Review

Special Issue: Zoonoses of people and pets in the USA

Toxoplasma gondii: epidemiology, feline clinical aspects, and prevention

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***Toxoplasma gondii* is a parasite of birds and mammals. Cats are the only definitive host and thus the only source of infective oocysts, but other mammals and birds can develop tissue cysts. Although feline infections are typically asymptomatic, infection during human pregnancy can cause severe disease in the fetus. Cat owners can reduce their pets' exposure risk by keeping all cats indoors and not feeding them raw meat. Humans usually become infected through ingestion of oocyst-contaminated soil and water, tissue cysts in undercooked meat, or congenitally. Because of their fastidious nature, the passing of non-infective oocysts, and the short duration of oocyst shedding, direct contact with cats is not thought to be a primary risk for human infection.**

Toxoplasmosis in cats and other animals

Toxoplasma gondii is a widespread zoonotic protozoan that infects most, if not all, species of birds and mammals. As the definitive hosts for this organism, felines are the only animals that pass oocysts in their feces (Table 1; Box 1), although intermediate hosts can harbor infective tissue cysts. Most feline infections occur post-natally through ingestion of infected tissue cysts or rarely oocysts, although congenital infections can occur [1]. Feline infections are typically subclinical; congenitally infected kittens are the most likely to have clinical signs of infection, but previously clinically healthy adult cats may can also be affected [1,4]. Common symptoms of *T. gondii* infection in cats can include fever, ocular inflammation, anorexia, lethargy, abdominal discomfort and neurologic abnormalities [4]. Cats are more likely to shed oocysts following ingestion of tissue cysts rather than tachyzoites or oocysts [5]. The ingestion of one bradyzoite will lead to feline infection, whereas a feline must ingest 1000 oocysts to develop an infection [5]. Following the ingestion of tissue cysts containing bradyzoites, some bradyzoites convert to tachyzoites and some to *T. gondii* schizonts, which replicate asexually in the intestinal tissue before beginning sexual reproduction (Figure 1) [5]. Although most cats only shed oocysts once in their lives, following infection, experimental infection and immunosuppression resulted in repeated shedding by kittens 20–21 days following the immunosuppressive event [6]. In addition, oocyst shedding was induced in a non-immunosuppressed kitten following a second inoculation with infected mouse brain homogenate [6]. It is thought that infection to one genotype of *T. gondii* confers immunity to all genotypes, but this phenomenon has not been explored in cats. In a mouse model, immunity was more effective using homologous rather than heterologous strains of the parasite [7,8].

Toxoplasma gondii infection can cause severe neurologic or ocular disease in the fetus during human pregnancy. Humans acquire their infections from ingestion of oocyst-contaminated soil and water, from tissue cysts in undercooked meat, by transplantation, blood transfusion, laboratory accidents, or congenitally [1] (Figure 1). Most people infected after birth are asymptomatic; however, some may develop fever, malaise, and lymphadenopathy. Congenital toxoplasmosis often results in debilitating ocular disease, causing (among other manifestations) retinochoroiditis and anterior uveitis [9]. Historically, women demonstrating exposure to *T. gondii* prior to pregnancy through serology were considered safe from future infection and risk to the fetus. However, apparent recrudescent infections during pregnancy can occur in immunocompetent mother although few cases have been reported [10]. A case of maternal *T. gondii* infection and subsequent fetal infection was reported in a 31-year old French woman with serological evidence of previous *T. gondii* exposure. The strain isolated from this pregnancy is likely to be the same strain as in the original infection [10], suggesting that exposure prior to pregnancy does not automatically confer protection from future infections that occur following recrudescence of *T. gondii* [10]. Post-natally infected humans, especially those with immunosuppression, can develop ocular complications such as retinochoroiditis.

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1471-4922/$ – see front matter doi:10.1016/j.pt.2010.01.009 Available online 2 March 2010

Table 1. Wild felids as definitive host for *Toxoplasma gondii*[a].

Definitive host	Oocyst shedding		Refs
	Expermental	Natural	
African wild cat (*Felis lybica*)	Yes[b]	No	[32]
Amur leopard cat (*Felis euptilurus*)	No	Yes[b]	[33]
Asian leopard (*Felis bengalensis*)	Yes	No	[34]
	Yes[b]	No	[35]
Bobcat (*Lynx rufus*)	No	Yes[b]	[36]
	Yes[b]	No	[35]
Cheetah (*Acinonyx jubatus*)	No	Yes[b]	[36]
	Yes[b]	No	[32]
Cougar (Felis concolor)	No	Yes[b]	[36]
	Yes[b]	No	[35]
Cougar (*Felis concolor vancouverensis*)	No	Yes[b]	[37]
Geoffroy's cat (*Oncifelis geoffroyi*)	No	Yes[c]	[38]
	No	Yes[b]	[33]
Iriomote cat (*Felis iriomotensis*)	No	Yes[b]	[39]
Jaguarundi (*Felis yagouaroundi*)	Yes[b]	No	[40]
Lion (*Panthera leo*)	No	Yes[d]	[41]
	Yes[b]	No	[32]
Mountain lion (*Felis concolar*)	No	Yes[b]	[36]
Ocelot (*Felis pardalis*)	Yes[b]	No	[40]
	No	Yes	[42]
Pallas cat (*Felis manul*)	No	Yes[b]	[43]
	No	Yes[d]	[44]
	Yes[b]	No	[32]
Pampas cat (*Oncifelis colocolo*)	No	Yes[c]	[38]
Siberian tiger (*Panthera tigris altaica*)	No	Yes[b]	[45]
Wild cat (*Felis silvestris*)	No	Yes[b]	[33]

[a]Data from Ref. [5].
[b]Confirmed by bioassay in mice.
[c]Confirmed by bioassay in pigs.
[d]Immunohistochemical post-mortem examination.

Toxoplasmic encephalitis, pulmonitis, or other systemic disease can be seen in patients with immunosuppressive disorders and those undergoing immunosuppressive therapy for circumstances such as organ transplantation.

Prevalence and risk factors for feline and human infection

The estimated seroprevalance for *T. gondii* in domestic cats (*Felis catus*), worldwide, is 30–40% [2]. There has been no national estimate of *T. gondii* prevalence in cats in the USA; however, local seroprevalences have varied from ~16% to 80% [3]. Serologic testing of clinically ill cats with toxoplasmosis as a differential diagnosis resulted in an overall seroprevalence of 31.6% [4]. Regional prevalences varied in conformance with different climates; prevalence in the drier southwest (New Mexico, Utah and Arizona) was lower (16.1%), whereas prevalence in humid climates was much higher (59.2% in Hawaii). These studies demonstrate that cats in the USA are commonly exposed to *T. gondii*. However, because of their fastidious nature, the fact that oocysts are not infective when passed, and the short duration of oocyst shedding, direct contact with cats is not thought to be a risk for human infection [1,4]. Cat owners can reduce their pets' exposure risk by: (i) keeping all cats indoors so they do not become infected by ingesting rodents and birds; (ii) not feeding cats raw meat; and (iii) controlling potential intermediate hosts such as rodents [10]. In cat populations in North Carolina, Nutter *et al.* [11] reported a higher seroprevalence for *T. gondii* in feral cats than in pet cats, and higher seroprevalence in pet cats that had access to outdoors than those that did not, thus supporting the case for restricting outdoor access for pet cats.

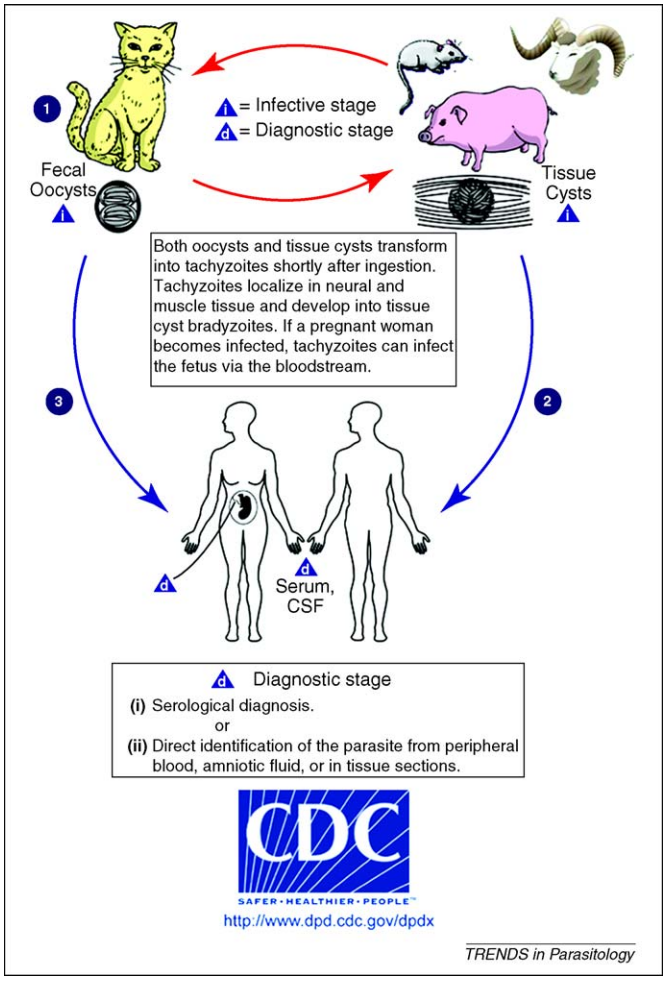

Figure 1. Transmission of *Toxoplasma gondii*. Members of the cat family (Felidae) are the only known definitive hosts for the sexual stages of *Toxoplasma gondii*, and thus are the main reservoirs of infection. Cats become infected with *T. gondii* by carnivorism (1). After tissue cysts or oocysts are ingested by the cat, viable organisms are released and invade epithelial cells of the small intestine, where they undergo an asexual followed by a sexual cycle and then form oocysts, which are excreted. The unsporulated oocyst takes one to five days after excretion to sporulate (become infective). Although cats shed oocysts for only one to two weeks, large numbers may be shed. Oocysts can survive in the environment for several months and are remarkably resistant to disinfectants, freezing, and drying, but are killed by heating to 70 °C for 10 minutes. Human infection may be acquired in several ways: A) ingestion of undercooked infected meat containing *Toxoplasma* cysts (2); B) ingestion of the oocyst from fecally contaminated hands or food (3); C) organ transplantation or blood transfusion; D) transplacental transmission; E) accidental inoculation of tachyzoites. The parasites form tissue cysts, most commonly in skeletal muscle, myocardium, and brain; these cysts may remain throughout the life of the host. This figure is an adaptation of the *Toxoplasma gondii* life cycle generated by the Centers for Disease Control and Prevention, http://www.dpd.cdc.gov/dpdx/HTML/Toxoplasmosis.htm.

Toxoplasma gondii is found in humans, worldwide, under a variety of climates and socio-economic circumstances. In the USA, the seroprevalence of *T. gondii* appears to be declining. A seroprevalence of 14.1% was reported during 1988–1994 in persons 12–49 years of age, whereas a seroprevalence of 9.0% was reported during 1999–2004 in this same age group (the prevalence values for these two time periods were age-adjusted and standardized to the US population for the comparison) [12]. A primary risk factor for human toxoplasmosis is contact with infective oocysts (Figure 1), which are found in cat feces (>1 day after shed), contaminated soil, and water [13]. Thus, gardening and playing in sandboxes increases

Box 1. Cats, clonality of *Toxoplasma gondii* and disease

Cats and wild felids are essential to the persistence of *T. gondii* in hosts such as grazing animals (e.g. sheep and deer) because they serve as the sole source of the infectious oocysts that contaminate the environment. At the same time, it is only in the feline host that sexual multiplication of this parasite takes place, so cats serve as the only site wherein genetic recombination and re-assortment of this parasite can occur. For the past several decades, it has been considered that, in spite of sexual recombination, there is only minimal genetic diversity among different isolates of *T. gondii* from around the world, with isolates being placed mainly in one of three genetic strains, Type I, II, and III [28]. However, it has recently been shown that there is significantly more genetic diversity in isolates from Brazil [29]. In one recent study, viable *T. gondii* isolates recovered from tissues of five to seven naturally infected cats from St Kitts, West Indies, had mixed infections with different genotypes [30]. The work indicated that the environment there was already contaminated with *T. gondii* oocysts shed by seropositive cats, suggesting that, in St Kitts at least, there is perhaps a high percentage of mixed infections in feral cats. No information yet exists to explain the drivers for this genetic diversity on St Kitts; additionally, no information exists to describe variability on other islands of the archipelago. We are only beginning to unravel the actual genetic relationship between virulence and the genetic makeup of the different strains of *T. gondii* present in the environment and in cases of human and animal disease [28]. Work on the genetics of virulence has indicated that some rhoptry proteins (held in the rhoptry organelles and released at the time of host cell penetration), for example ROP18, are linked to virulence in different genetic strains irrespective of their genetic strain assortment. Thus, work is moving towards a better understanding as to how to recognize the potential pathogenicity of isolates, which is important in a parasite that appears to vary in pathogenicity in different cases. It is also apparent that we are getting closer to being able to identify the specific sources of *T. gondii* responsible for outbreaks and individual cases of *T. gondii* infection [31].

the risk for humans of exposure to sporulated oocysts. Ways to minimize exposure risk include wearing gloves while gardening or otherwise interacting with soil, wearing gloves when disposing of cat litter, and washing hands after these activities [14]. Ingesting undercooked meat also provides an important avenue for *T. gondii* infection through the ingestion of viable tissue cysts. Undercooked pork is another common risk factor for *T. gondii* exposure; beef is not believed to be a major player, however, raw ground beef was found to be a risk factor in a US epidemiological study [13]. Other risk factors for infection include not washing kitchen knives after they have been used to cut raw meat, fruits, and vegetables, as well as infrequent hand washing [15].

Environmental contamination by *T. gondii* oocysts is a concern from both public health and biodiversity perspectives. Surface run-off containing feline feces containing *T. gondii* oocysts enters aquatic ecosystems, such as the marine habitat on the California coast [16]. An unusual genotype (Type X) of *T. gondii* has been identified to cause mortality in sea otters; infection with this genotype has also been found in several other coastal dwelling species, including marine bivalves and other filter-feeding invertebrates [16,17]. Sea otters (*Enhydra lutris nereis*) in these contaminated habitats might ingest invertebrates that concentrate *T. gondii* oocysts as a consequence of their feeding habits and as a result, prevalence in sea otters is high [16]. Sea otters that died of clinical toxoplasmosis had CNS abnormalities, behavioral changes, and reproductive problems such as abortion or neonatal death [16].

Diagnosis and treatment of infection in cats

Options for ante-mortem diagnosis in cats include fecal examination for oocysts (Figure 2) and serologic testing; definitive diagnosis of toxoplasmosis can be difficult to accomplish [18]. Most infected cats will shed oocysts only at a single point in their lifetime, generally for a period of one to two weeks, and it has been estimated that only ~1% of cats at any given time are actively shedding (Table 2) [1]; this estimate was supported through the observation of *T. gondii*-like oocysts in a population of Californian cats [19], although tests to confirm the oocyst identity were not reported. The probability of finding oocysts in the feces of a cat is low; a survey of cats in Germany and other European countries recently reported detection of *T. gondii* oocysts (Figure 2) in 26 of 24,106 (0.11%) fecal samples [1]. Complicating fecal detection is the fact that oocysts of other coccidia morphologically resemble those of *T. gondii* (*Hammondia hammondi* and *Besnoitia* spp); molecular and bioassay techniques are used to distinguish between organisms, and mouse bioassay is the only definitive confirmation method (Table 2) [5]. Serology can also be used as a diagnostic tool; however, positive results must be properly interpreted. Although a single positive IgG titer indicates exposure, clinical toxoplasmosis is indicated by a positive IgM titer or a fourfold increase in IgG levels in paired serum samples taken 2–4 weeks apart [4,18]. Because most cats seroconvert after they have finished shedding oocysts, the use of serology in pet cats as an indication of exposure risk to humans is limited [1,4].

There is no approved treatment for clinical toxoplasmosis in cats. Sulphonamides, trimethoprim, pyrimethamine, and clindamycin, either alone or in combination, have been used to treat cats with clinical toxoplasmosis, with varying results [20]. Ponazuril, an approved treatment for equine protozoal myeloencephalitis caused by *Sarcocystis neurona* in horses, is excellent in treating acute toxoplasmosis in mice [20] and in preventing recrudescent encephalitis

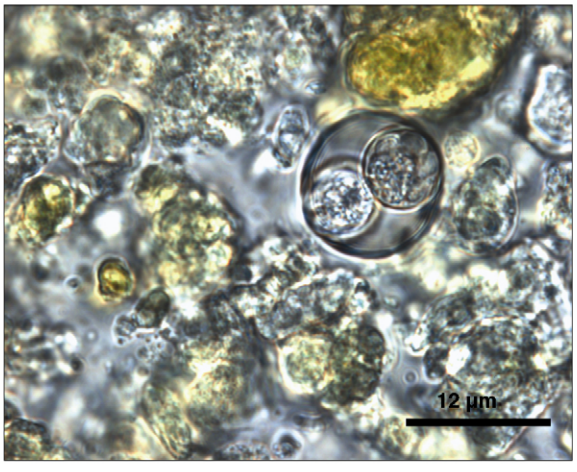

Figure 2. Sporulated oocyst of *Toxoplasma gondii* in sugar flotation of cat feces (dimensions 12 × 12.5 µm. Average size of *T. gondii* oocyst is 10–12 µm [27]. Photo courtesy of A. Lucio-Forster, Cornell University, USA.

Table 2. Prevalence of *T. gondii*-like oocysts in feces of cats (principally 1988–2008)[a]

Country	No. of cats	Grams of feces tested	Methods			No. pos (%)	Refs
			Micro	Bioassay	PCR		
Argentina	50	NS	Yes	Yes	No	**1(2.0)**[d]	[46,47]
Austria	1368	NS	Yes	No	No	27(2)	[48]
Belgium	30	NS	Yes	No	No	0	[49]
Brazil	237	1	Yes	Yes	Yes	3(1.2)	[50]
Colombia	18	NS	Yes	No	No	12 (66.6)	[51]
	143	2-10	Yes	Yes	No	0	[52]
Czec Republic	390	NS	Yes	Yes	No	0	[53]
France	322	NS	Yes	No	No	0	[54]
Germany	264	NS	Yes	No	No	0	[55]
	70[b]	NS	Yes	No	No	(17.1)	[56]
	24106	NS	Yes	No	Yes	26(0.11)	[57]
	2473	NS	Yes	No	No	22 (0.9)	[58]
	2472	NS	Yes	No	No	26 (1.0)	[59]
	441	NS	Yes	No	No	3 (0.7)	[60]
India	9	NS	Yes	Yes	No	**1(11)**	[61]
Iran	50	NS	Yes	No	No	0	[62]
	100	1	Yes	No	No	0	[63]
Israel	122	3	Yes	No	Yes	11(9)[e]	[64]
Japan	335	NS	Yes	Yes	No	**1(0.3)**	[65]
Mexico	200	NS	Yes	Yes	No	**14 (7.0)**	[66]
Mexico	200	NS	Yes	No	No	0	[67]
New Zealand	63	NS	Yes	No	No	0	[68]
Nigeria	52	NS	Yes	No	No	0	[69]
Panama	383	NS	NS	Yes	No	**2 (0.5)**	[70]
People's Republic of China	26	10	Yes	Yes	No	0	[71]
Singapore	722	1-2	Yes	No	No	0	[72]
Spain	382	3	Yes	No	No	0	[73]
	592	NS	Yes	No	No	0	[74]
Taiwan	96	NS	Yes	No	No	0	[75]
Turkey	72	NS	Yes	No	No	0	[76]
USA	274[c]	NS	No	Yes	No	**5 (1.8)**	[77]
	206	NS	Yes	No	No	0	[78]
	450	NS	Yes	No	No	3 (0.7)	[79]
	326	NS	Yes	No	No[f]	3 (0.9)	[20]
	263	NS	Yes	No	No	3 (1.1)	[80]
	34	NS	Yes	yes	No	0	[81]

[a]Data from Ref. [82].
[b]Litters of kittens from farms.
[c]Cats from pig farms.
[d]Confirmed by bioassay in mice.
[e]Oocysts not found by microscopic examination.
[f]Negative by PCR.

in mice [21], and should be evaluated in domestic cats. The recommended treatment in cases of human cerebral toxoplasmosis is pyrimethamine and sulfadiazine (plus folinic acid) [1].

Prevention of infection in cats and humans

In the absence of an effective vaccine in humans, prevention of zoonotic transmission might be the best way to approach the problem of toxoplasmosis, and must be done by limiting exposure to oocysts or tissue cysts. Recommendations for accomplishing this include practicing good hygiene (e.g. hand washing after soil contact, washing fruits and vegetables that are eaten raw), freezing meat at −12 °C for 24 hours [13] and/or cooking meat until an internal temperature of 66 °C is reached, and not drinking untreated water [1]. It is also recommended to keep cats indoors, feed them commercially prepared diets, and clean their litter boxes daily, because it takes at least one day for the organisms to sporulate and become infectious after being shed [4]. Recommendations specifically for pregnant women include wearing gloves when gardening or being in contact with soil or sand, followed by thorough hand-washing [22]. In addition, pregnant women should avoid changing cat litter if possible. Owners should also be advised to keep dogs away from the litter box to prevent ingestion of oocysts [23]. Health education for women of childbearing age should include information about meat-, soil-, and cat feces-related toxoplasmosis prevention. Immunosuppressed persons, including those with HIV infection, should also be educated about how to prevent infection [24]. Veterinarians should educate their canine-owning clients of the importance in vaccinating against distemper, as most cases of toxoplasmosis in dogs are seen in those not vaccinated against this immunosuppressive virus [1].

Vaccine development to prevent feline oocyst shedding is ongoing, mostly involving live vaccines. There are some disadvantages, including its limited shelf life and the risk of infection to humans who are handling the vaccines [25]. The S48 strain Toxovax® is a live vaccine originally developed for use in sheep, but, when used in cats, inhibits sexual development of *T. gondii*. Thus, the immune system responds to the parasite strain, but cats are unable to produce oocysts [25]. In sheep, this vaccine is used to reduce tissue cyst development. The T-263 strain of *T. gondii* is a live mutant strain designed to reduce or prevent oocyst shedding by cats by developing only partially in the feline intestinal tract [25]. Field trials with this feline vaccine were conducted on US pig farms [25,26]. Cats were trapped, vaccinated, and released. Following the vaccination of the resident cats on the farms, *T. gondii* seroprevalence in the farmed pigs decreased, suggesting less environmental contamination with oocysts, and thus, less infection risk for the pigs [25,26]. There are reports of attempted vaccination of cats using *T. gondii* strains modified by irradiation, chemical treatments, selected recombinant antigens, and new delivery systems, including a feline herpes-virus type 1 vehicle for delivery. All of these vaccines supply a level of immunity to cats, but a need exists for a killed or recombinant vaccine that could serve as a model for human vaccination against *T. gondii*.

Future research

Toxoplasmosis is a potentially serious disease, but one that is largely preventable. Informed clinicians—both in the human and veterinary medical professions—can decrease disease transmission while recognizing and helping to maintain the relationships that many people have with their domestic cats. The increased understanding of *T. gondii* infection provides exciting new opportunities for research, both in the veterinary and human medical fields (Box 2). Because the ingestion of oocysts is an important route of infection, future research should address the development of new methods to detect and inactivate infective oocysts in feces, soil, and water. Studies evaluating the survival or inactivation of the organism in commercial cat litters would be extremely valuable. Currently there is no information regarding cat litter and oocyst inactivation. Large scale, national serologic and fecal surveys of indoor, indoor/outdoor, and feral cats could also be accomplished by obtaining blood and fecal samples from veterinary clinics and trap/neuter/release programs. Such information could provide insight into management strategies to mitigate environmental contamination caused by outdoor cat populations. Finally, future projects should commit to improving communication and education methods for veterinarians, physicians, cat owners, and the general public about toxoplasmosis risk factors and mechanisms of prevention. Such efforts, and a means of evaluating their impact, might promote a decline in toxoplasmosis and transmission of *T. gondii*.

Box 2. Future directions for Toxoplasmosis Research and Education

- Development of new detection methods to assess environmental contamination
- Evaluation of the survival or inactivation of the organism in commercial cat litters
- New treatment protocols for cats
- Methods to prevent oocyst shedding
- Development of a killed, inexpensive vaccine for both domestic and feral cats
- Management of outdoor cat populations and other means of decreasing environmental contamination
- Methods for distinguishing the source of human infections (oocysts versus tissue cyst)
- Further development of diagnostic tests for cats with clinical disease
- Exploration of better public and professional education methods
- Examination of host genetics on *T. gondii* infection
- Factors affecting the virulence of different *T. gondii* genotypes
- Continued study of the relationships between chronic toxoplasmosis and human behavior.

Acknowledgements

This material developed out of a workshop held at the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia in 2008 with invited representatives from the Companion Animal Parasite Council (CAPC), the CDC, the American Association of Veterinary Parasitologists (AAVP), and others. Many people made this publication possible; Lora Ballweber assisted with the original drafts of this document and critical help was provided by Lonnie King and Mary Bartlett of the CDC, and Sonya Hennessey of CAPC. The authors also thank Blaine Mathison for assistance in creating Figure 1.

The findings and conclusions in this manuscript are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

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