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ORIGINAL ARTICLE

Life Cycle of *Cystoisospora felis* (Coccidia: Apicomplexa) in Cats and Mice

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ABSTRACT

Cystoisospora felis is a ubiquitous apicomplexan protozoon of cats. The endogenous development of *C. felis* was studied in cats after feeding them infected mice. For this, five newborn cats were killed at 24, 48, 72, 96, and 120 h after having been fed mesenteric lymph nodes and spleens of mice that were inoculated with *C. felis* sporulated sporocysts. Asexual and sexual development occurred in enterocytes throughout the villi of the small intestine. The number of asexual generations was not determined with certainty, but there were different sized merozoites. At 24 h, merogony was seen only in the duodenum and the jejunum. Beginning at 48 h, the entire small intestine was parasitized. At 24 h, meronts contained 1–4 zoites, and at 48 h up to 12 zoites. Beginning with 72 h, the ileum was more heavily parasitized than the jejunum. At 96 and 120 h, meronts contained many zoites in various stages of development; some divided by endodyogeny. The multiplication was asynchronous, thus both immature multinucleated meronts and mature merozoites were seen in the same parasitophorous vacuole. Gametogony occurred between 96 and 120 h, and oocysts were present at 120 h. For the study of the development of *C. felis* in murine tissues, mice were killed from day 1 to 720 d after having been fed 10^5 sporocysts, and their tissues were examined for the parasites microscopically, and by bioassay in cats. The following conclusions were drawn. (1) *Cystoisospora felis* most frequently invaded the mesenteric lymph nodes of mice and remained there for at least 23 mo. (2) It also invaded the spleen, liver, brain, lung, and skeletal muscle of mice, but division was not seen based on microscopical examination. (3) This species could not be passed from mouse to mouse.

CYSTOISOSPORA felis is the most common coccidian in feline feces (Levine 1973). Until 1970, coccidian of cats were considered to have 1-host fecal-oral-intestinal life cycle of little biological and public health importance. In 1970, the life cycle of *Toxoplasma gondii* was discovered; felids were found to be its only definitive hosts and all other mammals and birds were intermediate hosts. Soon after that, it was discovered that two other feline coccidians, *C. felis* and *Cystoisospora rivolta* could infect other hosts (Dubey and Frenkel 1972a; Frenkel and Dubey 1972). Both of these parasites could invade extra-intestinal organs of cats and mice, and they formed tissue cysts in murine tissues.

Before the discovery of the life cycle stages of *T. gondii* in the intestine of cats (Dubey and Frenkel 1972b), coccidians were considered to follow a rhythmic, timed cycle with fixed generations of schizogonic multiplication and morphologically distinct merozoites of each generation (Levine 1973). In the schizogonic cycle the sporozoites or the merozoites round up (called trophozoites) after entering an enterocyte, and the nucleus divides in to four or more nuclei before merozoites are formed. *Toxoplasma gondii* was found to divide by endodyogeny, multiple endodyogeny, and meronts often retained the merozoite shape (Dubey and Frenkel 1972a). Soon it was found that other coccidians of cats, including *C. rivolta* of cats and *Cystoisospora ohioensis* of dogs followed the same pattern of development as seen in the intestines of cats infected with *T. gondii* (Dubey 1978, 1979a,b). Fayer and Thompson (1974) demonstrated that *C. felis* divided into two by endodyogeny in cell culture. Ferguson et al. (1980) using the transmission electron microscopy demonstrated

that *C. felis* divided by endodyogeny in enterocytes of a weaned cat 6 d after feeding oocysts. They further indicated that the daughters undergo a further cycle of endodyogeny within the same parasitophorous vacuole (Ferguson et al. 1980). Even large schizonts seen were the product of endodyogeny (Ferguson, D. J. P., pers. commun. to JPD, May 2014).

Shah (1971) described in detail asexual and sexual cycle of *C. felis* in weaned cats after feeding them oocysts. He found three generations of schizonts with morphologically distinct merozoites. First generation merozoites were 11–15 × 3–5 μm; these were found at 96–120 h postinoculation (PI). The second generation merozoites were structurally similar to first generation merozoites and were seen 120–144 h PI. Third generation merozoites (6–8 × 1–2 μm) were about half the size of first generation merozoites. These merozoites were seen 144–216 h. Oocysts were first excreted at 168 h. Thus, asexual and sexual cycle continued simultaneously after 144 h.

Here, I describe the tissue cyst-induced life cycle of *C. felis* in cats and oocyst-induced infections in mice. Because coccidian of cats and dogs divide by several modes of division, division is asynchronous, some merons are merozoite shaped, and multiple cycles can occur in one entrocyte, I have called asexual stages as meronts.

MATERIALS AND METHODS

Experimental cats

This study was performed in 1972–1973 in the laboratory of Dr. J. K. Frenkel, Department of Pathology and Oncology, Kansas University Medical Center, Kansas City, Kansas. Newborn cats were used for all experiments to minimize the chances of spontaneous *C. felis* infection. Pregnant cats were obtained from homes in Kansas City. Feces of mother cats were examined for coccidian oocysts daily for the detection of spontaneous coccidial infection. Only cats without demonstrable oocysts were used in this study. The kittens were raised by their queens. In total, 18 newborn kittens from four litters were used for transmission experiments. In addition, two weaned cats were used as donors of oocysts.

Fecal flotation on the feces of queens were examined daily beginning 3–7 d before feeding, and lasting 1–10 d after feeding *C. felis* to their kittens. Feces of newborn kittens were collected by giving enemas using 3–5 ml of saline (0.9%, w/v NaCl solution). Feces of kittens were smeared on microscope slides and examined. After washing in water, the oocysts were sporulated in 2.5% (w/v) potassium dichromate at 22–26 °C for 1 wk and then stored at 4 °C. Before inoculation, the oocysts were washed free of potassium dichromate. The outer oocyst wall was then removed by treatment with 5.25% (w/v) sodium hypochlorite solution for 10–15 min and this, in turn, was removed from the oocyst suspension by repeated washing with distilled water and centrifugation. The washed oocysts were suspended in saline and shaken vigorously without glass beads until the sporocysts were released from the oocysts. Free, fully sporulated sporocysts, instead of intact oocysts, were fed to mice to facilitate excystation.

Experimental transmission of *C. felis* from mice to cats

It was shown previously that *C. felis* can invade extraintestinal organs of mice (Frenkel and Dubey 1972). The objectives of the present experiments were to determine what organs are invaded and to study its persistence in tissues, and whether *C. felis* can be passed from mouse to mouse, similar to *T. gondii*. Three experiments were performed. In the first experiment, five 1-d-old littermate cats were used. Brains, skeletal muscle, lungs, livers, and mesenteric lymph nodes of six mice fed *C. felis* oocysts 15 mo prior to their dissection were removed, homogenized and inoculated orally (referred as fed) by stomach tube to each of five referred cats. In the second experiment, brain, skeletal muscle, and brain were homogenized separately and each homogenate fed to 1-d-old cats (Table 1). The mice had been fed *C. felis* oocysts 24 mo prior to their feeding to cats. In the third experiment, five 1-d-cats were used to test the infectivity of infected murine tissues to other mice. For this, six mice were fed 10^5 *C. felis* sporocysts referred to as group A. Two mice were killed 5 d later and their internal organs (mesenteric lymph nodes, spleen, liver, lungs) were homogenized and inoculated intraperitoneally into another group of cortisonized (2.5 mg cortisol + 2.5 mg of cortisone acetate twice weekly for 2 wk) outbred Swiss Webster mice, referred to as group B. Tissues from group B were inoculated in to another group of cortisonized mice (group C). Mice were given cortisone to increase their susceptibility to *C. felis*. Mice from groups A, B, and C were killed on the day 30 of the experiment and their mesenteric lymph nodes, spleen, liver, and lungs were homogenized and fed to five 1-d-old cats. One cat was fed tissues from group A, two cats were fed tissues from group B, and two cats were fed tissues from group C mice.

Endogenous enteric stages in cats

For the study of murine-induced endogenous stages in cats, spleens, and mesenteric lymph nodes of 50 mice were pooled, homogenized in saline, and equal aliquots fed to each of five 1-d-old cats. The mice had been fed 2×10^5 sporocysts 11 d prior to their feeding to cats. The cats were killed 24, 48, 72, 96, and 120 h PI and necropsied (Table 2). The small intestine was removed and divided into five equal segments. The large intestine was the sixth segment. Each intestinal segment was flushed with ice-cold Zenker-formol solution. After a few minutes, each intestinal segment was cut into about 1 cm pieces and left in the fixative overnight. Tissues were washed in running water the next day, dehydrated and embedded in paraffin. Histologic 5 μm thick sections were examined after staining with hematoxylin-eosin, periodic acid Schiff hematoxylin counter stained with hematoxylin (PASH) or

Table 1. Prepatent period in newborn cats after ingesting individual organs of mice chronically infected with *Cystoisospora felis*[a]

No. of newborn cats used	Duration of infection in mice (months)	Prepatent period after ingesting				
		Brain	Skeletal muscle	Mesenteric lymph nodes	Lung	Liver
3	24	6	9	6	ND	ND
5	15	6	No ooctysts	7	7	6

[a]Experiment terminated 14 d after feeding mouse tissues to cats.

Table 2. Number of parasites in different levels of small intestine in relation to duration of infection with *Cystoisospora felis*

Hours after inoculation (ID of each cat) USNM.	No. of parasites[a] in small intestinal segments						
	1	2	3	4	5	Total	Gamonts
24 (J-73-165) USNM 1249757	3	2	0	0	0	5	0
48 (J-73-166) USNM 1249758	3	1	4	4	1	13	0
72 (J-73-167) USNM 1249759	1	0	4	18	36	59	0
96 (J-73-168) USNM 1249760	0	15	80	73	441	609	0
120 (J-73-169) USNM 1249761	156	588	255	699	3,978	5,676	2,932

[a]Counts based on the examination of six cross sections of intestine from each level of intestine, includes meronts and single intracellular zoites.

Giemsa's stain. Impression smears were made from intestines, air-dried, fixed in methanol, and examined microscopically after staining with Giemsa's stain. For study of the enteric stages, 6–12 cross sections were examined from each of the five small intestinal segments and three to six cross sections from the large intestine. Parasites were counted in six stained cross sections of intestines from each of five small intestinal segments under 1,000X magnification. Specimens were photographed in 2014.

Cystoisospora felis stages in tissues of mice

Mice were killed 1, 2, 3, 4, 5, 7, 10, 11, 14, 26, 71, 111, 360, 370, and 720 d after inoculation with 10^5–10^6 sporocysts and their internal organs were fixed in 10% formalin or Zenker-formal solutions for histology. Impression smears were made from mesenteric lymph nodes of each mouse and examined either fresh in saline or after staining with PASH or Giemsa's stain.

RESULTS

Life cycle of *C. felis* in mice

Only single parasites with a single nucleus were found in tissues of mice fed oocysts; dividing organisms were not seen, irrespective of duration of infection. Tissue cysts were found most often in mesenteric lymph nodes (Fig. 1A). The tissue cyst consisted of a sporozoite enclosed in PAS-positive cyst wall; these were identical to those described previously (Frenkel and Dubey 1972; Lindsay et al. 2014).

The cats fed individual tissues of mice that were fed *C. felis* oocysts 15 and 24 mo previously shed *C. felis* oocysts with a prepatent period of 6–9 d (Table 1).

Of the five newborn cats fed tissues of mice after different passages, the cat fed the mice from the first passage (group A fed *C. felis* sporocysts) shed *C. felis* oocysts beginning 7 d. The remaining four littermate cats fed tissues from second and third passages (group B and C, inoculated with tissues) did not shed *C. felis* oocysts.

Endogenous stages C. felis in cats

In general, *C. felis* stages were structurally consistent with those described by Shah (1971) in cats fed oocysts. *Cystoisospora felis* multiplied throughout the small intestine but the intensity and location of stages varied in relation to duration of infection (Tables 2 and 3; Fig. 1–3). Ileum was the most heavily parasitized. *Cystoisospora felis* stages were located in surface enterocytes throughout the villus, including the base, even in light infection. The lamina propria and the glands of Lieberkühn were not parasitized. The parasitized enterocyte nuclei were not hypertrophied. Endogenous stages were not found in the large intestine. At 24 h, meronts were seen only in the jejunum and contained 1–4 zoites. At 48 h, meronts contained 2–12 zoites. Beginning at 72 h, the ileum was the most heavily parasitized region. Multiple infections of enterocytes were seen beginning at 96 h, and profuse growth at 120 h (Fig. 3), thus it was difficult to determine generations and size of meronts and merozoites in histological sections. Representative different sized merozoites and meronts seen in the ileum of cat at 120 h are shown in Fig. 2. Merozoites varied in shape and size (Fig. 2A). A dividing merozoite/meront with a horse-shoe shaped nucleus (Fig. 2B), with two separate nuclei (Fig. 2C) and two merozoites (Fig. 2D), indicate division by endodyogeny. Male and female gamonts, and fully developed unsporulated oocysts were seen in the cat at 120 h; these were similar to those described by Shah (1971).

DISCUSSION

Results of this investigation showed that *C. felis* can persist in mice for 2 yr, but do not divide in murine tissues.

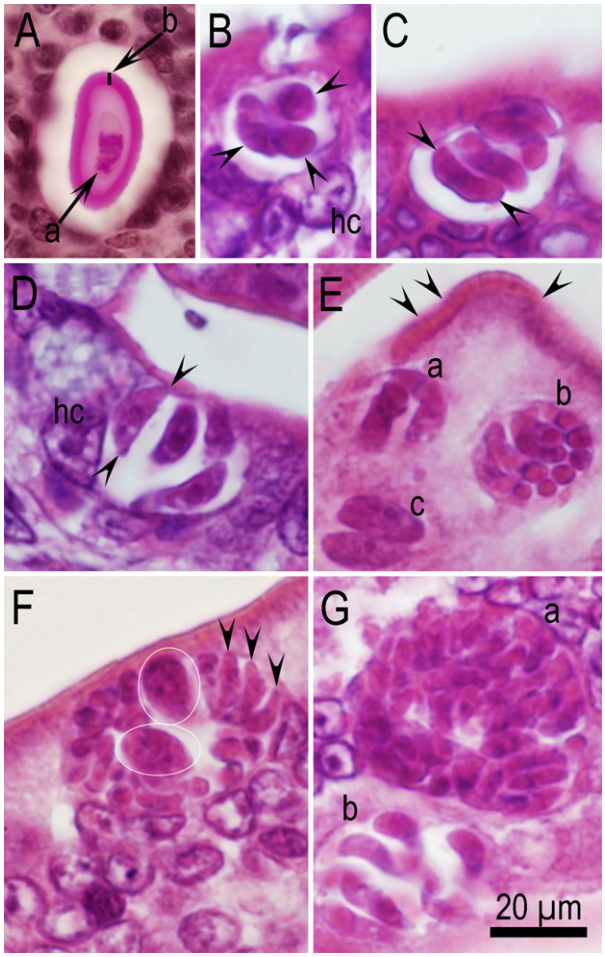

Figure 1 *Cystoisospora felis* stages in smear of mesenteric lymph node of a mouse (A) and histological sections of small intestines of cats (B–G). Periodic acid Schiff (PAS) reaction counter stained with hematoxylin (A) or hematoxylin and eosin (B–G). Scale bar applies to all parts. **A.** Tissue cyst in mesenteric lymph node, 32 mo after feeding oocysts. The tissue cyst contains one sporozoite (a) with PAS-positive central area. The zoite is enclosed in PAS-positive cyst wall (b). A hallow surround the cyst and is considered artifact of fixation. **B.** Four zoites (arrowheads) in a vacuole, only three are visible at this focus. Jejunum, 24 h PI. **C.** Eight zoites (arrowheads) in a vacuole, only four are visible at this focus. Jejunum, 48 h PI. **D.** Four multinucleated meronts (arrowheads) in a vacuole. Ileum, 72 h PI. **E.** Two meronts (a, b) and two big zoites, possibly macrogamonts (c) below the brush border (arrowheads). Ileum, 120 h PI. **F.** A large group of meronts. Two multinucleated meronts are circled. Arrowheads point to longitudinally cut merozoites.Ileum,120 h PI. **G.** Two different sized meronts. The large meront (a) contains numerous mature merozoites. The smaller meront (b) has larger merozoites. Ileum, 120 h PI.

Thus, the mice are paratenic, rather than intermediate, hosts in the life cycle of *C. felis*. In the original report Frenkel and Dubey (1972) indicated that *C. felis* persisted in tissues of rodents up to 67 d when the experiment was terminated. Other investigators have shown that *C. felis* infects even large animals. Fayer and Frenkel (1979) demonstrated that calves (*Bos taurus*) could be experimentally infected with *C. felis*. Six calves were killed 26–115 d after feeding *C. felis* oocysts, and their tissues were fed to cats; the cats shed oocysts with prepatent period of 4–10 d (Fayer and Frenkel 1979). Wolters et al. (1980) in Germany fed naturally exposed raw beef (diaphragm) to eight cats, all of them shed *C. felis* oocysts with a prepatent period of 5 or 6 d. They further demonstrated that freezing (−30 °C) killed *C. felis*; two cats fed same frozen beef did not shed oocysts (Wolters et al. 1980). Melo et al. (2003) detected tissue cysts in viscera of pigs fed 3.5×10^5 *C. felis* oocysts.

Using bioassay in cats, it was shown here that *C. felis* remained viable in several tissues of mice. This experiment was performed in 1973. However, bioassays in cats

Table 3. Asexual and sexual stages in different levels of small intestine of the cat 5 d after feeding infected mice

Intestinal level	Single	Meronts	Female gamonts	Male gamonts	Oocysts
1	28	138	0	0	0
2	46	542	71	22	0
3	23	232	50	17	0
4	87	612	642	42	0
5	1,434	2,544	1,872	180	36

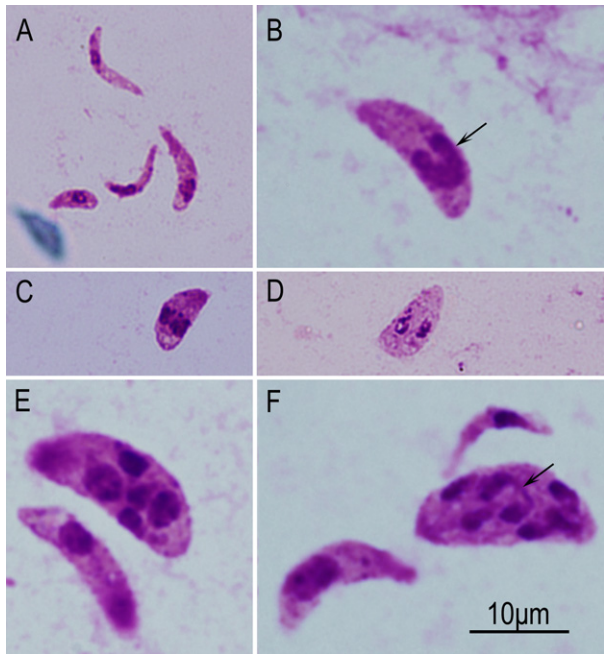

Figure 2 *Cystoisospora felis* meronts and merozoites in smears of ileum of the cat. 120 h PI. Giemsa stain. Scale bar applies to all parts. Note different sized merozoites and merozoite shaped meronts. **A.** Four merozoites of different shapes and sizes. **B.** Zoite with a horse-shoe shaped dividing nucleus (arrow). **C.** Zoite with two nuclei. **D**. Meront with two daughter zoites. **E.** A uninucleate meront and a meront with five nuclei. **F.** Three zoites. The large meront has seven nuclei, one of which is dividing (arrow).

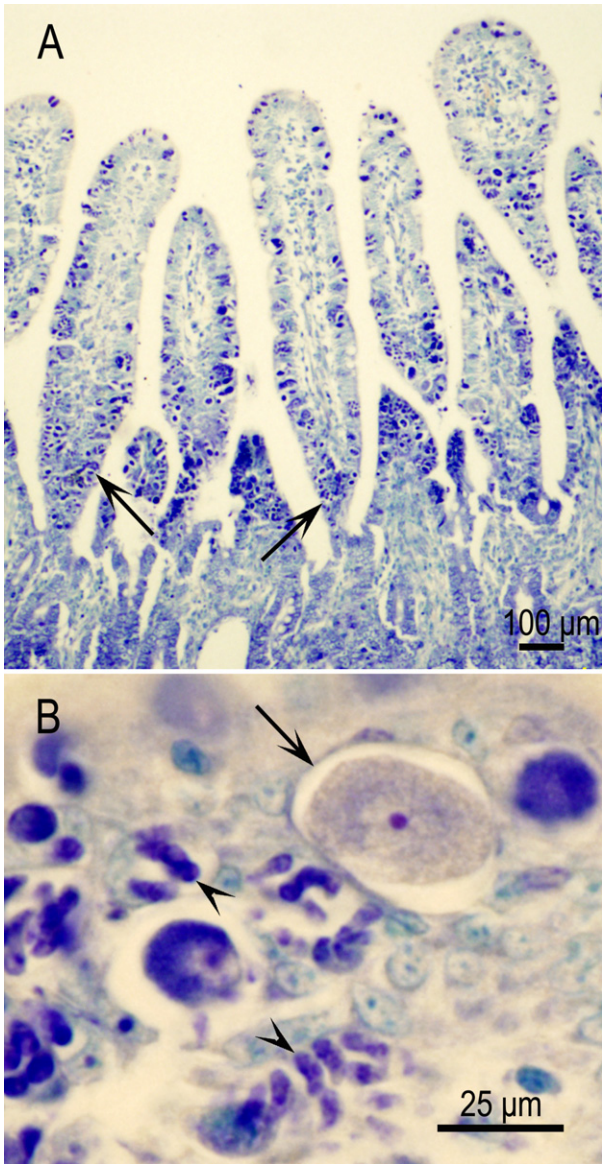

Figure 3 *Cystoisospora felis* infected section of the ileum of a cat. 120 h PI. Giemsa stain. **A.** The entire villi including the base (arrows) are parasitized (all deep staining areas) with *C. felis*. **B.** Higher magnification to show a macrogamont/oocyst (arrow) and numerous merozoites (arrowheads).

are now very expensive. More recent studies indicated that digestion in trypsin or pepsin could be used to locate *C. felis* cysts in tissues (Freire and Lopes 1995, 1996; Heine 1981). Freire and Lopes (1996) fed 10^6 *C. felis* oocysts to 1,000 mice. They killed 10 mice at each of the 0, 3, 6, 7, 8, 9, 16, 21, 28, and 30 d PI. Tissues were digested in pepsin and *C. felis* zoites were counted in the digest. The following conclusions were drawn from this study: (1) Zoites increased in size from 14.5 × 5.7 µm at day 7 PI to 35.8 × 12.3 at day 21 PI. (2) The mesenteric lymph node was the main organ infected; 91.5% of zoites recovered from mouse tissues at day 8 were from lymph nodes. (3) The number of zoites in mouse tissues decreased progressively from days 9 to 30. In my experience, *C. felis* is nonpathogenic to outbred mice, irrespective of the dose. Loss and Lopes (1992b) reported that mice fed 10^5 *C. felis* oocysts became ill and lost weight; this report needs confirmation.

The biological significance of these partenic hosts in the natural epidemiology of *C. felis* is unknown because the parasite is transmitted very efficiently via fecal-oral route. Among all known coccidian parasites, *C. felis* is the most efficiently transmitted organism. Virtually all cats, even those raised in strictly controlled environment, become infected with *C. felis* (Dubey, J. P., own observ.). *Cystoisospora felis* oocysts are highly infectious to cats; it can sporulate within 8 h at 30 °C (Shah 1970). Newborn uninfected kittens begin shedding *C. felis* 7 d after contact with kittens that are passing *C. felis* oocysts. Although *C. rivolta* has a similar life cycle, it is not as infectious as *C. felis* (Dubey, J. P., own observ.).

Cystoisospora felis is biologically important with respect to certain other protozoan infections. It can modify *T. gondii* oocyst shedding and oocysts are key in the transmission of the parasite to humans and other hosts. Cats shed *T. gondii* oocysts for 1–2 wk after eating infected tissues and seldom re-shed oocysts (Dubey 2010). This immunity to re-shedding of oocysts is normally not affected by super-infection even with the Feline Immnuodeficient Virus infection or Feline Retrovirus infection nor the administration of pharmacological doses of exogenous corticosteroids (Dubey 2010). However, infection of cats chronically infected with *T. gondii* with *C. felis* induces re-excretion of *T. gondii* oocysts in the absence of any clinical signs (Chessum 1972; Dubey 1976). The *C. felis* induced relapse is specific because *C. rivolta* does not induce relapse of *T. gondii* infection and the effect can be blocked by immunization of cats with *C. felis* before *T. gondii* infection (Dubey 1976, 1978). Both *C. felis* and *T. gondii* can be acquired simultaneously if the intermediate hosts are infected with these coccidians. Similar phenomenon has been observed with mice infected with *C. felis* and a blood protozoan, *Babesia microti* (Takahashi et al. 1993). Mice infected with *C. felis* were protected against *B. microti* infection and the protection could be transferred through immune cells and protection could be abolished via injection of monoclonal antibodies against lymphocytes (Takahashi et al. 1993).

In the present study, the number of asexual generations in the intestines of cats fed *C. felis* infected mice was not determined. Also I did not find the rounded uninucleate meronts (trophozoites) here or in the intestines of cats infected with *C. rivolta*, *T. gondii*, or any other *Cystoisospora* of dogs (Lindsay et al. 1997, 2014). In the present study, the mode of division of *C. felis* was not studied in detail. Simultaneous occurrence of merozoites of different sizes on day 5 PI (Fig. 2) made it difficult to determine different generations. In the present study, both mature and immature meronts were found in the same vacuole (Fig. 1F); Shah (1971) called these as "cysts" containing

third generation schizonts and merozoites. These structures are similar to Type B schizonts/meronts of *T. gondii* (Dubey and Frenkel 1972b; Speer and Dubey 2005).

Shah (1971) stated that in cats fed *C. felis* oocysts the parasite was located in the distal portion of the intestinal villus. In the present study, *C. felis* multiplied in enterocytes throughout the villus, and meronts were found at the base of the villus even in light infections during the early part of the cycle. These differences may be related to the inocula (oocysts vs. tissue cysts) and the age of the cats; Shah (1971) used weaned kittens (6 wk or older) and inoculated them with oocysts, whereas I used newborn kittens and inoculated them with infected mouse tissues. The number of infectious organisms present in inocula fed to cats was not known because they were given homogenate of tissues. The finding of only few parasites in numerous sections of intestines of cats at 1–3 d PI, indicates only a few parasites in the inocula.

In the present study, the minimum prepatent period of *C. felis* after feeding infected tissues was 5–9 d. Shah (1971) mentioned that the minimum prepatent period in cats after feeding oocysts was 7 d. It appears that the strain of *C. felis* could be a factor. Wolters et al. (1980) mentioned that prepatent period could be shortened by continuous passage of *C. felis* in cats. de Medeiros et al. (2007) stated that oocysts excreted by cats after feeding infected tissues were more variable in size compared with oocyst-induced infections.

There are conflicting reports concerning the pathogenicity of *C. felis* infection in cats. I consider *C. felis* to be not important clinically. In the present study, I did not find any host reaction around parasitized tissue, and in previous studies weaned kittens that excreted millions of *C. felis* oocysts were not ill (Dubey 1976, 1978). Hutchison et al. (1981) observed by scanning electron microscopy alterations in intestine of cats 6 d after feeding *C. felis* oocysts. The infected enterocytes were swollen, and the villi were blunt and shorter than villi in the intestines of uninfected cats. Loss and Lopes (1992a,c) described severe illness, and even mortality in cats inoculated with *C. felis*. Whether these results are related to parasite strains or concurrent infection with other pathogens needs further investigation.

Specimens deposited

Histological sections of intestines are deposited in the US National Parasite Collection, in the Division of Invertebrate Zoology and National Museum of Natural History, Smithsonian Institution, Washington, DC under USNAM 1249757–1249761 (see Table 2).

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I thank Dr. J. K. Frenkel (deceased in 2013) for guiding this investigation. He had permitted me to be the sole author of this article, and take histological sections of intestines and smears with me when I moved from his laboratory in 1973; paraffin blocks he retained have been destroyed. During my moves, the box of slides containing this material was misplaced and hence the 40-yr delay in publication. I thank Dr. S. K. Verma for his help with illustrations.

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