

## **Experimentally Induced Clinical *Cystoisospora canis* Coccidiosis in Dogs with Prior Natural Patent *Cystoisospora ohioensis*-like or *C. canis* Infections**

Author(s): Alice E. Houk , Thomas O'Connor , Hilda F. J. Pena , Solange Maria Gennari , Anne M. Zajac , and David S. Lindsay

Source: Journal of Parasitology, 99(5):892-895. 2013.

Published By: American Society of Parasitologists

DOI: <http://dx.doi.org/10.1645/13-197.1>

URL: <http://www.bioone.org/doi/full/10.1645/13-197.1>

---

BioOne ([www.bioone.org](http://www.bioone.org)) is a nonprofit, online aggregation of core research in the biological, ecological, and environmental sciences. BioOne provides a sustainable online platform for over 170 journals and books published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Web site, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at [www.bioone.org/page/terms\\_of\\_use](http://www.bioone.org/page/terms_of_use).

Usage of BioOne content is strictly limited to personal, educational, and non-commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

## RESEARCH NOTES

*J. Parasitol.*, 99(5), 2013, pp. 892-895  
© American Society of Parasitologists 2013

### Experimentally Induced Clinical *Cystoisospora canis* Coccidiosis in Dogs with Prior Natural Patent *Cystoisospora ohioensis*-like or *C. canis* Infections

Alice E. Houk, Thomas O'Connor\*, Hilda F. J. Pena†, Solange Maria Gennari†, Anne M. Zajac, and David S. Lindsay, Center for Molecular Medicine and Infectious Diseases, Department of Biomedical Sciences and Pathobiology, Virginia-Maryland College of Veterinary Medicine, Virginia Tech, 1410 Prices Fork Road, Blacksburg, Virginia 24061-0342; \*IDEXX Laboratories, Inc., 1 Idexx Drive, Westbrook, Maine 04092; †Departamento de Medicina Veterinária Preventiva e Saúde Animal, Faculdade de Veterinária e Zootecnia (FMVZ), Universidade de São Paulo, SP, Brazil. Correspondence should be sent to: [lindsayd@vt.edu](mailto:lindsayd@vt.edu)

**ABSTRACT:** Diarrhea caused by intestinal coccidia (*Cystoisospora* species) is a common problem in pet dogs and in dogs in animal shelters. *Cystoisospora canis* has the largest oocysts of the 4 named species of coccidia infecting dogs. The present study examined an isolate of *C. canis* obtained from a dog from São Paulo, SP, Brazil. Oocysts sporulated within 2 days at room temperature, and 20 sporulated oocysts were measured at 37.6 by 28.6  $\mu\text{m}$  (range 35–42 by 26–31  $\mu\text{m}$ ). Most sporulated oocysts contained 2 sporocysts, each with 4 sporozoites, although a few (<1%) were *Caryospora*-like and contained 1 sporocyst with 8 sporozoites. Two experiments using a total of 11 female 6-wk-old beagles were conducted to determine the pathogenicity of oral infection with  $5 \times 10^4$  sporulated oocysts of this isolate of *C. canis*. Five of the 11 dogs had natural infections with *Cystoisospora ohioensis*-like ( $n = 4$ ) or *C. canis* ( $n = 1$ ) species prior to the predicted patent period of 9–10 days. Ten of the dogs developed diarrhea with occasional blood, and 3 dogs were affected to the extent that clinical treatment for coccidiosis using sulfadimethoxine was recommended. Dog CRU had a natural *C. canis* infection and did not develop clinical disease after oral infection with *C. canis* oocysts. This dog had a prepatent period of 9 days and a patent period of 3 days, corresponding to experimental infection with the new isolate of *C. canis*. It excreted fewer *C. canis* oocysts than did the other dogs. The 4 dogs with natural *C. ohioensis*-like infection all developed clinical disease, and 1 required treatment. The prepatent period was 9–10 days, and the patent period was 10–11 days in these dogs. All 6 dogs not naturally infected with *Cystoisospora* developed clinical disease, and 2 required treatment. The prepatent period was 9–10 days, and the patent period was 8–12 days. The present study confirms that *C. canis* is a primary pathogen for young dogs. It demonstrates that prior infection with *C. canis* but not *C. ohioensis*-like coccidia confers some resistance to clinical diseases and a decrease in oocyst production in dogs challenged with *C. canis*.

Coccidia of the genus *Cystoisospora* are common intestinal parasites of dogs, cats, and humans worldwide (Frenkel, 1977). Dogs are hosts for 4 named species of *Cystoisospora*, and oocysts of *C. canis* can be definitively identified based on their structure in fecal samples because of their large size (>33  $\mu\text{m}$ ) when compared to the oocysts of *Cystoisospora ohioensis*, *Cystoisospora neorivolta*, and *Cystoisospora burrowsi*, which are smaller and structurally similar (<30  $\mu\text{m}$ ) to each other (see 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 (see Lindsay et al., 1997). New methods using PCR and sequencing or RFLP are being developed and will help better define these *C. ohioensis*-like parasites from dogs in the future (Samarasinghe et al., 2008; Matsubayashia et al., 2011; He et al., 2012).

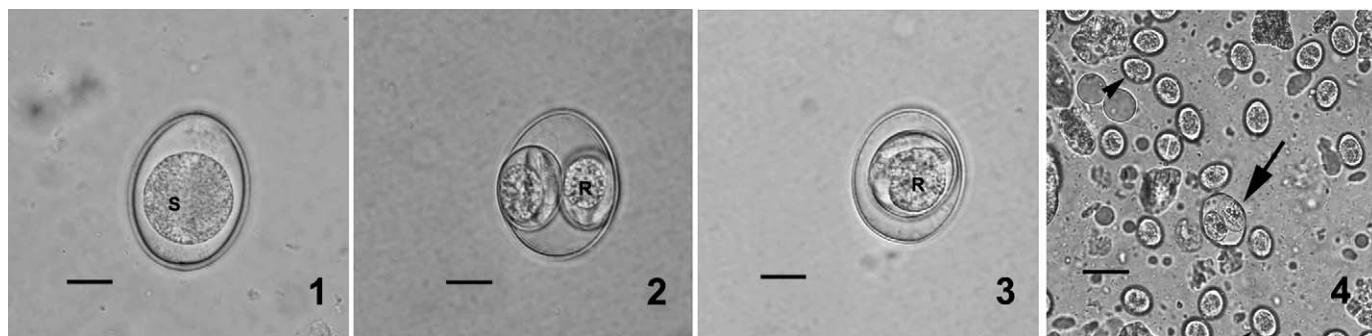
Little is known about immunity or resistance to intestinal coccidial infection caused by *Cystoisospora* species in dogs. This is because it is

difficult to obtain coccidia-free dogs from commercial suppliers and the logistics of keeping dogs coccidia free prior to experimental and challenge infections. In 1 study, solid immunity followed primary *C. canis* infection, and no *C. canis* oocysts were excreted after challenge (Becker et al., 1981). Eight of 22 dogs examined in a second study were excreting *C. ohioensis*-like oocysts in their feces prior to experimental infection with sporulated oocysts of *C. canis*. All 22 dogs developed clinical signs of coccidiosis and excreted *C. canis* oocysts in their feces (Mitchell et al., 2007).

We previously demonstrated that oocysts of *C. canis* isolated from pitbull puppies from Virginia are pathogenic for young beagle dogs (Mitchell et al., 2007) and that this isolate could be used to demonstrate drug efficacy in experimentally infected beagles (Reinemeyer et al., 2007). Unfortunately, this line of *C. canis* was lost due to a normal decline in infectivity with oocyst age. The present study describes the oocysts of a new isolate of *C. canis* obtained from a dog from Brazil and their pathogenicity for beagle puppies. We demonstrate that this isolate is pathogenic for beagle puppies and that preexisting *C. ohioensis*-like infection does not prevent clinical disease due to *C. canis*. We also are able to present results from a puppy naturally infected with *C. canis* that demonstrate some protection from homologous infection.

Oocysts larger than 33  $\mu\text{m}$  in length were detected in the feces of a 7-month-old female dog (Fig. 1) from the Departamento de Medicina Veterinária Preventiva e Saúde Animal, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, SP, Brazil, by 1 of us (H.F.J.P.) in April 2011. Some blood was present, but the feces were not diarrheic. Feces from this dog were collected and sent to the Department of Biomedical Sciences and Pathobiology, Center for Molecular Medicine and Infectious Diseases, Virginia-Maryland College of Veterinary Medicine (VAMDRCM), Virginia Tech, Blacksburg, Virginia, for propagation and further study of canine coccidiosis. Oocysts were sporulated in 2% (v/v) sulfuric acid solution at room temperature for 4 days (Figs. 2, 3). Twenty oocysts were examined and measured using a calibrated ocular micrometer and an Olympus BX60 epifluorescent microscope equipped with differential contrast optics (Olympus America Inc., Center Valley, Pennsylvania). Concentrated oocysts were stored in 2% sulfuric acid solution at 4 C. Oocysts were sterilized using exposure to 100% commercial bleach solution for 10 min. The oocysts were washed free of bleach using sterile Hanks balanced salt solution without calcium or magnesium (HBSS). The numbers of oocysts present were determined by counting in a hemocytometer. Oocysts were 3 mo old when used for experiment 1. Oocysts collected from dogs in experiment 1 were used to infect dogs in experiment 2, and the sporulated oocysts were 3 mo old.

Two experiments using a total of 11 female beagles were conducted (Table 1). Dogs were obtained at 6 wk of age (Covance, Cumberland, Virginia). The protocols used in the present report were approved by the Institutional Animal Care and Use Committee, Virginia Tech. All dogs were vaccinated and neutered and then adopted by members of the community following guidelines of Virginia Tech at the end of the study. Fecal samples were examined using centrifugal flotation in Sheathers' sugar solution and the McMaster egg counting technique. Fecal samples were examined daily or every other day until dogs were orally infected (if feces were available). Fecal oocyst numbers were determined by the



FIGURES 1–4. Photomicrographs of fecal flotations from dogs experimentally infected with *Cystoisospora canis* obtained from a dog from Brazil. (1) Unsporulated oocyst of *C. canis* demonstrating contracted sporont (S). Bar = 10 µm. (2) Sporulated *C. canis* oocyst containing 2 sporocysts each with 4 sporozoites. Note the sporocyst residum (R). Bar = 10 µm. (3) *Caryospora*-type sporulated oocyst of *C. canis* containing 8 sporozoites and a single sporocyst. A sporocyst residum (R) is present. Bar = 10 µm. (4) Sample from dog CXR fed sporulated *C. canis* oocysts demonstrating the inoculated sporulated *C. canis* oocyst (arrow) from the inoculum and many smaller nonsporulated *C. ohioensis*-like oocysts (arrowheads) from a natural infection. Bar = 20 µm.

McMaster method using 2 g of feces and mixing this sample in 28 ml of Sheathers' sugar solution. Both sides of a McMaster counting slide were loaded with the mixture. McMaster slides were allowed to sit for 5 min, and then all oocysts present were counted. The total number of oocysts counted was determined by multiplying the number counted by 50.

An inoculum dose of  $5 \times 10^4$  sporulated *C. canis* oocysts in 0.25 ml of HBSS was used. Dogs were orally infected by mixing the *C. canis* oocysts and HBSS solution in an appropriate amount (25 g) of commercial dog food (Hills Science Diet A/D, Topeka, Kansas) and placing the food in a bowl and allowing the puppies to consume the dog food. All dogs readily ate this mixture within 3–5 min, and none vomited the inoculum.

All puppies were examined daily, their health condition noted, and fecal samples were collected. Toys were provided to each pup, and they were housed so they could see, hear, and smell each other. Pups were allowed to exercise outside of their cage on the floor during the 15–20 min it took to clean their cage and collect fecal samples. An attempt was made to collect large amounts of feces during the patent period to provide a source of oocysts for future studies.

Dogs that developed severe diarrhea (watery and/or bloody), inappetence >24 hr, discomfort, or dehydration were treated medically by the clinical veterinarian for coccidiosis with sulfadimethoxine (Albon 5% suspension; Pfizer Inc., New York, New York) dosage at 55 mg/kg orally

on the first day, and then 27.5 mg/kg orally once daily for 3–5 days, and given supportive care (subcutaneous fluid boluses).

Oocysts survived shipping from Brazil and sporulated when placed in 2% sulfuric acid solution. Twenty oocysts were measured at  $37.6$  by  $28.6$  µm (range 35–42 by 26–31 µm) (Figs. 1–4). Oocysts in fresh samples contained a contracted sporont, and many had developed to the 2-sporoblast stage. Oocysts sporulated within 2 days at room temperature and contained 2 sporocysts each with 4 sporozoites (Fig. 2). *Caryospora*-like oocysts containing a single sporocyst enclosing 8 sporozoites (Fig. 3) were frequently present in samples sporulated for experimental studies. Autofluorescence of the oocyst and sporocysts was observed when samples were examined using UV light (Lindquist et al., 2003). In experiment 2, fully sporulated *C. canis* oocysts present from the infecting dose of bleach-treated *C. canis* oocysts were seen in the feces of a dog (CXR). This was 2 day postinoculation (PI), and dog CXR was excreting unsporulated *C. ohioensis*-like oocysts from a preexisting natural infection (Fig. 4).

Diarrhea was not present in the 5 pups in experiment 1 when they were examined the day of arrival at VAMDRCM. Three of 5 dogs in this group excreted *C. ohioensis*-like oocysts prior to infection with *C. canis* oocysts (Table I; Fig. 5). The prepatent period was 9 days, and the patent period was 8–10 days in these dogs. One dog (VG) was treated for intestinal coccidiosis with sulfadimethoxine and fluids beginning day 12 postinfect-

TABLE I. History of *Cystoisospora* oocyst excretion, results of clinical signs, and prepatent and patent periods following oral infection of dogs with 50,000 sporulated *C. canis* oocysts.

Experiment	Dog	Prior oocysts present	Clinical signs	<i>Cystoisospora canis</i>	
				Prepatent period (days)	Patent period (days)
1	VL	No	Yes	9	8
1	VI	No	Yes	9	9
1	VG	<i>C. ohioensis</i>	Yes*	9	10
1	VH	<i>C. ohioensis</i>	Yes	9	10
1	VJ	<i>C. ohioensis</i>	Yes	9	10
2	CTZ	No	Yes	10	10
2	CPX	No	Yes*	9	12
2	CUA	No	Yes*	8	12
2	CRW	No	Yes	10	10
2	CXR	<i>C. ohioensis</i>	Yes	10	11
2	CRU	<i>C. canis</i>	No	9	3

\* Treated with sulfadimethoxine for coccidiosis.

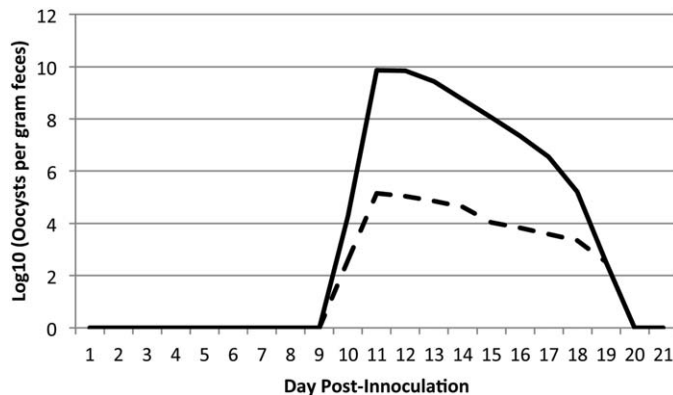


FIGURE 5. Results of *C. canis* oocyst counts from dogs in experiment 1. Dogs VL and VI are represented with solid line (no prior infection), and dogs VG, VH, and VJ are represented with the dashed line (prior *C. ohioensis*-like infection).

tion. Clinical signs of diarrhea were first observed 9 days PI, and fecal samples became formed again on day 14 PI.

Diarrhea was not present in the 6 pups in experiment 2 when they were examined the day of arrival at VAMDRCM. One of 6 dogs (CXR) in experiment 2 excreted *C. ohioensis*-like oocysts prior to infection with *C. canis* oocysts (Table I; Fig. 6). The prepatent period for *C. canis* was 10 days, and the patent period was 11 days in CXR. One of 6 dogs (CRU) excreted oocysts of *C. canis* oocysts prior to oral inoculation with *C. canis* oocysts (Table I). Two dogs (CUA and CPX) were treated for intestinal coccidiosis with sulfadimethoxine day 9 PI.

*Cystoisospora* species oocysts are most prevalent in dogs under 1 yr of age (Beuhl et al., 2006; Fontanarrosa et al., 2006; Little et al., 2009; Barutzki and Schaper, 2011). A survey of 1,199,293 canine fecal samples from the United States in 2006 demonstrated that 4.4% of the samples contained oocysts of *Cystoisospora* species (Little et al., 2009). This value was similar to the 2.6% to 4.8% prevalence previously found in surveys ( $n = 7$ ) from dogs from the United States (reviewed by Little et al., 2009). Beuhl et al. (2006) found that 8.7% of 3,590 diagnostic samples from Austrian dogs less than or equal to 2 yr old contained *Cystoisospora* oocysts. They found that 78% of the positive dogs were 4 mo old or less. Barutzki and Schaper (2011) found *Cystoisospora* oocysts in 5.6% of 24,677 dogs from Germany, and Fontanarrosa et al. (2006) found that 12% of 1,293 samples from dogs contained *Cystoisospora* oocysts.

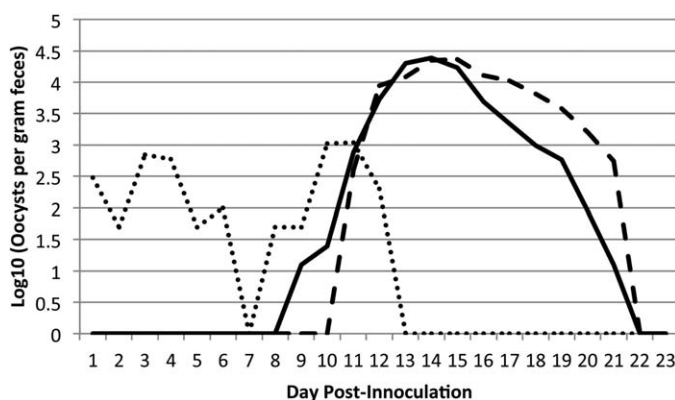


Figure 6. Results of *Cystoisospora canis* oocyst counts from dogs in Experiment 2. Dogs CPX, CUA, and CRW are represented with the solid line group (no prior infection); dog CXR is the dashed line (prior *Cystoisospora ohioensis*-like infection), and dog CRU is the dotted line (prior *C. canis* infection).

Diagnosis of the cause of diarrhea is often a challenge to veterinarians attending animal shelters because dogs enter the shelters with a variety of enteropathogens, many of which are pathogenic or zoonotic under defined circumstances (Tupler et al., 2012). The large size of *C. canis* oocysts makes them easily identified in fecal preparations from dogs with diarrhea. However, care should be taken to demonstrate other pathogenic microorganisms, physiological conditions, environmental conditions, age, and immune status of dogs prior to making a definitive diagnosis of cystoisosporiasis (Lindsay et al., 1997). It is interesting to note that Dauschies et al. (2000) reported that natural *Cystoisospora* infections were regularly found in 3–4-wk-old pups in dog breeding facilities in Austria and that they were not always associated with diarrhea. The age of dogs used in our study was 6 wk old, and this would be at the end of or near the end of a patent *C. ohioensis*-like coccidial infection that was acquired at 4 wk old. No surveys of dogs in breeding facilities have been reported from the United States, but the finding that 6-wk-old pups came naturally infected indicates that dogs in breeding colonies in the United States most likely acquire primary infections at a young age. Severe diarrhea was not present in any of the puppies in our study naturally infected with *C. ohioensis*-like oocysts.

*Caryospora*-like oocysts have been observed in many studies on *Cystoisospora* species in mammals (Lindsay et al., 1997). Little is known about factors that induce the development of *Caryospora*-like oocysts of what would normally be *Cystoisospora* species oocysts. Matsui et al. (1993) demonstrated that heat treatment of unsporulated *Cystoisospora rivolta* oocysts from cats at 50 °C for 5 min caused an increase in the numbers of *Caryospora*-like oocysts that were produced after sporulation. Oocysts collected from cats inoculated with *Caryospora*-like oocysts of *C. rivolta* were *Cystoisospora*-like after sporulation, indicating that heat treatment did not induce a stable mutation. The biological importance of these *Caryospora*-like oocysts is unknown. The heat stress treatment method of Matsui et al. (1993) may prove to be a useful method to examine gene expression associated with sporogony of *C. canis* oocysts.

Oral inoculation of  $5 \times 10^4$  oocysts of our isolate of *C. canis* used in the present study from Brazil caused similar clinical signs and prepatent and patent periods in 6-wk-old recipients, as did inoculation of  $5 \times 10^4$  oocysts of an isolate of *C. canis* obtained from dogs from Virginia (Mitchell et al., 2007; Reinemeyer et al., 2007) in 6-wk-old pups. An isolate of *C. canis* from Illinois was not pathogenic for 6-wk-old pups inoculated with  $1 \times 10^5$  sporulated oocysts (Lepp and Todd, 1974). Beuhl et al. (2006) reported that  $2 \times 10^4$  oocysts of *C. canis* obtained from dogs in Austria were pathogenic for 3-wk-old beagle puppies. Results of the present study support those of Beuhl et al. (2006), Mitchell et al. (2007), and Reinemeyer et al. (2007) and confirm that *C. canis* is a primary pathogen in young dogs.

These studies were supported by grants from IDEXX to DSL and AMZ.

## LITERATURE CITED

- BARUTZKI, D., AND R. SCHAPER. 2011. Results of parasitological examinations of faecal samples from cats and dogs in Germany between 2003 and 2010. *Parasitology Research* **109**: S45–S60.
- BECKER, C., J. HEINE, AND J. BOCH. 1981. Experimentelle *Cystoisospora canis*- und *C. ohioensis*-Infektionen beim Hund. *Tierärztliche Umschau* **36**: 336–341.
- BUEHL, I. E., H. PROSL, H.-C. MUNDT, A. G. TICHY, AND A. JOACHIM. 2006. Canine isosporosis—Epidemiology of field and experimental infections. *Journal of Veterinary Medicine B* **53**: 482–487.
- DAUSCHIES, A., H. C. MUNDT, AND V. LETKOVA. 2000. Toltrazuril treatment of cystoisosporosis in dogs under experimental and field conditions. *Parasitology Research* **86**: 797–799.
- FONTANARROSA, M. F., D. VEZZANI, J. BASABE, AND D. F. EIRAS. 2006. An epidemiological study of gastrointestinal parasites of dogs from southern Greater Buenos Aires (Argentina): Age, gender, breed, mixed infections, and seasonal and spatial patterns. *Veterinary Parasitology* **136**: 283–295.



- FRENKEL, J. K. 1977. *Besnoitia wallacei* of cats and rodents: With a reclassification of other cyst-forming isosporid coccidia. *Journal of Parasitology* **63**: 611–628.
- HE, P., J. LI, P. GONG, J. HUANG, AND X. ZHANG. 2012. *Cystoisospora* spp. from dogs in China and phylogenetic analysis of its 18S and ITS1 gene. *Veterinary Parasitology* **190**: 254–258.
- LEPP, D. L., AND K. S. TODD JR. 1974. Life cycle of *Isospora canis* Nemeseri, 1959 in the dog. *Journal of Protozoology* **21**: 199–206.
- , AND ———. 1976. Sporogony of the oocysts of *Isospora canis*. *Transactions of the American Microscopical Society* **95**: 98–103.
- LINDQUIST, H. D., J. W. BENNETT, J. D. HESTER, M. W. WARE, J. P. DUBEY, AND W. V. EVERSON. 2003. Autofluorescence of *Toxoplasma gondii* and related coccidian oocysts. *Journal of Parasitology* **89**: 865–867.
- LINDSAY, D. S., J. P. DUBEY, AND B. L. BLAGBURN. 1997. Biology of *Isospora* spp. from humans, nonhuman primates, and domestic animals. *Clinical Microbiology Reviews* **10**: 19–34.
- LITTLE, S. E., E. M. JOHNSON, D. LEWIS, R. P. JAKLITSCH, M. E. PAYTON, B. L. BLAGBURN, D. D. BOWMAN, S. MOROFF, T. TAMS, L. RICH, AND D. AUCOIN. 2009. Prevalence of intestinal parasites in pet dogs in the United States. *Veterinary Parasitology* **166**: 144–152.
- MATSUBAYASHIA, M., R. A. CARRENO, H. TANI, R. YOSHIUCHI, T. KANAI, I. KIMATA, S. UNI, M. FURUYAD, AND K. SASAID. 2011. Phylogenetic identification of *Cystoisospora* spp. from dogs, cats, and raccoon dogs in Japan. *Veterinary Parasitology* **176**: 270–274.
- MATSUI, T., S. ITO, T. FUJINIO, AND T. MORII. 1993. Infectivity and sporogony of *Caryospora*-type oocysts of *Isospora rivolta* obtained by heating. *Parasitology Research* **79**: 599–602.
- MITCHELL, S. M., A. M. ZAJAC, S. CHARLES, R. B. DUNCAN, AND D. S. LINDSAY. 2007. *Cystoisospora canis* Nemeséri, 1959 (syn. *Isospora canis*) infections in dogs: Clinical signs, pathogenesis and reproducible clinical disease in beagle dogs fed oocysts. *Journal of Parasitology* **93**: 345–352.
- REINEMEYER, C. R., D. S. LINDSAY, S. M. MITCHELL, H. C. MUNDT, S. D. CHARLES, R. G. ARTHUR, AND T. L. SETTJE. 2007. Development of experimental *Cystoisospora canis* infection models in beagle puppies and efficacy evaluation of 5% ponazuril (toltrazuril sulfone) oral suspension. *Parasitology Research* **101**: S129–S136.
- SAMARASINGHE, B., J. JOHNSON, AND U. RYAN. 2008. Phylogenetic analysis of *Cystoisospora* species at the rRNA ITS1 locus and development of a PCR-RFLP assay. *Experimental Parasitology* **118**: 592–595.
- TUPLER, T., J. K. LEVY, S. J. SABSHIN, S. J. TUCKER, E. C. GREINER, AND C. M. LEUTENEGGER. 2012. Enteropathogens identified in dogs entering a Florida animal shelter with normal feces or diarrhea. *Journal of the American Medical Association* **241**: 338–343.