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Toxoplasma gondii as a Parasite in Food: Analysis and Control

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ABSTRACT Foodborne infections are a significant cause of morbidity and mortality worldwide, and foodborne parasitic diseases, though not as widespread as bacterial and viral infections, are common on all continents and in most ecosystems, including arctic, temperate, and tropical regions. Outbreaks of disease resulting from foodstuffs contaminated by parasitic protozoa have become increasingly recognized as a problem in the United States and globally. Increased international trade in food products has made movement of these organisms across national boundaries more frequent, and the risks associated with infections have become apparent in nations with well-developed food safety apparatus in place.

Common parasitic protozoal infections are frequently transmitted by food containing fecally contaminated soil or water, which may carry the environmentally resistant oocyst stage of the parasites *Cyclospora cayetanensis*, *Cryptosporidium* spp., *Giardia* spp., *Toxoplasma gondii*, or *Sarcocystis* spp. However, both *T. gondii* and *Sarcocystis* can also be transmitted by consumption of a cyst stage of the parasite which is present in the meat of infected animals; currently, the extent of *Toxoplasma* and *Sarcocystis* infection due to foodborne transmission is unknown (1–5). Differences in the definitive and intermediate hosts exist between these pathogens which impact their abundance and geographical distribution in the environment (Table 1). As an example, because *Giardia* and *Cryptosporidium* spp. oocysts are excreted in large numbers by cattle, other ruminants, and a wide variety of other species (more than 10^9 per day), oocysts are very commonly found in the environment, while oocysts of *Toxoplasma*, which are exclusively excreted by felids, are restricted to areas inhabited by wild and domestic cats (6, 7).

This review will focus on *T. gondii*, since it encompasses both meat-borne transmission and environmental contamination (fresh produce/soil/surface water); however, significant differences between *Toxoplasma* and the other protozoans of food safety importance will be highlighted.

T. gondii is a coccidian parasite with an unusually wide range of intermediate hosts. Felids serve as definitive hosts and produce the environmentally resistant oocyst stage. *Toxoplasma* is one of the most common parasitic infections of humans, though its prevalence varies widely from place to place. Toxoplasmosis continues to be a significant public health problem in the United States, where 8 to 22% of people are infected; a similar prevalence is seen in the United Kingdom (8–13). In Central America, South America, and continental Europe, estimates of infection range from 30 to 90% (9, 14, 15). Most infections in humans are asymptomatic, but at times the parasite can produce devastating disease. Infection may be congenitally or postnatally acquired. In the United States, nationwide serological surveys demonstrated that seroprevalence in people remained stable at 23% from 1990 until 1998 (10), while recent surveys have demonstrated a significant

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TABLE 1 Protozoans of food safety importance and their hosts

Protozoan	Intermediate hosts	Definitive host
Cyclospora cayetanensis	N/A	Humans, other primates
Cryptosporidium spp.	N/A	Canids, birds, rodents, humans, ruminants, pigs, reptiles
Giardia duodenalis/Giardia lamblia	N/A	Humans, other mammals
Toxoplasma gondii	Warm-blooded animals, birds	Felids
Sarcocystis spp.	Mammals, birds, poikilothermic	Canids, birds, raccoons, primates, felids, reptiles

decrease in age-adjusted seroprevalence over the past 15 years to 10.8% (12) and 12.4% (13). Similar decreases in seroprevalence have been observed in many European countries (14).

It is estimated that 1,075,242 people are infected with *T. gondii* each year in the United States, and approximately 2,839 people develop symptomatic ocular disease annually (16). The cost of illness caused by *Toxoplasma* in the United States has been estimated to be nearly $3 billion, with an $11,000 quality-adjusted life year loss annually (17, 18). Recent publications have linked suicide and schizophrenia to *Toxoplasma* infection (19, 20).

T. gondii also infects food animals, including sheep, goats, pigs, chickens, and many game animal species. Infected animals harbor tissue cysts, and human consumers can be infected by ingestion of these cysts in raw or undercooked meat. Virtually all edible portions of an animal can harbor viable *T. gondii* tissue cysts (21), and tissue cysts can survive in food animals for years.

Unlike *T. gondii*, which is the only species in the genus, and whose only definitive hosts are members of the *Felidae*, there are perhaps hundreds of species of *Sarcocystis*. *Sarcocystis* spp. infect all vertebrate animals; these animals can serve as both intermediate or definitive hosts (22). Humans act as intermediate hosts for an unknown number of *Sarcocystis* spp. and act as definitive hosts for at least two species of *Sarcocystis*: *Sarcocystis hominis* and *Sarcocystis suihominis*, which are acquired by eating infectious sarcocysts in undercooked beef and pork muscle, respectively (23).

Cyclospora, *Giardia*, and *Cryptosporidium* spp. require only one host to complete their life cycles, and infections are transmitted to humans strictly through inadvertent consumption of infectious oocysts in contaminated food, water, or soil.

MORPHOLOGY, STRUCTURE, AND LIFE CYCLE

T. gondii belongs to phylum *Apicomplexa* (Levine, 1970), class *Sporozoasida* (Leukart, 1879), subclass *Coccidiasina* (Leukart, 1879), order *Eimeriorina* (Leger, 1911), and family *Toxoplasmatidae* (Biocca, 1956). There is only one species of *Toxoplasma*, *T. gondii*. Coccidia in general have complicated life cycles. Most coccidia are host-specific and are transmitted via a fecal–oral route. Transmission of *T. gondii* occurs via the fecal–oral route (Fig. 1), as well as through consumption of infected meat and by transplacental transfer from mother to fetus (8, 24).

The name *Toxoplasma* (toxon = arc, plasma = form) is derived from the crescent shape of the tachyzoite stage (Fig. 2). There are three infectious stages of *T. gondii*: the tachyzoites (in groups) (Fig. 3A), the bradyzoites (in tissue cysts) (Fig. 3B, C), and the sporozoites (in oocysts) (Fig. 3G). The tachyzoite is often crescent-shaped and is approximately the size (2 x 6 μm) of a red blood cell (Fig. 4). The anterior end of the tachyzoite is pointed, and the posterior end is round. It has a pellicle (outer covering), several organelles including subpellicular microtubules, mitochondrium, smooth and rough endoplasmic reticulum, a Golgi apparatus, an apicoplast, ribosomes, a micropore, and a well-defined nucleus. The nucleus is usually situated toward the posterior end or in the central area of the cell. The tachyzoite enters the host cell by active penetration of the host-cell membrane and can tilt, extend, and retract as it searches for a host cell. After entering the host cell, the tachyzoite becomes ovoid in shape and becomes surrounded by a parasitophorous vacuole (Pv in Fig. 4). *T. gondii* in a parasitophorous vacuole is protected from host defense mechanisms. The tachyzoite multiplies asexually within the host cell by repeated divisions in which two progenies form within the parent parasite, consuming it (Fig. 5A–D). Tachyzoites continue to divide until the host cell is filled with parasites.

After a few divisions, *T. gondii* forms tissue cysts that vary in size from 5 to 70 μm and remain intracellular (Fig. 6A–F). The tissue cyst wall is elastic, thin (<0.5 μm), and may enclose hundreds of the crescent-shaped, slender *T. gondii* stage known as bradyzoites (Fig. 7). The bradyzoites are approximately 7 x 1.5 μm in size and differ structurally only slightly from tachyzoites. They have a nucleus situated toward the posterior end, whereas the nucleus in tachyzoites is more centrally located.

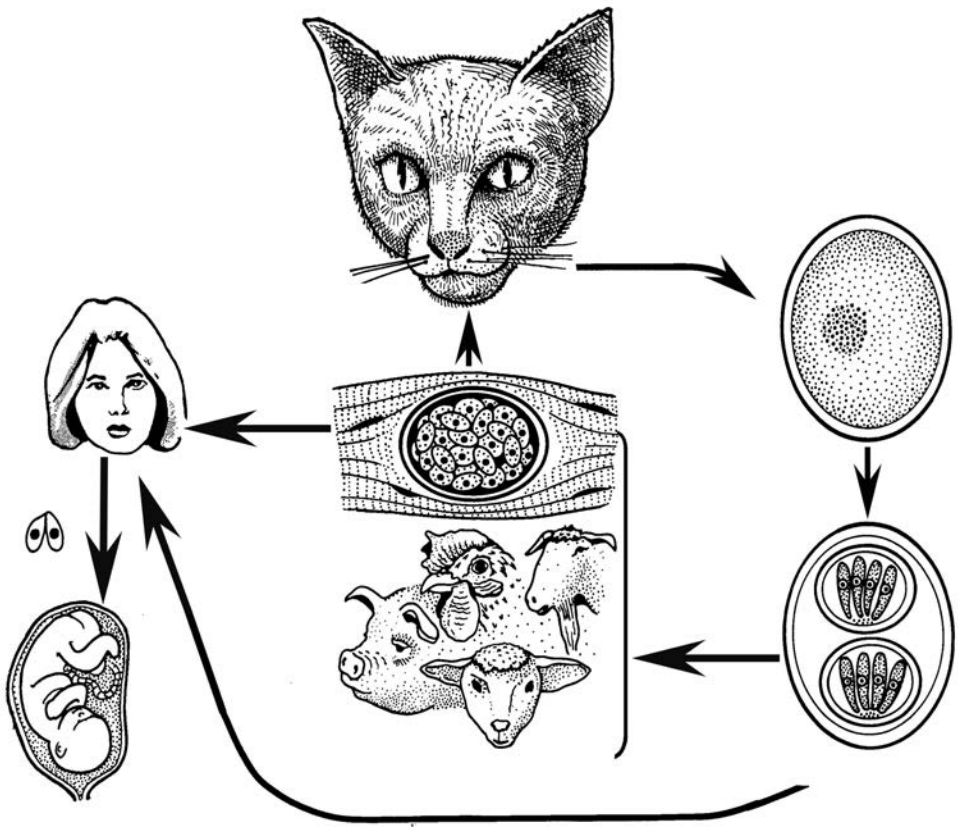

FIGURE 1 Life cycle of *Toxoplasma gondii*.

Bradyzoites are more slender and less susceptible to destruction by proteolytic enzymes than tachyzoites. Although tissue cysts containing bradyzoites may develop in visceral organs, including lungs, liver, and kidneys, they are more prevalent in muscular and neural tissues, including the brain (Fig. 6A–F), eye, and skeletal and cardiac muscle. Intact tissue cysts probably do not cause any harm and can persist for the life of the host (Table 2).

After the ingestion of tissue cysts by cats, the tissue cyst wall is dissolved by proteolytic enzymes in the stomach and small intestine. The released bradyzoites penetrate the epithelial cells of the small intestine and initiate development of numerous generations of asexual and sexual cycles of *T. gondii* (25). Bradyzoites penetrate the lamina propria of the feline intestine and multiply as tachyzoites. Within a few hours after infection of cats, *T. gondii* may disseminate to extraintestinal tissues. *T. gondii* persists in the intestinal and extraintestinal tissues of cats for at least several months and possibly for the life of the cat.

As the enteroepithelial cycle progresses, *T. gondii* multiplies profusely in the intestinal epithelial cells of cats (enteroepithelial cycle), and these stages, represented by five distinct morphological types (types A to E), are known as schizonts (Fig. 3D). Several generations of each type are produced, and daughter organisms known as merozoites are formed coincident with the last nuclear division. Merozoites give rise to gametes, the sexual stages of the organism. The microgamont (male gamont) has two flagella (Fig. 3E), and it swims to, enters, and fertilizes the macrogamont (female gamont), forming a zygote. After fertilization, oocyst wall formation begins around the zygote. Oocysts are discharged into the intestinal lumen by the rupture of intestinal epithelial cells.

Oocysts are environmentally resistant and are formed only in felids, probably in all members of the *Felidae* (Fig. 3G). Cats shed oocysts after ingesting any of the three infectious stages of *T. gondii*, i.e., tachyzoites, bradyzoites, and sporozoites (25–28). Prepatent periods (time to the shedding of oocysts after initial infection) and frequency of oocyst shedding vary according to the stage of *T. gondii* ingested (Table 3). Prepatent periods occur 3 to 10 days after ingesting tissue cysts and 19 days or more after ingesting tachyzoites or oocysts (25–27). Less than 50% of cats shed oocysts after ingesting tachyzoites or oocysts, whereas nearly all cats shed oocysts after ingesting tissue cysts (26). In freshly passed

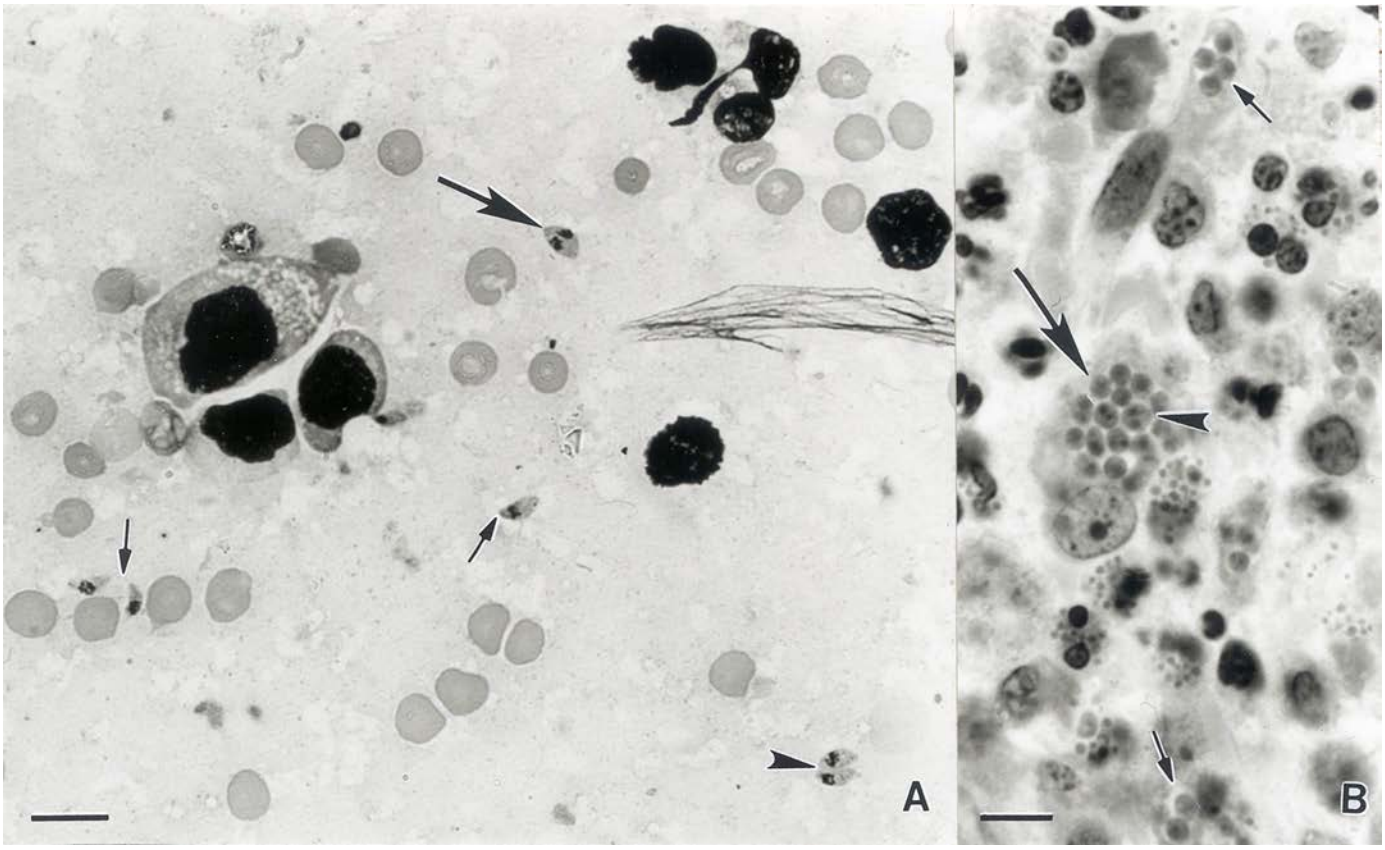

FIGURE 2 Tachyzoites of *Toxoplasma gondii*. Bars = 10 μm. **(A)** Individual (small arrows), binucleate (large arrow), and divided (arrowhead) tachyzoites. Impression smear of lung. Compare size with red blood cells and leukocytes. Giemsa stain. **(B)** Tachyzoites in a group (large arrow) and in pairs (small arrows) in section of a mesenteric lymph node. Note that organisms are located in parasitophorous vacuoles and some are dividing (arrowhead). Hematoxylin and eosin stain.

feces, oocysts are unsporulated (noninfective; Fig. 3F). Unsporulated oocysts are subspherical to spherical and are 10 x 12 μm in diameter. They sporulate (become infectious) outside the cat within 1 to 5 days depending upon aeration and temperature. Sporulated oocysts contain two ellipsoidal sporocysts (Fig. 3G), and each sporocyst contains four sporozoites. The sporozoites are 2 x 6 to 8 μm in size. The nucleus of *T. gondii* is haploid except during sexual recombination in the gut of the cat. Fourteen chromosomes are present, encompassing a 65-Mb genome (14, 29).

Most *T. gondii* isolates from human and animal sources in North America, Europe, and Africa have been grouped into one of three clonal lineages (types I, II, and III) (29–35) and are biologically and genetically different from isolates from Brazil and Columbia (36–40). A number of recent studies suggest that only a few ancestral strains have given rise to the three dominant clonal lineages and the existing genetic diversity seen in various geographic regions through a process of limited, mostly asexual, recombination (31, 34, 40, 41). Recent genotyping studies of isolates from pigs, lambs, and goats demonstrate that the type II lineage predominates in food animals in the United States, followed by type III isolates and atypical genotypes; type I isolates have rarely been found in farm animals (35, 42–44).

EPIDEMIOLOGY

Toxoplasmosis may be acquired by ingestion of oocysts or by ingestion of tissue-inhabiting stages of the parasite. Contamination of the environment by oocysts is widespread, a result of fecal contamination of soil and ground water by the estimated 140 million domestic and feral cats in the United States and other members of the *Felidae*. After ingesting as few as one bradyzoite or one tissue cyst (and many tissue cysts may be present in one infected mouse), each cat can deposit hundreds of

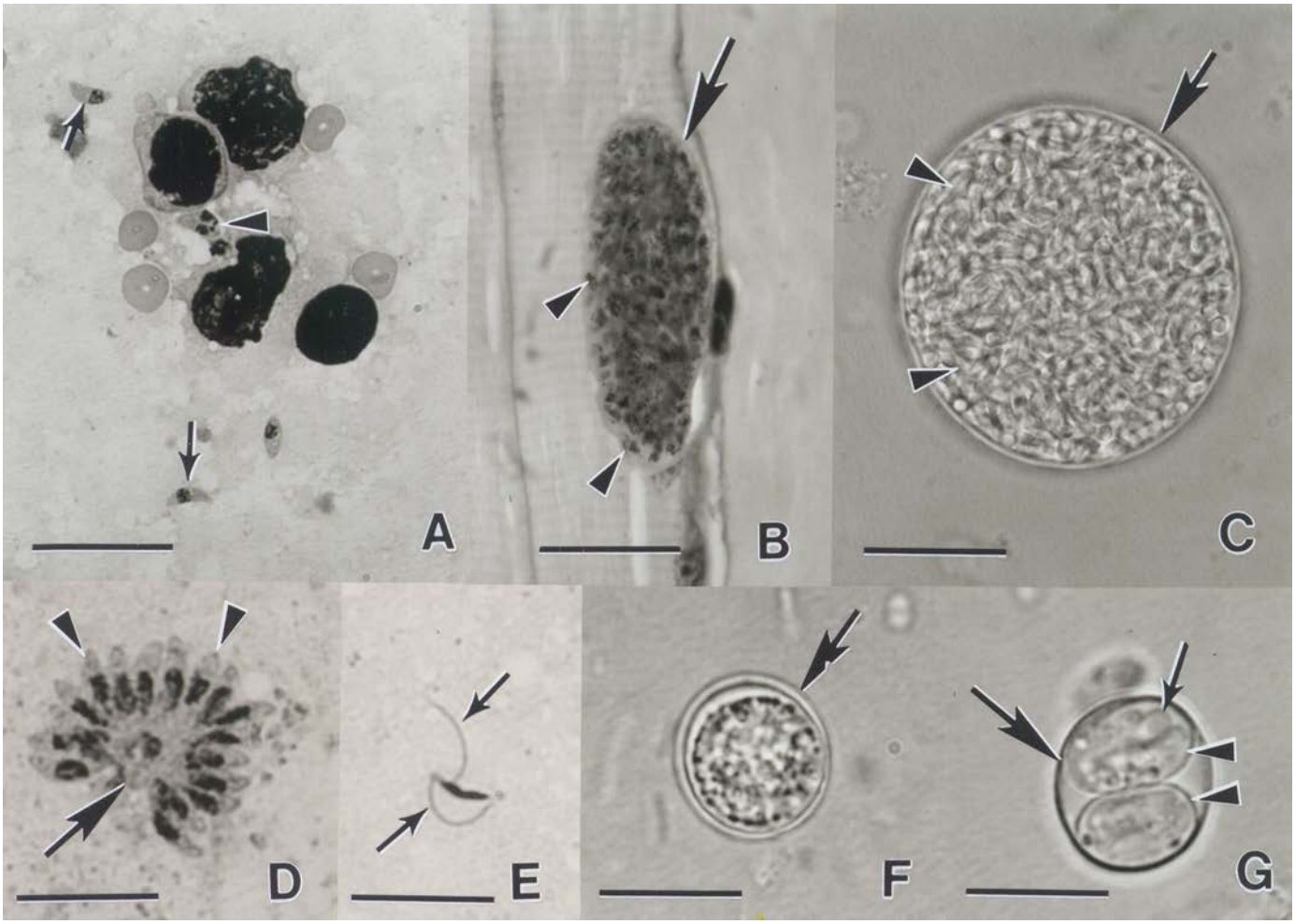

FIGURE 3 Stages of *Toxoplasma gondii*. Bars in A–D = 20 µm, in E–G = 10 µm. **(A)** Tachyzoites in impression smear of lung. Note crescent-shaped individual tachyzoites (arrows) and dividing tachyzoites (arrowheads) compared with size of host red blood cells and leukocytes. Giemsa stain. **(B)** Tissue cysts in section of muscle. The tissue cyst wall is very thin (arrow) and encloses many tiny bradyzoites (arrowheads). Hematoxylin 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 fecal 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) and two sporocysts (arrowheads). Each sporocyst has four sporozoites (small arrow), which are not in complete focus. Unstained.

millions of oocysts in feces during infection (8, 14, 24, 45–47). Domestic cats are the major source of contamination because oocyst formation is greatest in domestic cats, and cats are extremely common. Sporulated oocysts survive for long periods under most ordinary environmental conditions and even in harsh environments for months. They can survive in moist soil, for example, for months and even years (8).

Oocysts in soil can be mechanically transmitted by invertebrates such as flies, cockroaches, dung beetles, and earthworms, which can spread oocysts into human food and animal feeds. Infection rates in cats are determined by the rate of infection in local avian and rodent populations because cats typically become infected by eating these animals. The more oocysts there are in the environment, the more likely it is that prey animals

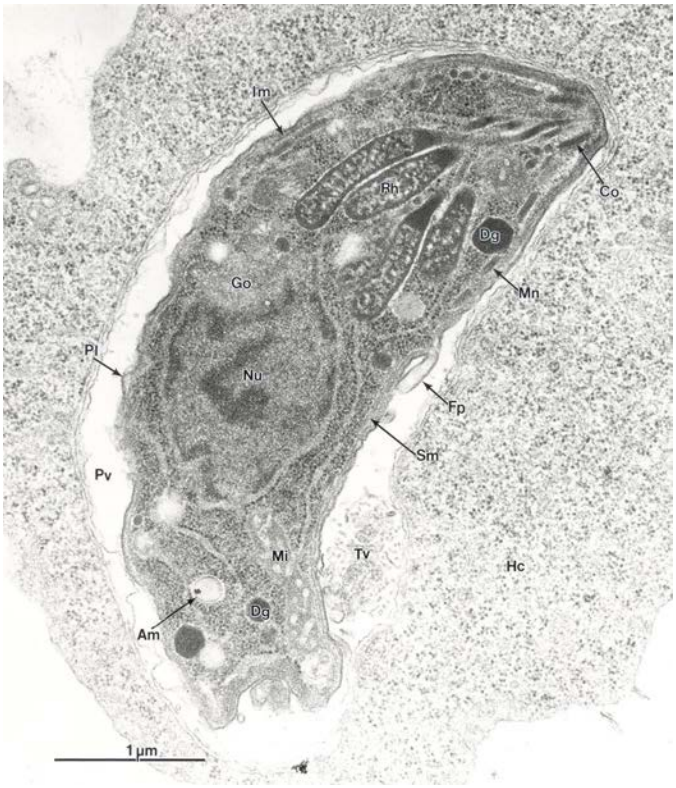

FIGURE 4 Transmission electron micrograph of a tachyzoite of *Toxoplasma gondii* in a mouse peritoneal exudate cell. Am, amylopectin granule; Co, conoid; Dg, electron-dense granule; Fp, finger-like projection of tachyzoite plasmalemma; Go, Golgi complex; Hc, host cell cytoplasm; Im, inner membrane complex; Mi, mitochondrion; Mn, microneme; Nu, nucleus; Pl, plasmalemma; Pv, parasitophorous vacuole; Rh, rhoptry; Sm, subpellicular microtubule; Tv, tubulovesicular membranes. Bar = 1 µm.

would be infected, and this in turn would increase the infection rate in cats. In certain areas of Brazil, up to 50% of 6- to 8-year-old children have antibodies to *T. gondii* linked to the ingestion of oocysts from an environment heavily contaminated with *T. gondii* oocysts (48, 49). The largest recorded outbreak of clinical toxoplasmosis in humans in North America was epidemiologically linked to drinking water from a municipal water reservoir in British Columbia, Canada (50, 51). This water reservoir was supposedly contaminated with *T. gondii* oocysts excreted by cougars (*Felis concolor*). Although attempts to recover *T. gondii* oocysts from water samples in the British Columbia outbreak were unsuccessful, methods to detect oocysts were reported (52). At present, no commercial reagents are available to reliably detect *T. gondii* oocysts in the environment.

Widespread infection in aquatic mammals indicates contamination and survival of oocysts in sea-water contaminated by runoff surface water entering the marine environment (53, 54). Wild populations of endangered southern sea otters off the west coast of the United States have been significantly impacted by exposure to *Toxoplasma* oocysts, presumably by eating filter-feeding mollusks in near-shore environments, resulting in devastating disease (55–57). Given the levels of oocyst contamination in surface waters, the potential for contamination of produce with oocysts is high, especially where surface waters are used for irrigation purposes. However, little is known of the contribution of oocyst-contaminated fruits and vegetables to human infection in the United States, but recent studies (4, 58) have suggested that oocyst exposure is the predominate route of *Toxoplasma* transmission to humans in the United States. Animal infections with *Toxoplasma*, especially infections in non-meat-eating ruminants, birds, wild herbivores, and pigs raised in confinement, also likely result from environmental exposure to *T. gondii* oocysts (59).

FOODBORNE TRANSMISSION

The relative contribution of meat-borne sources of *Toxoplasma* infection versus oocyst transmission of *Toxoplasma* to human infection is unknown and difficult to quantify, since meat from infected animals may undergo postharvest treatments such as heating, freezing, salting, or pumping (injection of water and salt-based solutions to retard microbial growth) that can render the parasite nonviable (60, 61), and few comprehensive assessments have been completed in meat available for retail purchase. Complicating matters is the fact that the number of *T. gondii* organisms in meat from naturally infected food animals is very low, making the parasite difficult and expensive to detect by direct methods. It is estimated that as few as one tissue cyst may be present in 100 g of meat (62). In addition, there is no predilection site for *Toxoplasma* in meat animals; virtually all edible portions of an animal can harbor viable *T. gondii* tissue cysts (21), and tissue cysts can remain viable in food animals for years.

Despite the uncertainty of human infection sources, *Toxoplasma* is increasingly recognized as a foodborne risk, and various studies have suggested widely disparate estimates of foodborne transmission. Mead et al. (63) suggested that *T. gondii* is one of three pathogens (along with *Salmonella* and *Listeria*) which account for >75% of all deaths due to foodborne disease in the United States. Roghmann et al. (64) suggested that 50% of *Toxoplasma* infections in the United States could be ascribed to foodborne transmission. Scallan et al. (65)

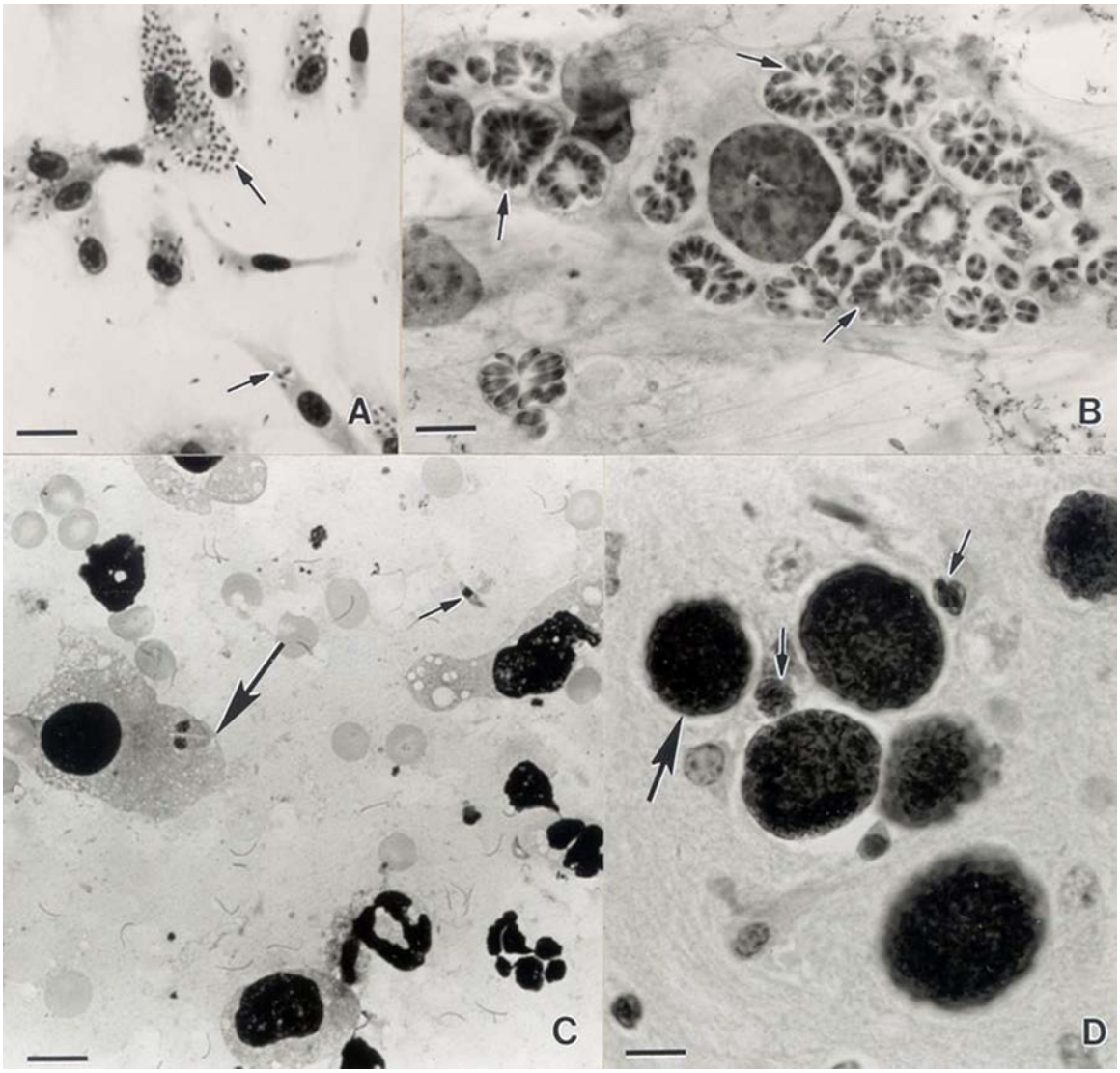

FIGURE 5 *Toxoplasma gondii* stages in *in vitro* and *in vivo* preparations. **(A)** Tachyzoites in culture of human foreskin fibroblast cells. Giemsa stain. Bar = 25 μm. **(B)** Rosettes of tachyzoites in human foreskin fibroblasts. Immunohistochemical stain with antitachyzoite-specific antibody. Smear. Bar = 10 μm. **(C)** Tachyzoites in a cytospin smear of pleural fluid from a cat with pneumonia. Giemsa stain. Compare the size of tachyzoites (arrow) with host cells. Giemsa stain. Bar = 10 μm. **(D)** Tachyzoites (arrows) and tissue cysts (large arrow) in section of mouse brain. Immunohistochemical stain with *T. gondii*–specific antibody. Bar = 10 μm.

estimated that *Toxoplasma* caused 8% of hospitalizations and 24% of deaths resulting from foodborne illnesses.

Dubey et al. (43) determined the prevalence of *T. gondii* in 383 U.S. lambs (<1 year old) from Maryland, Virginia, and West Virginia. Hearts of 383 lambs were obtained from a slaughter house on the day of killing, and blood was removed from each heart and tested for antibodies to *T. gondii* using the modified agglutination test (MAT). Antibodies (MAT, 1:25 or higher) to *T. gondii* were found in 104 (27.1%) of 383 lambs. Hearts of 68 seropositive lambs were used for isolation of viable *T. gondii* by bioassay in cats, mice, or both. For bioassays in cats, the entire myocardium was chopped and fed to one cat per heart, and feces of the recipient cats were examined for shedding of *T. gondii* oocysts. For bioassays in mice, 50 g of the myocardium was digested in an acid pepsin solution, and the digest was

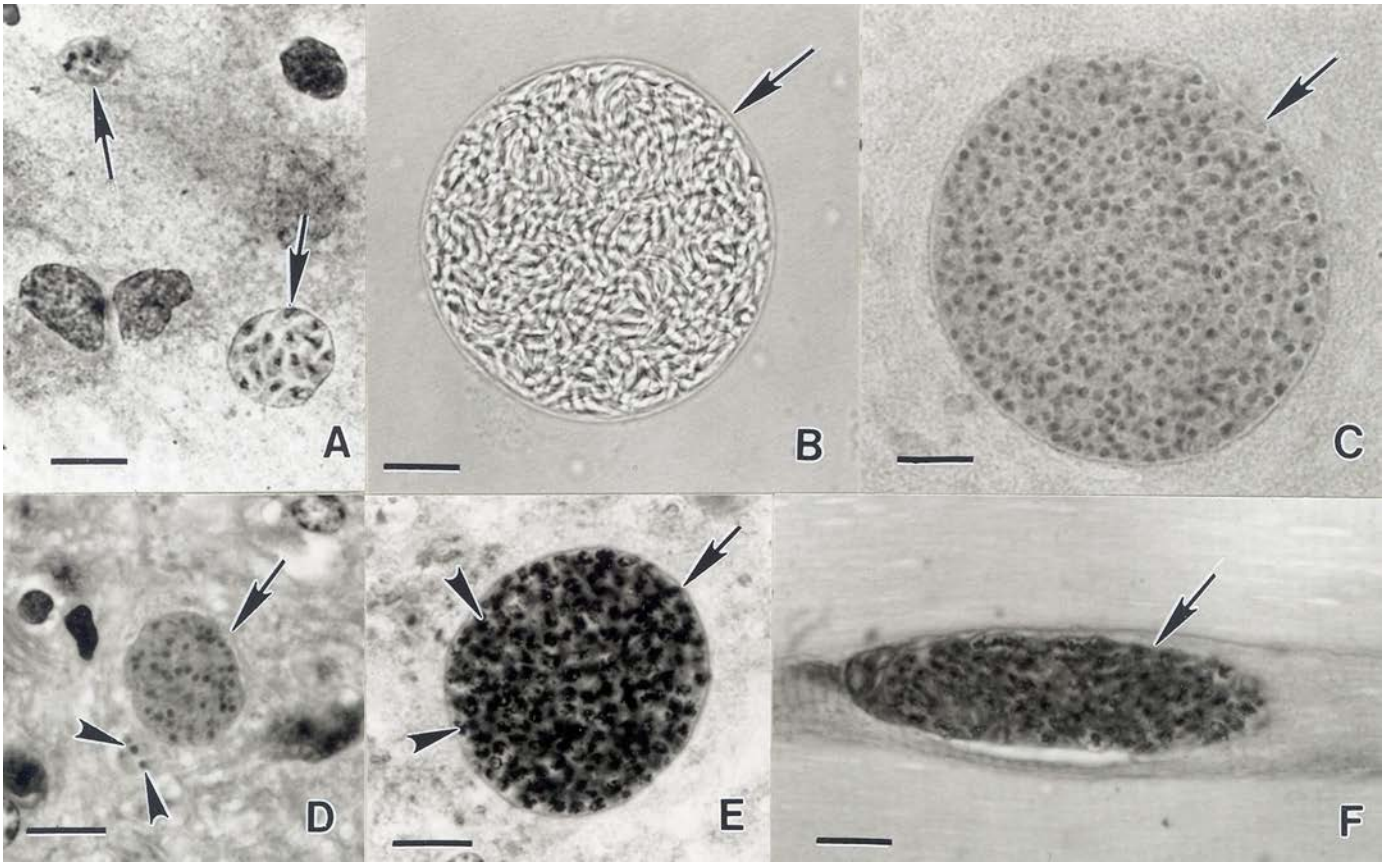

FIGURE 6 Tissue cysts of *Toxoplasma gondii*. Bar = 10 μm. **(A)** Two tissue cysts (arrows). Note the thin cyst wall enclosing bradyzoites. Impression smear of mouse brain. Silver impregnation and Giemsa stain. **(B)** A tissue cyst freed from mouse brain by homogenization in saline. Note the thin cyst wall (arrow) enclosing many bradyzoites. Unstained. **(C)** A large tissue cyst in a section of rat brain 14 months postinfection. Note the thin cyst wall (arrow). Hematoxylin and eosin stain. **(D)** A small tissue cyst with intact cyst wall (arrow) and four bradyzoites (arrowheads) with terminal nuclei adjacent to it. Section of mouse brain 8 months postinfection. Hematoxylin and eosin stain. **(E)** A tissue cyst in a section of mouse brain. Note Periodic acid Schiff (PAS)–negative cyst wall (arrow) enclosing many PAS-positive bradyzoites (arrowheads). The bradyzoites stain bright red with PAS but they appear black in this photograph. PAS hematoxylin stain. **(F)** An elongated tissue cyst (arrow) in a section of skeletal muscle of a mouse. PASH stain.

inoculated into mice; the recipient mice were then examined for *T. gondii* infection. In total, 53 isolates of *T. gondii* were obtained from 68 seropositive lambs. A similar study was carried out using hearts from 234 goats (44). Antibodies to *T. gondii* were found in 125 (53.4%) of 234 goats. Hearts of 112 goats were used for isolation of viable *T. gondii* by bioassays in mice. *T. gondii* was isolated from 29 goats. Taken together, these results indicate high parasite prevalence of *T. gondii* in lambs and goats destined for human consumption in the United States.

Chickens are considered one of the most important hosts in the epidemiology of *T. gondii* infection because they are an efficient source of infection for cats that excrete the environmentally resistant oocysts and because humans may become infected with this parasite after eating undercooked infected chicken meat. In a recent review of *T. gondii* infection in chickens worldwide (66), a very high prevalence of the parasite was found in chickens raised in backyards (up to 100%) and free-range organic (30 to 50%) establishments.

Beef, chicken, and pork are the main meat types consumed in the United States. In a case control study of 148 recently (<6 months) infected individuals, Jones et al. (67) identified an elevated risk of infection associated with eating raw ground beef, rare lamb, locally

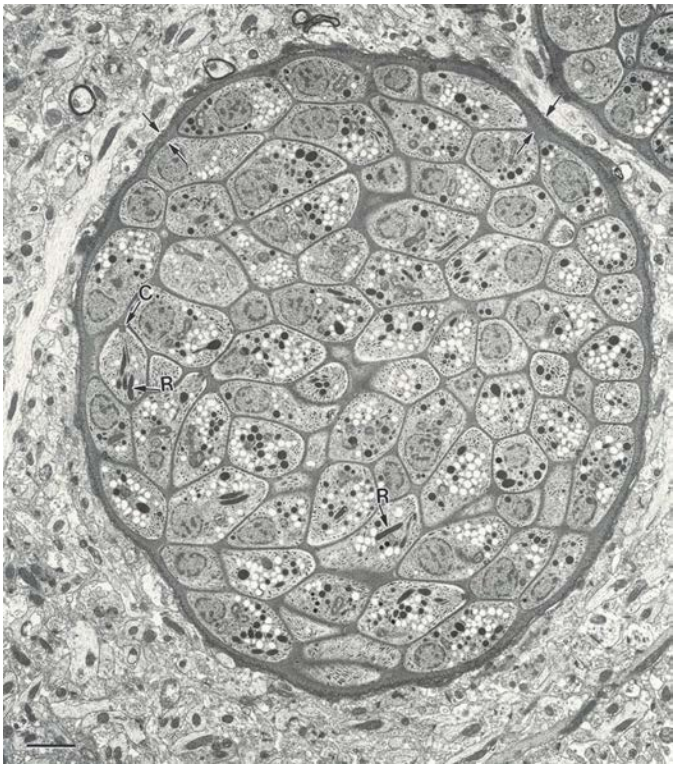

FIGURE 7 Transmission electron micrograph of a tissue cyst in the brain of a mouse 6 months postinfection. Note the thin cyst wall (opposing arrows), numerous bradyzoites each with a conoid (C), and electron-dense rhoptries (R). Bar = 3.0 μm.

produced cured, dried, or smoked meat, raw oysters, clams, or mussels; working with meat; and drinking unpasteurized goat's milk. The relative risk to U.S. consumers of acquiring *T. gondii* infection from undercooked meat was recently determined in a nationwide survey of retail chicken, beef, and pork (68). The survey of 698 retail outlets in 28 metropolitan statistical areas (MSAs as defined by the U.S. Census Bureau) covered 80% of the U.S. population. No viable *Toxoplasma* was found in any of 2,094 beef or 2,094 chicken samples by bioassay. Only pork was found to harbor viable *T. gondii* tissue cysts, which were isolated from 0.38% of samples (7/2,094) by cat bioassay, and 0.57% of pork samples were suspected to be infected based on positive enzyme-linked immunosorbent assay (ELISA) results. No beef samples were positive by ELISA, while 1.4% of chickens were positive by ELISA only. The northeastern United States had a higher number of positive pork samples than other regions of the country, reflecting the higher risk of pig infection due to regional management practices (outdoor versus confinement rearing).

Modern biosecure management practices on swine farms, which include restriction of human entry into pig barns, stringent rodent control, secure feed, and exclusion of cats and other wildlife, have resulted in reduced levels of *Toxoplasma* infection in confinement-raised swine over the past 20 years from 23% to 2.7% in the United States (69, 70).

While *Toxoplasma* infection in confinement-raised market pigs has decreased significantly, infection levels in pigs with access to the outdoors can be quite high, reaching 50 to 90% in recent studies (71, 72). In the U.S. national meat survey mentioned above, two of seven *Toxoplasma*-positive pork samples were derived from "naturally raised" pigs. An upsurge in consumer demand for "organically raised," "humanely raised," and "free-range" pork products has resulted in increasing numbers of hogs being raised in nonconfinement systems (73). Swine producers have been recruited to produce animals for the organic market to fulfill a consumer demand that has increased 20% per year in sales since 1990 (74). National Organic Program standards (http://www.ams.usda.gov/nop/) require that all organically raised animals must have access to the outdoors. Though humanely raised and free-range products have standards that are less stringently defined, outdoor access is also considered a requirement for labeling.

Few studies have determined the prevalence of *Toxoplasma* infection in swine raised in organic management systems. Kijlstra et al. (75) found 0 of 621 conventionally raised pigs to be seropositive for *Toxoplasma*, while 38 of 1,295 (2.9%) pigs raised in "animal friendly"

TABLE 2 Host tissues invaded by protozoan parasites

Species	Tissue parasitized in intermediate host	Tissue parasitized in definitive host
Cyclospora cayetanensis	N/A	Intestinal lining
Cryptosporidium spp.	N/A	Epithelial cells of the respiratory system or the intestine
Giardia duodenalis/Giardia lamblia	N/A	Brush border villous epithelium surface of small intestinal mucosa
Toxoplasma gondii	Many types of tissue, including muscle and epithelial cells	Intestinal epithelial cells followed by invasion of extraintestinal tissues and organs
Sarcocystis spp.	Meronts in endothelial cells of organ blood vessels, sarcocysts in muscle	Lamina propria of the small intestine

TABLE 3 Oocyst characteristics of protozoans of food safety importance

Species	Oocyst size	Number of organisms per cyst in feces	Days to infectivity
Cyclospora cayetanensis	8–10 μm	4	~14 days
Cryptosporidium spp.	4–6 μm	4	Immediately infective
Giardia duodenalis/Giardia lamblia	9–12 μm	2	Immediately infective
Toxoplasma gondii	10–12 μm	8	1–7 days
Sarcocystis spp.	12.3–14.6 μm	8 in oocyst/4 in sporocyst	Immediately infective

management systems were seropositive for *Toxoplasma*. In another study, 22 of 324 (6.8%) free-range pigs in North Carolina were seropositive for *Toxoplasma*, while 3 of 292 conventionally raised pigs (1.1%) tested were seropositive (76). Dubey et al. (77), determined *T. gondii* prevalence in organically raised pigs from two farms in Michigan. Serum and tissue samples from 33 pigs from the farms were available for *T. gondii* evaluation at slaughter. Serological testing was performed using both ELISA and MAT. Antibodies to *T. gondii* were detected by both ELISA and MAT in 30 of 33 animals, with MAT titers of 1:25 in 3, 1:50 in 6, 1:100 in 7, 1:200 in 13, and 1:400 in 1. The hearts of all 33 pigs were bioassayed for *T. gondii* in mice; *T. gondii* was isolated from 17 pigs including one from a seronegative (both ELISA and MAT) pig, revealing very high prevalence of *T. gondii* in organic pigs for the first time in the United States, indicating potentially increased health risk of consuming organic swine products.

Access to organic material contaminated with cat feces or to rodents or wildlife potentially infected with *Toxoplasma* during outdoor pasturage substantially increases the risk of exposure of pigs to *Toxoplasma* (42, 75, 78). These pigs enter the food chain, since there is no national system of identifying individual pigs slaughtered in the United States and no routine testing for *Toxoplasma*. Commercial meat cuts may therefore contain viable *T. gondii* tissue cysts; Dubey et al. (21, 79) demonstrated that virtually every edible portion of an infected pig carcass may contain viable tissue cysts. A single *T. gondii*–infected pig can be a source of infection for many people, since one market-weight hog (100 kg or more) can yield over 600 individual servings of meat. Prevention of exposure to infectious stages is the only way to stop infection of pigs, since there is no vaccine or treatment available, and once infected, tissue cysts persist in pig tissues for the life of the hog.

S. hominis (with its thick-walled cyst) is commonly found as infectious cysts in the muscles of slaughtered cattle worldwide (80, 81). Numerous studies of slaughtered cattle in Europe, South America, and Asia have demonstrated prevalence in cattle of over 40% in some countries (82–85). Tissue prevalence of *S. suihominis* in pigs is also high, especially in Asian countries and Europe (86, 87). Human intestinal *Sarcocystis* accompanied by the production of sporocysts has been shown to range from 1 to 23%, depending upon the country surveyed (5, 86–88).

C. cayetanensis has a direct fecal–oral transmission route, most likely from consumption of fecally contaminated food or water (89, 90). Extraintestinal phases are not present, and noninfectious oocysts are excreted in human feces; complete sporulation occurs within 1 to 2 weeks. Humans appear to be the only host of *C. cayetanensis*. Prevalence surveys of humans in South American and Asian countries demonstrated fecal oocysts in between 0.5 and 6% of the population, while fecal oocysts have not been found in surveys of domestic animals (22, 91).

Nearly two dozen species of *Cryptosporidium* occur in wild and domestic animals and humans worldwide. *Cryptosporidium hominis* and *Cryptosporidium parvum* are the most common cause of infections in humans. Like *Cyclospora*, transmission occurs through a direct fecal–oral route, and oocysts are immediately infective upon release in feces. Huge outbreaks of human infection have occurred in the United States as a result of contamination of municipal drinking water (92, 93), and consumption of fresh produce contaminated with oocysts has also been identified as a source of infection (94, 95).

Giardia duodenalis is a common cause of foodborne illness, even in developed nations. Host specificity has recently been clarified (96, 97), resulting in taxonomic separation of a number of species with more or less broad host ranges. *G. duodenalis* (group A or B) infects humans and other animals, while five other species do not appear to be zoonotic (98). The direct, one-host life cycle and immediate infectivity of the cyst upon release in feces results in millions of human infections with *Giardia* spp. each year worldwide. Transmission results from inadvertent consumption of contaminated soil or water and from direct contamination from feces of infected humans or animals.

PATHOGENESIS AND CLINICAL FEATURES

T. gondii usually parasitizes the host, definitive and intermediate, without producing clinical disease. Only rarely does it produce severe clinical manifestations (Table 4). The bradyzoites from the tissue cysts, or sporozoites from the oocyst, penetrate intestinal epithelial cells and multiply in the intestine as tachyzoites within 24 h of infection. *T. gondii* may spread first to mesenteric lymph nodes and then to distant organs by invasion of lymphatics, and blood and can multiply in virtually any cell in the body. All extracellular forms of the parasite are directly affected by antibody, but intracellular forms are not. More virulent strains of *Toxoplasma* have developed effective defensive mechanisms using ROP18, a rhoptry-associated serine/threonine kinase, to inactivate p47 GTPases, which are generated by the infected cell to rupture the vacuole containing the parasite, resulting in digestion of the organism (99). It is believed that cellular factors, including lymphocytes and lymphokines, are more important than humoral factors in immune-mediated destruction of *T. gondii* (100–102). Immunity does not eradicate infection. *T. gondii* tissue cysts persist for years after acute infection. The fate of tissue cysts is not fully known. Whether bradyzoites can form new tissue cysts directly without transforming into tachyzoites is not known. It has been proposed that tissue cysts may at times rupture during the life of the host. The released bradyzoites may be destroyed by the host's immune responses or there may be formation of new tissue cysts. In immunosuppressed patients, such as those given large doses of immunosuppressive agents in preparation for organ transplants and in those with acquired immunodeficiency syndrome (AIDS), rupture of a tissue cyst may result in transformation of bradyzoites into tachyzoites and renewed multiplication. The immunosuppressed host may die from toxoplasmosis unless treated. It is not known how corticosteroids cause relapse, but it is unlikely that they directly cause rupture of the tissue cysts.

TABLE 4 Clinical signs in humans[a]

Species	Signs
Cyclospora cayetanensis	Watery diarrhea, nausea, cramping, weight loss; symptoms can persist or relapse for weeks or months
Cryptosporidium spp.	Profuse diarrhea, nausea, cramping which lasts for 1–2 weeks; life threatening infections can occur in immunocompromised individuals
Giardia duodenalis/ *Giardia lamblia*	Asymptomatic to abdominal cramps, flatulence, foul smelling, greasy diarrhea, nausea, malabsorption of nutrients and failure to thrive, especially in young children (98)
Sarcocystis spp.	Tissue *Sarcocystis* may result in fever, swelling, muscle pain; intestinal *Sarcocystis* may result in abdominal pain, nausea, and diarrhea; both are frequently asymptomatic

[a]Table information (except *Giardia*) derived from reference 22.

Pathogenicity of *T. gondii* is determined by the virulence of the strain and the susceptibility of the host species (103). *T. gondii* strains may vary in their pathogenicity in a given host. Certain strains of mice are more susceptible than others, and the severity of infection in individual mice within the same strain may vary. Mice of any age are susceptible to clinical *T. gondii* infection (14). However, adult rats do not become ill, while young rats can die of toxoplasmosis. Adult dogs, like adult rats, are resistant, whereas puppies are fully susceptible to clinical toxoplasmosis. Certain species are genetically resistant to clinical toxoplasmosis. Cattle and horses are among the hosts more resistant to clinical toxoplasmosis, whereas certain marsupials and New World monkeys are highly susceptible to *T. gondii* infection (8, 14).

Nothing is known concerning genetically determined susceptibility to clinical toxoplasmosis in higher mammals, including humans. Infection in humans may be congenitally or postnatally acquired. Congenital infection occurs 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 trimesters (104, 105). 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 (104). Mild disease may consist of slightly diminished vision, whereas severely diseased children may have the full tetrad of lesions of the eye, hydrocephalus, convulsions, and intracerebral calcification. Of these, hydrocephalus is the least common but most significant lesion of toxoplasmosis. So far, the most common sequela of congenital toxoplasmosis is ocular disease (104, 105). The socioeconomic impact of toxoplasmosis in human suffering and the cost of care of sick children, especially those with mental retardation and blindness, are enormous (106, 107). The testing of all pregnant women for *T. gondii* infection is compulsory in some European countries, including France and Austria (108, 109).

The cost benefits of such mass screening are being debated in many other countries (105, 110, 111). Recently, Stillwaggon et al. (112) provided an extensive guideline for estimating costs of preventive maternal screening for and the social costs resulting from toxoplasmosis based on studies in Europe and the United States. When estimating these costs, the value of all resources used or lost should be considered, including the cost of medical and nonmedical services, wages lost, cost of in-home care, and indirect costs of psychological impacts borne by the family for lifetime care of a substantially cognitively impaired child; the cost of a fetal death was estimated to be $5 million dollars (112).

Postnatally acquired infection may be localized or generalized. Infection may occur in any organ. Oocyst-transmitted infections may be more severe than tissue cyst-induced infections (8, 113–117). Enlarged lymph nodes are the most frequently observed clinical form of toxoplasmosis in humans (113, 114). 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 a British Columbia outbreak, of the 100 people who were diagnosed with acute infection, 51 had lymphadenopathy and 20 had retinitis (50, 51). Encephalitis is the most important manifestation of toxoplasmosis in immunosuppressed patients because it causes the most severe damage to the patient (8, 118). 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 ranked high on the list of diseases which lead to the death of patients with AIDS, and approximately 10% of AIDS patients in the United States and up to 30% in Europe have died from toxoplasmosis (118). In AIDS patients, although any organ may be involved, including the testis, dermis, and spinal cord, infection of the brain is most frequently reported. Most AIDS patients suffering from toxoplasmosis have bilateral, severe, and persistent headaches which respond poorly to analgesics. As the disease progresses, the headaches 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 (119). Since the advent of highly active antiretroviral therapy (HAART) in the mid-1990s, the number of AIDS patients suffering from toxoplasmic encephalitis has fallen dramatically, at least partially due to the impact of protease inhibitors used in HAART on *Toxoplasma* proteases (120–122).

DIAGNOSIS AND TREATMENT

Diagnosis is made by biologic, serologic, or histologic methods or by some combination of the above (Table 5). Clinical signs of toxoplasmosis are nonspecific and are not sufficiently characteristic for a definite diagnosis. Toxoplasmosis mimics several other infectious diseases.

Detection of *T. gondii* antibody in patients may aid diagnosis. Numerous serologic procedures are available for the detection of humoral antibodies; these include the Sabin-Feldman dye test, MAT, the indirect hemagglutination test, the indirect fluorescent antibody assay (IFA), the direct agglutination test, the latex agglutination test, ELISA, and the immunosorbent agglutination assay test (IAAT). The IFA, IAAT, and ELISA have been modified to detect IgM antibodies (105). The IgM antibodies appear sooner after infection than the IgG antibodies and disappear faster than IgG antibodies after recovery, though a small percentage of infected people produce IgG first (105, 123). Progress has been made in the diagnosis of human infection with *Toxoplasma* using PCR (124). Infection has been diagnosed using nested, stage-specific primers and cerebrospinal fluid from AIDS patients with suspected toxoplasmic encephalitis (125,

TABLE 5 Diagnosis and treatment[a]

Species	Diagnosis	Treatment
Cyclospora cayetanensis	Oocysts in stool most common; PCR assays have been designed and used successfully for diagnosis	Most commonly treated with trimethoprim-sulfamethoxazole or ciprofloxacin
Cryptosporidium spp.	Oocysts in stool	Nitazoxanide is the only FDA-approved treatment for immunocompetent patients, along with supportive therapy
Giardia duodenalis/ *Giardia lamblia*	Oocysts in stool	5-nitroimidazole and benzimidazole derivatives, quinacrin, furazolidone, paromomycin, and nitazoxanide are all approved for treatment
Sarcocystis spp.	Oocysts in stool	Anticoccidials such as sulfanilamides are thought to be effective

[a]Table information derived from reference 140.

126), in immunocompromised patients undergoing hematopoietic stem cell transplantation (127), and in suspected cases of fetal toxoplasmosis using amniotic fluid (128). Improved sensitivity and performance standards for in-house methods and commercially available PCR kits is needed, because recent studies have shown that these PCR tests may not perform well using experimental or clinical samples (129–131).

Sulfadiazine and pyrimethamine (Daraprim) are two drugs widely used for the treatment of toxoplasmosis (132, 133). 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 do not usually 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 sulfonamides. Certain other drugs, such as diaminodiphenylsulfone, atovaquone, spiramycin, and clindamycin are also used to treat toxoplasmosis in difficult cases.

PREVENTION

To prevent infection of human beings by *Toxoplasma*, *Cryptosporidium*, *Cyclospora*, *Sarcocystis*, and *Giardia*, precautions should be taken to boil surface water before drinking to kill oocysts. General good hygiene should be observed, with frequent hand washing or wearing of gloves when in contact with soil or feces from animals or humans. Vegetables should be washed thoroughly before eating since they may have been contaminated with animal or human feces in irrigation water or soil.

Additionally, for *Toxoplasma* and *Sarcocystis*, the hands of people handling meat should be washed thoroughly with soap and water before they go to other tasks (8, 134). All cutting boards, sink tops, knives, and other materials coming in contact with uncooked meat should be washed with soap and water. *T. gondii* organisms in meat can be killed by exposure to extreme cold or heat. Tissue cysts in meat are killed by heating the meat throughout to 67°C (135) and by cooling to –13°C (136). *Toxoplasma* in tissue cysts is also killed by exposure to 0.5 kilorads of gamma irradiation (137). Meat of any animal should be cooked to at least 67°C before consumption, and tasting meat while cooking or while seasoning should be avoided. Thermal death curves for *Sarcocystis* have not yet been generated.

For *Toxoplasma*, due to the risk to the fetus, pregnant women 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, preferably not by a pregnant woman. Gloves should be worn while gardening. Expectant mothers should be made aware of the dangers of toxoplasmosis (138, 139).

At present there is no vaccine to prevent infection with *Toxoplasma*, *Cyclospora*, *Cryptosporidium*, *Giardia*, or *Sarcocystis* in humans.

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