-time PCR and fluorogenic probe was found to detect *T. gondii* DNA from as few as four bradyzoites [30].

Cultural habits of a population may affect the acquisition of *T. gondii* infection from ingested tissue cysts in undercooked meat. For example, in France the prevalence of antibody to *T. gondii* is very high in humans. Though 84% of pregnant women in Paris have antibodies to *T. gondii*, only 32% in New York City and 22% in London have such antibodies [1]. The high incidence of *T. gondii* infection in humans in France appears to be related in part to the French habit of eating some meat products raw or undercooked. In contrast, the high prevalence of *T. gondii* infection in Central and South America is probably due to high levels of contamination of the environment by oocysts [1]. As stated above, the relative frequency of acquisition of toxoplasmosis from eating raw meat and that due to ingestion of oocysts from cat feces is impossible to determine, and as a result, statements on the subject are at best controversial.

There is little, if any, danger of *T. gondii* infection by drinking cows' milk and, in any case, cows' milk is generally pasteurized or boiled, but infection has followed drinking unboiled goats' milk [1]. Raw hens' eggs, although an important source of *Salmonella* infection, are extremely unlikely to transmit *T. gondii* infection. Transmission by sexual activity including kissing is probably rare and epidemiologically unimportant [1].

Transmission of *T. gondii* may also occur through blood transfusions and organ transplants. Of these routes, transmission by transplantation is most important. Toxoplasmosis may arise in two ways in people undergoing transplantation: (i) from implantation of an organ or bone marrow from an infected donor into a non-immune, immunocompromised recipient and (ii) from induction of disease in an immunocompromised, latently infected recipient. Tissue cysts in the transplanted tissue or in the latently infected transplant patient are probably the source of the infection. In both cases, the cytotoxic and immunosuppressive therapy given to the transplant recipient is the cause of the induction of the active infection and disease [1,31].

Diagnosis of toxoplasmosis in humans is made by biological, serological, histological, or molecular methods, or by some combination of the above. Clinical signs of toxoplasmosis are non-specific and are not sufficiently characteristic for a definite diagnosis. Toxoplasmosis in fact mimics several other infectious diseases.

Detection of *T. gondii* antibody in patients may aid diagnosis. There are numerous serological procedures available for the detection of humoral antibodies; these include the Sabin–Feldman dye test, the indirect hemagglutination assay, the indirect fluorescent antibody assay (IFA), the direct agglutination test, the latex agglutination test (LAT), the enzyme-linked immunosorbent assay (ELISA), and the immunosorbent agglutination assay test (IAAT). The IFA, IAAT and ELISA have been modified to detect immunoglobulin M (IgM) antibodies [3,13]. The IgM antibodies appear sooner after infection than the IgG antibodies and disappear faster than IgG antibodies after recovery [3].

The finding of antibodies to *T. gondii* in one serum sample only establishes that the host has been infected at some time in the past. It is best to collect two samples from the same individual, the second collected 2–4 weeks after the first. A 16-fold higher antibody titer in the second sample indicates an acute infection. A high antibody titer sometimes persists for months after infection. A rise in antibody titer may not be associated with clinical symptoms because, as indicated earlier, most infections in humans are asymptomatic. The fact that titers persist in infected people after clinical recovery complicates the interpretation of the results of serological tests. Establishing recency of infection in pregnancy is of clinical importance with respect to medical intervention to minimize damage to the fetus, and there is not one test that can achieve this at the present time.

Toxoplasma gondii can be isolated from patients by inoculation of laboratory animals and tissue cultures with secretions, excretions, body fluids, tissues taken by biopsy, and tissues with macroscopic lesions taken post mortem. Using such specimens, one may not only attempt isolation of *T. gondii*, but one may search for *T. gondii* microscopically or for toxoplasmal DNA using PCR [32]. Recent studies have shown that monoplex and multiplex PCR can be useful for specifically identifying *T. gondii* (using the B1 gene as the target sequence) from tissue biopsies, cerebrosp-

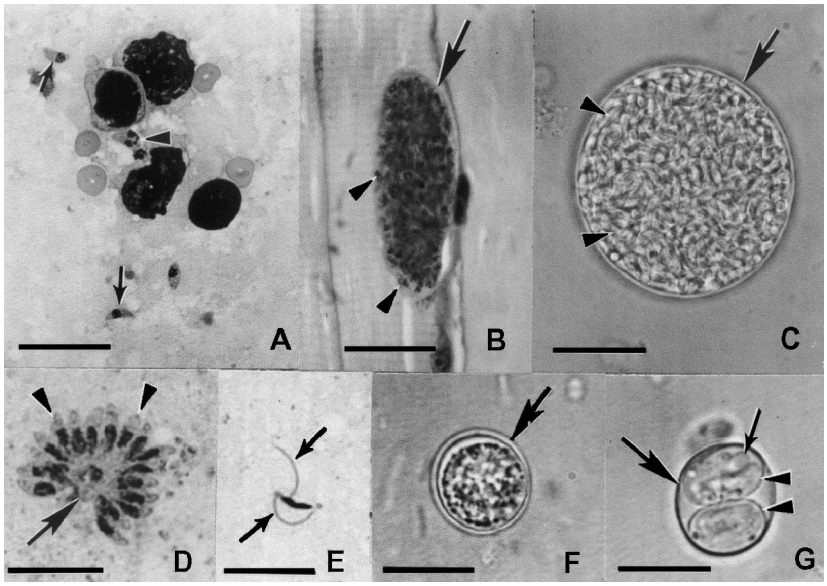

Figure 2 Stages of *Toxoplasma gondii*. Scale bar in (A) to (D) = 20 μm, in (E) to (G) = 10 μm. (A) Tachyzoites in impression smear of lung. Note crescent-shaped individual tachyzoites (arrows), dividing tachyzoites (arrowheads) compared with size of host red blood cells and leukocytes; Giemsa stain. (B) Tissue cysts in section of muscle. The tissue cyst wall is very thin (arrow) and encloses many tiny bradyzoites (arrowheads); haematoxylin and eosin stain. (C) Tissue cyst separated from host tissue by homogenization of infected brain. Note tissue cyst wall (arrow) and hundreds of bradyzoites (arrowheads); unstained. (D) Schizont (arrow) with several merozoites (arrowheads) separating from the main mass; impression smear of infected cat intestine, Giemsa stain. (E) A male gamete with two flagella (arrows); impression smear of infected cat intestine, Giemsa stain. (F) Unsporulated oocyst in faecal float of cat feces; unstained. Note double-layered oocyst wall (arrow) enclosing a central undivided mass. (G) Sporulated oocyst with a thin oocyst wall (large arrow), two sporocysts (arrowheads). Each sporocyst has four sporozoites (small arrow) which are not in complete focus; unstained.

inal fluid or vitreous body from patients with undiagnosed uveitis, fetal blood and amniotic fluid [33–37].

As just noted, diagnosis can be made by finding *T. gondii* in host tissue removed by biopsy or at necropsy. A rapid diagnosis may be made by microscopic examination of impression smears of lesions. After drying for 10–30 min, the smears are fixed in methyl alcohol and stained with one of the Romanowsky strains, the Giemsa stain being very satisfactory. Well-preserved *T. gondii* are crescent-shaped (Figure 2). In sections, the tachyzoites usually appear round to oval. Electron microscopy can aid diagnosis. *T. gondii* tachyzoites are always located in vacuoles. Tissue cysts are usually spherical, lack septa, and the cyst wall can be stained with a silver stain. The bradyzoites are strongly positive on periodic acid Schiff (PAS) staining. Immunohistochemical staining of parasites with fluorescent or other types of labelled *T. gondii* antisera can aid in diagnosis.

Sulphadiazine and pyrimethamine (Daraprim) are two drugs widely used for treatment of toxoplasmosis [38]. While these drugs have a beneficial action when given in the acute stage of the disease process when there is active multiplication of the parasite, they will usually not eradicate infection. It is believed that these drugs have little effect on subclinical infections, but the growth of tissue cysts in mice has been restrained with sulphonamides. Certain other drugs, diaminodiphenylsulphone, atovaquone, spiramycin and clindamycin, are also used to treat toxoplasmosis in difficult cases.

To prevent infection of human beings by *T. gondii*, the hands of people handling meat should be washed thoroughly with soap and water before they begin other tasks [1,23]. All cutting boards, sink tops, knives and other materials coming in contact with uncooked meat should be washed with soap and water also. Washing is effective because the stages of *T. gondii* in meat are killed by contact with soap and water [1].

T. gondii organisms in meat can be killed by exposure to extreme heat or cold. Tissue cysts in meat are killed by heating the meat throughout

to 67 °C [39] or by cooling to −13 °C [40]. Toxoplasma in tissue cysts are also killed by exposure to 0.5 kilorads of gamma irradiation [41]. Meat of any animal should be cooked to 67 °C before consumption, and tasting meat while cooking or while seasoning should be avoided.

Pregnant women, especially, should avoid contact with cats, soil and raw meat. Pet cats should be fed only dry, canned, or cooked food. The cat litter box should be emptied every day, a task to be avoided by pregnant women. Gloves should be worn while gardening. Vegetables should be washed thoroughly before eating because they may have been contaminated with cat feces. Expectant mothers should be aware of the dangers of toxoplasmosis [42]. At present there is no vaccine to prevent toxoplasmosis in humans.

In conclusion, infection by the protozoan parasite *T. gondii* is widely prevalent in humans and animals. Although it causes asymptomatic infection in immune competent adults, *T. gondii* can cause devastating disease in congenitally infected children and those with depressed immunity. To prevent human infection, all meat should be cooked well before consumption. Gloves should be worn while gardening, and sandboxes used by children should be covered when not in use to prevent exposure to soil contaminated with *T. gondii* oocysts excreted in cat feces. Extreme care should be used in handling litterboxes used by cats; and pregnant women, children and immunocompromised individuals should avoid litterboxes altogether.

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