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Immunoassay for detection of *Dipylidium caninum* coproantigen in dogs and cats

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Abstract. *Dipylidium caninum* infections in dogs and cats are underestimated because of a lack of proglottid observations and poor recovery of parasite elements by centrifugal flotation. We developed an immunoassay that employs a pair of monoclonal antibodies to capture *D. caninum*–specific coproantigen in fecal extracts from dogs and cats. Real-time PCR for *D. caninum* DNA in perianal swabs and observation of proglottids were used as reference methods. In 6 experimentally infected dogs, parasite DNA, coproantigen, and proglottid segments were first detected at 22, 23, and 26 d post-infection, respectively. Praziquantel treatment of 3 experimentally infected dogs resulted in the elimination of both coproantigen and proglottid shedding within 1–5 d post-treatment; however, parasite DNA persisted for 14 d. Immunohistochemistry on immature and mature tapeworm segments using an antibody against the coproantigen supports the premise that the antigen is produced in mature segments. We assessed the performance of our coproantigen test in natural infections in 78 dogs from a flea-endemic area. Of the 12 antigen-positive samples, 11 were confirmed with a positive PCR test and/or proglottid observation. Finally, we evaluated a convenience sample set of 730 canine and 163 feline fecal samples obtained from a commercial diagnostic laboratory; *D. caninum* antigen was detected in 4.1% of the canine and 12.9% of the feline samples, whereas parasite elements were observed in only 0.028% of samples. Our coproantigen immunoassay provides a sensitive method for the detection of *D. caninum* infection in dogs and cats.

Keywords: antigen; canine; cestode; feline; flea; tapeworm.

Dogs and cats are definitive hosts for the tapeworm *Dipylidium caninum*. Flea larvae (*Ctenocephalides felis*) ingest tapeworm eggs in the egg packets released by gravid proglottids. The hexacanth embryo remains in the flea throughout its metamorphosis into an adult flea. Cysticercoid elements are present 2–3 d after emergence of the adult flea and are infective if ingested by a cat or dog. The cysticercoid continues development in the small intestines of the host to a segment-shedding adult tapeworm in 2–3 wk.[4] Gravid segments are shed from the strobilus in feces and exit the host into the environment to continue the life cycle. Humans are accidental hosts if they ingest infected fleas. Tapeworm control is accomplished by treatment with praziquantel or epsiprantel and through proper flea prevention and/or control.

D. caninum is distributed worldwide as are its intermediate hosts. Globally, estimates of *D. caninum* prevalence in dogs and cats vary from < 1% to > 60%.[22] Infection rates are influenced by the environment, intensity of flea exposure, and grooming habits of the host. Animals not receiving routine veterinary care and regular flea control have been documented to have higher rates of tapeworm infection. In the United States, the prevalence reported in owned dogs is < 1–41.7%[12,20] and 2–49.5% in shelter and/or stray dogs.[1,24] Owned cats have a lower reported prevalence of 1.1%[21] compared to 1 to > 30% in shelter and/or stray cats.[13,17]

Limitations of existing detection techniques for *D. caninum* may contribute to the variation in observed prevalence. Shedding of proglottids is a macroscopic indication of tapeworm infection. Mobile proglottid segments may be seen on the fur at the perianal regions, on furnishings, or on fresh feces. However, segments are fragile and susceptible to desiccation, potentially preventing identification in fecal samples. Tapeworm speciation is not often performed on proglottids, although *D. caninum* proglottids resemble cucumber seeds and have bilateral genital pores. Traditional coprologic methods of detection include fecal smears, centrifugal flotation, and sedimentation. Centrifugal flotation assays, although generally preferred to sedimentation, tend to be insensitive given the density of tapeworm parasitic elements relative to the specific gravity of most flotation solutions, which impedes recovery. Molecular tests have been developed to detect *D. caninum*–specific DNA from fleas, feces, and perianal swabs.[15] Immunologic tests detect host antibodies to *D. caninum*, although the assays

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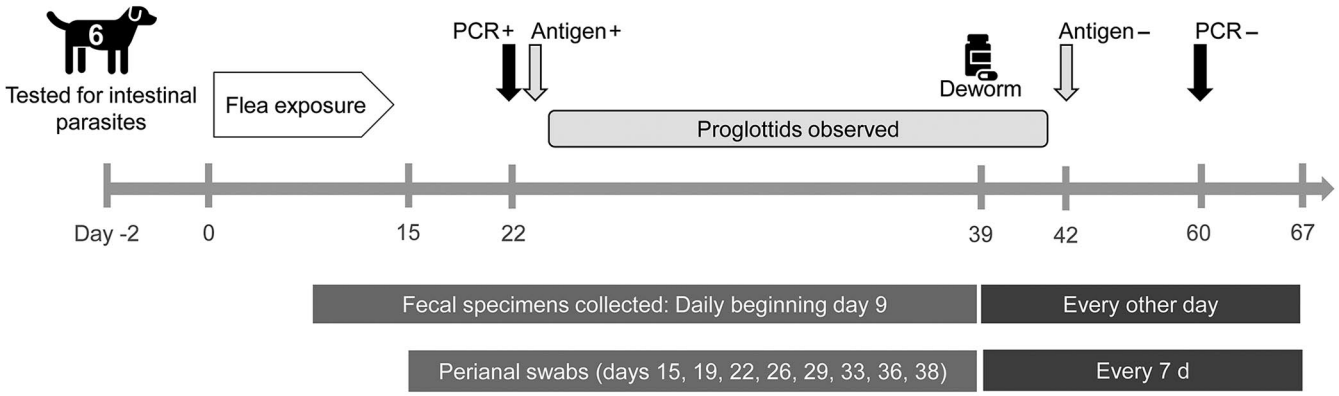

Figure 1. Schematic of *Dipylidium caninum* experimental infection protocol. Six dogs were infested with 3 batches of infected fleas by topical administration and gavage. Animals were dewormed at day 39. Proglottid shedding period is indicated by the gray box. First and last day of *D. caninum* detection by antigen (gray arrows) and PCR (black arrows) are indicated. Frequency of fecal specimen and perianal swab collection is indicated below the timeline.

cannot differentiate active infection from past tapeworm exposure.[23]

We describe here an immunoassay for the detection of *D. caninum* antigen in feces, similar to the antigen-detection tests developed for intestinal nematodes of cats and dogs,[7,8] as well as our validation using an experimental, canine infection model, naturally infected dogs with an elevated risk of *D. caninum* infection because of flea infestation, and a convenience sample set from dogs and cats obtained from a commercial diagnostic laboratory.

Material and methods

Canine and feline samples

The experimental infection animal care and use protocols were reviewed and approved by the vendor's Institutional Animal Care and Use Committee in accordance with the Guide for the Care and Use of Laboratory Animals (Ethics approval CG657-CV19/360; 2019 Oct 22). Six purpose-bred, Beagle dogs were dewormed with a short-acting anthelmintic (Milbemax; Elanco) at the recommended dosages (5 mg/kg praziquantel, 0.5 mg/kg milbemycin oxime), as described on the package insert, 20 d before experimental infestation with infected fleas. Two days prior to infestation, dogs were screened for helminth infections by fecal flotation. At the same time, the flea compatibility of each dog was assessed for 24 h.[10] *D. caninum*–infected fleas were generated.[2] The level of infectivity was evaluated by microscopically examining 100 fleas for *D. caninum* cysticercoids with a typical infection range of 25–50%. On study day 0, dogs were infested topically with ~250 *D. caninum*–infected fleas and allowed to groom naturally. On study day 4, fleas were collected with a comb and administered orally to the dogs. This cycle of topical infected flea infestation (study days 5 and 9) and oral administration (study days 9 and 13) was repeated 2 more times with new batches of fleas.[10] Fecal samples were collected daily for study days 9–39 and every other day for study days 40–67. Perianal swabs were collected twice a week from study days 15–38, and once a week for study days 40–67. From study days 14–37, *D. caninum* infection was assessed by observation of proglottids in voided fecal samples. On study day 39, 3 dogs were retreated with the anthelmintic, and the treatment was repeated on study day 53 (Fig. 1).

Voided fecal samples obtained from purpose-bred, Beagle dogs infected with *Ancylostoma caninum*, *Toxocara canis*, or *Trichuris vulpis* had been stored at −20°C.[7,8] Each nematode infection time course consisted of 5 dogs with fecal samples collected from day 0 to day 111 during which egg-positive and coproantigen-positive intervals were observed. A set of 100 voided fecal samples from a colony of specific pathogen–free (SPF) dogs (Marshall BioResources) was also obtained and stored at −20°C.

Working with 2 shelters in an area of Florida endemic for fleas, dogs >2-mo-old were identified for fecal sample collection if they had evidence of current or previous flea infestation or had proglottids found. Speciation of the tapeworm based on proglottid morphology was not performed. Perianal swabs and first-voided fecal samples were collected upon entry to the shelter and prior to routine deworming, when possible.[15] A cotton swab was rubbed around the perianal region, including the haircoat surrounding the anus, and along the external anal folds. The swab was inserted into a sterile tube and refrigerated. Ethical review and approval for sample collection in these shelters were provided by an internal review board (Idexx).

A convenience set of fecal samples submitted by practicing veterinarians to a commercial laboratory (Idexx) for examination by centrifugal flotation was obtained following the completion of all requested testing. According to terms of use, remnant samples become the property of the

commercial laboratory. If at least 2 g of fecal material remained, it was stored at −20°C.

Immunohistochemistry

D. caninum cestodes were obtained from a commercial source (Bioreed). Segments were dissected from a 40-cm tapeworm. Immature and mature segments were collected 3 cm and 20 cm distal to the scolex, respectively. Segments were embedded in cutting compound (OCT; Sakura Finetek) separately and sectioned at 5 µm on a cryostat. Sections were mounted on slides and fixed for 1 min with 90% ethanol–10% formalin. Slides were washed 4 times with PBS containing 0.05% Tween 20 (EMD-Millipore) for 5 min each time between incubation steps. Sections were rehydrated, and nonspecific binding of antibody was blocked with 2% goat serum (Jackson ImmunoResearch) and 1% bovine serum albumin (BSA; Sigma) in PBS, 0.05% Tween 20 for 30 min before being incubated with a mouse monoclonal antibody against *D. caninum* (IgG1) diluted to a final concentration of 3.2 µg/mL in PBS, 0.05% Tween 20 with 1% BSA overnight at 4°C. Slides were incubated with the secondary biotinylated goat anti-mouse antibody diluted 1:500 for 1 h followed by Avidin/Biotin Block (Vector) to block all endogenous biotin, biotin receptor, and avidin-binding sites present in the proglottids. Endogenous peroxidase was blocked by incubating the slides in 3% hydrogen peroxide in PBS for 15 min and then slides were incubated with a Vectastain Elite ABC HRP reagent (Vector) for 30 min as described by the kit manufacturer. Finally, slides were incubated in diaminobenzidine solution, which was used as a chromogen, for 5 min before microscopy.

Fecal extract preparation

Each fecal sample (~0.1 g) was added to 0.5 mL of sample buffer (Tris buffer, pH 7.2 [Sigma], supplemented with Tween 20, 5% mouse serum [Equitech], 10% rabbit serum [Pel-Freez], and 40% fetal bovine serum [Life Technologies]).[8] The sample was homogenized by stirring with a disposable stick for 5 s. The slurry was clarified by centrifugation at 1,300 × *g* for 5 min. Clarified fecal extract was tested immediately.

Dipylidium caninum immunoassay. We developed monoclonal antibodies (mAbs) against a homogenate preparation of whole *D. caninum* cestodes.[11] Antibodies were screened for their ability to bind the immunogen and produce a positive signal with fecal material from infected dogs and cats. The discrete antibody target is not known. An antigen capture immunoassay was developed using barcoded, magnetic bead (BMB) technology (Applied BioCode) with the antigen capture, negative control, and positive control BMBs each having a unique barcode.[9] Briefly, the BMB used for the *D. caninum* immunoassay was coated with the first mAb. The detection phase of the assay consisted of the second anti–*D. caninum* mAb covalently attached to biotin (Thermo Fisher) diluted in PBS containing 1% Tween 20 buffer, pH 7.4, 1% BSA, and 0.05% ProClin950 (Sigma-Aldrich). Assay signal fluorescence was achieved with an 8 µg/mL solution of streptavidin, R-phycoerythrin conjugate (Moss). The assay protocol was performed, and a median fluorescent intensity (MFI) was calculated for each BMB in the microwell.[9] The final assay result represents the background-corrected median MFI of all beads of the same barcode. The *D. caninum* assay cutoff value of 500 MFI was determined by taking 10× the SD of the grand $\bar{x}$ for the 100 SPF canine fecal samples.

Real-time PCR

Nucleic acid extraction of the canine perianal swabs and fecal samples was performed (High pure PCR template preparation kit; Roche) according to the manufacturer's instructions. Two modifications were applied to the fecal samples: the heating step was extended to 20 min, and the fecal suspension was clarified by centrifugation before transfer to the spin column.

A hydrolysis probe real-time PCR (rtPCR) was used for the molecular detection of *D. caninum* DNA. The primers were designed to the *D. caninum* 28S ribosomal RNA gene (GenBank MH040852). Primers DC28S-2F (5′-GATCCC GTTGTTAGGCA-3′) and DC28S-2R (5′-TCGATGACCA CACCATG-3′), at a final concentration of 0.83 µM, were paired with probe *D. caninum* dog (5′-FAM-GTGTGTGCA CAGTC-MGB-NFQ-3′)[15] at a final concentration of 0.16 µM. The primers and probe were added to the 1× master mix (LightCycler 480 probes master; Roche) in a 15-µL volume to which 5 µL of template was added. Cycling parameters for the LC480 II (Roche) were 95°C for 10 min, then 45 cycles of 95°C for 15 s and 60°C for 1 min.[15] Data analysis was performed with the LightCycler 480 software v.1.5.1.62 (Roche). Each PCR test run included a PCR-positive control consisting of a plasmid containing a single copy of the *D. caninum* target amplicon and a no-template water control.

A glyceraldehyde-3-phosphate dehydrogenase (GAPDH) rtPCR was used as a control for detection of canine DNA on perianal swabs. The primers and fluorescence resonance energy transfer (FRET) hybridization probes were designed to the *Canis lupus familiaris* GAPDH gene (GenBank AB038240). Primers GAPDH caFP (5′-GCCATCAAT GACCCCTTC-3′) and GAPDH caRP (5′-TCCACAA CATACTCAGCAC-3′) at final concentrations of 0.3 µM and 0.5 µM, respectively, were paired with FRET probes GAPDH caFL (5′-TCAAGGCTGAGAACGGGAAACTTGTCATC -FL-3′) and GAPDH caLC640 (5′-LC640-CGGGAAGTC CATCTCCATTCTTCCAGG-PH-3′) at a final concentration of 0.3 µM. The primers and FRET probes were added to 1× master mix (LightCycler 480 genotyping master; Roche) in a 15-µL volume to which 5 µL of template was added. Cycling parameters for the LC480 II (Roche) were 95°C for 10 min

Table 1. Coproantigen detection, proglottid observations, and rtPCR results for 6 experimentally infected dogs through 39 d post-infection. Fecal samples were collected daily, and perianal swabs were collected at 15, 19, 22, 26, 29, 33, 36, and 38 dpi.

Dog ID	Coproantigen positive, dpi	Proglottid positive, dpi	rtPCR positive, dpi
1	24–39	26–36	26, 29, 33, 36, 38
2	23–39	26–39	26, 29, 33, 36, 38
3	25–26, 31–36	26, 34–39	26, 29, 33, 36
4	27–42	27, 29–40, 44	22, 26, 29, 33, 36, 38
5	24–40	26–39	26, 29, 33, 36, 38
6	25, 28, 31–40	36–42	29, 36, 38

Dog 3 did not have a swab collected on day 38.

followed by 45 cycles of 95°C for 20 s, 60°C for 30 s, and 72°C for 20 s. Each PCR test run included a PCR-positive control of canine genomic DNA and a no-template water control.

The positive control plasmid and a subset of samples from dogs with either experimental ($n = 12$) or natural ($n = 3$) infections were selected for sequencing. In addition, 2 proglottid segments obtained from the feces of an experimentally infected dog were extracted, amplified, and sequenced. Previously published primers (DC28S-1F: 5′-GCATGCAAGT CAAAGGGTCCTACG-3′; DC28S-1R: 5′-CACATTCAA CGCCCGACTCCTGTAG-3′)[3] were added to a reaction mix consisting of 2.5 µL of 10× PCR buffer without $MgCl_2$ (Roche), 3 mmol of $MgCl_2$ (Roche), and 200 µmol of PCR nucleotide mix (Roche) in a 23-µL volume to which 2 µL of template was added for a final reaction volume of 25 µL. Cycling parameters were identical to those used for the GAPDH rtPCR. Amplicons were submitted to an academic center for purification and Sanger sequencing (University of Delaware DNA Sequencing and Genotyping Center, Newark, DE, USA). The amplicon sequences were aligned to GenBank MH040852 using SeqBuilder Pro v.17.2.1 (DNAstar).

Results

D. caninum proglottid segments and coproantigen were observed for all 6 experimentally infected dogs (Table 1). During the initial time course (0–39 d post-infection [dpi]), proglottid count and antigen signal varied within and between infected dogs. Proglottid shedding was observed at 26 dpi for 4 dogs, at 27 dpi for 1 dog, and at 36 dpi for 1 dog. Proglottid segments were observed continuously once shedding commenced for 4 dogs. Dogs 3 and 4 had intermittent shedding after initial proglottid observation. Dog 3 had a 7-d interval, and dog 4 had a single day and a 3-d interval in which shedding of proglottid segments was not observed. *D. caninum* antigen was observed as early as 23 dpi and as late as 27 dpi. Steady-state antigen detection was observed for 4 dogs. Two dogs, 3 and 6, displayed intermittent antigen detection with a 4- or 2-d interval in which antigen was below detectable levels.

Perianal swabs were collected twice weekly from all 6 experimentally infected dogs beginning at 15 dpi. One dog was PCR positive at day 22, another 4 became positive when samples were collected on day 26, and the final dog tested positive when a sample was tested on day 29 (Table 1). Apart from this last dog, all subsequent perianal swabs collected from the infected dogs tested positive through day 38. Positive and negative agreement between the PCR and coproantigen immunoassay results for 44 paired swab and fecal samples was 88% and 100%, respectively.

Three dogs were treated with praziquantel at 39 dpi (Fig. 2). Proglottid observations were noted for dog 4 until 44 dpi and for dog 6 until 42 dpi. Dog 5 did not shed proglottids past the treatment day (39 dpi). In 2 dogs, antigen was detected through 40 dpi, and for dog 4, through 42 dpi. Following praziquantel treatment at day 39, perianal swabs were collected weekly, and all 3 dogs remained PCR positive at the end of the first week; only 2 were PCR positive at the end of the second week. No parasite DNA was detected from any perianal swabs collected after the second praziquantel treatment on day 53.

Immunohistochemistry (IHC) of immature and mature *D. caninum* proglottid segments only revealed significant positive labeling of the tegument in mature segments (Fig. 3). Little-to-no significant positive antigen labeling was noted in immature segments.

No cross-reactivity was observed for any of the fecal samples from dogs experimentally infected with *A. caninum*, *T. canis*, and *T. vulpis* tested with our immunoassay, even when nematode-specific coproantigen and eggs were present (*A. caninum*, 23–87 dpi; *T. canis*, 38–87 dpi; *T. vulpis*, 69–89 dpi). Fecal samples from dogs and cats experimentally infected with other tapeworm species were not available.

We tested 78 dogs entering Florida shelters with evidence of fleas or having proglottids observed by both the *D. caninum* immunoassay and the *D. caninum* rtPCR from perianal swabs. Samples from 7 dogs were excluded because of insufficient sampling with the perianal swab as indicated by a GAPDH rtPCR Ct value of ≥35. Samples from 19 dogs had either coproantigen, *D. caninum*–specific DNA, and/or proglottids observed (Table 2). The remaining samples ($n = 52$) were negative for antigen and DNA and had no proglottids observed. *D. caninum* coproantigen was detected in 12 sam-

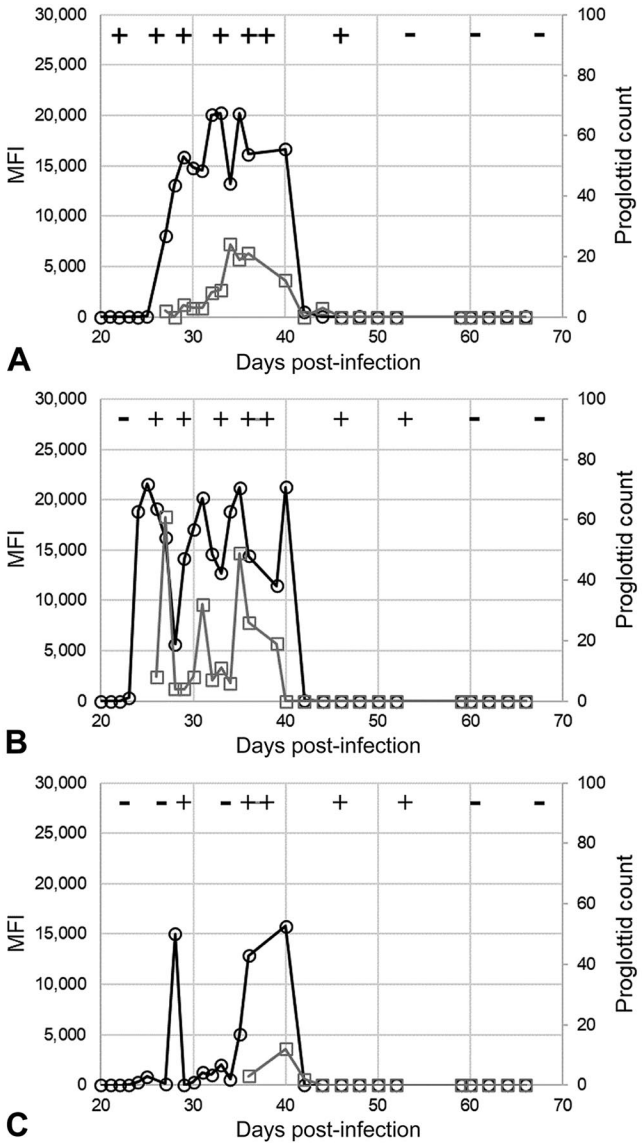

Figure 2. Time course of *Dipylidium caninum* experimental infection. Dogs were treated with praziquantel 39 d post-infection. Coproantigen assay median fluorescence intensity (MFI) results (black line and open circles), proglottid counts (gray line and open squares), and perianal swab rtPCR results (+ = positive; − = negative) are indicated for 3 dogs: (**A**) 4, (**B**) 5, (**C**) 6.

ples (16.9%) from this collection. Assay signal intensities were 541–9,474 MFI. Of these 12 antigen-positive samples, 11 also had a positive PCR result and/or proglottids observed. One coproantigen-positive sample had neither a positive PCR result nor proglottids observed. Three samples had negative antigen test results but were positive by PCR; proglottid segments were observed for 1 of these samples. Four samples had proglottids observed in the absence of antigen- or PCR-positive test results.

To verify the fidelity of the rtPCR assay, 17 amplicons from either proglottids or perianal swab samples, along with the plasmid control, were submitted for DNA sequencing. All sequences were 100% homologous to the *D. caninum* large-subunit ribosomal RNA gene (GenBank MH040852), including those obtained from the 3 dogs in the Florida shelter. To verify that the perianal swab provided a reliable sample for detection of *D. caninum* DNA, 28 nucleic acid extracts from paired fecal samples were also tested for molecular evidence of *D. caninum*. Of the 16 rtPCR-positive perianal swab samples obtained from the 6 experimentally infected dogs, 12 paired fecal samples were also positive by rtPCR. The average Ct in the rtPCR assay was lower when the perianal swab extract (30.7; range: 28.1–36.4) was used compared to fecal extracts (34.1; range: 30.3–37.9). Similar results were obtained from 12 shelter samples with positive perianal swabs; 7 fecal samples had molecular evidence of *D. caninum*, and the perianal swab extracts had a lower Ct compared to feces (swab 28.4–40.0 vs. fecal 35.3–38.9).

The *D. caninum* immunoassay was performed on a convenience set of 893 fecal samples that had been submitted by practicing veterinarians to a commercial laboratory (Idexx) for testing by centrifugal flotation. The 730 canine and 163 feline samples were submitted from CA, CO, CT, HI, MA, ME, NH, NV, NY, OR, RI, and TX. *D. caninum* eggs were observed in 2 canine samples. No proglottid observations were noted for any fecal samples. Of the 730 canine fecal samples, 31 samples tested positive in the *D. caninum* coproantigen immunoassay with MFI signals of 517–15,100. The 2 canine samples with *D. caninum* eggs observed were both positive in the antigen assay (852 and 3,800 MFI). Of the 163 feline samples, 21 were positive with the *D. caninum* coproantigen test; the assay signal was 622–14,300 MFI (Fig. 4). Overall, the *D. caninum* assay detected antigen in 52 of the 893 (5.8%) fecal samples tested. Antigen was detected at a higher rate in cats (12.9%) compared to dogs (4.1%).

Discussion

We found that our coproantigen immunoassay could detect mature *D. caninum* parasites in dogs and cats, and that this method is more sensitive than the commonly used techniques of proglottid observations and recovery of parasite elements by centrifugal flotation. In our experimental infections with *D. caninum*, coproantigen could be detected before proglottid shedding by 1–3 d for 4 dogs, and 10 d for 1 dog. Results of the *D. caninum* coproantigen test were 100% concordant with proglottid observation for all 6 dogs up to 39 dpi. The timing of antigen and proglottid detection is consistent with the IHC results in which the antigen does not appear to be produced in immature tapeworm segments but is present in mature segments being expelled from the host. Initiation of the segment-shedding process may release antigen, making it available in the feces for immunoassay detection prior to when proglottids appear in the feces. A similar finding has been reported for an *Echinococcus granulosus* coproantigen ELISA that was designed

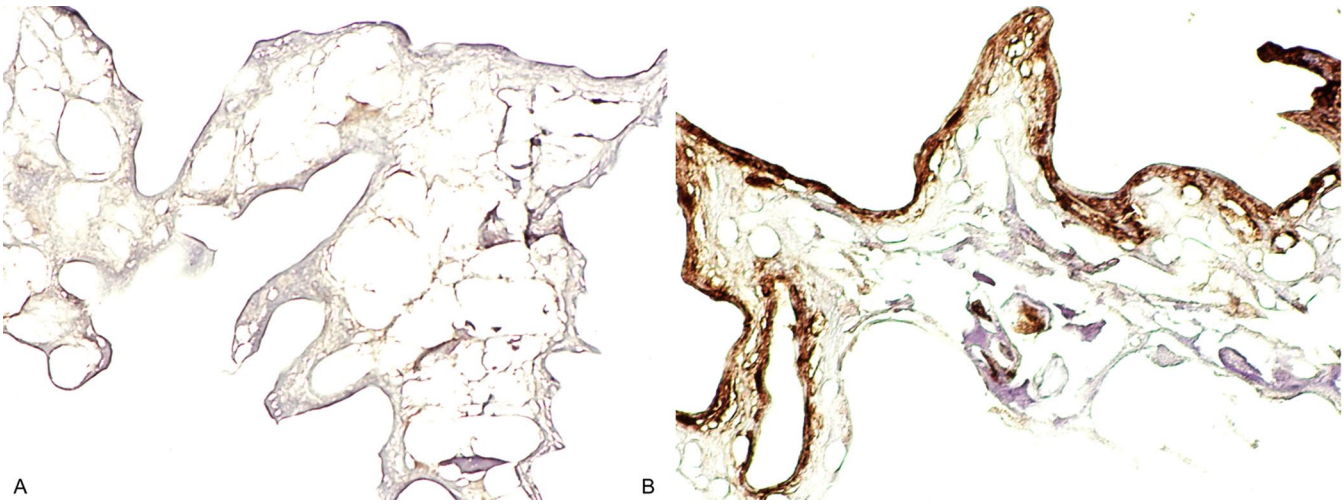

Figure 3. Immunohistochemistry of *Dipylidium caninum* proglottid segments. **A**. Little-to-no significant detection of the antigen in an immature proglottid segment. **B**. Positive antigen labeling in the tegument of a mature segment.

Table 2. Coproantigen, perianal swab rtPCR, and proglottid observations for 71 shelter dogs included in the analysis.

	rtPCR	
Coproantigen	Positive	Negative
Positive	10 (4)	2 (1)
Negative	3 (1)	56 (4)

The number of samples containing proglottids for each category is indicated in parentheses.

to detect a tegument-derived membrane antigen.[14] Additional IHC experiments are needed to explore the expression of the *D. caninum* target antigen in full-length parasites as well as to correlate the observed immunoreactivity with coproantigen immunoassay results using segment-specific parasite extracts.

Recognizing the limitations of the existing methods for the detection of *D. caninum* infections, we adopted a rtPCR for DNA from the 28S ribosomal RNA gene from the literature to help assess the performance of the coproantigen assay. Similar to the coproantigen assay, DNA was detected prior to proglottid shedding in 2 of 6 dogs, coincident with shedding in 3 dogs, and followed shedding in 1 dog. DNA detection by PCR occurred after the detection of coproantigen in 5 of 6 dogs. However, estimating the timing of PCR-positive results relative to either proglottid shedding or coproantigen detection was limited by the twice-weekly collection of perianal swabs compared to daily fecal sampling. When considering the results of the PCR and coproantigen testing before anthelminthic treatment, good overall agreement was observed between the presence of parasite DNA and coproantigen in paired samples, supporting the use of the PCR as a reference method in field populations, as described previously.[15] Continued detection of parasite DNA for up to 2 wk following treatment was an unexpected finding, but similar results have been obtained with fecal samples from dogs treated with lower doses of praziquantel and fenbendazole for asymptomatic *Heterobilharzia americana* infections.[5] In the case of *D. caninum* infection, it is possible that the DNA was from a scolex that may have remained attached to the host's intestines after treatment, an insufficient dose of cestocide to eliminate all parasites, or from environmental contamination.

Despite having only 6 dogs available for evaluating the coproantigen immunoassay relative to proglottid observations and perianal swab PCR, testing of serial samples over 39 d provided a valuable opportunity to assess the relative performance of the different methods. A substantial amount of variability occurred not only for proglottid shedding patterns but also for coproantigen levels as indicated by assay MFI values. Previous experimental infection studies have not demonstrated a strong correlation between worm burden and the onset of proglottid shedding.[10] Evaluating samples post-treatment informed us of a time course for the discontinuation of proglottid shedding relative to coproantigen and revealed the persistence of DNA on perianal swabs. More reliable detection of *D. caninum* DNA along with lower Cts occurred with perianal swabs compared to nucleic acid extracts from feces, possibly because there was more nucleic acid template in the swab extract or fewer PCR inhibitors than in fecal extracts.[18] The life cycle of tapeworms may also contribute to the sensitivity of the perianal swab method, which has been used successfully in the detection of *Taenia* spp. in foxes,[19] and may reflect the residual egg packets found in the perianal area historically reported with the sellotape method.[6]

Using these detection techniques with field samples revealed a similar pattern of *D. caninum* coproantigen detection relative to the rtPCR. Of 71 fecal samples collected upon intake from dogs at risk for *D. caninum* infection given the presence of fleas, the coproantigen and perianal swab rtPCR results had a positive and negative agreement of 77% and

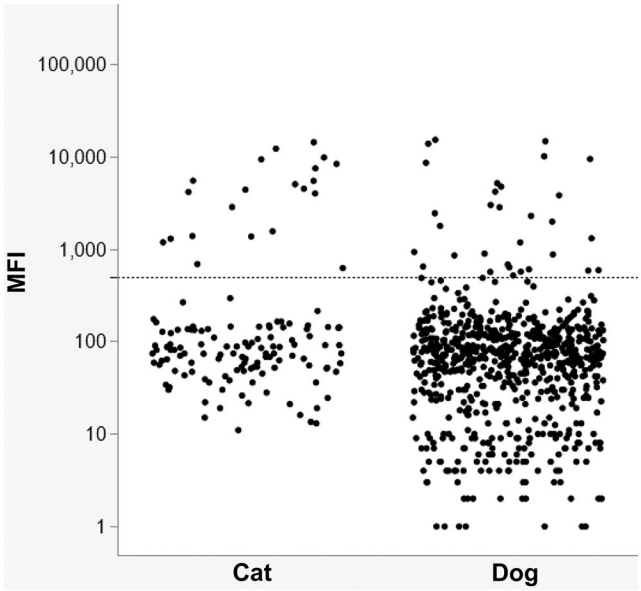

Figure 4. *Dipylidium caninum* immunoassay results for antigen-positive fecal samples from 730 naturally infected dogs and 163 cats. Dotted line is the immunoassay cutoff of 500 median fluorescence intensity (MFI).

97%, respectively, with a prevalence of 17% based on antigen detection. However, proglottids were observed in 4 dogs in the absence of positive antigen or PCR results. These cases may reflect false-negative results of the assays or identification of tapeworm segments from a different species (e.g., *Taenia* spp.) given that the proglottid segments were not characterized microscopically.

Using a convenience set of canine and feline fecal samples obtained from a commercial laboratory to represent a broader geographic distribution, *D. caninum* egg results were confirmed by centrifugal flotation in only 2 canine samples. No samples were reported to have proglottids present by gross examination and this may point to the fragility of these structures throughout the transit period from veterinarian to reference laboratory. Coproantigen was detected in 12.9% of the feline samples and in 4.1% of canine samples. This level of infection is greater than what could have been estimated from egg recovery or proglottid observations in this sample set. The higher proportion of antigen detection in feline fecal samples may reflect a greater susceptibility of cats to *D. caninum* infection given the fewer average doses of flea prevention purchased annually compared to dogs[16] and the fastidious grooming habits of cats. It is interesting to note that the assay detected the coproantigen in both canine and feline samples even though there are reports that different genotypes of *D. caninum* may infect these hosts.[3] One limitation of using this convenience set was that we were unable to confirm *D. caninum* infections in these canine and feline samples because matched perianal swabs were not available. Future studies may require the use of fecal samples for rtPCR despite the recognized lack of sensitivity compared to the perianal swab as described in our study. Additionally, further research is needed to demonstrate the performance of the coproantigen assay for the detection of *D. caninum* antigen in feline fecal samples and to understand how quickly coproantigen levels decrease following anthelmintic treatment in cats.

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Declaration of conflicting interests

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