

## STANDARD ARTICLE

# Comparison of diagnostic techniques for detection of *Giardia duodenalis* in dogs and cats

Meriam N. Saleh<sup>1,3</sup>  | Jack R. Heptinstall<sup>2</sup> | Eileen M. Johnson<sup>3</sup> |  
 Lora R. Ballweber<sup>4</sup> | David S. Lindsay<sup>1</sup> | Stephen Werre<sup>1</sup> | Joel F. Herbein<sup>2</sup> |  
 Anne M. Zajac<sup>1</sup>

<sup>1</sup>Department of Biomedical Sciences & Pathobiology, Virginia Tech, Blacksburg, Virginia

<sup>2</sup>Scientific Affairs, TECHLAB Inc., Blacksburg, Virginia

<sup>3</sup>Department of Veterinary Pathobiology, Oklahoma State University, Stillwater, Oklahoma

<sup>4</sup>Department of Microbiology, Immunology & Pathology, Colorado State University, Fort Collins, Colorado

**Correspondence**

Meriam N. Saleh, 250 McElroy Hall, Stillwater, OK 74078,  
 Email: meriam.saleh@okstate.edu

**Present address**

Meriam N. Saleh, Oklahoma State University, Stillwater, Oklahoma.

**Abstract**

**Background:** An evaluation of currently available in-clinic diagnostic tests for *Giardia duodenalis* infection of dogs and cats has not been performed. In addition, there is discordance among published diagnostic comparisons. The absence of a true gold standard for detecting *Giardia duodenalis* also complicates diagnostic evaluations.

**Objectives:** To evaluate diagnostic tests commercially available in the United States for detecting *Giardia duodenalis* in dogs and cats, in comparison to a widely used reference test, the direct immunofluorescent assay (IFA), and also to compare the results of 2 methods of analysis: comparison of diagnostic tests to a reference test (IFA) and Bayesian analysis.

**Animals:** Fecal samples from a convenience sample of 388 cats and dogs located in Colorado, Oklahoma, and Virginia.

**Methods:** Fecal samples were tested for *Giardia duodenalis* by zinc sulfate centrifugal fecal flotation and 4 different commercial diagnostic immunoassays. Results were analyzed via Bayesian analysis and by comparison to the IFA as the reference test.

**Results:** Sensitivity and specificity by comparison to IFA was  $\geq 82\%$  and  $\geq 90\%$ , respectively, for all diagnostic tests in dogs and cats. When analyzed via Bayesian analysis, sensitivity and specificity were  $\geq 83\%$  and  $\geq 95\%$ , respectively. When ZnSO<sub>4</sub> centrifugal fecal flotation results were combined with immunoassay results, there was no longer a significant difference between the sensitivities of the commercial in-clinic immunoassays.

**Conclusion and Clinical Relevance:** The Bayesian analysis validates using IFA as the reference test. Differences in commercial in-clinic immunoassay sensitivities can be mitigated when the results are combined with ZnSO<sub>4</sub> centrifugal fecal flotation results.

**KEYWORDS**

canine, direct immunofluorescent, fecal flotation, feline, immunoassay, protozoa

**Abbreviations:** CAPC, Companion Animal Parasite Council; IFA, MERIFLUOR *Cryptosporidium/Giardia* direct immunofluorescent assay; SNAP, IDEXX *Giardia* Antigen Test (IDEXX Laboratories, Westbrook, ME); VetChek, TECHLAB VETCHEK ELISA (TechLab Inc., Blacksburg, VA); VetScan, Abaxis VetScan *Giardia* Antigen test (Abaxis, Inc., Union City, CA); ZnSO<sub>4</sub>, Zinc sulfate centrifugal fecal flotation.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2019 The Authors. *Journal of Veterinary Internal Medicine* published by Wiley Periodicals, Inc. on behalf of the American College of Veterinary Internal Medicine.

## 1 | INTRODUCTION

*Giardia duodenalis* is a common enteric protozoan parasite of dogs and cats. Most infections are subclinical, but acute or chronic diarrhea can also occur.<sup>1–3</sup> The prevalence of *G. duodenalis* infection varies depending on the age, clinical status, housing, and geographic region of the animals surveyed and is influenced by the detection method used.<sup>1,4</sup> Historically, diagnosis of *G. duodenalis* in dogs and cats has been via microscopic examination of feces for trophozoites or cysts.<sup>5</sup> However, accuracy of microscopic diagnosis of *G. duodenalis* is limited by the infrequent presence of trophozoites in diarrheic feces, intermittent passage of cysts, and the requirement that an experienced technician perform the examination.<sup>6–8</sup> The direct immunofluorescent assay (IFA) is more sensitive and specific for diagnosing *G. duodenalis* than conventional flotation tests<sup>9,10</sup> and is the reference test for evaluating *G. duodenalis* tests in companion animals.<sup>4,11,12</sup> Immunoassays are also available that detect a soluble cyst antigen of *G. duodenalis*. The Companion Animal Parasite Council (CAPC), which is widely cited as a source of guidelines for parasite control in the United States, recommends that centrifugal fecal flotation be used in conjunction with an immunoassay for diagnosing *G. duodenalis* infections in veterinary practices ([www.capcvet.org](http://www.capcvet.org)). This is supported by the evidence that the sensitivity of fecal flotation is improved when used with a commercial immunoassay.<sup>13</sup>

Diagnostic test evaluation is performed by comparison to a gold standard reference test. However, there is often not a true gold standard for test comparison, and this is the case for *G. duodenalis*. The IFA is widely accepted as the most sensitive and specific test for *G. duodenalis* and is often used as the reference test.<sup>9,10</sup> But, it is not a true gold standard (ie, “...absolutely accurate”),<sup>14</sup> and using an imperfect reference test as the gold standard for diagnostic comparison can lead to miscalculation of test performance for the test(s) being evaluated. To overcome this problem, a Bayesian analysis can be performed. This statistical method allows for diagnostic test evaluation in the absence of a gold standard and has been used for *G. duodenalis* diagnostic test evaluations.<sup>10,14–17</sup>

Various *G. duodenalis* diagnostic tests have been evaluated for detection of infection in small animals, but not all comparisons have included a reference test.<sup>4,10,11,13,15</sup> The purpose of this study was to evaluate diagnostic tests commercially available in the United States that are optimized for detecting *G. duodenalis* in dogs and cats, by comparison to a reference test (IFA) and also to compare all the diagnostic tests, including the IFA, using a Bayesian analysis.

## 2 | MATERIALS AND METHODS

### 2.1 | Fecal specimen collection and screening

Fecal samples (n = 388) were collected from dogs and cats at 3 distinct study sites from 2012 to 2014. These sites were parasitology laboratories at veterinary teaching hospitals in Fort Collins, Colorado; Stillwater, Oklahoma; and Blacksburg, Virginia. Samples at all 3 sites consisted of

hospital submissions (wellness examinations and clinical cases), plus collection surveys from animal shelters, and rescue organizations.

At each study site, samples were screened for the presence of *G. duodenalis* cysts using zinc sulfate centrifugal fecal flotation (ZnSO<sub>4</sub>) as outlined below. *Giardia duodenalis* positive samples of sufficient quantity to perform all diagnostic tests were included in the study. Fecal consistency and the presence of other parasites were recorded for each sample. For each *G. duodenalis* positive sample, a matching *G. duodenalis* negative sample (by ZnSO<sub>4</sub> flotation) of the same fecal consistency from the same geographic location was included in the study. This selection procedure was used to ensure an adequate number of *G. duodenalis* positive samples for meaningful analysis, because prevalence in the general dog and cat population is relatively low. Near the end of the study period, all fecal samples from shelter collections in Virginia, regardless of positive or negative status, were included in the sample pool to increase the overall sample size. Samples collected from Oklahoma and Colorado were refrigerated and shipped weekly with ice packs to the main study site. Sample selection and fecal flotations were performed at each study site, and all immunoassays were performed weekly at the main study site (Blacksburg, Virginia) upon sample arrival. Zinc sulfate fecal flotations were not repeated at the main study site.

### 2.2 | Fecal flotation

Zinc sulfate centrifugal fecal flotation was performed as described by Zajac and Conboy<sup>18</sup> using 2 to 4 g of feces. The feces and zinc sulfate solution (specific gravity 1.18) were centrifuged with a coverslip in place for 5 minutes at 200 × g. After centrifugation, the coverslip was removed to a glass slide and scanned for *G. duodenalis* cysts with a compound microscope at 100×, and if no cysts were detected then also at 200×. *Giardia duodenalis* and any other parasite species present were recorded as present, but no quantitation was performed.

### 2.3 | Immunoassays

The VETCHEK ELISA (TECHLAB Inc, Blacksburg, Virginia) (VetChek) is an enzyme immunoassay developed for the qualitative detection of *G. duodenalis* cyst antigen in canine and feline fecal specimens. The test was performed following the manufacturer's instructions. This is the first well-plate ELISA that is commercially available and optimized for use in dogs and cats.

The SNAP *Giardia* Antigen Test (IDEXX Laboratories, Westbrook, Maine) (SNAP) is a rapid in-clinic enzyme immunoassay for the detection of *G. duodenalis* antigen in canine and feline feces. Tests were performed following the manufacturer's instructions.

The Abaxis VetScan *Giardia* Antigen test (VetScan), is a rapid in-clinic enzyme immunoassay for the detection of *G. duodenalis* antigen in canine feces only. Tests were performed following the manufacturer's instructions. Although this test is not intended for use in cats, it was included in this feline comparison because practitioners might consider using the VetScan for cats if the test is available in their clinic. The VetScan cat results were excluded from statistical analysis

because the manufacturer does not intend for the test to be used in cats.

The MERIFLUOR *Cryptosporidium/Giardia* direct immunofluorescent assay (Meridian Bioscience Inc, Cincinnati, Ohio) (IFA) was used as the reference test and performed following the manufacturer's instructions to identify *G. duodenalis* cysts in feces. Samples were run in batches, with positive and negative controls each time the test was run. Slides were examined at 100 $\times$ , and if no cysts were detected, then also at 200 $\times$  magnification using a fluorescence microscope. A sample was considered positive if any *G. duodenalis* cysts were detected. The presence of *Cryptosporidium* oocysts was also recorded if any were observed, but results are not reported here.

## 2.4 | Statistical analysis

The data was analyzed by multiple methods. Sensitivity and specificity of the fecal flotation and immunoassays were calculated by comparison to IFA.<sup>4,9,10</sup> The sensitivities and specificities of each diagnostic test were then compared for differences using McNemar's test for significance of changes.<sup>19</sup> These analyses were conducted on data from dogs and cats separately. Additionally, the in-clinic immunoassay results (SNAP and VetScan) were analyzed in combination with the ZnSO<sub>4</sub> fecal flotation results in dogs (ie, the sample is considered positive if at least 1 test is positive) to mimic their use in clinics as recommended by CAPC. The combined results were then compared for differences in sensitivities and specificities using McNemar's test for significance of changes. Lastly, a Bayesian analysis, in which no test is considered a reference standard, was performed to estimate the sensitivities and specificities of each diagnostic test for dogs and cats separately.<sup>10,20,21</sup>

The Bayesian analysis framework expanded the 3-test script BayesDiagnosticTests\src\tt3.txt by Lawrence Joseph and colleagues ([www.medicine.mcgill.ca/epidemiology/Joseph/Bayesian-Software-Diagnostic-Testing.html](http://www.medicine.mcgill.ca/epidemiology/Joseph/Bayesian-Software-Diagnostic-Testing.html)) to 5 tests for data from canine samples and to 4 tests for data from feline samples. Theoretical foundations of the actual software (BayesDiagnosticTests version 3.10.2, January 2016) have been reported.<sup>20,21</sup> The new scripts were implemented and executed using WinBugs version 1.4.3. Parameters to be estimated included overall prevalence for either canines or felines, and sensitivity and specificity for each of the diagnostic tests. Pairwise comparisons between diagnostic tests for sensitivity and specificity were included as differences within the scripts. Prior information for the model was obtained from published studies and manufacturers' information.<sup>4,10,11,13</sup> The prior information was collated and summarized as mean ( $\pm$ SD) that was subsequently converted into alpha and beta parameters (Table 1) of the beta prior density. Research evidence to update the prior information is presented in the form of cross-classification for canine and feline samples (Table 2). After initialization, all models were run for 25 000 iterations (5000 for burn-in and 20 000 for parameter estimation). Trace plots were inspected to verify convergence, and the Monte Carlo error for each parameter was also verified to be less than 5% of the sample SD. Finally, for each parameter of interest, a median

**TABLE 1** Prior information for each of the 11 parameters to be estimated by Bayesian analysis

Test	Parameter	Mean (%)	SD (%)	Alpha	Beta
ZnSO <sub>4</sub>	Prevalence	7.69	6.42	1.25	14.98
	Sensitivity	67.15	25.67	1.58	0.77
Vet Chek	Specificity	96.85	4.04	17.13	0.56
	Sensitivity	93	N/A	9.80	0.74
SNAP	Specificity	99.7	N/A	0.44	0.001
	Sensitivity	84.77	7.51	18.56	3.33
VetScan	Specificity	97.27	4.56	11.45	0.32
	Sensitivity	98.1	N/A	2.26	0.04
IFA	Specificity	99.3	N/A	2.33*	0.016*
	Sensitivity	100 <sup>†</sup>	0 <sup>†</sup>	997.00	1.00
	Specificity	100 <sup>†</sup>	0 <sup>†</sup>	997.00	1.00

N/A, not available. N/A values were replaced with 7.51 and 4.56 for sensitivity and specificity, respectively. These values were approximately mid-way for all SDs.

\*The model would not estimate this parameter. As such noninformative values of (1) were used instead.

<sup>†</sup>To compute alpha and beta, a mean of 99.9% and an SD of 0.1% were used.

**TABLE 2** Results from 5 diagnostic tests for the detection of *G. duodenalis*

ZnSO <sub>4</sub>	VetChek	SNAP	VetScan	IFA	Number of samples		
					Canine	Feline	Total
-	-	-	-	-	128	82	210
+	+	+	+	+	84	24	108
+	+	+	-	+	10	11	21
-	+	+	+	+	9	0	9
+	-	-	-	-	4	1	5
-	+	-	-	-	2	3	5
+	+	-	+	+	4	0	4
+	+	+	+	-	4	0	4
+	-	-	-	+	3	1	4
+	+	-	-	+	2	1	3
-	-	+	-	+	2	1	3
-	+	+	-	-	2	1	3
-	+	-	-	+	2	0	2
+	+	-	-	-	2	0	2
-	-	-	-	+	1	1	2
-	+	+	-	+	0	1	1
+	-	+	-	+	1	0	1
+	+	+	-	-	1	0	1
Total					261	127	388

Abbreviations: +, positive result; -, negative result; IFA, MERIFLUOR *Cryptosporidium/Giardia* direct immunofluorescent assay; SNAP, IDEXX *Giardia* Antigen Test; VetChek, TECHLAB VETCHEK ELISA; VetScan, Abaxis *Giardia* Antigen test; ZnSO<sub>4</sub>, zinc sulfate centrifugal fecal flotation.

together with the 2.5 and 97.5 percentiles were obtained from the posterior distribution.

### 3 | RESULTS

Overall 388 samples (127 feline and 261 canine) were included in the study and evaluated for the presence of *G. duodenalis* by each diagnostic test. The cross-classified results from each diagnostic test are outlined in Table 2. Of the 388 samples tested, 108 (24 feline and 84 canine) were positive for *G. duodenalis* on all 5 diagnostic tests, whereas 210 (82 feline and 128 canine) were negative on all diagnostic tests. In total, 318 of 388 samples (82%) had concordant results across all 5 diagnostic tests. With regards to the geographic origin of each sample, there was no difference in sensitivity or specificity of the initial ZnSO<sub>4</sub> fecal flotation when compared to IFA among the different study sites.

#### 3.1 | Comparison to IFA

The sensitivity and specificity of each test compared to the IFA reference test for dogs and cats are presented in Tables 3 and 4. Analysis of canine data alone shows that the test with the highest sensitivity when compared to IFA was VetChek (94.1%) followed by SNAP (89.8%). The results of the McNemar's test in dogs showed that the ZnSO<sub>4</sub> flotation, VetChek, and SNAP all had significantly higher sensitivities than the VetScan. When the in-clinic immunoassay results in dogs were combined with the ZnSO<sub>4</sub> fecal flotation result with a dog being considered positive if either immunoassay or flotation was positive, there was no longer a significant difference in sensitivity between the SNAP and VetScan immunoassays in dogs. There was no statistically significant difference in specificities between the in-clinic immunoassays (SNAP and VetScan) in dogs. VetScan (97.2%) and SNAP (95.1%) both had significantly higher specificities than the VetChek and ZnSO<sub>4</sub> fecal flotation (both 92.3%).

**TABLE 3** Diagnostic test performance in dogs compared to IFA

Diagnostic test	95% confidence interval	
	Sensitivity	Specificity
SNAP	89.8 <sup>b</sup> (83.1-94.1)	95.1 <sup>bc</sup> (90.2-97.6)
VetChek	94.1 <sup>b</sup> (88.3-97.1)	92.31 <sup>a</sup> (86.8-95.7)
VetScan	82.2 <sup>a</sup> (74.3-88.1)	97.2 <sup>b</sup> (93.0-98.9)
SNAP w/ZnSO <sub>4</sub>	97.5 <sup>ab</sup> (92.8-99.1)	90.9 <sup>abc</sup> (85.1-94.6)
VetScan w/ZnSO <sub>4</sub>	95.8 <sup>ab</sup> (90.5-98.2)	92.31 <sup>abc</sup> (86.8-95.7)
ZnSO <sub>4</sub>	88.1 <sup>ab</sup> (81.1-92.8)	92.3 <sup>ac</sup> (86.8-95.7)

The sensitivity and specificity of each test with 95% confidence intervals (CI) when compared to the IFA reference test in dogs. Within columns, different letters are significantly different (McNemar's  $P < .05$ ). Cells without superscripts had no significant differences.

Abbreviations: IFA, MERIFLUOR *Cryptosporidium/Giardia* direct immunofluorescent assay; SNAP, IDEXX *Giardia* Antigen Test; Vet Chek, TECHLAB VETCHEK ELISA; VetScan, Abaxis *Giardia* Antigen test; ZnSO<sub>4</sub>, zinc sulfate centrifugal fecal flotation.

**TABLE 4** Diagnostic test performance in cats compared to IFA

Diagnostic test	95% confidence interval	
	Sensitivity	Specificity
SNAP	92.5 (87.6-95.9)	98.9 (95.9-99.9)
Vet Chek	92.5 (92.9-99.1)	95.4 (90.2-97.8)
ZnSO <sub>4</sub>	92.5 (85.7-95.1)	98.9 (90.7-97.8)
SNAP w/ZnSO <sub>4</sub>	97.5 (87.1-99.6)	97.7 (92.0-99.4)

The sensitivity and specificity of each test with 95% confidence intervals (CI) when compared to the IFA reference test in cats. Within columns, different letters are significantly different (McNemar's  $P < .05$ ). Cells without superscripts had no significant differences.

Abbreviations: IFA, MERIFLUOR *Cryptosporidium/Giardia* direct immunofluorescent assay; SNAP, IDEXX *Giardia* Antigen Test; Vet Chek, TECHLAB VETCHEK ELISA; VetScan, Abaxis *Giardia* Antigen test; ZnSO<sub>4</sub>, zinc sulfate centrifugal fecal flotation.

In cats, all 3 tests had the same sensitivity (92.5%) when compared to the IFA (Table 4), and specificity was  $\geq 95\%$  for each diagnostic test. There were no statistically significant differences between the sensitivities and specificities of any of the tests in cats. When the immunoassay (SNAP) result for cats was combined with the ZnSO<sub>4</sub> fecal flotation result, sensitivity did improve from 92.5% to 97.5%, but this difference was not statistically significant.

#### 3.2 | Bayesian analysis

The estimated sensitivity, specificity, and 95% confidence interval of each diagnostic test for dogs using the Bayesian analysis are shown in Table 5. The most sensitive diagnostic test was the IFA (99.4%), which was also the most specific (99.7%). Although the VetScan had the lowest sensitivity (83.3%), it had the highest specificity (99.3%) after the IFA. Specificity was  $\geq 95\%$  for all other diagnostic tests.

The analysis of the feline data using the Bayesian analysis is presented in Table 6. The most sensitive and specific diagnostic test was the IFA (99.9% and 99.8%, respectively). In cats, the sensitivity of the ZnSO<sub>4</sub>

**TABLE 5** Bayesian analysis estimates of diagnostic test parameters in dogs

Diagnostic test	95% confidence interval	
	Sensitivity	Specificity
IFA	99.4 (98.86-99.7)	99.8 <sup>b</sup> (99.3-99.9)
SNAP	90.5 <sup>ab</sup> (85.0-94.9)	98.7 <sup>ab</sup> (95.9-100)
VetChek	94.5 <sup>a</sup> (90.9-98.7)	96.0 <sup>a</sup> (91.9-98.8)
VetScan	83.3 <sup>b</sup> (75.9-89.6)	99.3 <sup>b</sup> (97.4-99.9)
ZnSO <sub>4</sub>	88.7 <sup>b</sup> (82.3-93.7)	95.5 <sup>a</sup> (91.6-98.3)

The sensitivity and specificity of each test in dogs with 95% confidence intervals (CI) estimated by Bayesian analysis. Within columns, different letters are significantly different (McNemar's  $P < .05$ ). Cells without superscripts had no significant differences.

Abbreviations: IFA, MERIFLUOR *Cryptosporidium/Giardia* direct immunofluorescent assay; SNAP, IDEXX *Giardia* Antigen Test; Vet Chek, TECHLAB VETCHEK ELISA; VetScan, Abaxis *Giardia* Antigen test; ZnSO<sub>4</sub>, zinc sulfate centrifugal fecal flotation.

**TABLE 6** Bayesian analysis estimates of diagnostic test parameters in cats

Diagnostic test	95% confidence interval	
	Sensitivity	Specificity
IFA	99.9 (99.6-100)	99.8 <sup>b</sup> (99.8-99.9)
SNAP	91.1 <sup>a</sup> (82.7-96.9)	98.8 <sup>ab</sup> (95.7-100)
VetChek	94.4 <sup>a</sup> (86.4-99.1)	95.7 <sup>a</sup> (90.5-98.9)
ZnSO <sub>4</sub>	92.9 <sup>a</sup> (83.3-98.6)	98.5 <sup>ab</sup> (95.5-99.9)

The sensitivity and specificity of each test in cats with 95% confidence intervals (CI) estimated by Bayesian analysis. Within columns different letters are significantly different (McNemar's  $P < .05$ ). Cells without superscripts had no significant differences.

Abbreviations: IFA, MERIFLUOR *Cryptosporidium*/*Giardia* direct immunofluorescent assay; SNAP, IDEXX *Giardia* Antigen Test; Vet Chek, TECHLAB VETCHEK ELISA; VetScan, Abaxis *Giardia* Antigen test; ZnSO<sub>4</sub>, zinc sulfate centrifugal fecal flotation.

flotation and SNAP were similar (92.9% and 91.1%, respectively) as were their specificities (98.5% and 98.8%, respectively). The sensitivity of the VetChek was 94.4%, and specificity was 95.7%. Similar to its performance in dogs, the SNAP (98.8%) followed the IFA in specificity.

## 4 | DISCUSSION

### 4.1 | Importance of results

This study evaluated diagnostic tests for *G. duodenalis* detection in dogs and cats using both comparison to a reference test and the Bayesian methodology for data analysis. The Bayesian analysis in this study correlated well with the direct comparison to the IFA, which provides support for previous studies comparing results to the IFA. Overall, when the diagnostic tests were compared to the IFA as the reference test in both cats and dogs, no single test stood out as an obvious best diagnostic, as all of the tests had relatively high sensitivities and specificities. Although there were significant differences in sensitivity between the in-clinic immunoassays (SNAP and VetScan) when compared to the IFA in dogs, this difference was no longer significant when the antigen test results were combined with the ZnSO<sub>4</sub> flotation results, and it was not statistically significant on the Bayesian analysis either. This data strongly supports the recommendation to use centrifugal fecal flotation in conjunction with an immunoassay for diagnosing *G. duodenalis* infections in veterinary practices. Compared to other diagnostic evaluations in dogs, our study had a much higher ZnSO<sub>4</sub> flotation sensitivity.<sup>4,10,11</sup> However, because we screened samples using ZnSO<sub>4</sub> and included positives with a matching negative, this could have increased the ZnSO<sub>4</sub> sensitivity in this study compared to other studies. Additionally, positive and negative predictive values are not displayed for this study as these variables are reliant on the prevalence of the disease being tested for and can vary greatly among populations. This study had an artificially high prevalence of *G. duodenalis* as we selected positive samples for inclusion in the study to allow for a more robust comparison.

This study also confirms the value of the performing more than 1 test when *G. duodenalis* infection is suspected. There were several

samples with discordant results (Table 2). In particular, there were 5 samples (4 canine and 1 feline) positive by ZnSO<sub>4</sub> fecal flotation but negative on the IFA and all other immunoassays, and an additional 2 samples (both canine) that were positive on the ZnSO<sub>4</sub> fecal flotation and the VetChek but negative on the IFA, SNAP, and VetScan. There were also 4 samples (all canine) that were negative on the IFA but positive on all other diagnostic tests. It seems likely that for at least some of these samples, the IFA results are false negatives. However, it is also possible that the refrigeration, storage, and shipping of samples between the time of fecal flotation and the performance of the immunoassays contributed to some of the discrepancies between the positive ZnSO<sub>4</sub> fecal flotation and negative immunoassay results, particularly in samples with low cyst numbers. These discordant results demonstrate the difficulty in determining which diagnostic test is most accurate when comparing diagnostic tests without a true gold standard for detecting *G. duodenalis*.

### 4.2 | Comparison to IFA

VetChek, the most recently licensed immunoassay included in the study, was the most sensitive test in dogs when compared to IFA. When compared to IFA, the sensitivity of the SNAP test was determined to be 92.5% in cats and 89.8% in dogs, which is lower than reported on the package insert (95%) but is similar to other reports.<sup>4,10,11</sup>

### 4.3 | Bayesian analysis

Sensitivities and specificities for the IFA, ZnSO<sub>4</sub>, and SNAP were higher in the Bayesian analysis than have been reported by others.<sup>4,10,11</sup> Sensitivity for the SNAP test was 92.2%, which is much higher than the reported sensitivity (52% and 67%) in a previous analysis.<sup>10</sup> The estimated sensitivity of the ZnSO<sub>4</sub> from the Bayesian analysis was also much higher in this study (90.95%) compared to others (34% and 65%).<sup>10</sup> A possible explanation for these differences is that more noninformative priors were used in previous studies,<sup>10</sup> which has minimal impact on the variables in question when building a model. We were able to utilize prior information from the previous studies,<sup>4,10,11</sup> which could have resulted in a more rigorous analysis in the present study.

Other studies have also found the IFA to be the most sensitive test when comparing tests using a Bayesian analysis.<sup>10,17</sup> This underscores the high performance of the IFA and provides more evidence to support the use of IFA as the de facto reference test even though it is not a true gold standard with potential for false negatives/positives.

### 4.4 | Practical application of results

When evaluating diagnostic tests, it is important to consider the test purpose. The SNAP and VetScan are both rapid in-clinic tests that require no additional equipment. Although the ZnSO<sub>4</sub> centrifugal fecal flotation test has a short turnaround time, it does require a centrifuge. Although not designed as an in-clinic rapid test, VetChek performed as well as other currently available diagnostics by both direct comparison to the IFA and Bayesian analysis and can be considered a sensitive

and specific test for *G. duodenalis* detection. The IFA requires the most specialized equipment and training and is not available in the veterinary practice.

In conclusion, there are now several highly sensitive and specific antigen tests that are optimized for detecting *G. duodenalis* in companion animals. However, only centrifugal fecal flotation has the ability to detect other parasites that could be present, and a combination of both immunoassay and microscopic techniques provides the most sensitive procedure for detection of infection with *G. duodenalis* and other internal parasites.

## ACKNOWLEDGMENTS

This study was supported by funds from TechLab, Inc, Blacksburg, VA. Portions of this work were presented by Meriam Saleh at the 60th Annual Meeting of the American Association of Veterinary Parasitologists in Boston, MA, July 10-14, 2014, and the 64th Annual Meeting of the American Association of Veterinary Parasitologists in Denver CO July 14-17, 2018.

## CONFLICT OF INTEREST DECLARATION

Anne M. Zajac has received honoraria from IDEXX and research support from TechLab, Inc. Meriam N. Saleh received travel funds and partial stipend support from TechLab, Inc. David S. Lindsay has received research support from IDEXX. Joel F. Herbein is currently an employee of TechLab, Inc, and at the time the research was conducted, Jack R. Heptinstall was employed by TechLab, Inc.

## OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

## INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Authors declare no IACUC or other approval was needed. Fecal samples were collected by animal owners or environments without animal handling.

## HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

## ORCID

Meriam N. Saleh  <https://orcid.org/0000-0003-1397-8828>

## REFERENCES

1. Ballweber LR, Xiao LH, Bowman DD, Kahn G, Cama VA. Giardiasis in dogs and cats: update on epidemiology and public health significance. *Trends Parasitol.* 2010;26:180-189.

2. Leib MS, Zajac AM. Giardiasis in dogs and cats. *Vet Med.* 1999;94:793.
3. Thompson RCA, Palmer CS, O'Handley R. The public health and clinical significance of *Giardia* and *Cryptosporidium* in domestic animals. *Vet J.* 2008;177:18-25.
4. Rishniw M, Liotta J, Bellosa M, Bowman D, Simpson KW. Comparison of 4 *Giardia* diagnostic tests in diagnosis of naturally acquired canine chronic subclinical giardiasis. *J Vet Intern Med.* 2010;24:293-297.
5. Bowman DD. *Georgis' Parasitology for Veterinarians.* 10th ed. St. Louis, MO: Elsevier; 2014.
6. Zimmer JF, Burrington DB. Comparison of four protocols for the treatment of canine giardiasis. *J Am Anim Hosp Assoc.* 1986;22:168-172.
7. Kirkpatrick CE, Farrell JP. Feline giardiasis: observations on natural and induced infections. *Am J Vet Res.* 1984;45:2182-2188.
8. Barr SC, Bowman DD. Giardiasis in dogs and cats. *Comp Cont Educ Pract Vet.* 1994;16:603.
9. Alles AJ, Waldron MA, Sierra LS, Mattia AR. Prospective comparison of direct immunofluorescence and conventional staining methods for detection of *Giardia* and *Cryptosporidium* spp. in human fecal specimens. *J Clin Microbiol.* 1995;33:1632-1634.
10. Geurden T, Berkvens D, Casaert S, Vercruyse J, Claerebout E. A Bayesian evaluation of three diagnostic assays for the detection of *Giardia duodenalis* in symptomatic and asymptomatic dogs. *Vet Parasitol.* 2008;157:14-20.
11. Mekaru SR, Marks SL, Felley AJ, Chouicha N, Kass PH. Comparison of direct immunofluorescence, immunoassays, and fecal flotation for detection of *Cryptosporidium* spp. and *Giardia* spp. in naturally exposed cats in 4 northern California animal shelters. *J Vet Intern Med.* 2007;21:959-965.
12. Rimhanen-Finne R, Enemark HL, Kolehmainen J, Toropainen P, Hänninen ML. Evaluation of immunofluorescence microscopy and enzyme-linked immunosorbent assay in detection of *Cryptosporidium* and *Giardia* infections in asymptomatic dogs. *Vet Parasitol.* 2007;145:345-348.
13. Dryden MW, Payne PA, Smith V. Accurate diagnosis of *Giardia* spp and proper fecal examination procedures. *Vet Ther.* 2006;7:4-14.
14. Dohoo IR, Martin SW, Stryhn H. *Methods in Epidemiologic Research.* Charlottetown, Canada: VER Inc; 2012.
15. Papini R, Carreras G, Marangi M, Mancianti F, Giangaspero A. Use of a commercial enzyme-linked immunosorbent assay for rapid detection of *Giardia duodenalis* in dog stools in the environment: a Bayesian evaluation. *J Vet Diagn Invest.* 2013;25:418-422.
16. Tangtrongsup S, Scorza V. Update on the diagnosis and management of *Giardia* spp infections in dogs and cats. *Top Companion Anim Med.* 2010;25:155-162.
17. Geurden T, Levecke B, Pohle H, de Wilde N, Vercruyse J, Claerebout E. A Bayesian evaluation of two dip-stick assays for the on-site diagnosis of infection in calves suspected of clinical giardiasis. *Vet Parasitol.* 2010; 172:337-340.
18. Zajac AM, Conboy GA. *Veterinary Clinical Parasitology.* 8th ed. Chichester, West Sussex, England: John Wiley & Sons Inc; 2012:4-8.
19. Adedokun OA, Burgess WD. Analysis of paired dichotomous data: a gentle introduction to the McNemar test in SPSS. *J Multidiscip Eval.* 2012;8(17):2011.
20. Joseph L, Gyorkos TW. Inferences for likelihood ratios in the absence of a "gold standard". *Med Decis Making.* 1996;16:412-417.
21. Joseph L, Gyorkos TW, Coupal L. Bayesian estimation of disease prevalence and the parameters of diagnostic tests in the absence of a gold standard. *Am J Epidemiol.* 1995;141:263-272.

**How to cite this article:** Saleh MN, Heptinstall JR, Johnson EM, et al. Comparison of diagnostic techniques for detection of *Giardia duodenalis* in dogs and cats. *J Vet Intern Med.* 2019;33:1272-1277. <https://doi.org/10.1111/jvim.15491>