Toxoplasma gondii: Transmission through Feces in Absence of Toxocara cati Eggs

Abstract. *When incubated at room temperature (23°C) for 3 to 14 days, feces from cats infected 4 to 8 days with* Toxoplasma gondii, *and free of* Toxocara cati *eggs, produced toxoplasmosis in mice. Results indicate that the nematode egg is not necessary for transmission of the parasite.*

The protozoan *Toxoplasma gondii* infects man and animals, but little is known about its means of transmission from host to host. Attempts to transmit the organism with various invertebrate hosts serving as vectors, or by feeding feces of infected animals to other animals have yielded negative results (*1*). Hutchison (*2*) transmitted *T. gondii* by feeding feces from a cat infected with this protozoan and a nematode, *Toxocara cati*, to mice. He interpreted his results to indicate that *T. cati* eggs contained the infective form of *Toxoplasma gondii*. Subsequently, *Toxocara cati* larvae, isolated after induced hatching of eggs, produced toxoplasma infections when fed to mice (*3*). Jacobs and Melton (*1*) found that transmission was successful when feces from two cats in which no *T. cati* eggs could be found were used, but they could not account for these discrepancies. Transmission (in a helminth-free cat) was also reported by Dubey (*4*), but the significance of this finding was not discussed. In view of these positive results in cats which were apparently free of *T. cati*, we questioned the role of the nematode in toxoplasma transmission. Our data indicate that *Toxoplasma gondii* can be transmitted from cats to mice by way of the feces in the absence of *Toxocara cati* eggs.

Cats were infected with *Toxoplasma gondii* (strain M-4471, originally isolated from sheep) by feeding them mouse brain containing cysts. Feces were collected daily. Zinc sulfate flotation was used to concentrate the infective material in the feces. After flotation, the material was washed and incubated at room temperature (approximately 23°C) in tap water. Portions of this material were then given to mice by stomach tube at various times after collection. Successful toxoplasma transmission was determined by finding the organisms in mice that died or by obtaining a positive Sabin-Feldman dye test on serum from surviving mice approximately 30 days after feeding the flotation material.

In one experiment eggs of *Toxocara cati* were removed from the uteri of worms recovered from seven cats infected with *Toxoplasma gondii* 6 to 13 days previously. No transmissions occurred in mice fed the eggs after incubation from 0 to 6 months. On the other hand, flotations of feces collected from these cats between 4 and 10 days after infection and incubated from 5 to over 100 days produced toxoplasmosis. In another experiment two cats (Nos. 32 and 33) were treated with Vermiplex (Pitman-Moore) to remove *Toxocara cati*. In these and other cats, treatment was considered effective when eggs were absent in $ZnSO_4$ concentrations of four daily fecal collections. All collections during the transmission experiments were also examined for eggs. After infection with *Toxoplasma gondii*, feces from both cats produced toxoplasmosis in mice after incubation for from 6 to more than 27 days. When killed 17 days after infection, cat No. 32 contained one immature female *Toxocara cati*; cat No. 33 contained none. Results of these experiments indicated that the toxoplasma organisms might be in the feces rather than associated with the nematode egg.

Two experiments, each on two cats, were designed to determine the number of days after infection that feces could produce toxoplasmosis and the period of incubation necessary (Table 1). All cats were dewormed with piperazine citrate. Feces from cats Nos. 34 and 35 were collected on days 1 through 9. Flotation material was fed to mice after incubation for 3 to 14 days. Feces collected from cat No. 34 on days 4, 6, and 8 produced infection after all incubation periods. Material from collection on day 9 was only positive after 14 days of incubation. When the cat was killed 9 days after infection, one immature female *T. cati* was found. Most of the incubations from feces of cat No. 35 collected on days 4, 6, and 8 were positive. One incubation sample each of collection days 2 and 3 were also positive. When the cat was killed 9 days after infection no worms were found.

Feces from cats Nos. 36 and 37 were collected on 1 through 7 days after infection, and then incubated from 0 to 8 days. Nearly all samples incubated from 3 to 8 days after collection on days 6 and 7 of infection produced toxoplasmosis. *Toxocara cati* eggs, present before deworming, reappeared in the feces of cat No. 36 on day 10 after infection—2 days after the conclusion of the experiment. No eggs were seen in the 14 daily collections before day 10. No eggs were seen in the feces of cat No. 37 after deworming.

These data indicate that *Toxoplasma gondii* can be experimentally transmitted from cats to mice by the feces. The presence of *Toxocara cati* eggs in the feces, as previously reported necessary, is not essential for transmission (*5*). Of six cats whose feces produced infection, all dewormed prior to experimentation, three were free of *T. cati*, two had only a single immature female *T. cati* present after the experiment,

Table 1. Infectivity of feces of *Toxoplasma*-infected cats. A positive result indicates that at least one of the two mice used for each sample acquired toxoplasmosis. Feces were collected after infection on the days indicated.

Feces sample (days)	Isolation after incubation for the following number of days														
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
							Cat No. 34								
1–3				−	−	−	−	−	−	−	−	−	−	−	−
4, 6–8				+	+	+	+	+	+	+	+	+	+	+	+
9				−	−	−	−	−	−	−	−	−	−	−	+
							Cat No. 35								
2				−	−	−	−	−	−	−	−	−	−	−	−
3				−	−	−	−	−	+	−	−	−	−	−	−
4				+	+	−	+	+	+	+	+	+	+	+	+
6				+	+	+	+	+	+	+					
7				−	+	+	−	+	+	+	+	+	−	+	+
8				−	+	+	−	−	+	−	+	+	+	+	−
9				−	−	−	−	−	−	−	−	−	−	−	−
							Cat No. 36								
1–5	−	−	−	−	−	−	−	−	−						
6	−	−	−	+	−	+	+	+	+						
7	−	−	−	+	+	+	+	+	+						
							Cat No. 37								
1–5	−	−	−	−	−	−	−	−	−						
6	−	−	−	+	−	+	+	+	+						
7	−	−	−	−	+	+	+	+	+						

and one had *T. cati* eggs appearing in the stool 2 days after completion of the experiment. No *T. cati* eggs were found during the experimental period. Therefore, the infective form of *Toxoplasma gondii* was presumably present in the flotation material and unassociated with nematode eggs.

In 16 cats with which transmission was achieved with or without *Toxocara cati* infections, infection was not produced by feces collected before day 4 nor after day 11 with two exceptions as noted above. This indicates that the parasite must remain at least 3 days in the cat before its transmissible form can be passed in the feces. The parasite must then remain outside the host for a minimum of 3 days before becoming infective to mice, since we have not been able to get transmission before day 3 of incubation. The transmissible forms are no longer present in the feces approximately 12 days after infection. Some parasites, collected and incubated, retained their infectivity for more than 100 days.

In previous reports (*1, 2, 4, 6*) the role of the *T. cati* egg has been discussed as a protective habitat for the toxoplasma organism during exposure to unfavorable conditions outside the host. Our results do not necessarily rule out the possibility of *Toxoplasma gondii* being transmitted through nematode eggs but show that transmission can occur in their absence. A new resistant form of *T. gondii* evidently develops in the cat. Further studies are necessary to identify this form and describe its development.

Note added in proof: Hutchison *et al.* (*7*) have reported fecal transmission of *T. gondii*. After being fed cysts of *T. gondii*, 2 of 21 *Toxocara*-free cats passed feces which, after concentration and incubation, produced toxoplasmosis in mice.

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Toxoplasma gondii: Fecal Forms Separated from Eggs of the Nematode Toxocara cati

Abstract. *Cats excreted the protozoan* Toxoplasma gondii *in feces, generally between 5 to 12 days after ingesting mice with chronic toxoplasmosis.* Toxoplasma gondii *and eggs of the nematode* Toxocara cati *occurring together could be separated by washing them through sieves that retained the eggs. This finding negates the postulated role of* Toxocara cati *in the transmission of toxoplasmosis.*

Ever since Hutchison described transmission of *Toxoplasma gondii* through the cat ascarid *Toxocara cati* (*1*) we have tried to repeat his observations and to identify the forms of *Toxoplasma* within the nematode and its eggs. We found that several *Toxoplasma* strains, including one derived from the Beverley strain with which Hutchison worked, were not transmitted in the feces of cats. However, *Toxoplasma* strain M-7741, originally isolated from sheep, was recovered 41 times from the feces of ten cats that had been fed mice infected with *Toxoplasma* 2 to 18 days earlier. Between 12 and 15 days after the infectious meal, transmission became irregular. Mice fed fecal floats from cats prior to being fed *Toxoplasma* did not develop toxoplasmosis.

The feces, which generally contained *Toxocara* eggs also, were collected daily, and were subjected to flotation at specific gravity of 1.180 in a solution of zinc sulfate; the top layer was aspirated, diluted, and sedimented in tap water. Such sediments were maintained at 21° to 24°C in water without a preservative, and their infectivity was titrated by feeding to groups of two to six mice. The fecal preparations frequently caused death in 6 days, with *Toxoplasma* visible only in the lesions of advanced enteritis. Surviving mice developed antibody to *Toxoplasma*, as measured by the Sabin-Feldman dye test, and *Toxoplasma* cysts were found in the brain.

We noted that immature eggs and infertile eggs "transmitted" toxoplasmosis although devoid of the hatching enzymes normally secreted by the developed larva (*1*). Hence we titrated a fecal suspension with a known number of *Toxocara* eggs to determine their ability to transmit toxoplasmosis and found that a suspension containing one or two intact eggs produced toxoplasmosis in five out of six mice. The fact that an end point was not reached where expected suggested that *Toxoplasma* might not be "packaged" with *Toxocara* eggs.

Efforts to separate infectious fecal forms of *Toxoplasma* from *Toxocara* eggs were successful. By passing feces containing *Toxocara* eggs (60 to 80 μ) through a 44-μ U.S. standard sieve and flushing the retained portion with water, we obtained identical or ten times more *Toxoplasma* in the filtrate than in the retained portion. After straining feces through an 88-μ sieve, to remove assorted debris and fungal mycelia, we completely separated *Toxoplasma* from the accompanying *Toxocara* eggs by flushing one sample with running tap water for 5 minutes through a 44-μ sieve. The integrity of the sieve was indicated by the fact that it completely retained 40,000 eggs. The filtrate, 1.6 ml, examined drop by drop, contained between 20 and 200 *Toxoplasma*. In addition, 7000 larval *Toxocara* were completely separated from associated *Toxoplasma* by migration in agar. At the site of deposit 13,000 larvae remained with 10 to 100 *Toxoplasma*.

Feeding a *Toxocara*-free cat for three successive days with mice infected with *Toxoplasma* resulted in shedding of fecal forms of *Toxoplasma* at day 5 through day 14 thereafter (Table 1). Results were the same for two other cats. One of these cats shed *Toxoplasma* 2, 3, 5, 6, and 7 days after a contaminated meal.

The characteristics of one lot of fecal forms of *Toxoplasma* were compared. They were infectious to mice when administered orally, intraperitoneally, and subcutaneously. They were generally not infectious on the day that they were passed in the cat feces, but developed infectivity within 1 week. They could be stored at room temperature for at least 3 months. The fecal forms of *Toxoplasma* passed through 44- and 37-μ wire mesh sieves and Pyrex C fritted-glass filters (pore size, 40 to 60 μ) but were retained by a Pyrex M fritted-glass filter (pore size, 10 to 15 μ). Infectious fecal forms of *Toxoplasma* resisted 5 percent sodium hypochlorite for at least ½ hour and 1 percent formalin solution overnight.