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Toxoplasma oocysts as a public health problem

E. Fuller Torrey[1] and Robert H. Yolken[2]

[1] The Stanley Medical Research Institute, 8401 Connecticut Avenue, Suite 200, Chevy Chase, MD 20815, USA
[2] Johns Hopkins University Medical Center, The Stanley Laboratory of Developmental Neurovirology, 600 North Wolfe Street, Blalock 1105, Baltimore, MD 21287-0005, USA

Waterborne outbreaks of *Toxoplasma gondii* have focused attention on the importance of oocysts shed in the feces of infected cats. Cat feces deposited annually into the environment in the United States total approximately 1.2 million metric tons. The annual oocyst burden measured in community surveys is 3 to 434 oocysts per square foot and is greater in areas where cats selectively defecate. Because a single oocyst can possibly cause infection, this oocyst burden represents a major potential public health problem. The proper disposal of cat litter, keeping cats indoors, reducing the feral cat population, and protecting the play areas of children might potentially reduce the oocyst burden.

Why cats and *Toxoplasma* oocysts are important

Cats enrich the lives of many and have become increasingly popular as pets in many parts of the world. In the United States, between 1989 and 2006, cat ownership increased approximately 50% (from 54.6 to 81.7 million pet cats), whereas dog ownership increased by 38% (from 52.4 to 72.1 million dogs); yet, the human population increased by only 23% [1]. In addition to owned cats, the number of feral cats in the United States has been estimated to be between 25 and 60 million [2]. In the UK, the cat population was estimated to be 8.0 million in 2009, having increased from 4.5 million in 1990 [3,4]. Cat ownership has also increased in other parts of the world, especially Latin America and China. Cats have close contact with their owners in many families; one American study reported that 62% of cats slept with their adult owners and another 13% slept with children [5].

Cat feces are known to carry a variety of infectious agents, including the oocysts of *Toxoplasma gondii*, a coccidian protozoan of Phylum Apicomplexa. When *T. gondii* infects previously seronegative pregnant women it may cause a congenital syndrome that includes deafness, seizures, retinal damage, and mental retardation in the fetus or neonate. In immunocompromised individuals, such as those with HIV infection or undergoing immunosuppressive chemotherapy, it may produce severe central nervous system damage, seen less often since the introduction of effective antiretroviral treatment. Until recently, *T. gondii* infection was assumed to be largely asymptomatic in immunocompetent individuals. This notion is now under reconsideration following the outbreak of toxoplasmosis epidemics, including ocular toxoplasmosis, which are associated with *T. gondii* oocyst contamination of water [6–12]. Additional concerns have been raised by recent studies of schizophrenia [13], depression [14], suicidal behavior [15], obsessive–compulsive disorder [16], rheumatoid arthritis [17], brain cancer [18], and scholastic underachievement in children [19], which have reported correlations between such conditions and elevated *T. gondii* seropositivity rates as compared with those in control populations.

How infection occurs

Felines, including domestic cats, are the definitive hosts of *T. gondii*, and the organism can only complete its sexual cycle within feline hosts. Cats usually become infected with *T. gondii* when they initially begin to hunt and ingest an infected bird or small mammal [20]. When infected, the cats deposit fecal oocysts in the soil, grass, animal feed, water, or elsewhere. There they may be ingested by another animal and, as bradyzoites (see Glossary), become tissue cysts in that animal, especially in muscle tissue. If the infected tissue is eaten without being properly cooked, it may infect humans or other animals. Thus, *T. gondii* is a common infection of not only farm animals but also many wild animals such as raccoons, bears, and deer [21].

In addition to humans becoming infected by ingesting tissue cysts, they may also become infected by ingesting or inhaling the *T. gondii* oocysts directly. This may occur when they are changing the litter box of a cat, gardening, playing in a sandbox, eating unwashed fruits or vegetables, or drinking water containing oocysts. Studies have shown that cockroaches and flies may carry oocysts from cat feces to unprotected food [22,23]. *T. gondii* oocysts may even infect humans who pet dogs that have rolled in cat feces

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> **Glossary**
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> **Bradyzoites**: the slowly dividing form of *Toxoplasma gondii* which becomes encysted in the tissues of the host.
> **Sporozoite**: the form of *T. gondii* in which it is excreted by felids as oocysts. The sporozoites may then become tachyzoites or bradyzoites.
> **Tachyzoite**: the rapidly dividing form of *T. gondii*, which is crescent-shaped and approximately 2×6 μm in size.
> **Tissue cyst**: the vehicle which holds the bradyzoites, usually found in brain, muscle, or eye tissue. Tissue cysts vary in size depending on the number of bradyzoites they hold.

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[24]. Thus, there are many ways to become infected which do not involve a person having had any direct cat contact.

The number and viability of *T. gondii* oocysts are impressive. Studies have reported that approximately 1% of cats are shedding oocysts at any given time [3,25] and that they excrete oocysts for a median of 8 days with a total of up to 55 million oocysts per day [3,25]. The total number of oocysts shed by a single cat varies widely from 3 to 810 million [3]. The oocysts are remarkably stable, especially if they are deposited in shady, moist, and temperate conditions. In Texas, under outdoor shaded conditions with a mean air temperature of 19.5°C, oocysts remained viable during a 13-month experiment [26]. In Kansas, oocysts were buried in loose soil and remained viable for 18 months [27]. Oocysts maintained experimentally at 4°C in seawater or freshwater remained viable for 24 and 54 months, respectively [28,29]. Oocysts also survived for over a year in vials of 2% sulfuric acid at 4°C [30]. Because almost all of these studies were terminated while at least some of the oocysts were still viable, we do not yet know the outer limit of viability for *T. gondii* oocysts deposited in various environmental conditions (Box 1).

The percentage of human *T. gondii* infections acquired by tissue cysts versus oocysts is not known and probably varies depending on environmental conditions (Box 1). In a country such as Ethiopia, which has a very high rate of seropositivity and a custom of eating raw beef, it is assumed that tissue cysts are the main source of infection [31]. However, recent studies have reported that the majority of congenital infections [32] and postnatal acute infections [33] in the United States are from oocysts. Experimental observations raised an additional concern: non-dose-dependent ingestion of oocytes results in a more severe infection than those induced by tissue cysts and bradyzoites [25]. It is thus apparent that *T. gondii* oocysts are worthy of additional study.

Oocyst burden on the environment

Given the large number of oocysts excreted by infected cats, attempts have been made to calculate the oocyst burden in the environment. In California, a study of 12 244 households in three communities assessed the disposition of cat feces [34]. The study identified 7284 pet and 2046 feral cats. Among the owned cats, 48% used a litter box at least 75% of the time, 44% defecated outside at least 75% of the time, and 8% both used a litter box and defecated outdoors between 25% and 75% of the time. The litter boxes were disposed of as follows: (i) 4% of cat owners dumped the contents of the litter box on or near their property; (ii) 9% flushed litter down the toilet; and (iii) the remainder put the litter in the garbage. It was assumed that feral cats defecated outside all of the time. The final destination of cat feces was of particular interest in this study because it was attempting to determine the origin of cat fecal contamination thought to be responsible for the deaths of sea otters in the Morro Bay region of California [35].

Based on where the cat owners disposed of feces, the total fecal and oocyst burden on the environment was determined for the three communities [34,36]. In a related study, it was shown that the mean daily defecation per cat

totaled 40.2 g for adult cats and 31.7 g for kittens [34]. The environmental accumulation from pet cats was calculated to be 76.4 tons of feces each year in three communities, while feral cats deposited an additional 29.5 tons [34]. Insofar as these communities are representative of the United States population, the 81.7 million owned cats would produce 856 930 tons of outdoor cat feces each year. Assuming there are only 25 million feral cats, these would produce another 360 459 tons of cat feces, resulting in a total accumulation of 1 217 389 tons deposited annually in the environment of the United States.

The *T. gondii* annual oocyst burden in the three California communities was calculated by dividing the cat feces by the land area of residential housing. The communities differed by size and number of cats [34]. Depending on estimations of oocyst production by the cats, the number of *T. gondii* oocysts ranged from 9 to 434 per square foot [36]. A similar study was carried out in three communities in rural France, using comparable assumptions, and reported that the annual environmental oocyst burden varied from 3 to 335 oocysts per square foot [37]. In another French study, *T. gondii* oocysts were identified in 8 of 62 soil samples collected from cat defecation sites on the grounds of an urban hospital [38]. In Brazil, *T. gondii* oocysts were isolated from ten soil samples taken from the playgrounds of 31 elementary schools; the authors suggested that these results indicated a wide distribution of *T. gondii* oocysts around elementary schools in the region [39]. In a village in Panama, it was estimated that the oocyst burden in soil near houses where cats are fed varied from 18 to 72 per square foot [40]. In Poland, *T. gondii* oocysts were isolated from 18 of 101 soil samples taken from places thought to be favored by cats for defecation: sandboxes, playgrounds, parks, gardens, and areas around rubbish pits [41].

A study of *T. gondii* oocysts in public parks was also recently carried out in Wuhan, China [42]; under the regime of Mao Zedong, the keeping of pets was considered bourgeois and discouraged. Pet keeping only started to become prevalent after the death of Mao in 1976 and did not become common until recent years [43,44]. Yet, when 252 soil samples were taken from six public parks in Wuhan in 2009 and 2010, 58 samples (23%) contained *T. gondii* oocysts. The soil samples were taken from areas frequented by cats. The wider prevalence of *T. gondii* in modern China is also reflected in surveys of *T. gondii* seropositivity in pregnant women. In seven studies between 1996 and 2004, the average seropositivity rate was found to be 4.5%, but in six studies completed since 2004 the average rate was 10.2% [45].

Because cats do not defecate randomly but rather select places with loose soil so that they can cover their feces, gardens, children's play areas with loose soil, and especially sandboxes (also called sandpits and sand piles) are favored sites. A study in Japan quantified the frequency of cat defecations in three uncovered urban sandboxes by monitoring them for almost 5 months with night lights and camcorders [46]. The study measured *Toxocara* eggs, not *Toxoplasma* oocysts, as a measure of cat fecal contamination of public sandboxes, but it is useful in providing an accurate count of cat defecations. During this period, there were a total of 961 cat and 11 dog defecations, mostly

Table 1. Estimated accumulation of *Toxoplasma gondii* oocysts in sandboxes[a]

Sandbox[b]	Dimensions (square feet)[b]	Cat defecations observed in 20 weeks[b]	Infected cat defecations estimated after 18 months[a]	Number of oocysts in sandbox[a]	Oocysts per square foot[a]
A	344	96	3.8	19 million	55 184
B	247	201	7.8	39 million	157 575
C	194	664	26.0	325 million	1 677 852

[a]The authors provide a hypothetical calculation of *Toxoplasma* oocysts deposited by cats into sandboxes of children, extrapolated from a study of *Toxocara* cysts quantitated from urban sandboxes in Japan [46].

[b]Data from [46].

occurring at night, in the three sandboxes, which varied in size: 344, 247, and 194 square feet. If we use this number of cat defecations, assume that 1% of the cats were shedding *T. gondii* oocysts, assume that each of these cats shed a total of 5 million oocysts during the time they were shedding, and assume that the oocysts remained viable for 18 months (although specific sand survival studies have not been done), we estimated the hypothetical accumulation of oocysts per square foot in each sandbox. This estimate is 55 184, 157 575, and 1 677 852 oocysts per square foot in the three sandboxes (Table 1). The variation presumably was due to the residential density of that neighborhood and number of cats living nearby.

What are the chances of a child playing in such a sandbox becoming infected with *T. gondii*? One study of young children reported that children who are under 3 years of age put their hands or other objects in their mouths every 2–3 min [47]. Another study, which included 64 children between 1 and 4 years old, carried out in a Massachusetts daycare center, reported that the children ingested a median of 40 mg of soil per day; furthermore, one child consumed 5–8 g of soil per day on average [48]. Although there are no measurements of how many *T. gondii* oocysts are required to infect a child, for obvious reasons, a study that was conducted with pigs found that a single oocyst was sufficient to cause infection in 13 of 14 experimentally infected pigs [49]. Because *T. gondii* oocysts are known to become aerosolized when they dry out, it is also possible that a child playing in such a sandbox could become infected simply by breathing in oocysts [50].

A historical perspective
The relationship between *T. gondii* and the presence of cats has been clearly established for almost half a century. On an isolated Pacific island where cats were just being introduced, the *T. gondii* infection rate among inhabitants was zero except among inhabitants who had lived elsewhere. By contrast, on two neighboring islands where cats were present, the infection rates were 49% and 56% [51,52]. A similar situation was observed in parts of Papua New Guinea in that evidence of human infection was rare when there was a complete lack of felids or where domestic cats had been introduced recently; conversely, areas where cats were more numerous and had been present longer were associated with a higher rate of human infection [53].

The widespread keeping of cats as pets is a relatively recent historical development, starting in the late eighteenth century but becoming more prevalent in the late nineteenth century. Historians of pet keeping date 'the beginning of the cat fever in America' to the first cat show in the United States, held in New York's Madison Square

Garden on May 8, 1895 [54]. At that time, there was said to have been 'a rapid and promising growth of what disaffected and alliterative critics call the "cat cult," and poets and printers vie with one another in celebrating the charms of this long-neglected pet' [55].

The affluence that followed World War II in the United States resulted in another rise in pet keeping, mostly of dogs and cats. By the early 1970s, cats were being kept as pets even in Eskimo villages north of the Arctic Circle, purportedly arriving with school teachers from other areas of the United States [56]. Cats subsequently became common in Inuit villages in the Arctic and also became widespread on Maquarie Island in the Antarctic [57]. Recent studies have documented *T. gondii* infection among seals in both the Arctic and Antarctic, but whether the infection occurs there or elsewhere is unknown [57,58].

The most recent increase in pet keeping, including the 50% increase in owned cats, was reported between 1989 and 2006 in the United States [1]. There is no historical precedent for such numbers of cats. This should raise public health concerns about the number of *T. gondii* oocysts being distributed in the environment, especially as we do not yet know the limits of oocyst viability or the true relationship between these oocysts and the human diseases with which they have been recently associated.

Unanswered questions
A steady decrease in the incidence of human toxoplasmosis, as assessed by antibody levels, has been reported among adults over the past two decades in the United States and Northern Europe [59]. Given the increasingly extensive distribution of *T. gondii* oocysts, how can these two facts be reconciled (Box 1)? One possibility is that the decrease is being driven by improved methods of meat keeping, especially given the prevalence of freezers, which kill most of the *T. gondii* tissue cysts [60]. Another possibility is that the increased distribution of *T. gondii* oocysts in the environment is too recent to have been observed in epidemiological surveys. Many such surveys only included adults, whereas more recent investigations into contamination of sandboxes, play areas, and school grounds with *T. gondii* oocysts, which could be a source of infection for children, could perhaps indicate a wave of infections yet to be diagnosed. Still another possibility is that our present methods for detecting antibodies to *T. gondii* do not detect unusual strains or recent antigenic modifications of existing strains and thus are not an entirely accurate measure of *Toxoplasma* exposure. Clearly, these are questions needing additional research (Box 1).

Another interesting question involves studies that have reported a weak relationship and sometimes no

Box 1. Outstanding questions

- Why is the incidence of *Toxoplasma gondii* infection decreasing despite the increasing incidence of cat ownership?
- How accurate are our present methods for detecting antibodies to unusual strains of *T. gondii*?
- What are the limits for the viability of *T. gondii* oocysts under different climactic conditions?
- Are the consequences of being infected with *T. gondii* oocysts in humans different from being infected with tissue cysts?

relationship at all between cat ownership and *T. gondii* seropositivity [61–65]. Such studies should distinguish between exposure to indoor cats, which pose minimal risk, and outdoor cats. Such studies should also try and differentiate exposure risks for children, such as playing in a contaminated sandbox, from exposure risks for adults, such as eating raw meat (Box 1). It would also be very useful if we had an ability to distinguish whether the original infection was by tissue cyst or oocyst and to ascertain whether the outcomes are different. Because cats are now so ubiquitous in the environment, one may become infected by neighboring cats which defecate in one's garden or play area, or by playing in public areas such as parks or school grounds. Indeed, as cats increasingly contaminate public areas with *T. gondii* oocysts it will become progressively more difficult to avoid exposure.

Implications for public practice

Given the number of both feral and pet cats, the number of *T. gondii* oocysts they excrete while infectious and the longevity of the oocysts, there are several implications for public practice. First, it should be assumed that the play areas of children, especially sandboxes, are highly infectious unless they have been covered at all times when not in use or are located in a protected area not accessible to cats. If in doubt, sand in sandboxes should be replaced and protective barriers put in place. Covered and protected sandboxes have been demonstrated to remain uninfected [66]. Second, it should also be assumed that gardens to which cats have access are infectious, and gardeners should wear gloves and wash their hands after completing gardening. One research group reported that 7–13 mg of soil can be removed from under the fingernails after digging in the dirt; this quantity of soil could harbor up to 100 *Toxoplasma* oocysts [27]. Because of possible contamination, fruits and vegetables should be thoroughly washed.

If the oocyst stage of the disease can be modulated, then the disease cycle will be better controlled, especially if tissue cyst transmission can also be minimized by adequate cooking of meat and the control of infections among food animals in the farm environment [67]. Prevention can also be accomplished, in addition to the above suggestions, by educating the public regarding the proper disposal of cat litter, by keeping cats indoors to minimize their acquisition of infection from prey or the environment, and by reducing the feral cat population. Research directed at the prevention of *Toxoplasma* infection in cats by the use of immunization and other interventions should also be strongly encouraged.

Concluding remarks

There is evidence that accumulating *T. gondii* oocysts in the environment pose a significant public health hazard, especially in the sandboxes of children, gardens, and other places favored by cats for defecation. The increasing number of cats in the United States, enormous number of oocysts shed by each cat which becomes infected, unknown parameters for the viability of the oocysts, and the fact that mammals may become infected by a single oocyst should give us cause for concern. The potential magnitude of contamination of the environment by *T. gondii* oocysts is thus impressive. What is not known are the possible effects such oocysts may have on humans [13–19]. We should therefore implement practices to minimize *T. gondii* oocyst transmission to humans even as we simultaneously undertake more research to answer the outstanding questions.

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