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Comparison of 3 Diagnostic Tests for the Detection of *Giardia* and *Cryptosporidium* spp. in Asymptomatic Dogs (*Canis lupis familiaris*)

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After detecting *Giardia* and *Cryptosporidium* infections and coinfections in 2 litters of puppies in our vivarium, our team realized that we needed a simple, quick, and economical point-of-care test for concurrent screening of asymptomatic dogs for both organisms. Periodic screening of colony dogs and of all dogs introduced into a colony can prevent the spread of *Giardia* and *Cryptosporidium* to immunologically naïve animals and help keep staff safe from these zoonotic organisms. To compare methods for diagnosing *Giardia* and *Cryptosporidium* spp. in dogs, we used a convenience sampling of feces from 2 populations of dogs; samples were tested with a lateral-flow assay (QC), a commercially-available direct fluorescent assay (DFA), and an inhouse PCR test using established primers. QC results were analyzed in 2 ways: 1) relative to a reference standard that permitted comparative interpretation of DFA and PCR results; and 2) using Bayesian analysis for comparison independent of a reference standard. The QC test showed good specificity for the detection of *Giardia* according to both the reference standard (95%) and the Bayesian analysis (98%). Similarly, specificity of the QC for the detection of *Cryptosporidium* was 95% according to the reference standard and 97% according to Bayesian analysis. However, the sensitivity of the QC test was much lower for both *Giardia* (reference standard, 38%; Bayesian analysis, 48%) and *Cryptosporidium* (25% and 40%, respectively). This study demonstrates that the QC test can be used to detect both *Giardia* and *Cryptosporidium* in dogs and that positive results can be accepted with confidence, whereas negative tests should be confirmed through secondary testing methods.

Abbreviations: CI, confidence interval; DFA, direct immunofluorescent assay; QC, patient-side test; ROC, receiver operating characteristic

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Introduction

Giardia duodenalis and *Cryptosporidium* spp. are important gastrointestinal pathogens in humans and canids.[3,9] Coinfections with these 2 pathogens in canids have not been well documented, with disagreement in the literature regarding its significance.[20,32] *G. duodenalis* has multiple genetic assemblages, most of which are host adapted and have little to no clinical significance in humans.[7] Assemblages C and D are most commonly isolated in both clinical and asymptomatic dogs and cause little to no disease in humans, yet assemblages A and B occur in dogs and can cause clinical disease in humans and canids.[7,16] Similarly, the genus *Cryptosporidium* comprises more than 25 host-associated species,[34] of which *C. canis* is most commonly found in dogs; however, *C. parvum* and *C. hominis* have also been identified in dogs and are the most common species associated with disease in humans.[7,10,34,38]

A litter of puppies born at our institution in 2020 was diagnosed, via fecal PCR by a veterinary diagnostic laboratory, as having clinical coinfections of *Giardia* and *Cryptosporidium* spp. This diagnosis led us to realize the need for a dependable, quick screening test that could identify multiple assemblages and species of both of these organisms. Inhouse methods for the detection of *Giardia* and *Cryptosporidium* include direct fecal smear and centrifugal fecal flotation with either Sheather sucrose for *Cryptosporidium* or zinc sulfate for *Giardia*.[41] These techniques, although cost effective, require technicians with specialized training in the recognition of cysts and oocysts as well as the necessary laboratory equipment, such as centrifuges and microscopes, depending on the procedure.[30] For optimal accuracy, *Cryptosporidium* should be stained and viewed at 400× magnification, thus adding an another layer of technical complexity.[4,5] Wide ranges of sensitivity and specificity have been reported for these methods, with 34% to 88% sensitivity and 92% to 96% specificity of zinc sulfate fecal flotation for the detection of *Giardia*.[19,35,40] Fecal flotation methods to detect *Cryptosporidum* in dogs have not been well studied, but in other species reported sensitivity ranges from 21% to 68% with a specificity of 93% to 98%.[4,5,27,29]

These 2 pathogens can cause subclinical infections in animals, leading to potential zoonotic transmission, particularly in immunocompromised people.[23,39,40] Reported prevalence values for canine giardiasis are as high as 16% in the United States and 15% in dogs globally.[3,6] A recent global meta-analysis incorporated studies that determined *Giardia* prevalence based on microscopy, ELISA, direct immunofluorescent assay (DFA), and PCR analysis and found that prevalence varied with testing modality; microscopy performed poorly compared with the other testing methods.[6] Giardiasis in humans remains a concern, with an incidence rate of 6 per 100,000 population in the United States in 2019.[12] Between 2012 and 2017, 111 giardiasis

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outbreaks were reported in humans from 26 different states, with 760 primary cases documented.[14] *G. duodenalis* is the most common intestinal parasitic infection of humans in the United States.[14] A recent *Cryptosporidium* meta-analysis, using data from descriptive, cross-sectional, and case-control studies, with microscopic, molecular, and coproantigenic methods, cited 5% prevalence of cryptosporidiosis in dogs in North America and 8% globally[38] but did not report a link between testing modality and likelihood of identifying the organism.[6,38] *Cryptosporidium* remains a prominent gastrointestinal parasite in humans, with an overall incidence rate of 4 per 100,000 people in the United States in 2019; this value represents an increase in incidence of 47% over the last decade.[11]

Several tests are currently approved for *Giardia* detection in dogs, and a few are approved for the diagnosis of cryptosporidiosis. However, no approved tests are available for concurrent rapid diagnosis of both of these pathogens in dogs.[6,31,35] This lack of dependable and uncomplicated testing modalities led our team to search for an economic, sensitive, and specific test that our technicians could use to screen incoming dogs and suspected clinical cases for *Cryptosporidium* and *Giardia*. Given their zoonotic potential, screening for these parasites would promote the safety of both our dogs and their caretakers. To this end, we compared a commercial point-of-care test (QC test) with both DFA and PCR analysis to determine whether this test would be useful for screening both healthy incoming and symptomatic dogs for the presence of *Giardia* and *Cryptosporidium*. We hypothesized that the QC test would perform with a sensitivity of at least 50% and specificity of at least 90% for the detection of *Giardia* and *Cryptosporidium* in dogs.

Materials and Methods

Ethics statement. This study was designed and conducted in accordance with the Texas A&M University IACUC, and in accordance with the regulations of the Animal Welfare Act.[1] An animal use protocol was deemed unnecessary by the IACUC because none of the experiments directly affected the day-to-day activities of the dogs; all samples were voluntarily voided into the environment and were collected during routine cleaning of the dogs' standard enclosures.

Animals and sample collection. Fecal samples (n = 170) were collected from dogs housed at our research facility (population 1; n = 96; age, 3 mo to 10 y) and from a Texas supplier of dogs for research (population 2; n = 74; age 3 mo to 13 y) during March through October 2021. Samples were collected from all dogs in population 1 and from a convenience sample of dogs in population 2 during daily cleaning activities and routine yearly physical exams.

Experimental design. This study evaluated a lateral flow assay that is approved to detect coproantigens of both *Giardia* and *Cryptosporidium* in humans (Quik Chek [QC], TechLab, Blacksburg, VA). The test takes approximately 30 min to run and requires no specialized equipment.

All fecal samples were individually labeled and stored in sealed plastic bags at 4 to 8 °C for 24 to 48 h prior to analysis. The first analysis used the QC test according to the manufacturer's recommendations. All reagents and samples were brought to room temperature and a 1.5 µL microcentrifuge tube was prepared for each sample by adding 500 µL of manufacturer-provided diluent and one drop of conjugate. A small, approximately 2-mm diameter, portion of feces was added to this tube. This was emulsified using an applicator stick and vortexer. A 500-µL portion of the diluted-conjugate prepared sample was deposited in the sample well of the test

Figure 1. A QC test that was positive for *Giardia* (blue line) and negative for *Cryptosporidium* (no blue line), with 3 control dots in the middle.

and was incubated at room temperature for 15 min. Then, 300 µL of the provided wash buffer was added to the reaction window and allowed to fully absorb. Lastly, 2 drops of the provided substrate were applied to the reaction window and the test was incubated at room temperature for 10 min. The result was considered positive for an organism when a blue line appeared next to the corresponding indicator (Figure 1).

The samples were then divided, with approximately 0.1 g placed in a 1.5-mL microcentrifuge tube containing formalin and stored at room temperature for DFA. The remaining sample was stored in plastic specimen containers at −80 °C for PCR analysis.

Formalin-preserved samples were tested using DFA (Merifluor *Cryptosporidium/Giardia*, Meridian Bioscience, Cincinnati, OH) according to the manufacturer's instructions. Samples were examined at 200× and 400× by a single trained reviewer using a fluorescence microscope.

Prior to PCR analysis, all frozen fecal specimens were thawed, and cysts and oocysts were isolated by using a gradient centrifugation protocol. An emulsion was created by mixing 2 to 3 g of feces with approximately 12 mL of a PBS–EDTA, 0.01 M, solution and straining through a double layer of cheesecloth. A disposable plastic pipette was used to transfer the eluate into sucrose solution (specific gravity, 1.26) in a 15-mL conical tube. This mixture was centrifuged at 800 × g for 10 min at room temperature. The top layer and emulsion interface were then pipetted into a new tube and centrifuged for another 10 min at 1,200 × g, at room temperature. The supernatant was discarded, the pellet was washed twice with PBS-EDTA, and the final pellet, which contained oocytes and cysts, was resuspended in 1 mL of PBS-EDTA and stored at −80 °C until DNA extraction.[35,37] DNA was extracted from stored pellets by using a commercial kit (DNeasy Blood and Tissue Kit, Qiagen, Germantown, MD).[17,36] DNA was eluted in 100 µL of elution buffer and stored at −20 °C until PCR analysis.

Purified and extracted DNA samples were analyzed for *Giardia* by using a 2-step nested PCR assay (MyCycler, Bio-Rad, Hercules, CA), in which a 292-bp fragment of the 16S rRNA gene was amplified by using the primers 5′ AAG TGT GGT GCA GAC GGA CTC 3′ and 5′ CTG CTG CCG TCC TTG GAT GT 3′ for the primary reaction and 5′ CAT CCG GTC GAT CCT GCC 3′ and 5′ AGT CGA ACC CTG ATT CTC CGC CAG G 3′ for the secondary reaction.[2,22] The primary and secondary PCR master mixes each included 8.75 µL of molecular-grade water,

0.625 µL of 10-µM forward primer, 0.625 µL of 10-µM reverse primer, and 12.5 µL of GoTaq Green (Promega, Madison, WI) to which 2.5 µL of sample was added for a total of 25 µL. After a 2-min initiation at 95 °C for the primary reaction, 40 cycles were run at 95 °C for 30 s, 54.5 °C for 45 s, and 72 °C for 90 s, followed by a final extension at 72 °C for 90 s and storage at 4 °C. The secondary reaction differed in that the 40 cycles were run at 95 °C for 30 s, 56.5 °C for 45 s, and 72 °C for 90 s.

Samples were similarly analyzed for *Cryptosporidium* DNA by using a 2-step nested PCR assay. An 800-bp fragment of the SSU rRNA gene was amplified by using primers 5′ TTC TAG AGC TAA TAC ATG CG 3′ and 5′ CCC ATT TCC TTC GAA ACA GGA 3′ for the primary reaction and 5′ GGA AGG GTT GTA TTT ATT AGA TAA 3′ and 5′ CTC ATA AGG TGC TGA AGG AGT A 3′ for the secondary reaction.[33] The primary and secondary reaction master mixes were prepared as described above to a total of 25 µL. After a 2-min initiation at 95 °C for the primary reaction, 40 cycles were run at 95 °C for 30 s, 48 °C for 45 s, and 72 °C for 90 s, followed by a final extension at 72 °C for 90 s and storage at 4 °C. For the secondary reaction, 40 cycles were run at 95 °C for 30 s, 50 °C for 45 s, and 72 °C for 90 s. Negative and positive controls for both *Giardia* and *Cryptosporidium* were included in each batch run.

All secondary reaction PCR products underwent gel electrophoresis through a 1% agarose gel stained with GelRed (Biotium, Fremont, CA), with a 100-mV procedure for 45 to 75 min, DNA marker ladder (Quick-Load DNA Ladder, New England BioLabs, Ipswich, MA), and gel imaging system (Gel-Doc Go, Bio-Rad, Hercules, CA). Samples were considered conditionally positive when they had a band at approximately 800 bp for *Cryptosporidium* and approximately 300 bp for *Giardia*.

All conditionally positive PCR samples were purified (Omega EZNA Cycle Pure Kit, Norcross, GA, or Wizard Gel and PCR Clean-Up System, Promega) according to the manufacturer's recommendations, and the resulting product was submitted for confirmatory sequencing (Eurofins Genomics, Louisville, KY). All genetic sequences were queried in the Nucleotide collection database by using MegaBLAST (National Center for Biotechnology Information, National Library of Medicine). The sequences for *Giardia* were matched to accession numbers AF310725.1, KY783324.1, LC437354.1, LC437356.1, LC437360.1, LC437361.1, LC437365.1, MG972765.1, MN263895.1, MN593002.1, MT129490.1, and MT484087.1. The sequences for *Cryptosporidium* were matched to accession numbers KT749817.1 and MT329018.1 (Table 1).

Table 1. NCBI Megablast results

Sample ID*	% identity	Accession number	Sample ID*	% identity	Accession number
Giardia			*Giardia*		
2	99.06	LC437365.1	84	99.60	LC437356.1
3	87.50	MT129490.1	85	99.58	LC437361.1
4	98.33	MN263895.1	86	92.24	LC437361.1
6	96.61	MN263895.1	89	97.69	LC437361.1
7	91.95	LC437365.1	91	94.61	MT129478.1
10	98.37	MN263895.1	92	100.0	LC437360.1
15	81.15	MT129490.1	94	100.0	MN263895.1
16	98.72	LC437365.1	98	97.56	MN263895.1
18	93.88	LC437365.1	101	97.56	MN263895.1
19	96.49	MT484087.1	108	87.03	LC437360.1
29	96.67	MN263895.1	110	85.19	MT129478.1
31	88.27	MN593002.1	116	86.13	LC437365.1
32	90.43	MN263895.1	117	97.71	MN263895.1
34	98.63	LC437354.1	121	90.16	LC437365.1
35	96.61	MT484087.1	126	90.38	LC437360.1
36	94.74	MT484087.1	130	90.70	LC437365.1
39	95.87	MN263895.1	132	88.05	MN593002.1
41	100.0	LC437354.1	136	94.37	LC437360.1
43	98.73	LC437354.1	138	100.0	LC437365.1
44	98.73	LC437365.1	151	100.0	MG972765.1
72	92.31	LC437365.1	161	91.57	KY783324.1
75	92.70	AF310725.1	164	89.26	LC437365.1
79	84.30	LC437365.1	165	95.38	LC437365.1
81	97.50	MN263895.1	167	97.39	LC437365.1
83	92.99	LC437365.1	168	98.33	MN263895.1
Cryptosporidium					
103	99.62	KT749817.1			
125	98.55	MT329018.1			
151	100.0	KT749817.1			
152	97.84	KT749817.1			
163	95.57	KT749817.1			

*All sample numbers that are not shown correspond to samples that were PCR-negative for both organisms

Statistical analysis. All data were analyzed initially by using STATA SE 17.0 (STATA Corp, College Station, TX). The apparent prevalence of each organism was calculated for both test populations for each of the 3 testing modalities. Our testing methods of direct visualization of the organism on DFA and sequencing the products of PCR-positive samples with nucleotide bank verification allowed us to assume that the specificity of both tests closely approached 100%. Therefore, we created a reference standard for the QC test by using both the DFA and PCR results in order to improve overall sensitivity. Apparent prevalence values for each organism in each population were calculated by using this reference standard.

Differences in prevalence of *Giardia* and *Cryptosporidium* between and within populations were calculated by using a z-test statistic, with significance defined as $P < 0.05$. Using the diagt command in STATA, we calculated sensitivity and specificity values for the QC, DFA, and PCR tests by using the reference standard. Likelihood ratio analysis was also performed; this analysis provides the probability that a dog that tests positive truly has disease, whereas one that tests negative truly does not have the disease. Receiver operatoring characteristic (ROC) curves were calculated for each test compared with the defined reference test for both organisms. Bayesian analysis with the Markov Chain Monte Carlo process was then performed by using WinBugs (version 1.4.3, University of Cambridge, Cambridge, United Kingdom), with the assumption of complete independence and adaption of the code (Figure 2) from the Center for Animal Disease Modeling and Surveillance (University of California Davis, Davis, CA).[8,18] BetaBuster (version 1.0, Chun-Lung Su, Informer Technologies, Los Angeles, CA) was used to calculate all α and β priors from previously reported specificities from the literature.[4,5,13,15,21,31,36,40] Informed priors from the literature differed largely from the sensitivities obtained for DFA and PCR in the current study. Therefore, for Bayesian analysis, we used the sensitivities for DFA and PCR as compared with the reference standard in the current study in order to avoid overestimating the sensitivities of each of the tests during Bayesian analysis.

Results

In determining the best test to use as a reference standard, neither DFA nor PCR analysis emerged as the obvious choice for either *Giardia* or *Cryptosporidium* because both tests had low detection for both organisms. However, assigning a positive finding if either PCR or DFA results were positive provided the highest proportion of correct classification of positive samples.

The prevalence of *Giardia* was 38% in population 1 (institutional colony) and 49% in population 2 (vendor colony; Table 2). The prevalence of *Cryptosporidium* was 1% in population 1 and 9% in population 2 (Table 2). Overall *Giardia* was significantly ($P < 0.0000$) more prevalent than *Cryptosporidium* in both populations, and *Cryptosporidium* parasites were significantly more prevalent ($P = 0.0050$) in population 2 than population 1. *Giardia* prevalence was not significantly different between the 2 populations ($P = 0.0900$).

```
model {
y1[1:Q, 1:Q, 1:Q] approximately dmulti(p1[1:Q, 1:Q, 1:Q], n1)
y2[1:Q, 1:Q, 1:Q] approximately dmulti(p2[1:Q, 1:Q, 1:Q], n2)
p1[1,1,1] <- Prev1*(SeT1*SeT2*SeT3) + (1-Prev1)*((1-SpT1)*(1-SpT2))*(1-SpT3)
p1[1,1,2] <- Prev1*(SeT1*SeT2)*(1-SeT3) + (1-Prev1)*((1-SpT1)*(1-SpT2))*(SpT3)
p1[1,2,1] <- Prev1*(SeT1*(1-SeT2))*SeT3 + (1-Prev1)*((1-SpT1)*SpT2)*(1-SpT3)
p1[1,2,2] <- Prev1*(SeT1*(1-SeT2))*(1-SeT3) + (1-Prev1)*((1-SpT1)*SpT2)*(SpT3)
p1[2,1,1] <- Prev1*((1-SeT1)*SeT2)*SeT3 + (1-Prev1)*(SpT1*(1-SpT2))*(1-SpT3)
p1[2,1,2] <- Prev1*((1-SeT1)*SeT2)*(1-SeT3) + (1-Prev1)*(SpT1*(1-SpT2)*SpT3)
p1[2,2,1] <- Prev1*((1-SeT1)*(1-SeT2)*SeT3) + (1-Prev1)*(SpT1*SpT2)*(1-SpT3)
p1[2,2,2] <- Prev1*((1-SeT1)*(1-SeT2)*(1-SeT3))+ (1-Prev1)*(SpT1*SpT2*SpT3)
p2[1,1,1] <- Prev2*(SeT1*SeT2*SeT3) + (1-Prev2)*((1-SpT1)*(1-SpT2))*(1-SpT3)
p2[1,1,2] <- Prev2*(SeT1*SeT2)*(1-SeT3) + (1-Prev2)*((1-SpT1)*(1-SpT2))*(SpT3)
p2[1,2,1] <- Prev2*(SeT1*(1-SeT2))*SeT3 + (1-Prev2)*((1-SpT1)*SpT2)*(1-SpT3)
p2[1,2,2] <- Prev2*(SeT1*(1-SeT2))*(1-SeT3) + (1-Prev2)*((1-SpT1)*SpT2)*(SpT3)
p2[2,1,1] <- Prev2*((1-SeT1)*SeT2)*SeT3 + (1-Prev2)*(SpT1*(1-SpT2))*(1-SpT3)
p2[2,1,2] <- Prev2*((1-SeT1)*SeT2)*(1-SeT3) + (1-Prev2)*(SpT1*(1-SpT2))*SpT3
p2[2,2,1] <- Prev2*((1-SeT1)*(1-SeT2))*SeT3 + (1-Prev2)*(SpT1*SpT2)*(1-SpT3)
p2[2,2,2] <- Prev2*((1-SeT1)*(1-SeT2))*(1-SeT3) + (1-Prev2)*(SpT1*SpT2)*SpT3
SeT1approximately dbeta(1,1)
SpT1approximately dbeta(1,1)
SeT2approximately dbeta(1,1)
SpT2approximately dbeta(1,1)
SeT3approximately dbeta(92, 4.08) # mode = 0.98, 95% >0.95
SpT3approximately dbeta(152,4.08) # mode = 0.95, 95% >0.91
Prev1approximately dbeta(1,1)
Prev2approximately dbeta(1,1)
}
```

```
# data
# T1 = QC; T2 = DFA; T3 = PCR
# n1 = batch 1; n2 = batch 2
list(n1 = 96, n2 = 74, Q = 2, y1 = structure(.Data = c(0,0,0,2,0,1,0,93),.Dim = c(2,2,2)),
y2 = structure(.Data = c(1,0,1,2,1,1,3,65),.Dim = c(2,2,2)))
```

```
# initials 1
list(SeT1 = 0.86, SpT1 = 0.97, SeT2 = 0.64, SpT2 = 0.75, SeT3 = 0.96, SpT3 = 0.99, Prev1 = 0.010, Prev2 =
0.108)
```

Figure 2. WinBugs code for comparing 3 independent tests.

Table 2. Apparent prevalence of *Giardia* and *Cryptosporidium* in populations 1 and 2

	Giardia		*Cryptosporidium*	
	Population 1	**Population 2**	**Population 1**	**Population 2**
Reference standard	38.5 (29.3, 48.7)	48.6 (36.9, 60.6)	1.0 (0.1, 7.2)	9.5 (3.9, 18.5)
QC	13.5 (8.0, 22.1)	27.0 (18.0. 38.4)	2.1 (0.5, 8.1)	5.4 (2.0, 13.7)
DFA	14.6 (8.8, 23.3)	31.1 (21.5, 42.7)	1.0 (0.1, 7.2)	4.1 (1.2, 12.0)
PCR	33.3 (24.5, 43.5)	25.7 (16.9, 37.0)	0 (0,0)	8.1 (3.6, 17.1)

Data are given as proportion (95% CI)

Table 3. Evaluation of *Giardia* detection

	Sensitivity	**Specificity**	**PPV**	**NPV**	**LR(+)**	**LR(–)**
QC	38.4 [27.2, 50.5]	94.8 [88.4, 98.3]	84.8 [68.1, 94.9]	67.2 [58.6, 74.9]	7.44 [3.0, 18.3]	0.65 [0.5, 0.8
DFA	50.7 [38.7, 62.6]	100 [96.3, 100]	100 [90.5, 100]	72.9, [64.5, 80.3]	—	0.49 [0.4, 0.6]
PCR	69.9 [58.0, 80.1]	100 [96.3, 100]	100 [93.0, 100]	81.5 [73.4, 88.0]	—	0.30 [0.2, 0.4]

LR(–), negative likelihood ratio; LR(+), positive likelihood ratio; NPV, negative predictive value; PPV, positive predictive value. Data are given as mean percentage (95% CI).

Table 4. Evaluation of *Cryptosporidium* detection

	Sensitivity	**Specificity**	**PPV**	**NPV**	**LR(+)**	**LR(–)**
QC	25.0 (3.2, 65.1)	95.5 (93.8, 99.3)	33.3 (4.3, 77.7)	96.3 (92.2, 98.6)	10.13 (2.2, 47.3)	0.77 (0.5, 1.2)
DFA	50 (15.7, 84.3)	100 (97.7, 100)	100 (39.8, 100)	97.6 (93.9, 99.3)	—	0.5 (0.3, 1.0)
PCR	75.0 (34.9, 96.8)	100 (97.7, 100)	100 (54.1, 100)	98.8 (95.7, 99.9)	—	0.25 (0.1, 0.8)

LR(–), negative likelihood ratio; LR(+), positive likelihood ratio; NPV, negative predictive value; PPV, positive predictive value. Data are given as mean percentage (95% CI).

For the detection of *Giardia*, the sensitivity of the QC test was 38%, and specificity was 95% (Table 3). For the detection of *Cryptosporidium*, the sensitivity of the QC test was 25% and specificity was 95% (Table 4). The ROC area for QC detection was 0.67 for *Giardia* (Figure 3), and 0.61 for *Cryptosporidium* (Figure 4).

The prevalence of *Giardia* based on Bayesian analysis was 33% in population 1 and 51% in population 2, which falls within the confidence interval (CI) of our reference standard (Table 2). Bayesian analysis showed that for the QC test, the mean sensitivity was 48% and specificity was 98%; for the DFA test, the mean sensitivity was 51% and specificity was 99%; and for the PCR test the mean sensitivity was 63% and specificity was 92% (Table 5).

Bayesian analysis showed that prevalence of *Cryptosporidium* was 1% for population 1 and 9% for population 2. For the QC test, the mean sensitivity was 40% and specificity was 97%; for the DFA test, mean sensitivity was 38% and specificity was 99%; for the PCR test, mean sensitivity was 93% and specificity was 99% (Table 6).

Discussion

In this study, we evaluated the use in dogs of a QC diagnostic test originally developed for the detection of *Giardia* and *Cryptosporidium* in humans. We used frequentist statistics to perform standard comparison to a reference testing scheme and Bayesian

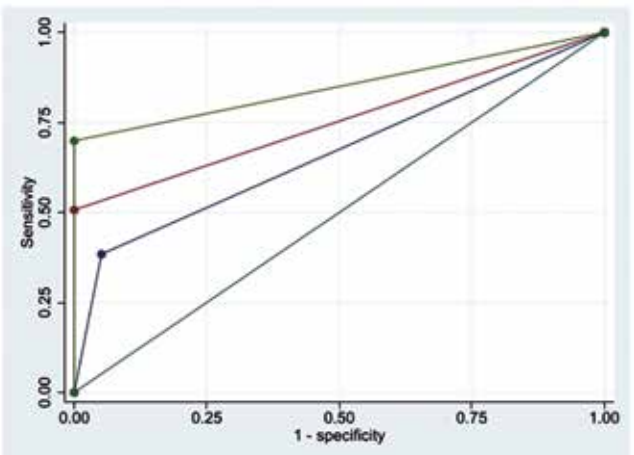

Figure 3. Receiver operating characteristic curve for *Giardia* analysis with QC (blue; area, 0.67), DFA (red; area, 0.75), and PCR (green; area, 0.85) assays compared with the reference standard (gray).

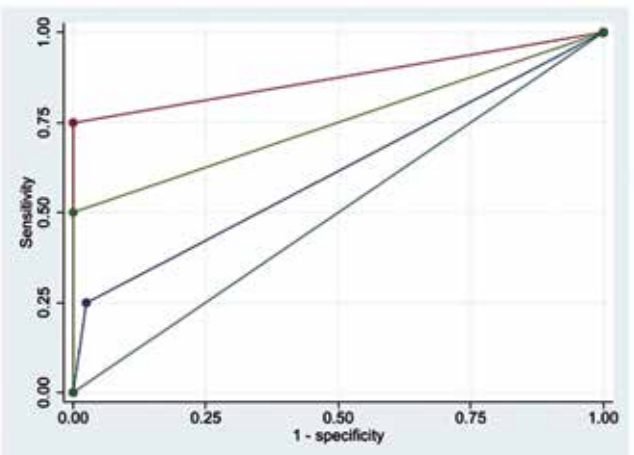

Figure 4. Receiver operating characteristic curve for *Cryptosporidium* analysis with QC (blue; area, 0.61), DFA (red; area, 0.75), and PCR (green; area, 0.88) assays compared with the reference standard (gray).

Table 5. Bayesian analysis for *Giardia*

	Mean %	Median % (95% CI)	1 SD	Monte Carlo error
Prevalence population 1	32.6	32.0 (16.1, 51.7]	0.0966	1.9×10^3
Prevalence population 2	50.6	50.7 (33.2, 68.0]	0.0896	1.4×10^3
Sensitivity QC	48.2	47.2 (30.4, 71.0]	0.1068	2.1×10^3
Specificity QC	97.9	98.4 (93.4, 99.9]	0.0363	2.0×10^4
Sensitivity DFA	51.4	51.4 (41.6, 61.8]	0.0515	8.7×10^4
Specificity DFA	99.2	99.4 (97.0, 100]	0.0081	9.4×10^5
Sensitivity PCR	62.8	62.9 (55.5, 69.7]	0.0363	2.9×10^4
Specificity PCR	92.5	92.5 (84.1, 99.7]	0.0450	9.5×10^4

Table 6. Bayesian analysis for *Cryptosporidium*

	Mean %	Median % (95% CI)	1 SD	Monte Carlo error
Prevalence population 1	1.1	0.7 (0.0, 3.9)	0.0173	1.4×10^4
Prevalence population 2	8.9	8.54 (3.0, 16.9)	0.0356	3.0×10^4
Sensitivity QC	40.5	38.97 (10.4, 78.5)	0.1784	1.3×10^3
Specificity QC	97.1	97.26 (94.0, 99.1)	0.0132	1.1×10^4
Sensitivity DFA	37.7	37.38 (21.4, 56.0)	0.0895	6.1×10^4
Specificity DFA	98.7	97.26 (96.7, 99.8)	0.0079	6.9×10^4
Sensitivity PCR	93.3	93.89 (84.6, 98.6)	0.0370	2.9×10^4
Specificity PCR	99.2	99.44 (97.3, 100)	0.0073	8.7×10^4

statistics for comparison of tests without using a 'gold standard' as a reference. Our results indicate that the QC test provides good certainty that a positive finding for either *Giardia* or *Cryptosporidium* is a true positive. However, because the QC test has low sensitivity, confirmatory testing should be performed before concluding that a dog is negative for the presence of either *Giardia* or *Cryptosporidium*.

We collected and analyzed samples from pathogen surveillance testing of clinically normal dogs maintained in institutional and vendor colony populations. No single test stood out as a true gold standard. We therefore analyzed the data by using 2 distinct statistical methods. First, we created the reference standard test by using the results from both of our 2 near-perfect specificity tests; this approach assigns the sample as positive if either of the tests were positive. This approach increases the sensitivity of the overall testing scheme when highly specific tests, such as DFA and PCR, are used to assess low prevalence populations. Although this approach provided concise and easily interpreted results, we further analyzed the data by using Bayesian analysis. Those results closely approximated the frequentist statistical analysis, giving us confidence in interpretating these diagnostic tests for detection of *Giardia* and *Cryptosporidium* in asymptomatic canine populations.

Evaluation of a diagnostic test in asymptomatic, subclinical, or carrier subjects is the most rigorous approach to assessment of the test. Subjects with such an infection status by definition have low concentrations of organisms in their stool relative to clinical cases. This can lead to test results that vary when different tests are applied to the same sample.[24] This variability is evident in the evaluation of our reference test in which we analyzed both PCR and DFA results in parallel. The low sensitivity demonstrated by PCR and DFA when compared with the reference standard supports the assumption that our samples had low concentrations of organisms. Furthermore, the asymptomatic status of our subjects may explain the lower sensitivity of PCR analysis and DFA in our study as compared with previous reports.[4,5,19,27,29,35,40]

Compared with the QC test, the PCR and DFA tests in our current study detected more cases of *Giardia*, whereas PCR analysis detected more cases of *Cryptosporidium*. However, given the extra expense, time, and specialized equipment needed for PCR and DFA tests, the QC test performed well. The overlap in the CIs of specificity of QC, DFA, and PCR tests for both *Giardia* and *Cryptosporidium* indicate that the 3 tests perform similarly in detecting a negative dog. The overlap in the CIs of sensitivity for QC, DFA, and PCR for *Cryptosporidium*, and of QC and DFA for *Giardia* indicate that the tests perform similarly in detecting an infected dog.

Bayesian analysis agreed with our standard, frequentist analysis in the current study, with the Bayesian mean prevalences of both organisms in populations 1 and 2 falling within the CI of the frequentist analysis. Similarly, the mean sensitivities of the QC, DFA, and PCR tests fell within the CIs of frequentist analyses for both *Giardia* and *Cryptosporidium*. The mean specificities in the Bayesian analysis of the QC, DFA, and PCR tests fell within the CIs of the frequentist analysis for *Cryptosporidium*, and the specificities of the QC and DFA tests fell within the CIs of the frequentist analyses for *Giardia.* In the current study, DFA sensitivity for *Cryptosporidium* did not approach what is reported in the literature.[42] Although this difference could be due to the low organism concentrations in our samples, another possibility is that the species of *Cryptosporidium* in our population is not *C. parvum* but rather the common dog species, *C. canis*.[21] Further analysis will be necessary to investigate this finding.

A limitation of our study is the assumption (for the Bayesian analysis) of independence of the 3 diagnostic tests, based on their biologic characteristics. Because we did not know the true infection status of each dog in our populations, we could not reliably assess conditional dependence and therefore assumed their independence in our Bayesian analysis, as has been done previously in other studies.[8,25,26,28] Other limitations of the current study include the low prevalence of *Cryptosporidium* in our samples and the variability between tests that traditionally have high sensitivity and specificity. These limitations could be mitigated in future studies by performing the tests in replicate, as suggested previously.[33]

In conclusion, we find the QC test is a simple, quick, and economical test that yields reliable results for both *Giardia* and *Cryptosporidium* in asymptomatic dogs. The QC test showed good specificity as compared with DFA and PCR analysis and achieved results that were close to our goals of sensitivity (50%) and specificity (90%) for both *Giardia* and *Cryptosporidium* detection.

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