

***CYSTOISOSPORA CANIS* NEMESÉRI, 1959 (SYN. *ISOSPORA CANIS*),
INFECTIONS IN DOGS: CLINICAL SIGNS, PATHOGENESIS, AND
REPRODUCIBLE CLINICAL DISEASE IN BEAGLE DOGS FED OOCYSTS**

Author(s): Sheila M. Mitchell , Anne M. Zajac , Sam Charles , Robert B. Duncan , and David S. Lindsay

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CYSTOISOSPORA CANIS NEMESÉRI, 1959 (SYN. ISOSPORA CANIS), INFECTIONS IN DOGS: CLINICAL SIGNS, PATHOGENESIS, AND REPRODUCIBLE CLINICAL DISEASE IN BEAGLE DOGS FED OOCYSTS

Sheila M. Mitchell, Anne M. Zajac, Sam Charles*, Robert B. Duncan, and David S. Lindsay†

Department of Biomedical Sciences and Pathobiology, Virginia Tech, 1410 Prices Fork Road, Blacksburg, Virginia 24061-0342.
e-mail: lindsayd@vt.edu

ABSTRACT: Canine intestinal coccidiosis is a cause of diarrhea in young dogs and dogs that are immunocompromised. Reports in the literature indicate that experimental reproduction of clinical coccidiosis with *Cystoisospora canis* (syn. *Isospora canis*) is difficult, and few studies have been done with *C. canis*. Experimental oral infections were attempted in 22, 6- to 8-wk-old female beagles with 5×10^4 (n = 2) or 1×10^5 (n = 20) sporulated *C. canis* oocysts. Diarrhea was observed in all inoculated dogs. Diarrhea began 2–3 days before oocyst excretion. Five of the 22 dogs were given an anticoccidial (sulfadimethoxine) because of their clinical signs. The mean prepatent period was 9.8 days (range, 9–11 days, n = 22 dogs), and the patent period was 8.9 days (range, 7–18 days, n = 20 dogs). Two dogs exhibiting clinical coccidiosis were examined at necropsy 10 days after infection. Developmental stages of *C. canis* were present in cells in the lamina propria throughout the entire small intestine in both dogs. Microscopic lesions observed in both of these dogs were villous atrophy, dilation of lacteals, and hyperplasia of lymph nodes in Peyer's patches. Results of bacterial and viral examinations of these 2 dogs were negative, indicating that intestinal coccidiosis was the cause of the diarrhea. Our study indicates that *C. canis* can be a primary cause of diarrhea in young dogs.

Coccidia are common parasites of dogs worldwide. Dogs are hosts for *Cystoisospora canis* Nemeséri, 1959; *Cystoisospora ohioensis* Dubey, 1975; *Cystoisospora burrowsi* Trayser and Todd, 1978; and *Cystoisospora neorivolta* Dubey and Mahrt, 1978. In dogs, oocysts of *C. canis* can be definitively identified based on their structure in fecal samples because of their large size (>33 μm) when compared with the oocysts of *C. ohioensis*, *C. neorivolta*, and *C. burrowsi*, which are structurally similar (<30 μm) (Lindsay et al., 1997). The oocysts of these 3 similar-sized coccidial species are often grouped together and termed *C. ohioensis*-like oocysts because detailed structural examinations and life-cycle studies are needed before a definitive diagnosis can be made. The life cycle and transmission of *C. canis* has been examined by several groups of researchers (Nemeséri, 1960; Lepp and Todd, 1974, 1976; Dubey, 1975b, 1982; Hilali et al., 1979; Becker et al., 1981). The life cycles and transmission of *C. ohioensis*, *C. neorivolta*, and *C. burrowsi*, have also been examined (Dubey, 1975a, 1978a, 1978b; Dubey and Mahrt, 1978; Dubey and Mehlhorn, 1978; Dubey et al., 1978; Trayser and Todd, 1978; Becker et al., 1981; Rommel and Zielasko, 1981.).

There is controversy over the pathogenicity of *C. canis* and other *Cystoisospora* species occurring in dogs. Severe clinical disease was not produced in 25, 6-wk-old or 6, 8-wk-old dogs inoculated with $1\text{--}1.5 \times 10^5$ *C. canis* oocysts of an Illinois isolate of the parasite (Lepp and Todd, 1974). Nemeséri (1960) found that 5×10^3 oocysts of a Hungarian isolate of *C. canis* were not pathogenic for dogs, but an inoculum of 5 or 8×10^4 oocysts produced clinical coccidiosis. The present study was done to evaluate the pathogenicity of an isolate of *C. canis* obtained from pit bull puppies. Additionally, the oocysts of *C. canis* are redescribed, and additional information on the life cycle of *C. canis* is presented.

MATERIALS AND METHODS

Source of oocysts

Oocysts consistent with the structure of *C. canis* were identified in the feces of 2 littermate pit bull puppies, housed at the Montgomery County animal shelter in Blacksburg, Virginia. The pups were 1–2 mo of age. Feces were collected from these puppies 1 or 3 times/wk from 26 February 2004 through 24 March 2004. These oocysts were mixed in 2% (v/v) sulfuric acid, filtered through 2 layers of cheesecloth, placed in a thin layer (4–6 mm) in 150-cm² tissue culture flasks with vented tops, and placed on a mechanical shaker for 4–6 days at room temperature. Oocysts were concentrated by flotation using Sheathers' sugar solution and stored at 4 C in 2% sulfuric acid until used. Oocysts were washed free of sulfuric acid in sterile Hanks balanced salt solution (HBSS) by centrifugation before use in experimental infections.

Dogs and fecal examinations

Five experiments using 22 female beagles were conducted (Table I). Dogs were obtained at 6–8 wk of age (Covance, Cumberland, Virginia). Weights were obtained upon the dogs' arrival at our facilities and at weekly intervals thereafter. Fecal samples were examined using centrifugal flotation in Sheathers' sugar solution. Fecal samples were examined daily until dogs were orally infected (if feces were available). Samples were examined on days -1, 0, and 1–29 for coccidial oocysts. Quantitative fecal oocyst counts using the McMaster method were done when a dog became positive for the *C. canis* oocyst (Tables II, III). Briefly, the McMaster method was conducted by mixing 2 g of feces with 28 ml of Sheathers' sugar solution. Both sides of a McMaster counting slide were loaded with the mixture. Slides were allowed to sit for 5 min, and then all oocysts present were counted. The total numbers of oocysts counted was determined by multiplying the number counted by 50. No. 1 was used if the McMaster exam was negative, but the fecal float was positive.

Clinical signs

Clinical signs were recorded for each dog daily after clinical signs became apparent. Temperatures were obtained when dogs became clinically ill (Experiments 1–3) or at weekly intervals (Experiments 4–5). Fecal samples were scored daily (Table IV). Briefly, a score of 1 = normal-formed feces; 2 = mixture of loose and formed; 3 = completely loose but not liquid; and 4 = liquid. A note was made whether blood or mucus was present.

Hematocrit and total protein values were examined weekly in dogs from Experiments 4 and 5.

Experimental infections

Experiments 1–5 used an inoculum dose of 1×10^5 sporulated *C. canis* oocysts, whereas Experiment 3 used an inoculum dose of $5 \times$

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* Bayer HealthCare Animal Health, Shawnee Mission, Kansas 66201-0390.

† To whom correspondence should be addressed.

TABLE I. Experimental protocol for oral infection of dogs with sporulated oocysts of *Cystoisospora canis*, clinical signs, and prepatent and patent periods in days.

Expt.*	Dog	Dose of Oocysts	Clinical signs	Prepatent period	Patent period
1	BAR†	1 × 10 ⁵	Yes	11	10
1	BAS†‡	1 × 10 ⁵	Yes	9	10
2	AHC	1 × 10 ⁵	Yes	10	5
2	ALF†	1 × 10 ⁵	Yes	10	8
2	AHP	1 × 10 ⁵	Yes	10	8
2	AIZ1	1 × 10 ⁵	Yes	10	8
3	BAG§	5 × 10 ⁴	Yes	9	9
3	AXY§	5 × 10 ⁴	Yes	10	7
3	BBH§	1 × 10 ⁵	Yes	10	18
3	AYF§	1 × 10 ⁵	Yes	10	7
4	ASF†§	1 × 10 ⁵	Yes	10	10
4	ASH	1 × 10 ⁵	Yes	10	11
4	ASI	1 × 10 ⁵	Yes	10	8
4	ASG§	1 × 10 ⁵	Yes	10	8
5	AIY§	1 × 10 ⁵	Yes	10	9
5	AJU	1 × 10 ⁵	Yes	10	8
5	AKA	1 × 10 ⁵	Yes	10	7
5	AJY†	1 × 10 ⁵	Yes	10	7
5	AJA	1 × 10 ⁵	Yes	10	7
5	AJZ§	1 × 10 ⁵	Yes	10	8
5	AJV	1 × 10 ⁵	Yes	9	NA
5	AIZ2	1 × 10 ⁵	Yes	9	NA

* Experiment number.

† Dog treated with sulfadimethoxine because of clinical coccidiosis.

‡ This dog was treated orally with 5 mg prednisone daily 3 days before infection and then daily on days 1–6 and 8–12 after infection.

§ *Cystoisospora ohioensis*-like oocysts observed in the feces of dog before experimental oral infection with *C. canis* oocysts.

10⁴ sporulated *C. canis* oocysts in 2 of the 4 dogs in addition to the dose listed above in the remaining 2 dogs. Dogs were orally infected by mixing the appropriate amount of sporulated oocysts in commercial dog food (Hills Science Diet A/D, Topeka, Kansas). All dogs readily ate this mixture within 3–5 min, and none vomited the inoculum.

One dog (BAS) in Experiment 1 was treated orally with 5 mg of prednisone daily for 3 days before infection and then daily on days 1–6 and 8–12 after infection (Table I). Results of Experiment 1 indicated that prednisone immunosuppression was not needed, and none of the other dogs was given this treatment. Dogs BAR, BAS, ALF, ASF, and AJY were treated with 25 mg/kg sulfadimethoxine (Pfizer Inc., Groton, Connecticut) for 2–3 days because of severe diarrhea (Table I).

Pathogenicity and development study (Experiment 5)

Experiment 5, using 8 dogs, was designed to determine the role of *C. canis* in the pathogenicity of diarrhea observed in the infected dogs and to rule out other causes, such as bacteria and viruses. The sporulated oocyst inoculum was treated with 50% v/v bleach solution for 5 min on an ice bath and then washed by centrifugation in cold sterile HBSS until the smell of bleach was no longer present. This inoculum was then streaked onto blood agar and TSA agar to detect bacteria that may have survived bleach treatment. This inoculum was used to infect 8 beagles.

Two dogs (AJV and AIZ-2) were killed 10 days postinoculation (PI). A board-certified pathologist (R.B.D) conducted the necropsy. Intestinal tissues were collected for bacteriological culture and histological examination. Additional tissues collected for histology only and fixed in 10% neutral buffered formalin solution were mesenteric lymph nodes, liver, and spleen. Formalin-fixed tissues were embedded in paraffin, sectioned at 6 µm, and stained with hematoxylin and eosin. Feces were collected for virology and examined by transmission electron microscopy (TEM) after negative staining at the Texas Veterinary Medical Diagnostic Laboratory, College Station, Texas. Additionally, portions of ileum were fixed in 3% (v/v) glutaraldehyde in phosphate buffer (PBS, pH 7.4). Tissues were postfixed in 1% (w/v) osmium tetroxide in 0.1 M phosphate buffer, dehydrated in a series of ethanols, passed through 2 changes of propylene oxide, and embedded in Poly/Bed 812 resin (Polysciences Inc., Warrington, Pennsylvania). Thin sections were stained with uranyl acetate and lead citrate and examined with a Zeiss

TABLE II. Daily McMaster's oocysts counts per gram of feces per dog (days 8–17).

ID	8	9	10	11	12	13	14	15	16	17
BAR*	0	0	0	30,500	22,000	118,400	82,750	33,200	204,600	134,200
BAS*†	0	12,300	0	0	43,450	77,250	77,900	59,700	39,150	3,450
AHC	0	0	1	28,800	0	23,300	4,300	0	0	0
ALF*	0	0	1	31,450	29,150	30,700	28,050	8,900	3,400	100
AHP	0	0	1	140,650	66,250	51,800	33,800	20,700	11,100	800
AIZ	0	0	1	137,950	58,350	83,500	54,750	18,800	4,800	1,250
BAG‡	0	250	81,650	200,250	215,650	29,050	7,000	2,800	150	50
BBH‡	0	0	14,600	137,300	1,370,300	98,400	118,900	47,250	27,950	24,500
AXY‡	0	0	5,450	123,050	64,250	28,100	6,450	1,700	100	0
AYF‡	0	0	36,700	125,000	113,350	64,750	38,050	6,700	50	0
ASF*‡	0	0	100	75,600	20,700	2,900	10,000	5,600	2,100	200
ASH	0	0	1,900	22,750	96,000	49,800	8,600	5,600	2,000	1,950
ASI	0	0	500	9,150	120,400	23,100	3,000	3,100	3,300	900
ASG‡	0	0	11,450	114,400	55,800	24,500	5,900	400	1,050	150
AIY‡	0	1	4,400	14,400	32,900	28,200	13,300	12,500	3,600	850
AJU	0	1	176,500	36,100	83,900	40,300	24,200	6,500	550	50
AKA	0	0	8,350	106,800	101,600	38,100	92,500	5,200	5,450	0
AJY*	0	0	22,400	122,600	125,100	92,500	124,400	22,000	1,100	0
AJA	0	0	38,700	47,500	122,500	59,900	16,000	20,400	6,050	0
AJZ‡	0	0	40,000	109,300	167,800	138,800	53,600	11,800	5,050	300
AJV	0	1	5,900	0	0	0	0	0	0	0
AIZ-2	0	1	17,550	0	0	0	0	0	0	0

* Dog treated with sulfadimethoxine because of clinical coccidiosis.

† This dog was treated orally with 5 mg prednisone daily 3 days before infection and then daily on days 1–6 and 8–12 after infection.

‡ *Cystoisospora ohioensis*-like oocysts observed in the feces of dog before experimental oral infection with *C. canis* oocysts.

TABLE III. Daily McMaster's oocysts counts per gram of feces per dog (days 18–27).

ID	18	19	20	21	22	23	24	25	26	27
BAR*	82,400	4,500	100	0	0	0	0	0	0	0
BAS*†	350	0	0	0	0	0	0	0	0	0
AHC	0	0	0	0	0	0	0	0	0	0
ALF*	0	0	0	0	0	0	0	650	1,250	600
AHP	0	0	0	0	0	0	0	0	0	0
AIZ	0	0	0	0	0	0	50	0	0	0
BAG‡	0	0	0	0	0	0	0	0	0	0
BBH‡	3,550	700	0	0	0	3,250	1,500	1	1	1
AXY‡	0	0	0	0	0	0	0	0	0	0
AYF‡	0	0	0	0	0	0	0	0	0	0
ASF*‡	850	250	0	0	0	0	0	0	0	0
ASH	1,150,200	200	50	0	0	0	0	0	0	0
ASI	0	0	0	0	0	0	0	0	0	0
ASG‡	0	0	0	0	0	0	0	0	0	0
AIY‡	100	0	0	0	0	0	0	0	0	0
AJU	0	0	0	0	0	0	0	0	0	0
AKA	0	0	0	0	0	0	0	0	0	0
AJY*	0	0	0	0	0	0	0	0	0	0
AJA	0	0	0	0	0	0	0	0	0	0
AJZ‡	0	0	0	0	0	0	0	0	0	0
AJV	0	0	0	0	0	0	0	0	0	0
AIZ-2	0	0	0	0	0	0	0	0	0	0

* Dog treated with sulfadimethoxine because of clinical coccidiosis.

† This dog was treated orally with 5 mg prednisone daily 3 days before infection and then daily on days 1–6 and 8–12 after infection.

‡ *Cystoisospora ohioensis*-like oocysts observed in the feces of dog before experimental oral infection with *C. canis* oocysts.

TABLE IV. Daily fecal scores* postinoculation (PI) (dogs BAR–AYF).

Days PI	BAR†	BAS†‡	AHC	ALF†	AHP	AIZ	BAG§	BBH§	AXY§	AYF§
–1	1	1	2	1	1	1	1	1	1	1
0	2	1	2	1	1	1	1	1	1	1
1	2	1	2	2	1	1	1	1	1	2
2	2	1	2	2	1	1	1	1	1	1
3	1	1	1	2	1	1	1	1	1	1
4	1	1	2	2	2	2	1	1	1	1
5	2	1	2	2	2	2	1	2	1	1
6	2	3	1	1	2	2	1	2	1	2
7	1	2	1	1	2	1	1	2	1	2
8	1	1	4	4	2	1	2	2	2	2
9	4	3	4	4	3	3	3	3	2	1
10	4	4	4	4	4	4	4	2	2	3
11	4	4	4	4	4	3	3	2	2	3
12	4	2	4	4	4	2	4	1	2	3
13	4	2	3	4	4	3	4	3	3	3
14	4	2	3	4	4	2	2	2	1	2
15	3	4	4	4	4	3	2	2	1	2
16	4	4	3	3	3	3	2	2	1	2
17	4	4	3	3	3	3	2	2	1	1
18	3	3	2	2	2	2	1	1	1	1
19	3	2	1	1	2	1	1	1	1	1
20	2	2	2	2	2	1	1	1	1	1
21	2	2	2	2	2	1	1	1	1	1
22	2	2	1	2	1	1	1	1	1	1
23	3	2	1	2	1	1	1	1	1	1
24	2	1	1	2	2	1	1	1	1	1

* A score of 1 = normal-formed feces; 2 = mixture of loose and formed; 3 = completely loose but not liquid; and 4 = liquid.

† Dog treated with sulfadimethoxine because of clinical coccidiosis.

‡ This dog was treated orally with 5 mg prednisone daily 3 days before infection and then daily on days 1–6 and 8–12 after infection.

§ *Cystoisospora ohioensis*-like oocysts observed in the feces of dog before experimental oral infection with *C. canis* oocysts.

TABLE V. Daily fecal scores* postinoculation (PI) (dogs ASF–AIZ-2).

Days PI	ASF†‡	ASH	ASI	ASG‡	AIY‡	AJU	AKA	AJY†	AJA	AJZ‡	AJV	AIZ-2
-1	1	1	1	1	3	2	3	2	2	2	2	2
0	1	1	1	1	2	1	1	1	1	1	1	1
1	1	1	1	1	3	1	1	1	1	1	1	1
2	1	1	1	1	3	2	2	3	3	3	3	2
3	1	1	1	1	3	3	1	3	1	3	3	3
4	1	1	1	1	3	2	2	2	2	3	3	2
5	1	1	1	1	3	3	2	2	2	3	3	2
6	1	1	1	1	3	3	2	2	2	3	3	2
7	1	1	1	2	4	2	1	1	1	2	2	1
8	1	1	2	2	NA§	4	2	2	1	3	3	3
9	3	2	1	4	4	4	4	4	4	4	4	4
10	4	4	4	4	4	4	4	4	4	4	4	4
11	4	4	4	4	4	4	4	4	4	4	NA	NA
12	4	4	4	4	4	4	2	4	4	4	NA	NA
13	NA	4	3	4	3	4	4	4	4	4	NA	NA
14	3	3	3	3	3	3	2	4	3	2	NA	NA
15	3	3	3	3	3	3	1	3	3	3	NA	NA
16	3	3	3	3	3	3	2	3	3	3	NA	NA
17	1	1	3	3	3	3	1	3	3	3	NA	NA
18	1	1	1	1	2	1	1	3	3	2	NA	NA
19	1	1	1	NA	3	3	2	3	3	3	NA	NA
20	1	1	2	1	2	3	1	1	2	1	NA	NA
21	1	1	1	1	1	1	2	1	2	1	NA	NA
22	2	1	1	2	1	1	1	1	2	1	NA	NA
23	1	1	1	1	3	1	1	1	2	2	NA	NA
24	1	1	1	1	1	2	1	1	1	1	NA	NA

* A score of 1 = normal-formed feces; 2 = mixture of loose and formed; 3 = completely loose but not liquid; and 4 = liquid.

† Dog treated with sulfadimethoxine because of clinical coccidiosis.

‡ *Cystoisospora ohioensis*-like oocysts observed in the feces of dog before experimental oral infection with *C. canis* oocysts.

§ Not applicable because no sample was obtained that day postinoculation.

10CA TEM operating at 60 kV. Digital images were captured using an ATM camera system (Advanced Microscopy Techniques Corp., Danvers, Massachusetts).

Thick sections of resin-embedded tissues were stained with methylene blue-Azure II-Basic fuchsin triple stain (Hayat, 1989) and mounted on glass slides for observation with light microscopy.

Immunohistochemistry

Immunohistochemistry was done to determine whether developmental stages of *C. canis* contained cross-reactive antigens to *Neospora caninum*, *Toxoplasma gondii*, or *Sarcocystis neurona*. Parasite-specific antisera were made in rabbits and used at dilutions of 1:500 and 1:1,000. Paraffin-embedded tissue sections of *C. canis*-infected ileum were cut at 6 μ m, mounted on glass slides, and used for immunohistochemical examinations using the avidin-biotin immunoperoxidase complex (ABC) test, as previously described by Lindsay and Dubey (1989). Positive controls for parasite cross-reactivity were tissue sections containing developmental stages of *T. gondii*, *N. caninum*, or *S. neurona*.

Redescription of *C. canis*

Sporulated oocysts from pit bull puppies were examined using an Olympus BX60 microscope equipped with differential contrast optics and a digital camera. Measurements were obtained from 25 oocysts using oil emersion and a calibrated ocular micrometer.

RESULTS

Cystoisospora ohioensis-like oocysts were observed in the feces of dogs in Experiment 3 (4 of 4 dogs), Experiment 4 (2 of 4 dogs), and Experiment 5 (2 of 8 dogs) before infection with *I. canis* oocysts (Tables I–III). Clinical signs were not as-

sociated with the presence of these *C. ohioensis*-like oocysts. All dogs that excreted *C. ohioensis*-like oocysts were susceptible to clinical coccidiosis when fed *C. canis* oocysts orally (Table I). The 2 dogs (AJV and AIZ2) used in Experiment 5 for histology and pathology studies never excreted *C. ohioensis*-like oocysts, and that was a selection criterion for their use in the studies. The *C. canis* oocyst counts for the dogs in Experiments 1–5 are presented in Tables II and III.

Clinical signs

Clinical coccidiosis was induced in all dogs in Experiments 1–5 (Table I). Fecal scores are presented in Tables IV–V. Fecal scores of 3 or 4, indicating severe diarrhea, were usually seen 2–3 days before oocyst excretion. Clinical signs were consistent with canine coccidiosis and included watery or bloody diarrhea, anorexia, weight loss, vomiting, and lethargy. Increased rectal temperatures were also noted in most dogs. Hematocrit and total protein values obtained from dogs in Experiments 4 and 5 were within normal ranges (37–55% hematocrit; 5.2–7.8 g/dl total protein) for dogs. Total weight gains for dogs ranged from 0.2 to 3.0 kg (Table VI).

All dogs excreted *C. canis* oocysts. The mean prepatent period was 9.8 days (range, 9–11 days, n = 22 dogs), and the patent period was 8.9 days (range, 7–18, n = 20 dogs).

Pathogenicity and development

Results of histopathological examination of small intestine documented asexual stages and sexual stages of *C. canis* within

TABLE VI. Beginning and ending weights of dogs in kilograms.

Expt.*	Dog	Oocysts	Beginning	Ending	Total gain
1	BAR†	1 × 10 ⁵	2.5	3.8	1.3
1	BAS†‡	1 × 10 ⁵	2.8	4.3	1.5
2	AHC	1 × 10 ⁵	2.6	2.8	0.2
2	ALF†	1 × 10 ⁵	2.0	3.1	1.1
2	AHP	1 × 10 ⁵	2.8	3.8	1.0
2	AIZ	1 × 10 ⁵	1.8	2.7	0.9
3	BAG§	5 × 10 ⁴	1.6	4.3	2.7
3	BBH§	5 × 10 ⁴	1.5	4.5	3.0
3	AXY§	1 × 10 ⁵	1.9	4.7	2.8
3	AYF§	1 × 10 ⁵	1.5	4.5	3.0
4	ASF†§	1 × 10 ⁵	1.0	2.5	1.5
4	ASH	1 × 10 ⁵	1.1	2.8	1.7
4	ASI	1 × 10 ⁵	1.2	3.0	1.8
4	ASG§	1 × 10 ⁵	1.0	2.5	1.5
5	AIY§	1 × 10 ⁵	1.5	3.0	1.5
5	AJU	1 × 10 ⁵	1.7	2.7	1.0
5	AKA	1 × 10 ⁵	2.0	3.2	1.2
5	AJY†	1 × 10 ⁵	1.7	2.7	1.0
5	AJA	1 × 10 ⁵	2.0	3.0	1.0
5	AJZ§	1 × 10 ⁵	1.8	3.2	1.4
5	AJV	1 × 10 ⁵	2.2	2.4	0.2
5	AIZ-2	1 × 10 ⁵	1.7	2.0	0.3

* Experiment number.

† Dog treated with sulfadimethoxine because of clinical coccidiosis.

‡ This dog was treated orally with 5 mg prednisone daily 3 days before infection and then daily on days 1–6 and 8–12 after infection.

§ *Cystoisospora ohioensis*-like oocysts observed in the feces of dog before experimental oral infection with *C. canis* oocysts.

|| Dog was killed and examined at necropsy.

the subepithelial lamina propria of intestinal villi (Figs. 1–4). There was mild villous atrophy; moderate, diffuse villous epithelial cell attenuation; moderate crypt epithelial cell hyperplasia; occasional widely scattered, mildly dilated lacteals; and marked lymphoid hyperplasia of the Peyer's patches (Fig. 5). Occasional crypts contained a few eosinophils, polymorphonuclear leukocytes, and necrotic epithelial cells. Rare sexual stages of *C. canis* were present in the colon. Extraintestinal stages of *C. canis* were not detected in the mesenteric lymph nodes, but there was moderate-to-marked lymphoid hyperplasia, mild sinus histiocytosis, and occasional scattered foci of neutrophils and eosinophils.

No bacterial growth was observed on the blood agar or TSA agar plates after 3 days of incubation with sterilized oocysts mixture used to infect dogs. No bacterial pathogens were isolated from the intestines of the 2 dogs killed and examined at necropsy. No viruses were detected by electron microscopy in the feces from these 2 dogs.

Schizonts, merozoites, macrogamonts, microgamonts, and oocysts were present in all sections of small intestines (Figs. 1–4) from both dogs. Developmental stages were located in a parasitophorous vacuole in host cells that were in the lamina propria. Different developmental stages appeared to be in the same host cell (Fig. 3). Immature schizonts and mature merozoites could also be seen in the same cell. Occasionally, macrogamonts and microgamonts were seen in the same host cell. Light microscopic observations on asexual stages occupying the same host cell were validated by examinations using TEM.

Immunohistochemistry

Developmental stages of *C. canis* did not react with antibodies to *T. gondii*, *N. caninum*, or *S. neurona*.

REDESCRIPTION

Cystoisospora canis

Diagnosis: Oocysts ovoid. Micropyle absent; oocyst residuum absent. Sporulated oocysts measure 37.2 ± 1.0 by 29.5 ± 1.2 μm (35–39 by 27–32 μm , $n = 25$); length to width ratio 1.3 ± 0.06 (1.16–1.38, $n = 25$). Two sporocysts present in each oocyst; sporocysts ellipsoidal, Stieda and substieda bodies absent, sporocyst residuum present, composed of a compact spherical mass or dispersed granules. Sporocysts measure 21.2 ± 0.9 by 16.3 ± 0.1 μm (19–23 by 15–18 μm , $n = 25$); length to width ratio 1.3 ± 0.08 (1.17–1.47, $n = 25$). Four sporozoites in each sporocyst.

Taxonomic summary

Type host: Domestic dog, *Canis familiaris*.

Other hosts: Coyotes, *Canis latrans*, are experimental (Loveless and Anderson, 1975; Dubey, 1982; Dunbar and Foreyt, 1985) and natural hosts (Dubey, Fayer et al., 1978).

Paratenic hosts: Mice, cats, dogs, swine, sheep, water buffalos, and camels (Dubey, 1975b; Hilali et al., 1992, 1995; Zayed and El-Ghaysh, 1998). These studies are based on feeding tissues of naturally or experimentally infected animals and finding oocysts of *C. canis* in canine feces after feeding of host tissues.

Location in host: Inside of host cells, within the lamina propria of the duodenum, jejunum, and ileum of the small intestine and rarely the colon.

Prepatent period: From 9 to 11 days (Nemeséri, 1960; Lepp and Todd, 1974; present study) if oocysts are used as inoculum. The prepatent period is 8–9 days in dogs fed *C. canis*-infected mice (Dubey, 1975b).

Patent period: Either 4 wk (Nemeséri, 1960) or 7–15 days (present study).

Sporulation time: Sporulation is complete in 48 hr at 20 C and 16 hr at 30 or 35 C (Lepp and Todd, 1976).

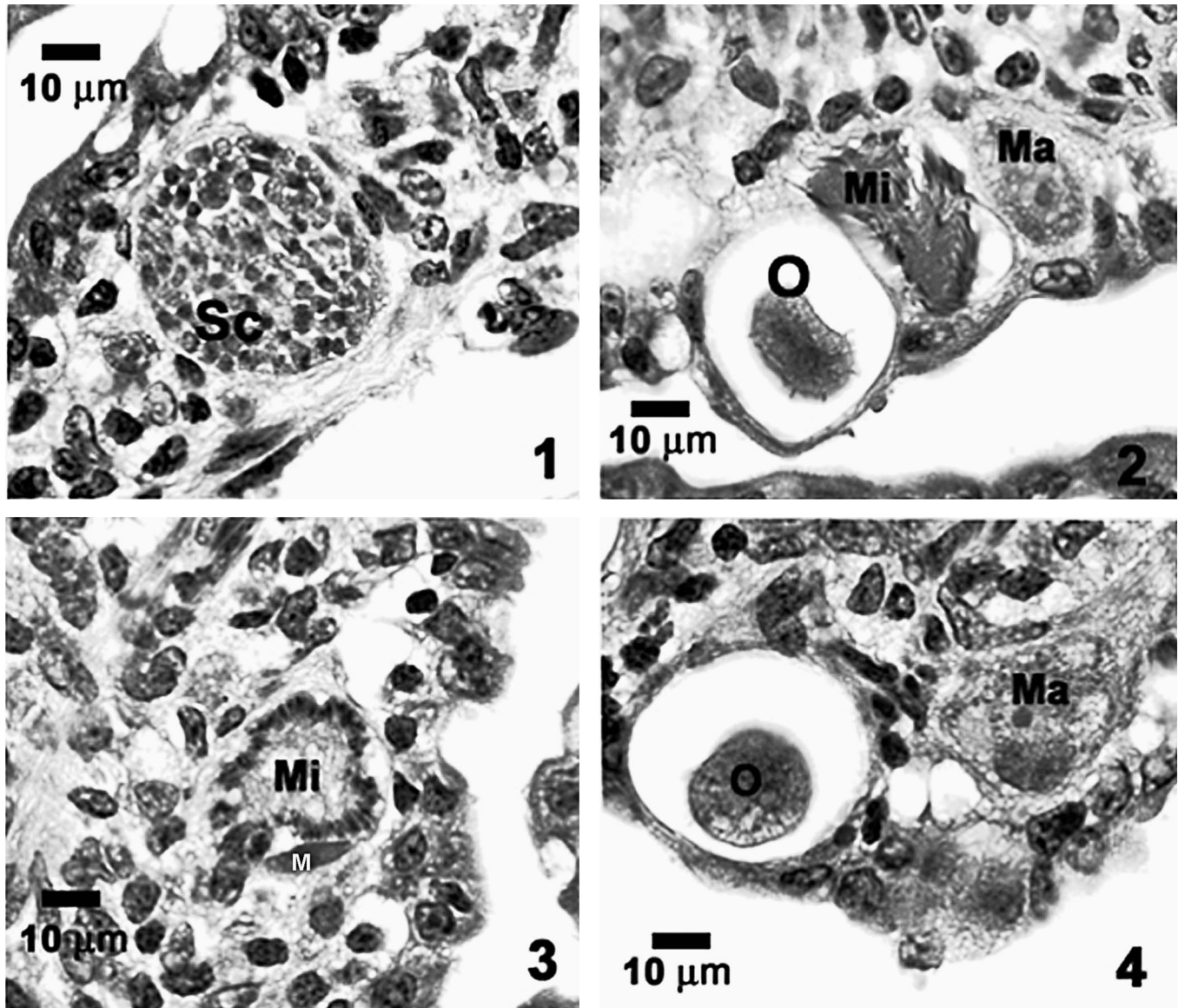
Material deposited: A phototype (see Bandoni and Duszynski, 1988) of sporulated oocysts is deposited in the U.S. National Parasite Collection (USNPC), Beltsville, Maryland. USNPC no. 097291.00.

Remarks

Amorphous inclusions were present between the sporont and oocyst wall of many unsporulated *C. canis* oocysts. These inclusions have been observed in unsporulated *Cystoisospora suis* oocysts from pigs (Biester and Murray, 1934; Lindsay et al., 1980, 1982) and unsporulated *Cystoisospora rivolta* oocysts from cats (Dubey, 1979). This material is not present in fully sporulated oocysts of these *Cystoisospora* species.

DISCUSSION

Intestinal coccidial infections in naturally infected dogs have been examined in many countries (Dubey, Weisbrode et al., 1978; Boch et al., 1981; Correa et al., 1983; Kirkpatrick and Dubey, 1987; Penzhorn et al., 1992; Dauschies et al., 2000; Junker and Houwers, 2000). It is difficult to attribute intestinal disease to coccidia unless other pathogens are ruled out in a thorough search for disease-causing agents (Lindsay et al., 1997). Most studies rely only on clinical signs and do not examine tissues for lesions or other pathogenic agents. Penzhorn et al. (1992) studied a commercial German Shepherd breeding kennel in South Africa and found *Cystoisospora* sp. oocysts in the feces of dogs with diarrhea, some of which were also hemorrhaging. These authors were not able to demonstrate canine pathogenic bacteria or viruses in the feces of these dogs (Penzhorn et al., 1992). They were not able to link oocyst excretion



FIGURES 1–4. Hematoxylin and eosin stained histological sections of the ileum of dog AIZ infected with 1×10^5 *Cystoisospora canis* oocysts 10 days previously and demonstrating developmental stages in the intestinal lamina propria. (1) A mature schizont (Sc) containing numerous merozoites is located in a host cell in the lamina propria. (2) Several sexual stages including an oocyst (O), a mature microgamont (Mi) with microgametes, and a macrogamont (Ma) are present in this section. (3) An immature microgamont (Mi) that appears to be in the same host cell as a merozoite (M). The infected cell is in the lamina propria. (4) An oocyst with a contracted sporont (O) and a macrogamont (Ma) in the lamina propria.

by bitches to coccidial infections in their puppies. Dauschies et al. (2000) reported that natural *Cystoisospora* sp. infections were regularly found in 3- to 4-wk-old pups in dog-breeding facilities and that they were not always associated with diarrhea.

Experimental studies on the pathogenicity of canine coccidia are few, and they often conflict each other. Dubey (1978b) found that 5×10^5 *C. ohioensis* oocysts (administered as 1×10^6 sporocysts in the original paper) caused diarrhea in experimentally infected 7-day-old pups but not weaned pups or young dogs. Microscopic changes associated with *C. ohioensis* infection included villous atrophy, necrosis of apical enterocytes, and cryptitis (Dubey, 1978b). Dauschies et al. (2000) reported puppies (age not given) experimentally infected with 4

$\times 10^4$ oocysts of the *C. ohioensis* group developed catarrhal-to-hemorrhagic diarrhea. Little is known about the pathogenicity of *C. neorivolta* (Mahrt, 1967; Dubey and Mahrt, 1978) or *C. burrowsi* (Trayser and Todd, 1978; Rommel and Zielasko, 1981).

Levine and Ivens (1981) suggested that strain differences in pathogenicity of *C. canis* could be present in dogs. Nemeséri (1960) found that 5×10^3 oocysts of a Hungarian isolate of *C. canis* were not pathogenic for dogs, but an inoculum of 5 or 8×10^4 oocysts produced clinical coccidiosis. In contrast, severe clinical disease was not produced in 25, 6-wk-old or 6, 8-wk-old pups inoculated with 1 – 1.5×10^5 *C. canis* oocysts (Lepp and Todd, 1974) isolated in dogs from Illinois. The pathoge-



FIGURE 5. Section of ileum from dog AIZ infected with 1×10^5 *Cystoisospora canis* oocysts 10 days previously. Note mild villous atrophy, dilated lacteals, and marked lymphoid hyperplasia of the Peyer's patches.

nicity of *C. canis* oocysts in the present study are more similar to what was reported by Nemeséri (1960), rather than what was reported by Lepp and Todd (1974).

The present study demonstrated that *C. canis* is a primary pathogen in young dogs. Our histological studies demonstrated lesions (Fig. 5) in the small intestine, which were associated with the presence of developmental stages (Figs. 1–4) of *C. canis* and clinical signs of diarrhea. Bleach treatment of the inoculum rendered it free of bacteria, indicating that bacteria were not responsible for causing the clinical signs. Our attempts to demonstrate pathogenic bacteria and viruses in the 2 experimentally infected dogs examined at necropsy were negative, indicating that the coccidia were responsible for the clinical signs and microscopic lesions in these animals.

Solid immunity follows a primary *C. canis* infection, and no oocysts are discharged after challenge (Becker et al., 1981). We used young (6- to 8-wk-old) dogs in hopes of obtaining them before they developed a natural *C. canis* infection. Fortunately, none of our dogs came infected with *C. canis* because preinoculation fecal examinations for *C. canis* were negative and the timing of the prepatent period was consistent with the literature (Nemeséri, 1960; Lepp and Todd, 1974; Levine and Ivens, 1981). Prior infection is always a problem when working with coccidia in animals. Some of our dogs harbored *C. ohioensis*-

like oocysts in their feces before infection (Tables I–III). However, this *C. ohioensis*-like infection did not prevent these dogs from being infected with *C. canis* nor did it preclude them from developing clinical signs. Neither of the 2 dogs used for microscopic lesion studies had prior infection with *C. ohioensis*-like coccidia.

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