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SPORULATION AND SURVIVAL OF *TOXOPLASMA GONDII* OOCYSTS IN DIFFERENT TYPES OF COMMERCIAL CAT LITTER

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ABSTRACT: *Toxoplasma gondii* oocysts are environmentally resistant and can survive outdoors for many months in dry and cold climates. In the present study, sporulation and survival of *T. gondii* oocysts was studied in different types of cat litters commercially available in the United States. Oocysts sporulated within 2–3 days in all types of cat litters and occasionally remained viable for 14 days. Results indicate that cat litter should be changed daily to prevent sporulation and infectivity to people.

Toxoplasma gondii infects virtually all warm-blooded animals, including humans, livestock, and marine mammals (Dubey, 2010). Various surveys have found that 10–50% of the adult population in the United States has been exposed to this parasite (Dubey and Jones, 2008). Humans become infected postnatally by ingesting tissue cysts from undercooked meat, or consuming food or drink contaminated with oocysts. The proportion of the human population that acquires infection by ingestion of oocysts in the environment or by eating contaminated meat is not known and currently there are no tests available that can determine the source of infection.

Cats are considered the key hosts in the epidemiology of *T. gondii* infection because they are the only species that can excrete the environmentally resistant oocysts. Cats have a worldwide distribution, except the frozen Arctic (Dubey, 2010). For example, approximately 1/3 of households in the U.S. own a cat, and this number is steadily increasing. There are approximately 78 million domestic cats and 73 million feral cats in the United States (Conrad et al., 2005). The fate of cat feces disposed of in the toilet or in domestic trash destined for landfills is unknown (Elmore et al., 2010). It is anticipated that the heat generated and lack of oxygen in landfills will kill some, or all, oocysts, depending on the conditions. It is likely that oocysts are carried into our homes on shoes contaminated with oocysts on street pavement. If one assumes a 30% seropositivity of 151 (78 domestic and 73 feral) million cats and a conservative shedding of a total of 1 million oocysts per cat, then there will be enormous numbers of oocysts (50 million × 1 million) in the environment. Dabritz et al. (2007) estimated an annual number of 94 to 4,671 oocysts/m^2 in California.

Cats can shed millions of oocysts after ingesting a few *T. gondii* bradyzoites (Dubey, 2001). *Toxoplasma gondii* oocysts are shed unsporulated in freshly passed cat feces and they can sporulate in 1 day under ideal environmental conditions of humidity, aeration, and ambient temperature (Dubey et al., 1970). The environmental resistance of *T. gondii* has been amply documented (Yilmaz and Hopkins, 1972; Frenkel et al., 1975; Dubey, 2010; Elmore et al., 2010; Jones and Dubey, 2010). However, little is known of the sporulation rate of *T. gondii* in cat feces in nature. The environmental resistance of *T. gondii* depends upon the stage of the development of the oocyst. For example, unsporulated oocysts are killed by 8 hr exposure to 37 C, whereas sporulated oocysts remain viable at 40 C for 28 days (Dubey et al., 1970; Dubey, 1998).

Many homeowners use litter pans and cat litter in which cats defecate. There are numerous brands of cat litter on the market (Table I), with various recommendations for disposal of feces ranging from changing of litter daily to once a week. These commercial products have a warning label concerning the transmission of *T. gondii* to humans, especially pregnant women. However, we are not aware of any studies on survival of oocysts in cat litter. The objective of the present investigation was to examine sporulation and survival of *T. gondii* oocysts in commercial cat litter.

MATERIALS AND METHODS

Toxoplasma gondii oocysts

Cats were infected by feeding *T. gondii* tissue cysts of 4 strains (CT-1,TgCatBr1, TgCatBr2, and TgGoatUS4) as described (Dubey, 1992, 1995; Dubey et al., 2004, 2011). Feces were collected daily from litter pans of cats, 5–7 days after feeding *T. gondii* tissue cysts and emulsified with enough water to make a thick paste. An aliquot (7–8 g, experiments 1 and 2, 10 g in experiment 3) was transferred to a sample of different types of cat litter (Table I) in disposable plastic 11 × 8–cm, 400-ml cups; a cup was filled to 75% capacity and the fecal aliquot was covered such that a small layer of litter covered the feces completely. These infected cat feces in different types of cat litters were left uncovered in a secure building. Temperature and humidity were recorded daily; they varied from 17 to 23 C and 20 to 26%, respectively. For control, an aliquot of feces (7–8 g, experiments 1 and 2, 10 g in experiment 3) was suspended in 50 ml of 2% H_2SO_4 and placed on a shaker for 1 wk at room temperature (20–23 C); 2% H_2SO_4 prevents microbial growth without affecting the oocysts.

Sporulation and viability of oocysts

After designated exposure intervals, feces were removed from the fecal cups, suspended in 50 ml 2% H_2SO_4, incubated on a shaker at room temperature, and, after 1 wk, filtered through gauze and centrifuged; the supernatant was discarded. The sediment was suspended in 45 ml of 33% sucrose solution (sp. gr. 1.15) and centrifuged for 10 min; then 5 ml of the supernatant were removed, mixed with 45 ml of water and centrifuged, and the sediment was suspended in 10 ml of 2% H_2SO_4, and stored at 4 C until evaluated (designated sample A). Each sample was bioassayed in mice to determine *T. gondii* infectivity. For this, 2 ml of each sample A was neutralized with 3.3% NaOH and centrifuged. The pellet was suspended in 1 ml of saline (0.85% NaCl) and inoculated orally into 2 mice. The inoculated mice were observed for 2 mo. Impression smears of tissues (mesenteric lymph nodes, lungs) of dead mice were examined microscopically for *T. gondii* tachyzoites. The survivors were bled 7–8 wk postinoculation (PI) and a 1:25 dilution of their sera was tested for *T. gondii* antibodies with the use of the modified agglutination test (MAT) (Dubey and Desmonts, 1987). Mice were killed 8 wk PI and their brains were examined for tissue cysts (Dubey, 2010). Mice were considered infected when *T. gondii* was demonstrable in their tissues.

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TABLE I. Details of cat litters used.

Product	Contents and notes
1. Special Kitty* Scoopable, marketed by Wal-Mart Stores, Inc., Bentonville, AR 72716	Natural clumping, 99% dust free
2. Tidy Cats® Scoop* for multiple cats, 24/7 Performance, Nestlé Purina Pet Care Company, St. Louis, MO 63164	Natural clay product and deodorizing system, 99% dust free
3. Super Scoop, Arm & Hammer® Baking Soda, Church & Dwight Co., Inc., Princeton, NJ 08543-5297	Clumping, 99% dust free, with ammonia baking soda crystals block formula, destroys odors instantly
4. Fresh Step®, manufactured for the Clorox Pet Products Company, 1221 Broadway, Oakland, CA 94612	Clumping with odor-eliminating carbon
5. Tidy Cats® Clay Cat Litter* for multiple cats, Nestlé Purina Pet Care Company, St. Louis, MO 63164	Natural clay product and deodorizing, 99% dust free
6. Fresh Step® Crystal* premium, manufactured for the Clorox Pet Products Company, 1221 Broadway, Oakland, CA 94612	Silica gel, long-lasting odor-control fresh formula
7. Corn Cob–Small, 1/8-inch product,7092 Harlan Laboratories, Inc., Madison, WI 53744	Fragrance-free
8. Harlan paper product, Harlan Laboratories, Inc., Madison, WI 53744	None
9. Essentials™ Natural,* clumping cat litter, Arm & Hammer®, Church & Dwight Co., Inc., Princeton, NJ 08543-5297	Odor-eliminating baking soda
10. Special Kitty* Scoopable for multiple cats, marketed by Wal-Mart Stores, Inc., Bentonville, AR 72716	Clay with absorbing crystals
11. Fresh Step® Premium, for multiple cats, manufactured for the Clorox Pet Products Company, 1221 Broadway, Oakland, CA 94612	Clumping carbon granules, more ingredients; odor eliminating that inhibits growth of bacteria
12. Fresh Results* (PRO-PEP), Eight in One Pet Products, distributed by United Pet Group, Cincinnati, OH 45230	Natural, corn cob, pine, 99% dust free
13. Fresh Step® Clay,* manufactured for the Clorox Pet Products Co., 1221 Broadway, Oakland, CA 94612	Clay with carbon granules
14. Fresh Kitty,* marketed by Wal-Mart Stores, Inc. Bentonville, AR 72716	Ground clay, fragrance free

* Scoop away daily.

Three experiments were performed. In experiments 1 and 2, feces from fecal cups were removed at designated intervals (Tables II and III), suspended in 2% H_2SO_4, and aerated on a shaker for 7 days before bioassay in mice. Success in transmission indicated survival of oocysts in different types of cat litter.

In experiment 3, in addition to survival, sporulation of oocysts in different cat litters was studied. Feces from 3 cats collected 5–7 days after being fed the CT-1 strain of *T. gondii* (Dubey, 1992) were refrigerated, pooled (total weight 600 g), emulsified with water, and mixed with 200 g of uninfected cat feces. The 600 g of pooled feces were thoroughly mixed, and aliquoted into 10-g samples (samples designated as A). Thirty-six A samples were incubated with 12 types of cat litter, and 3 samples were dispersed in cups without litter (Table IV); these samples were removed from litters after incubation for 2, 7, and 10 days. As a control, 2 A samples were mixed with 40 ml of 2% H_2SO_4 and incubated on a shaker. Another 10-g aliquot was mixed with 40 ml of sucrose solution in a 50-ml tube and centrifuged for 10 min. Five milliliters of the float from the very top of the tube was mixed with 45 ml of water (sample B), and oocysts were counted in a hemacytometer; counts indicated that each 10-g sample of feces contained 5×10^6 oocysts. Five milliliters of sample B were fed to 5 mice to verify that oocysts were unsporulated and not infective before they were incubated with different types of cat litters. Sample B was centrifuged for 10 min, and the sediment was suspended in 5 ml of 2% H_2SO_4 and aerated on a shaker (sample C).

TABLE II. Sporulation and survival of *Toxoplasma gondii* oocysts in 7 types of cat litters (Experiment 1).

Cat litter	*T. gondii* strain (incubation period)	
	TgCatBr2 (day3)	TgCatBr1 (day 5)
1. Special Kitty	0*	2 (10, 11)
2. Tidy Cats	2 (9, 9)	0
3. Super Scoop	2 (11, 11)	0
4. Fresh Step	2 (11, 20)	0
5. Tidy Cats	2 (8, 9)	0
6. Fresh Step Crystal	2 (8, 9)	0
7. Corn Cob–Small	2 (8, 8)	0
Control	2 (5, 5)	2 (5, 5)

* Two mice were inoculated with each sample. Day of death is in parentheses.

TABLE III. Sporulation and survival of *Toxoplasma gondii* oocysts (TgGoatUS4) in 14 types of cat litter (Experiment 2).

Cat litter	Days incubated in cat litters		
	2	7	14
1. Special Kitty	2 (10, 13)*	1†	2†
2. Tidy Cats	1 (10)	0	2†
3. Super Scoop	2 (10, 14)	0	0
4. Fresh Step	2 (7, 7)	0	1†
5. Tidy Cats	2 (8, 9)	0	0
6. Fresh Step Crystal	2 (13, 14)	0	0
7. Corn Cob–Small	2 (6, 6)	0	0
8. Harlan	2 (8, 8)	0	0
9. Essentials™ Natural	2 (5, 6)	0	2†
10. Special Kitty	2 (9, 9)	1†	2†
11. Fresh Step Premium	2 (9, 9)	0	2†
12. Fresh Results	2 (5, 6)	0	0
13. Fresh Step Clay	1†	0	0
14. Fresh Kitty	1 (9)	0	2†
15. Control	2 (6, 7)	0	2†

* Two mice were inoculated. Day of death is in parentheses.
† Mice survived and tissue cysts were found in their brains.

TABLE IV. Effect on sporulation and survival of *Toxoplasma gondii* oocysts in cat feces in different cat litters (Experiment 3).

	Day 2		Day 7		Day 10	
Cat litter	Before incubation*	After incubation†	Before incubation*	After incubation†	Before incubation*	After incubation†
1. Special Kitty	0‡	2 (5, 5)	0	0	0	0
2. Tidy Cats	0	2 (5, 5)	0	0	0	0
3. Super Scoop	1(18)	2 (5, 5)	0	0	0	0
4. Fresh Step	0	2 (7, 7)	0	0	0	0
5. Tidy Cats	1(13)	2 (5, 5)	0	0	0	0
6. Fresh Step Crystal	1(22)	2 (5, 5)	0	0	0	0
9. Essentials Natural	1(29)	2 (5, 5)	0	0	0	0
10. Special Kitty	0	2 (5, 5)	0	0	0	0
11. Fresh Step Premium	1(27)	2 (5, 5)	0	0	0	0
12. Fresh Results	0	2 (5, 5)	0	0	0	0
13. Fresh Step Clay	0	2 (5, 5)	0	0	0	0
14. Fresh Kitty	0	2 (5, 5)	0	0	0	0
15. Control (without cat litter)	0	2 (5, 5)	0	0	0	0
Control (shaker)	2 (6, 6)	ND¶	2 (5, 7)	ND	2 (6, 6)	ND

* Samples bioassayed on the day of removal from cat litter.
† Samples incubated in 2% H_2SO_4 for 1 wk and then bioassayed.
‡ Of 2 mice inoculated, day of death is in parentheses.
¶ ND = not done.

After designated times, fecal samples in experiment 3 were removed, mixed with 40 ml of cold water in a plastic cup, and stored at 4 C to prevent any sporulation (sample D). After overnight soaking, feces in sample D were mixed with water, filtered through gauze and centrifuged; the supernatant was discarded. The sediment was floated in 40 ml of sucrose and centrifuged, and 5 ml of the float from the very top of the tube was mixed with 45 ml of water and centrifuged; the sediment was suspended in 5 ml of water; 1 ml of this mixture was fed to 2 SW mice. Two milliliters were mixed with 2 ml of 4% sulfuric acid (final concentration 2% H_2SO_4), incubated on a shaker for 1 wk, and bioassayed in 2 SW mice to determine if the oocysts were viable.

RESULTS

Oocysts incubated in cat litters for 2 days were infective for mice, but incubation for 5 days (Table II), or longer (Table III), was often lethal for oocysts.

In experiment 3, a few oocysts sporulated within 2 days in 5 litters as indicated by bioassays in mice on the day of removal from the cat litter (Table IV). However, oocysts in all cat litters for 2 days remained potentially viable as indicated by bioassay in mice after incubation of the feces at room temperature for 1 wk (Table IV, day 2, column 2). The bioassay data indicated that oocysts were generally killed in all cat litters between 2 and 7 days of incubation; all mice fed incubated feces remained *T. gondii*–free. Bioassays of samples of oocysts confirmed that more than 100,000 oocysts were present in each sample used for testing in different cat litters. With this strain of *T. gondii* (CT1), all mice fed 1–100,000 oocysts died between 5 and 12 days. Day of death of mice incubated with 1, 10, 100, 1,000, 10,000, and 100,000 oocysts was 5, 7–8, 8, 8–9, 9–10, and 12, respectively.

DISCUSSION

There were several shortcomings in the experimental design in that it is difficult to duplicate conditions in a home or in a cattery. The size of the litter pan and depth of the litter, frequency of changing litter, clinical status, and defecation patterns of individual cats will have an effect on sporulation of *T. gondii* oocysts. In the present study, a thick paste, instead of solid cat feces, was used because we wanted to distribute equal aliquots for each one tested. Moreover, some cats bury their feces; others do not. The frequency of urination also alters the moisture content in the litter pan. Cat feces are generally hard, and expelled in a few lumps, but it will vary if the cat has diarrhea. Most litters absorb water from feces, especially if they are hygroscopic, such as silica gel.

In the present study, all fecal samples were bioassayed in mice because it is difficult to differentiate microscopically live from dead sporulated *T. gondii* oocysts due to their small size. Although titrations were not performed to determine the number of viable oocysts, the day mice died after feeding fecal samples was recorded; there is good correlation between the day of death of mice and the number of *T. gondii* oocysts after oral inoculation (Dubey and Frenkel, 1973).

Mouse mortality data in Tables II and III indicate that oocysts generally lost infectivity 2–3 days after incubation in cat litter. The sporulation of *T. gondii* is markedly reduced at temperatures higher than 30 C (Dubey et al., 1970). Therefore, it would be ideal if cat litter could be heated to 40 C or higher during the night, but it is impractical to train cats to defecate at specific times, and the foul smell created during heating would make this approach impractical.

Toxoplasma gondii oocysts are resistant to disinfectants unless they are used in concentrations that are likely to harm cats or their owners. However, we continue to search for safe methods that will inactivate *T. gondii*. Until then, cat litter should be changed daily, and pregnant women should delegate this task to others. Many scoopable cat litter manufacturers suggest scooping fecal lumps daily and changing litter weekly, but this is likely to give a false sense of security because the oocysts might sporulate in the leftover feces; in addition, the scooping spoon is likely to become contaminated with oocysts; hundreds of oocysts may be present in a milligram of infected feces. There is also the risk of cats tracking infected feces throughout the house. We do not currently have a practical recommendation for safe disposal of cat

litter other than disposing it in a heavy duty plastic bag with the hope that anoxia will kill *T. gondii* oocysts.

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