A Comparative Study of Two Methods for the Identification of Gastrointestinal Parasites in Domestic Dogs and Cats in North America

BACKGROUND

Gastrointestinal (GI) parasitism is a common problem in domestic dogs and cats in North America.[1] Infections may result in significant clinical disease, especially in puppies and kittens. Additionally, dogs and cats may act as carriers for zoonotic infections, often without clinical signs to alert pet owners or veterinarians. Diagnosing and treating zoonotic parasites is essential and part of the One Health Initiative.[2,6]

To reduce the potential health risk to pets and humans, the Companion Animal Parasite Council (CAPC) recommends screening for fecal parasites at least four times during the first year of life and at least twice per year thereafter.[3]

Traditionally, microscopic centrifugal flotation (ova and parasite; O&P) examination has been used for parasite identification. Accuracy requires significant experience and proficiency. Despite this, some parasites and their ova are rare or difficult to identify — this poses challenges. Additionally, microscopic evidence may not be consistently present in the stool sample of infected patients. Some smaller parasites cannot be visualized by routine microscopy.

More recently, microplate enzyme-linked immunosorbent assays (ELISA) for parasite-specific antigen in feces (coproantigen assay) have been suggested to improve sensitivity and specificity.[4] Coproantigen ELISA assays have some limitations: low burden parasites may be below the detection limit, and the tests currently available are only limited to a few parasites.[5]

Molecular diagnostic methods using real-time PCR (qPCR) have been recommended to maximize sensitivity and specificity of parasite detection, provide information on zoonotic risk, and provide potential resistance to anthelmintic treatments.[8] As parasitic nucleic acid is stable in feces, the age and storage of the sample are less likely to influence the results of PCR testing.[7] Historically, the expense and long turnaround time for qPCR tests have previously limited their clinical utility.

However, recent proprietary advances in PCR technology (Antech's KeyScreen™ GI Parasite PCR) have provided the opportunity to use PCR testing for gastrointestinal parasites in routine wellness examinations, as well as during the investigation of clinical disease.

OBJECTIVES

Use canine and feline stool sample submissions from veterinary general practices across North America to determine:

- Fecal testing positivity for two methods of parasite identification: O&P and KeyScreen GI Parasite PCR
- The proportion of all *Giardia* isolates with zoonotic potential
- The number of hookworm (*Ancylostoma caninum*) isolates that are resistant to benzimidazoles (e.g., fenbendazole and febantel)
- The proportion of samples with co-infection (i.e. positive for two or more parasites in O&P compared to KeyScreen GI Parasite PCR)

METHODS

Fecal tests

O&P testing per the standard operating procedure (SOP) for Antech's network of veterinary reference laboratories, using zinc sulfate centrifugal flotation.

KeyScreen GI Parasite PCR testing per the SOP for Antech's network of veterinary reference laboratories, using a panel of qPCR tests for 20 parasites, a marker for benzimidazole resistance in *A. caninum*, and identification of *Giardia* strains with zoonotic potential. (See Table 1 and Figure 2 for details.)

Stool samples

One hundred and twenty-five veterinary hospitals distributed across North America were recruited for the study during two weeks in February 2022. The practices submitted all canine and feline samples for O&P testing with Antech per their usual routine. For all submissions, the sample was also tested using KeyScreen GI Parasite PCR. In total, 2293 samples were received and tested with both methods (1957 canine and 336 feline; no more than one sample per patient).

RESULTS

See Tables 1 and 2; and Figures 1 and 2.

Table 1
Number of samples positive for gastrointenstinal parasites by methodology [stool ova and parasite examination at Antech Diagnostics (O&P); parasite-specific qPCR assay (KeyScreen GI Parasite PCR)] and species. Samples from 125 general practices across North America.

Z! = zoonotic potential; NA = not distinguished by microscopy

	CANINE (1957 SAMPLES)		FELINE (336 SAMPLES)	
	O&P	KeyScreen GI Parasite PCR	O&P	KeyScreen GI Parasite PCR
Hookworms (includes *Ancylostoma* spp. Z!; *Uncinaria stenocephala*)	34	48	1	1
Ancyclostoma caninum with benzimidazole (e.g., fenbendazole) resistance	NA	3	NA	0
Roundworms (includes *Toxocara* spp.; *Toxocara canis* Z!; *Toxocara cati* Z!; *Toxascaris leonina*; *Baylisascaris procyonis* Z!)	24	26	10	13
Whipworms (*Trichuris vulpis*)	7	17	0	0
Tapeworms	0	12	0	10
Dipylidium caninum		12		10
Echinococcus granulosus Z!		0		0
Echinococcus multilocularis Z!		0		0
Taenia spp. Z!		0		0
Giardia				
Giardia duodenalis Z!	71	257	7	26
Giardia zoonotic strains [Subtypes A & B] Z!	NA	23	NA	3
Coccidia				
Cystoisospora spp. (formerly *Isospora* spp.)	16	19	2	5
Eimeria spp.	32	120	0	0
Other protozoa	NA	45	NA	8
Cryptosporidium canis		45		0
Cryptosporidium felis		0		6
Toxoplasma gondii Z!		0		1
Neospora caninum		0		0
Tritrichomonas blagburni		0		1

Table 2
The number (and percentage) of samples with a positive result.

	O&P	KeyScreen GI Parasite PCR
Infection (1 or more parasites)	151 (6.6%)	373 (16.3%)
Co-Infection (2 or more parasites)	23 (1.0%)	76 (3.3%)

Figure 1
The number of stool samples (dog and cat combined) that were positive for gastrointestinal parasites by methodology.

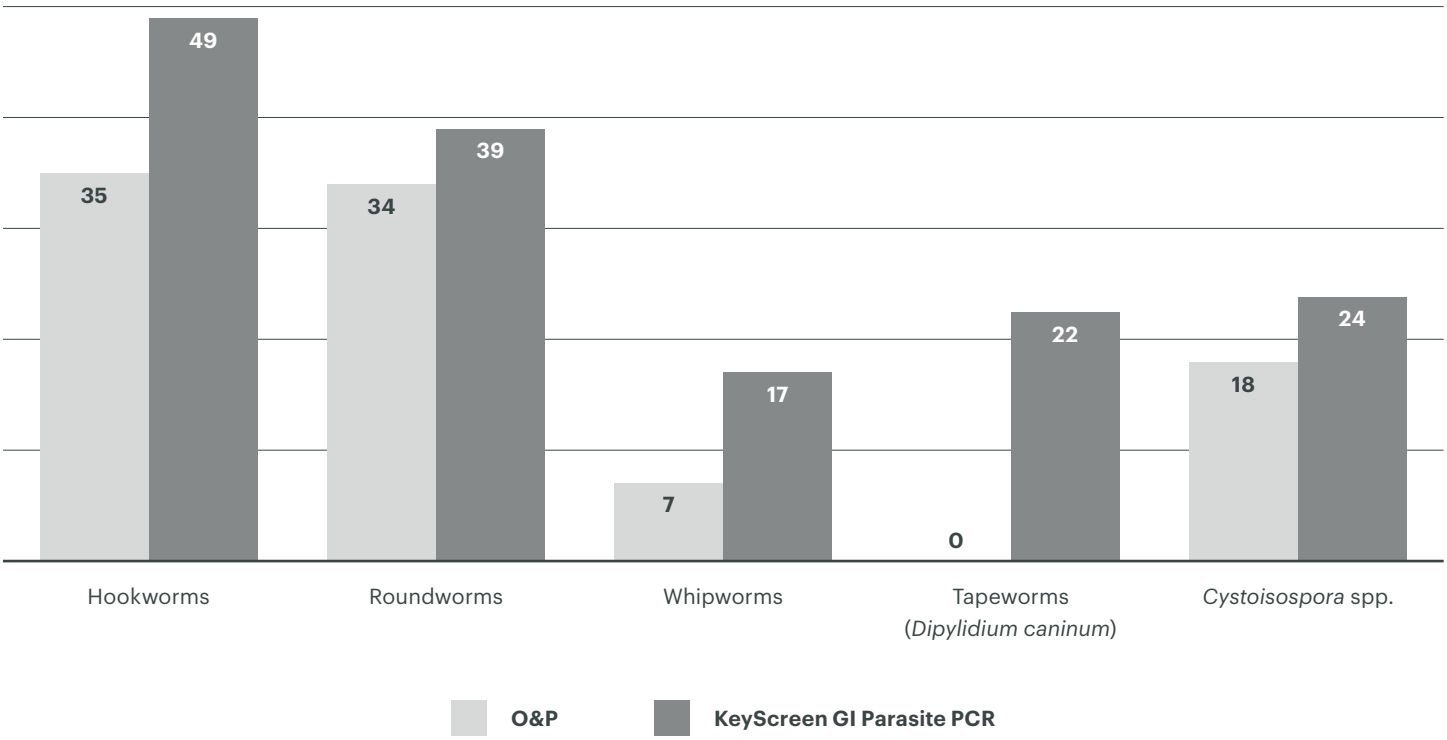
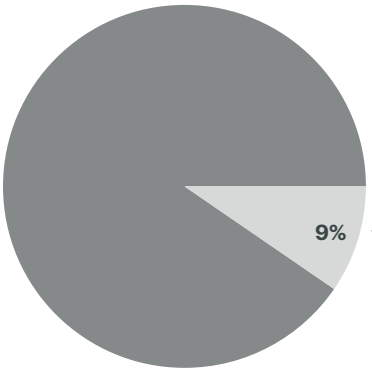


Figure 2
Pie chart showing the percentage of potentially zoonotic *Giardia* isolates in the study population. *Giardia duodenalis* has several genotypes and is grouped into subtypes ("assemblages") A to F. Only assemblages A and B are potentially associated with zoonosis.[9] KeyScreen GI Parasite PCR includes a marker that distinguishes these from the other subtypes.



Approximately one in 10 *Giardia* strains were potentially zoonotic (Assemblages A and B).

DISCUSSION

Compared to traditional O&P, KeyScreen GI Parasite PCR significantly increased the sensitivity for the detection of parasites that were identified by microscopy, including hook-, round-, whip-, tapeworms, Coccidia, and Giardia. In addition, KeyScreen GI Parasite PCR identified important parasites that are difficult to identify by microscopy, such as *Cryptosporidium*, *Tritrichomonas blagburni*, and *Toxoplasma gondii*.

Overall, KeyScreen GI Parasite PCR more than doubled the number of pets identified with parasitic infections. For co-infections, the number of patients with two or more parasites more than tripled when using PCR. These findings are based on a combination of increased sensitivity and broader detection of parasites that cannot be identified with O&P or ELISA tests.

KeyScreen GI Parasite PCR provides additional information that allows veterinarians to take into account pharmacological stewardship — an important element of the One Health initiative. Notably, in this study, one in 10 *Giardia duodenalis* strains were potentially zoonotic — an important consideration when making treatment decisions. KeyScreen GI Parasite PCR also includes a test for benzimidazole resistance in hookworms, allowing veterinarians to limit the use of anthelmintics and choose the correct drug the first time. Three samples contained *A. caninum* with benzimidazole resistance. Furthermore, the detection of *Echinococcus multilocularis* enables vigilance to an emerging problem of the parasite's prevalence.[10]

The recent advancements in PCR technology allow for faster and broader recognition of parasites in routine veterinary care. KeyScreen GI Parasite PCR also provides clinically useful information that cannot be obtained by centrifugal flotation or ELISA testing.

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