J. Parasitol., 88(4), 2002, pp. 713–717

TACHYZOITE-INDUCED LIFE CYCLE OF *TOXOPLASMA GONDII* IN CATS

J. P. Dubey

Parasite Biology, Epidemiology and Systematics Laboratory, Building 1001, Animal and Natural Resources Institute, Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland 20705-2350. *e-mail: jdubey@anri.barc.usda.gov*

ABSTRACT: The tachyzoite-induced cycle of *Toxoplasma gondii* was studied in 46 cats. Tachyzoites of the M-7741 or Me-49 strain of *T. gondii* were administered orally to cats by pouring into the mouth or by stomach tube, or by intraintestinal inoculation. Ten weaned cats that had been inoculated with tachyzoites directly in the intestine were killed 1, 3, 6, 9, 12, 15, 18, or 25 days later, and their tissues were studied histologically and bioassayed in mice. *Toxoplasma gondii* was demonstrable in the blood of 8 cats and in other tissues of all these 10. Four out of five 1- to 8-day-old cats fed tachyzoites by stomach tube became infected with *T. gondii*, and 1 became ill because of toxoplasmosis. All 19 weaned cats fed tachyzoites (poured into the mouth) became infected, and 6 died of acute toxoplasmosis 9–15 days after being fed *T. gondii*. Six out of 12 weaned cats fed tachyzoites by stomach tube became infected but were asymptomatic. Overall, 12 out of 26 cats observed for 19 days or more shed oocysts with a prepatent period (pp) of 19 days or more, with the sole exception of 1 cat that shed oocysts with a pp of 5 days. Enteroepithelial stages of *T. gondii* were not found in any cat before oocysts were shed. Cats shed up to 360 million oocysts in a day, and oocysts were shed for 4–6 days.

Cats shed oocysts after ingesting any of the 3 infectious stages of *T. gondii*: tachyzoites in groups, bradyzoites in tissue cysts, and sporozoites in oocysts. The prepatent period (pp, days to shedding of oocysts) is different after ingesting bradyzoites versus tachyzoites or oocysts (Dubey and Frenkel, 1976). The pp after ingesting bradyzoites is 3–10 days (Dubey and Frenkel, 1976; Dubey, 2001) and 18 days or longer after ingesting oocysts or tachyzoites (Dubey and Frenkel, 1976; Freyre et al., 1989; Dubey, 1996). The life cycle of *T. gondii* after ingesting bradyzoites or tissue cysts was described in detail by Dubey and Frenkel (1972). In addition to the extraintestinal cycle observed in all intermediate hosts of *T. gondii*, 5 asexual types (types A–E) were observed before the development of the sexual cycle in the intestine of cats (Dubey and Frenkel, 1972). The oocyst-induced cycle has also been studied previously (Freyre et al., 1989; Dubey, 1996). The objective of the present paper was to study the tachyzoite-induced cycle in cats and to determine oocyst shedding after feeding tachyzoites.

MATERIALS AND METHODS

Toxoplasma gondii strains and production of tachyzoites

Two strains of *T. gondii* (M-7741 and Me-49) originally isolated from sheep diaphragms were used. Strain M-7741 was the same as that used for studying the tissue cyst-induced cycle of *T. gondii* in cats (Dubey and Frenkel, 1972). The Me-49 strain was the same as that used by Lunde and Jacobs (1983). Both strains produce many tissue cysts in the brains of outbred Swiss Webster (SW) mice.

Tachyzoites were obtained from the peritoneal cavities of mice inoculated intraperitoneally (i.p.); this is the only reliable method for producing tachyzoites because bradyzoites are formed in virtually all tissues of mice and also in cell culture. Bradyzoites or tissue cysts have never been demonstrated conclusively in the peritoneal exudate (pex) of mice. The M-7741 and Me-49 strains of *T. gondii* grow slowly in the peritoneal cavity of mice, and it is difficult to obtain large numbers of tachyzoites of these strains unless the mice are immune-compromised (Dubey, 1977). Different procedures were used to immune-suppress mice (Table I).

To obtain tachyzoites, mice were initially inoculated i.p. with *T. gondii* tissue cysts. For this, brains of mice inoculated 2 mo previously with *T. gondii* were homogenized in 0.9% sodium chloride solution (saline) using a pestle and mortar, and the homogenate was inoculated i.p. into immune-compromised mice. In certain trials, mice were inoculated with free bradyzoites. To release bradyzoites from tissue cysts, Percoll-cleaned tissue cysts were incubated in pepsin solution for 1–5 min and then neutralized with 1.2% sodium bicarbonate (Cornelissen et al., 1981; Popiel et al., 1996). To obtain clean bradyzoites, bradyzoite suspension was passed through a 3-μm membrane filter (Nuclepore, Pleasanton, California). After 5 days or more, saline was gavaged into the peritoneal cavities of mice, and the aspirate was examined microscopically to ascertain the presence of *T. gondii*. Organisms so obtained were considered as passage 1. Organisms (10^5–10^6 tachyzoites) from passage 1 were inoculated either into cats or into mice.

Inoculation of cats

Eight trials were performed over a period of 22 yr (1979–2001). *Toxoplasma*-free cats originally derived from 1 source were used; the management and the history of these cats were described earlier (Dubey, 1995).

Tachyzoites were introduced into cats by various routes (Table I). In trial 1, cats were inoculated intraintestinally to bypass the stomach because tachyzoites are susceptible to gastric juice. For this, cats were anesthetized with ketamine hydrochloride. After performing laparotomy, tachyzoites were inoculated directly into the lumen of the upper part of the jejunum. In trials 2, 3, 7, and 8, cats were inoculated by stomach tube. For feeding newborn kittens, a 7-cm needle with bulbous round end was used as a stomach tube. For weaned cats, a 20-cm-long stomach tube was used, and cats were anesthetized with ketamine hydrochloride. In trials 4–6, tachyzoites were poured into the mouth of the cat while the cat's head was held in an upward position.

Mouse bioassay for *Toxoplasma gondii* infection

Blood (~1 ml) was collected from each cat in trial 1 before necropsy in a tube containing ethylenediaminetetraacetic acid and inoculated subcutaneously into 2 SW mice to determine parasitemia. Specimens of adrenal glands, brain, diaphragm, eye, heart, intestines, kidneys, liver, lung, mesenteric lymph nodes, spinal cord, skeletal muscle, spleen, and thymus were removed for bioassay (Table II). Tissues were homogenized in saline and either inoculated directly (adrenal, 1 eye, mesenteric lymph nodes) or after digestion in 1% trypsin solution, as described previously (Sharma and Dubey, 1981). Tissues of newborn kittens of trials 2 and 3 and of their dam were bioassayed in mice with or without digestion in pepsin (Dubey and Beattie, 1988).

All mice used for bioassay were examined for *T. gondii* infection. Impression smears of lungs or brains of mice that died were examined for *T. gondii* stages. Survivors were bled 8 wk later, and a 1:25 dilution of serum from each mouse was tested for antibodies to *T. gondii* using modified agglutination test (MAT). Surviving mice were killed 2 mo postinoculation, and a 2-mm^2 piece of the cerebrum was placed between a glass slide and coverslip and examined microscopically for tissue cysts (Dubey and Beattie, 1988). Mice were considered infected with *T. gondii* if the organisms were found in their tissues.

Histologic and immunohistochemical examinations

All cats were examined at necropsy. In trial 1, samples of all tissues were fixed in neutral buffered 10% formalin (NBF). The entire small

Received 8 October 2001; revised 8 January 2002; accepted 8 January 2002.

TABLE I. Details of procedures used to inoculate cats with *Toxoplasma gondii* tachyzoites.

Trial no.	Mice and procedure for tachyzoites*	Tachyzoite passage	No. of cats	Age of cats (days)	Route
1	SW, cortisone†	2–4	10	50–70	Intrajejunal
2	BALB/c, IFNG antibody‡	1	4	1–8	Stomach tube
3	SW, methyl prednisolone acetate§	2	2	1	Stomach tube
4	BALB/c, IFNG antibody‡	1	5	70–84	Poured into the mouth
5	BALB/c, KO‖	3	10	89–95	Poured into the mouth
6	SW, dexamethasone#	1	4	89–93	Poured into the mouth
7	KO, SW, dexamethasone#	1	6	66–100	Stomach tube
8	KO, SW, dexamethasone#	2	6	85–106	Stomach tube

* KO, knockout mice; SW, Swiss Webster mice; IFNG, interferon-gamma.
† 2.5 mg cortisone acetate, twice weekly before and after *T. gondii* inoculation.
‡ 2.5 mg of anti-interferon gamma monoclonal antibody (Suzuki et al. 1988).
§ 4 mg methyl prednisolone acetate (UpJohn, Kalamazoo, Michigan).
‖ Interferon-gamma gene knockout mice (Dubey and Lindsay, 1998).
1 mg/ml of dexamethasone in drinking water (Sigma, St. Louis, Missouri).

intestine was divided into 5 equal parts. After removing a small section from each of the 5 segments for bioassay, the remaining portions were immersed in NBF, cut into 5-mm portions, and most of it blocked in paraffin for histology. Formalin-fixed tissues were embedded in paraffin, cut into sections of 5-μm thickness, stained with hematoxylin and eosin, and examined microscopically.

Selected deparaffinized sections were stained with anti-*T. gondii* antibodies prepared in rabbits. Two types of antibodies were used. Polyclonal antibodies were prepared in a rabbit fed *T. gondii* oocysts; these antibodies stain both tachyzoites and bradyzoites. Bradyzoite-specific antibodies were also prepared in rabbits inoculated with purified proteins (BAG-1, BAG-5, or heat shock protein) as described by McAllister et al. (1996). A 1:10,000 serum dilution was used; this antibody does not stain tachyzoites. The immunohistochemical procedures were essentially those described previously by Lindsay and Dubey (1989) and Dubey et al. (2001). Livers and spleens of mice inoculated with the RH strain of *T. gondii* (Sabin, 1941) were used as tachyzoite control, and tissue cysts from the brains of mice were used as bradyzoite control; the RH strain used does not produce tissue cysts (Dubey et al., 1999). Tissues from 27 cats (all 10 in trial 1 and 17 in trials 2–8) were selected for immunohistochemical staining by both polyclonal and anti-BAG-1 antibodies.

TABLE II. Toxoplasmosis in kittens inoculated intraintestinally with tachyzoites of the M-7741 strain of *Toxoplasma gondii*.*

Day killed	MAT titer	Tissues of cats positive for *T. gondii* by mouse bioassay
1	<25	Bl, Lu
3	<25	I, Li, Ml, Sp
6	<25	A, Bl, Br, D, H, I†, K, Lu, Li, Ml, Sm, Sp
9	50	Bl, Br, E, H, I†, Lu, Sc, T
12	400	Bl, Br, D, E, H, I† K, Li, Lu, Me‡, Ml, Sc, Sm, Sp, T
12	25	Bl, Br, D, I, K, Li, Lu, Ml, Sp, T
15	200	A, Bl, Br, Di, H, I‡, K, Li, Lu‡, Ml, S, Sm, Sp, T
18	200	Bl, D, I, K, Li, Lu, Ml, T
21	200	D, K, Lu, Ml, T
25§	3,200	Bl, Br, D, H, I, K, Li, Lu, Ml, Sc, Sm, Sp, T

* Abbreviations: A, adrenal; Bl, blood; Br, brain; D, diaphragm; E, eye; H, heart; I, intestine; K, kidneys; Li, liver; Lu, lung; Me, mesentery; Ml, mesenteric lymph nodes; Sc, spinal cord; Sm, skeletal muscle; Sp, spleen; T, thymus.
† Intestinal serositis with *T. gondii* tachyzoites in intestinal serosa and muscular layers.
‡ BAG-1 positive.
§ Shed oocysts days 23–25.

Serologic examination

Serum samples from mice and cats were examined for antibodies to *T. gondii* by MAT, as described previously (Dubey and Desmonts, 1987).

Fecal examination

Weaned cats were housed individually. Newborn kittens were housed with their mothers. Feces from each mother were examined daily for oocyst excretion. Because the mother eats the kittens' excreta, oocysts excreted by kittens and their mother can be detected by examining the mother's feces.

Feces of each weaned cat were examined microscopically for oocysts. For this the entire daily feces were emulsified with a small volume of water and then ~10 g of the emulsion was mixed with ~40 ml of sucrose solution (specific gravity 1.18), filtered through gauze, and centrifuged in a 50-ml tube at 2,000 rpm (~1,200 *g*) for 10 min. A drop of the float from the meniscus was examined microscopically for oocysts. If oocysts were detected, the entire daily feces were mixed with sucrose solution in 50-ml tubes and centrifuged at 2,000 rpm for 10 min. Then the entire supernatant (~40 ml) was mixed with 200 ml of water and centrifuged. The supernatant was discarded and the sediment was mixed with water, and all samples from each cat for each day were pooled, centrifuged, and finally suspended in water to make a final volume of 100 ml. Oocysts were counted in the 4 WBC chambers of a hemacytometer. The number of oocysts in the 4 WBC chambers was multiplied by 2,500 × 100 (total volume). Thus, the sensitivity of counting oocysts was 250,000 oocysts/fecal sample.

RESULTS

Clinical signs

All cats in trials 1, 3, 7, and 8 remained clinically normal. In trial 2, 1 kitten was found comatose on the 15th day, with its mouth dry and apparently not nursing; histologically, it had generalized toxoplasmosis. In trials 4–6, 6 out of 19 cats fed tachyzoites died (or were euthanized) 9–15 days later because of toxoplasmic pneumonia.

Histopathologic examination and *Toxoplasma gondii* demonstration

In trial 1, *T. gondii* was isolated from the blood of 8 out of 10 cats inoculated intraintestinally; it was isolated from several tissues of each of these cats (Table I). In trial 2, *T. gondii* was isolated or demonstrated histologically in tissues of all 5 kittens

TABLE III. Identification of *Toxoplasma gondii* stages in tissues of cat fed Me-49 strain tachyzoites.

Trial no.	Cat no.	Age (days)	Day of death or killed*	Immunohistochemical positive tissues	
				Polyclonal	BAG-1
2	K1	8	K35	NS†	NS
	K2	8	K35	B‡	NS
	K3	8	K35	T	B, T
	K5	1	K20	B, T	B, H, Sc, T
3	K1	1	DK15	B, H, M, T	NS
	K2	1	K31	NS	NS
	97	736	K31	NS	NS
4	219	84	K26	NS	NS
	234	81	K21	B, I, M, T	B, M, T
	238	70	D13	A, B, E, H, I, Lu, Sp, T	H, M, T
	239	70	K26	NS	NS
	240	70	D14	A, B, B1, E, I, K, M1, T	K, M, T
5	569	84	D9	B, H, Li, Lu	Lu, T
	563	81	D15	B, H, I, Li, Lu, M	B, H, T
	566	82	D11	B, H, Ml, Li, Lu, Sp, T	B, T
6	608		D10	A, B, Di, Li, Lu, K, Sp, St, Int. P, Ml, H, Sk, T	B, M
	615		K20	B, M, T, I	B, M, T

* D, died; K, killed; Dk, killed when ill.
† NS, not seen.
‡ A, adrenal; B, brain; Bl, bronchial lymph node; Di, diaphragm; E, eye; H, heart, I, intestine; Li, liver; Lu, lung; M, muscle; Ml, mesenteric lymph node; K, kidney; P, pancreas; Sc, spinal cord; Sk, skeletal muscle; Sp, spleen; St, stomach; T, tongue.

fed tachyzoites and in the central kitten. In trail 3, *T. gondii* was found in tissues of 1 out of the 2 kittens fed tachyzoites. In trials 4–6, *T. gondii* was found in several tissues of 6 cats that died; all the remaining cats developed antibodies to *T. gondii* in titers of 1:500 or more. In trial 7, all cats developed high titers to *T. gondii* (≥1:800). In trial 8, antibodies were not detected in 1:25 dilutions of the sera of all 8 cats killed 31 days after inoculation, indicating they were not infected.

Bradyzoite formation

Bradyzoites (BAG-1 positive) were detected in tissues of 2 cats in trial 1 (Table II) and in 10 out of 17 cats in trials 2–8 (Table II).

TABLE IV. Oocyst shedding by cats fed tachyzoites of the Me-49 strain of *Toxoplasma gondii.*

Trial no.	No. of cats fed tachyzoites	No. of cats died and day of death	No. of cats infected with *T. gondii**	No. of cats shed oocysts†	Prepatent period
2	4	0	4	2/4	30, 30
3	2	1 (15)	1	0/1	NA‡
4	5	2 (13, 14)	5	3/3	20, 25, 26
5	10	2 (9, 15)	1	4/8	19, 20, 23, 28
6	4	1 (10)	3	2/3	21, 24
7	6	0	6	2/6	5, 21
8	6	0	0	0	NA

* Based on isolation of *T. gondii* or seroconversion.
† Number of cats observed for oocyst shedding beyond 19 days postinoculation with tachyzoites.
‡ NA, not applicable.

Entero-epithelial stages

Entero-epithelial stages (schizonts, gamonts) of *T. gondii* were found only in 1 cat (no. 234, trial 4) killed 21 days after being fed tachyzoites. These stages were present in surface epithelial cells. The submucosa and intestinal musculature had focal inflammatory lesions and tachyzoites.

Oocyst shedding

In trial 1, of the cats inoculated with tachyzoites directly in the intestines, 1 cat shed oocysts with a pp of 23 days. In trial 2, *T. gondii* oocysts were found in feces of the dam of the 3 kittens fed tachyzoites, 30 days after they were fed tachyzoites. Because kittens were housed with their mother, the exact pp was not determined. The kittens and their mother were killed on the 30th day; oocysts were found in rectal contents of 2 kittens but not in their 2 littermates. Of the two 1-day-old kittens fed tachyzoites, oocysts were found in 1 kitten (kitten no. 5, Table III) on the 20th day after being fed tachyzoites. Although the kitten was killed on the 20th day, its mother continued shedding oocysts until the 27th day.

In trials 3–8, 12 out of 26 cats fed tachyzoites and observed beyond 19 days shed *T. gondii* oocysts with a pp of 19 days in 1 cat, 20 days in 2, 21 days in 2, and 23 days or more in 6 (Table IV). Peak oocyst shedding occurred on days 2–4 of the patency (Table V). The number of oocysts shed varied from 3×10^6 to 7×10^7.

DISCUSSION

In the present study, 12 out of 26 cats given tachyzoites and observed for more than 19 days shed oocysts with a pp of 19 days or more, with the exception of 1 cat (to be discussed later). These results are similar to those obtained previously using the

TABLE V. Number ($\times 10^5$) of *Toxoplasma gondii* oocysts shed by cats fed tachyzoites of the Me-49 strain.

Day*	Cat 615 (trial 6)	Cat 614 (trial 6)	Cat 626 (trial 7)
20	0	0	0
21	Few†	0	Few†
22	360‡	0	12‡
23	300	0	8
24	45	30‡	4
25	0	22	6
26	0	16	0
27	0	13	0
28	0	3	0
29	0	1	0
30	0	0	0

* Day after feeding tachyzoites.
† <100,000.
‡ ×100,000.

M-7741 strain of *T. gondii*. Dubey and Frenkel (1976) directly inoculated tachyzoites from pex in the intestinal lumen of 6 cats; 5 shed oocysts with a pp of 21–34 days. They also inoculated mice with tachyzoites by 4 parenteral routes and then fed mice to cats. Of the 24 cats fed mice infected with tachyzoites 2 days previously, 4 cats shed oocysts with a pp of 27–40 days. Of the 19 cats fed mice infected for 3 days, 4 shed oocysts, 2 with a short pp (6 and 8 days) and 2 with a long pp of 37 days. Of the 10 cats fed mice infected for 4 days, 8 shed oocysts after a short pp (4–7 days). On the basis of these findings it was proposed that the short pp (3–10 days) was related to the ingestion of bradyzoites and the long pp (>19 days) to the ingestion of tachyzoites. In addition, none of the 32 cats fed mice parenterally injected with sporozoites or bradyzoites 6 days earlier shed oocysts with a short pp (Dubey and Frenkel, 1976).

One cat in trial 7 fed tachyzoites of the 1st passage shed oocysts with a pp of 5 days. The shedding of oocysts by this cat suggests that bradyzoites were present in the inoculum. The inoculum fed to cats was derived from the pex of knockout mice injected 7 days earlier with brain homogenate containing tissue cysts. There are 2 likely explanations. Firstly, a few bradyzoites survived 7 days in the peritoneal cavity of mice; the brain homogenate had not been digested in vitro before mouse inoculation. Secondly, a few bradyzoites were formed in the mouse peritoneal cavity or shed into the cavity from the surface of visceral tissues. Cats fed as few as 1 bradyzoite have shed millions of oocysts (Dubey, 2001).

The primary objective of the present study was to investigate the entero-epithelial cycle of *T. gondii* after infection with tachyzoites. Therefore, tachyzoites were inoculated directly in the intestine to bypass the stomach. Cats inoculated intraintestinally with tachyzoites developed generalized *T. gondii* infection. Although *T. gondii* was demonstrated in many tissues, including blood, entero-epithelial stages were not found in any cats examined 1–19 days after being fed tachyzoites. Because of the surgical procedure involved, this trial was performed in weaned cats. Finding early entero-epithelial stages is difficult in weaned cats in spite of a heavy dose (Davis and Dubey, 1995). Therefore, in trials 2 and 3, newborn kittens were fed tachyzoites by stomach tube, but the kittens had to be housed with their mothers. Kittens fed tachyzoites became infected with *T. gondii*. In addition, the dam of these kittens became infected with *T. gondii* although it was not fed tachyzoites. It is likely that the dam became infected by licking the kittens and some of the inoculum that might have been regurgitated; the dam subsequently shed *T. gondii* oocysts. This approach of studying localization of *T. gondii* after feeding tachyzoites was abandoned because the dam had to be killed with each experiment and thus had to be removed from our breeding colony.

In trials 4–6, weaned cats were fed tachyzoites by mouth; one-third of these cats developed respiratory distress because of toxoplasmic pneumonia. It is likely that a few tachyzoites were inhaled or that tachyzoites penetrated the oral–pharyngeal mucosa. Whatever the mechanism, cats became infected by the tachyzoites they were fed. The volume of the inoculum was not a factor because only 1–5 ml of the inoculum was poured into the mouth of cats. Entero-epithelial stages were not found even in cats that had severe toxoplasmosis involving several tissues (Table III).

In trials 7 and 8, cats were inoculated orally using a 20-cm stomach tube. Care was taken not to spill inoculum while withdrawing the stomach tube; the stomach tube was emptied by means of a bolus of air before withdrawing the tube. Six out of 12 cats fed tachyzoites by stomach tube became infected although only 2 shed oocysts.

The frequency and duration of oocyst shedding in cats fed tachyzoites were unpredictable; therefore, cats were not observed for more than 1 mo. Usually oocyst shedding peaked 1–3 days after the cat started shedding oocysts.

The pp in cats fed tachyzoites (19 days or more) is the same as after being fed oocysts (21 days or more) using the M-7741 or Me-49 strain of *T. gondii* (Freyre et al., 1989; Dubey, 1996). In 1 cat fed oocysts of the VEG strain, the pp was 18 days (Dubey, 1996). Thus, it is assumed that the development of the entero-epithelial cycle is the same as after being fed oocysts or tachyzoites.

Attempts to find entero-epithelial stages of *T. gondii* in cats fed oocysts were unsuccessful, in spite of the large numbers of oocysts fed to them (Freyre et al., 1989). One hypothesis proposed is that after ingestion of oocysts or tachyzoites, *T. gondii* is disseminated into tissues of cats. Bradyzoites and tissue cysts are formed during the second week after infection and possibly return to the small intestine via parasitemia when bradyzoites are released periodically from tissue cysts. Because the rupture of tissue cysts, parasitemia, and return to the gut is unpredictable, there is no fixed pp. However, it must take a minimum of 18 days for this cycle to be completed because oocysts were not excreted sooner than 18 days. In this respect the results of the present study demonstrate that parasitemia can occur at various intervals after ingestion of tachyzoites. In trial 1, parasitemia was detected 1–25 days after tachyzoites were introduced into the intestinal lumen. Using the bradyzoite-specific BAG-1 antibodies in the present study, bradyzoites were demonstrable in the intestinal musculature, mesentery, lung, kidneys, tongue, quadriceps, spinal cord, and brain. The earliest time bradyzoites were demonstrable was 15 days in the intestinal musculature, 9 days in the lungs and tongue, 11 days in the brain, 13 days in the heart, and 20 days in the spinal cord.

Although lactogenic transmission of *T. gondii* in cats has not

been demonstrated, there was suggestive evidence that it may occur in cats (Dubey et al., 1995). *Toxoplasma gondii* has been found in milk of experimentally infected cats (Powell et al., 2001), and the most likely stage excreted in milk is the tachyzoite. The results of the present study indicate that cats fed tachyzoites can shed large numbers of oocysts.

ACKNOWLEDGMENTS

I would like to thank S. K. Shen, O. C. H. Kwok, and D. Hawkins-Cooper for technical assistance and Y. Suzuki for the gift of anti-interferon gamma antibody.

LITERATURE CITED

CORNELISSEN, A. W. C. A., J. P. OVERDULVE, AND J. M. HOENDERBOOM. 1981. Separation of *Isospora* (*Toxoplasma*) *gondii* cysts and cystozoites from mouse brain tissue by continuous density-gradient centrifugation. Parasitology **83:** 103–108.

DAVIS, S. W., AND J. P. DUBEY. 1995. Mediation of immunity to *Toxoplasma gondii* oocyst shedding in cats. Journal of Parasitology **81:** 882–886.

DUBEY, J. P. 1977. *Toxoplasma*, *Hammondia*, *Besnoitia*, *Sarcocystis*, and other tissue cyst-forming coccidia of man and animals. *In* Parasitic protozoa, J. P. Kreier (ed.). Academic Press, New York, New York, p. 101–237.

———. 1995. Duration of immunity to shedding of *Toxoplasma gondii* oocysts by cats. Journal of Parasitology **81:** 410–415.

———. 1996. Infectivity and pathogenicity of *Toxoplasma gondii* oocysts for cats. Journal of Parasitology **82:** 957–960.

———. 2001. Oocyst shedding by cats fed isolated bradyzoites and comparison of infectivity of bradyzoites of the VEG strain *Toxoplasma gondii* to cats and mice. Journal of Parasitology **87:** 215–219.

———, AND C. P. BEATTIE. 1988. Toxoplasmosis of animals and man, CRC Press, Boca Raton, Florida, 220 p.

———, AND G. DESMONTS. 1987. Serological responses of equids fed *Toxoplasma gondii* oocysts. Equine Veterinary Journal **19:** 337–339.

———, AND J. K. FRENKEL. 1972. Cyst-induced toxoplasmosis in cats. Journal of Protozoology **19:** 155–177.

———, AND ———. 1976. Feline toxoplasmosis from acutely infected mice and the development of *Toxoplasma* cycts. Journal of Protozoology **23:** 537–546.

———, M. M. GARNER, M. D. STETTER, A. E. MARSH, AND B. C. BARR. 2001. Acute *Sarcocystis falcatula*-like infection in a carmine bee-eater (*Merops nubicus*) and immunohistochemical cross reactivity between *S. falcatula* and *Sarcocystis neurona*. Journal of Parasitology **87:** 824–831.

———, M. R. LAPPIN, AND P. THULLIEZ. 1995. Diagnosis of induced toxoplasmosis in neonatal cats. Journal of the American Veterinary Medical Association **207:** 179–185.

———, AND D. S. LINDSAY. 1998. Isolation in immunodeficient mice of *Sarcocystis neurona* from opossum (*Didelphis virginiana*) faeces, and its differentiation from *Sarcocystis falcatula*. International Journal for Parasitology **28:** 1823–1828.

———, S. K. SHEN, O. C. H. KWOK, AND J. K. FRENKEL. 1999. Infection and immunity with the RH strain of *Toxoplasma gondii* in rats and mice. Journal of Parasitology **85:** 657–662.

FREYRE, A., J. P. DUBEY, D. D. SMITH, AND J. K. FRENKEL. 1989. Oocyst-induced *Toxoplasma gondii* infections in cats. Journal of Parasitology **75:** 750–755.

LINDSAY, D. S., AND J. P. DUBEY. 1989. Immunohistochemical diagnosis of *Neospora caninum* in tissue sections. American Journal of Veterinary Research **50:** 1981–1983.

LUNDE, M. N., AND L. JACOBS. 1983. Antigenic differences between endozoites and cystozoites of *Toxoplasma gondii*. Journal of Parasitology **69:** 806–808.

MCALLISTER, M. M., S. F. PARMLEY, L. M. WEISS, V. J. WELCH, AND A. M. MCGUIRE. 1996. An immunohistochemical method for detecting bradyzoite antigen (BAG5) in *Toxoplasma gondii*-infected tissues cross-reacts with a *Neospora caninum* bradyzoite antigen. Journal of Parasitology **82:** 354–355.

POPIEL, I., M. C. GOLD, AND K. S. BOOTH. 1996. Quantification of *Toxoplasma gondii* bradyzoites. Journal of Parasitology **82:** 330–332.

POWELL, C. C., M. BREWER, AND M. R. LAPPIN. 2001. Detection of *Toxoplasma gondii* in the milk of experimentally infected lactating cats. Veterinary Parasitology **102:** 29–33.

SABIN, A. B. 1941. Toxoplasmic encephalitis in children. Journal of the American Medical Association **116:** 801–807.

SHARMA, S. P., AND J. P. DUBEY. 1981. Quantitative survival of *Toxoplasma gondii* tachyzoites and bradyzoites in pepsin and in trypsin solutions. American Journal of Veterinary Research **42:** 128–130.

SUZUKI, Y., M. A. ORELLANA, R. D. SCHREIBER, AND J. S. REMINGTON. 1988. Interferon-gamma: The major mediator of resistance against *Toxoplasma gondii*. Science **240:** 516–518.