PAPER

Diagnostic accuracy of two point-of-care kits for the diagnosis of *Giardia* species infection in dogs

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Objectives: The objective of this study was to compare results obtained by $ZnSO_4$ Flotation and SNAP® Giardia to those generated by the new point-of-care tests Single and Triple Rapid.

Methods: Prospective study evaluating 51 canine faecal samples submitted at a reference laboratory for the presence of *Giardia* spp. Kappa statistics, specificity, sensitivity, positive predictive value (PPV) and negative predictive value (NPV) were calculated by comparing the new tests to the combined results of $ZnSO_4$ and SNAP tests.

Results: There was fair (Single Rapid, κ=0·434) to good (Triple Rapid, κ=0·797) agreement with the reference tests. At this study's prevalence (59 to 61%), specificities and PPV were high (1·00) with both Rapid tests, but sensitivities and NPV were lower for the Single than for the Triple (0·48 *vs* 0·83 and 0·55 *vs* 0·80) tests. At lower prevalence rates, both tests exhibited a high PPV (1·00), but the NPV were higher with the Triple (0·96 to 0·99) than the Single (0·88 to 0·96) Rapid test.

Clinical Significance: Both tests exhibited excellent PPV values at all prevalence rates but an excellent NPV only at low prevalence. As the prevalence is likely to be low (<15%) in clinical settings, we propose that these tests may be helpful in the in-house diagnosis of *Giardia* spp infection. However, they exhibit lower sensitivity than the combined sensitivity of $ZnSO_4$ and SNAP tests, particularly in high prevalence settings.

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INTRODUCTION

Giardia spp is a Protozoan parasite capable of causing clinical and subclinical infection in various species including dogs, cats and humans (Ballweber *et al.* 2010). In dogs, infections are more common in young animals (less than a year old) with small intestinal diarrhoea as the main clinical sign (Gates & Nolan 2009, Epe *et al.* 2010). Infection prevalence rates have been reported to range from 2·3% to 74.3%, with the higher rates being found in sheltered animals (Papini *et al.* 2005, Guest *et al.* 2007, Batchelor *et al.* 2008, Tzannes *et al.* 2008, Gates & Nolan 2009, Epe *et al.* 2010, Olson *et al.* 2010, Upjohn *et al.* 2010).

Detection of *Giardia* spp is not only important for the management of clinical disease but also for limiting environmental contamination with cysts and preventing zoonosis. Various laboratory-based methods such as zinc sulphate flotation ($ZnSO_4$) (Decock *et al.* 2003, Rishniw *et al.* 2010), direct immunofluorescence (Rimhanen-Finne *et al.* 2007, Geurden *et al.* 2008), enzyme-linked immunosorbance (ELISA) (Decock *et al.* 2003, Cirak & Bauer 2004, Papini *et al.* 2005, Rimhanen-Finne *et al.* 2007, Rishniw *et al.* 2010, Papini *et al.* 2013) and polymerase chain reaction (PCR) (Rimhanen-Finne *et al.* 2007, Sotiriadou *et al.* 2013) are available for the detection of *Giardia* spp in faecal samples.

In recent years, various point-of-care kits have also been developed using immunochromatography (FASTest®, ImmunoCard STAT!®, X/pect®) or ELISA (SNAP®) for the in-clinic detection of *Giardia* spp, and various studies have evaluated their diagnostic performance in dogs and cats (Gundłach *et al.* 2005, Dryden *et al.* 2006, Mekaru *et al.* 2007, Geurden *et al.* 2008, Olson *et al.* 2010, Rishniw *et al.* 2010). Of these kits, the SNAP® test is

commonly used in studies investigating the prevalence of *Giardia* spp infection in dogs (Carlin *et al.* 2006, Epe *et al.* 2010, Olson *et al.* 2010, Becker *et al.* 2012).

Recently, a new point-of-care immunochromatography test (Anigen Rapid Ag Test, BioNote, Korea) has become commercially available for the detection of *Giardia* spp in canine faecal samples. However, there are no published studies in the veterinary literature using this test in clinical practice. There is a need to evaluate the diagnostic performance of new coproantigenic detection tests as there are studies showing that assays that can be used for the detection of *Giardia* spp in human samples do not detect the presence of cysts in animal samples (Mekaru *et al.* 2007, Gow *et al.* 2009) and studies that demonstrate the lack of reactivity when the same tests are used in different animal species (Wilson & Hankenson 2010).

The aim of this study was to compare the results obtained by a cyst detection method (Faecal $ZnSO_4$ Flotation Coproscopy) and the SNAP® Giardia antigen test (Idexx Laboratories Inc, Netherlands) to those generated by the Single Anigen Rapid Giardia Ag and the Triple Anigen Rapid CPV-CCV-Giardia Ag (BioNote) point-of-care tests.

MATERIALS AND METHODS

Tests were performed on 51 canine faecal samples submitted to the Diagnostic Laboratories for coproanalysis (Faecal $ZnSO_4$ Flotation Coproscopy and faecal culture) between November 2009 and July 2010. A spare sample on each of these submissions was analysed on the same day by experienced microbiology technicians using the three coproantigenic detection tests. The tests were performed sequentially ($ZnSO_4$ first and then SNAP, Single Rapid and Triple Rapid) by the same technician. As the $ZnSO_4$ is part of the requested coproanalysis, the technician was aware of the results of this test prior to performing the remaining tests, which were done sequentially but started at similar times (no prior knowledge of results). This procedure allowed for a selection of some samples which were positive by the $ZnSO_4$ as initially submitted samples were negative by all four tests.

Faecal $ZnSO_4$ flotation coproscopy ($ZnSO_4$)

The $ZnSO_4$ was performed as described previously (Dryden *et al.* 2006, Gow *et al.* 2009). Briefly, 2 to 3 grams of faeces were mixed with 10 mL of distilled water, strained through a 0·15 mm mesh sieve and centrifuged at 2000 rpm for 1 minute. The supernatant was discarded and the pellet re-suspended in a $ZnSO_4$ solution (specific gravity of 1·18) and centrifuged at 2000 rpm for 1 minute. A 10 µL-inoculating loop was used to transfer a small portion of the surface of positive meniscus of the faecal suspension to a clean slide for microscopy examination (×400 magnification).

Inexperienced observers performing the $ZnSO_4$ method have been reported to increase the numbers of false negatives (Dryden *et al.* 2006). However, in the present study, all slides were examined by two experienced technicians, and the test was considered positive (or negative) when both observers reported the presence (or absence) of one or more *Giardia* spp cysts.

SNAP® Giardia test (SNAP)

The test was performed according to manufacturer's instructions. In brief, the swab provided with the assay was coated with a thin layer of faecal material, inserted into a reagent tube and mixed with the conjugate solution. This solution was then transferred to the SNAP testing device and allowed to flow across the test area of the device. The test was considered positive if any blue colour developed in the test area and the internal control spot was also positive at five minutes. A result was recorded as "weak positive" when the test spot had a faint blue colour.

Anigen rapid Giardia Ag test (single rapid) and anigen rapid CPV-CCV-Giardia Ag test (triple rapid)

The Triple Rapid test is identical to the Single Rapid Giardia test, but the device also includes two additional sample and test reaction areas – one sample and reaction area for the detection of canine parvovirus (CPV) and another sample and reaction area for the detection of canine coronavirus (CCV) antigen. In the present study, only the *Giardia* species area of the Triple Rapid kit was assessed.

The Giardia test on the Single and Triple Rapid kits was performed following an identical methodology and according to the manufacturer's instructions. Briefly, the swab provided was coated with a thin layer of faecal sample and mixed with 1 mL of the assay diluent. Four drops of the mixture were then placed on the sample area using the disposable pipette supplied with the kit, and the material was allowed to flow across the device. Results were read after 5 and 10 minutes as recommended by the manufacturer. A test was considered positive when red bands developed both in the test area and the control area. A result was recorded as a "weak positive" when the test band had a faint red colour.

Statistical analysis

Results were recorded in a spread sheet (Microsoft Excel, Washington, USA), and a statistical package (GraphPad Prism, GraphPad Software, La Jolla, USA) or methods available at VassarStats:Website for Statistical Computation (http://vassarstats.net/ assessed October 2011 and July 2015) were used for statistical analysis.

Laboratory test results were compared for overall agreement using Cohen's Unweighted Kappa. Sensitivity, specificity, positive and negative predictive values for the Rapid tests were determined by considering the combined $ZnSO_4$ and SNAP test results as the "reference method;" a sample with a positive $ZnSO_4$ and/or SNAP test result was considered a true positive. The results of all tests were used to estimate the prevalence rate of disease in the present study. Predictive values for each of the Rapid tests were also estimated at the prevalence rates of 7, 15 and 20% using the clinical calculator 2 available at the VassarStats: Website for Statistical Computation. These rates were selected as they have been previously described in clinical studies reporting the prevalence of *Giardia* spp infection in dogs in the UK (Guest *et al.* 2007, Batchelor *et al.* 2008, Epe *et al.* 2010, Upjohn *et al.* 2010).

RESULTS

The submitted faecal samples were collected from dogs with ages ranging from 8 weeks to 13 years. There were 28 samples from female dogs (three of which neutered) and 23 from male dogs (two of which neutered). The presence of diarrhoea was recorded on 41 of the 51 accompanied submission forms. No clinical information was available on the remaining forms.

When tested with the three point-of-care tests, all samples generated a valid result. The Triple Rapid test was not performed in two cases due to insufficient sample volume. The SNAP, Single Rapid and Triple Rapid tests generated four (7·8%), nine (17·6%) and 15 (29·4%) results that were recorded as "weak positives." In the statistical analysis, all "weak positive" results were considered positives.

Thirty-one samples were defined as true positives (29 positive with $ZnSO_4$ and SNAP, two samples positive only with $ZnSO_4$) and 20 samples were defined as true negatives (all $ZnSO_4$ and SNAP negative).

There was fair agreement between the Single Rapid and the combined $ZnSO_4$/SNAP tests (κ=0·424) and good agreement between the Triple Rapid and the combined $ZnSO_4$/SNAP tests (κ=0·797). Thirty-five of the 51 samples (68·6%) generated concordant results. Of these, 20 (39·2%) were negative and 15 (29·4%) positive (Table 1). Discordant results were obtained from 16 samples (31·4%), and these were all positive with the $ZnSO_4$ and/or SNAP tests. All 16 samples tested negative with the Single Rapid; five were also negative when analysed with the Triple Rapid; and in two of these samples, there was insufficient sample to perform the Triple Rapid test (Table 1).

When the high prevalence rate of the present study (59 to 60%) was taken into account, the specificities and PPV (positive predictive value) were high (Sp/PPV: 1) with both Rapid tests, but the sensitivity and NPV (negative predictive value) with the Single Rapid were markedly lower than those obtained with the Triple Rapid test (0·48 *vs* 0·83 and 0·55 *vs* 0·8, respectively) (Table 2). At the pre-selected lower prevalence rates (20, 15, 7%), the PPV for both tests were high (PPV: 1), but the NPV were higher with the Triple (0·96 to 0·99) than the Single Rapid test (0·88 to 0·96) (Table 3).

Table 1. Cross-classified positive and negative test results obtained with each one of the rapid tests

	$ZnSO_4$ and/or SNAP (n=51)	Single rapid (n=51)	Triple rapid (n=49)
Total positive	31	15	24
Total negative	20	36	25
No of samples	$ZnSO_4$ and/or SNAP	Single rapid	Triple rapid
15	+	+	+
20	–	–	–
9	+	–	+
5	+	–	–
2	+	–	nd

(+) positive result, (–) negative result, nd not done (insufficient sample)

DISCUSSION

To the authors' knowledge, this is the first study investigating the diagnostic performance of the Anigen Rapid point-of-care kits using clinical samples from dogs.

There was a marked difference in the performance of the Single and Triple tests, with the Single generating a lower number of positive results (n=15) than the Triple (n=24), resulting in a higher number of false negatives and a lower *kappa* value as measure of agreement (κ=0·424 *vs* κ=0·797). This difference is difficult to explain considering that both tests are produced by the same manufacturer, employ the same technology and almost certainly use the same antibody for the detection of *Giardia* spp, although this type of information is not specified by the manufacturer and could not be obtained. The Single test has been developed for use in dogs and cats, whereas the Triple test has been designed only for dogs; therefore, we propose that the employment of potentially different antibodies and/or buffers or differences in the limit of detection between the two tests may account for the lower sensitivity of the Single kit (El-Nahas *et al.* 2013).

As the selection of a "gold standard" method for the diagnosis of *Giardia* spp infection is not always possible, and cyst shedding is variable in infected animals (Lappin 2005, Vasilopulos & Mackin 2006), the clinical evaluation of new diagnostic tests using traditional contingency table analysis is difficult. Bayesian statistical approaches have been employed as a solution to the lack of a "gold standard" (Geurden *et al.* 2008, Papini *et al.* 2013), but these demand a high number of samples, which are frequently difficult to obtain in clinical settings. In the present study, both the $ZnSO_4$ and SNAP were selected as reference methods because they are commonly used tests by many veterinary diagnostic laboratories, have been included in numerous studies evaluating the diagnostic performance of novel assays and have been employed in various large-scale *Giardia* spp prevalence studies (Gundlach *et al.* 2005, Carlin *et al.* 2006, Dryden *et al.* 2006, Geurden *et al.* 2008, Epe *et al.* 2010, Rishniw *et al.* 2010, Becker *et al.* 2012). In addition, various studies have reported high specificities for both $ZnSO_4$ (Sp: 0·94 to 0·99) and SNAP (Sp: 0·92 to 1), although sensitivities have been reported to be reasonable but lower for

Table 2. Diagnostic performance of the rapid tests with the combined $ZnSO_4$ and snap as the "gold standard"

Test	Sensitivity (95% CI)	Specificity (95% CI)	Study estimated prevalence (%)	PPV (95% CI)	NPV (95% CI)
Single rapid	**0·48** (0·31 to 0·66)	**1** (0·80 to 1)	**61**	**1** (0·75 to 1)	**0·55** (0·38 to 0·71)
Triple rapid	**0·83** (0·64 to 0·93)	**1** (0·80 to 1)	**59**	**1** (0·83 to 1)	**0·80** (0·59 to 0·92)

CI confidence intervals, PPV positive predictive value, NPV negative predictive value

Table 3. Estimated predictive values of the rapid tests at different prevalence rates and with the combined $ZnSO_4$ and snap as the "gold standard"

Test	20% Prevalence		15% Prevalence		7% Prevalence	
	PPV	NPV	PPV	NPV	PPV	NPV
Single rapid	1	0·88	1	0·92	1	0·96
Triple rapid	1	0·96	1	0·97	1	0·99

PPV positive predictive value, NPV negative predictive value

both methods (Se: 0·65 to 0·85) (Mekaru *et al.* 2007, Geurden *et al.* 2008, Rishniw *et al.* 2010). However, it has been shown that when the SNAP is used in parallel with $ZnSO_4$, and the generated results are compared to those obtained by employing direct immunofluorescence as the "gold standard," the combined sensitivity improves to such a degree (from 0·85 to 0·98) that the SNAP becomes particularly useful in an in-hospital setting (Mekaru *et al.* 2007). For this reason, in the present study, we defined as true positive a result that was positive by the SNAP and/or $ZnSO_4$.

In the U.K., *Giardia* spp infection rates in dogs have been reported to vary from 8·4 % in symptomatic animals (Batchelor *et al.* 2008) to 21% in dogs admitted to a London rescue shelter (Upjohn *et al.* 2010). In addition, a recent European study on the presence of *Giardia* spp in samples collected from symptomatic dogs reported a prevalence in the UK of 14·6% (Epe *et al.* 2010). In the present study, approximately 50% of the samples were selected to be positive for *Giardia* spp. Although this intentional bias allowed an adequate evaluation of the diagnostic performance of the new Rapid point-of-care kits, it resulted in an artificially higher prevalence to those reported previously. Due to this bias, PPV and NPV were also estimated for different prevalence rates as recently suggested for the evaluation of new diagnostic tests (Erb 2011). The values of 7, 15 and 20% were selected in order to reflect an approximate range of the published prevalence rates for the *Giardia* spp in the U.K. (Batchelor *et al.* 2008, Epe *et al.* 2010, Upjohn *et al.* 2010). In the current study, the PPV for both Rapid tests were high at all prevalence rates, indicating that a positive result generated by the Single or Triple test is almost certainly a true positive. Conversely, the NPV were lower at the high prevalence rate but improved markedly almost to 100% as the prevalence decreased, especially when the samples were analysed with the Triple Rapid test. This means that the likelihood of a false negative is higher in the high prevalence setting. However, if the prior probability of infection is low, then a negative result is almost certainly a true negative, and therefore, either Rapid test will have a reasonable chance of ruling out *Giardia* species as the cause of the clinical signs.

There are some limitations to our study. The number of faecal samples analysed is relatively low, but financial constraints precluded the inclusion of more samples. Although the laboratory's current recommendation for parasitological testing is the submission of pooled samples collected over three days, we cannot assure that all samples included in this study were pooled as this information was not recorded on the submission form. For this reason, it is possible that some animals infected with *Giardia* spp might have been missed by the reference tests ($ZnSO_4$ and SNAP) as it has been shown that shedding of cysts is intermittent, and multiday faecal examinations are required. However, we believe that combining the $ZnSO_4$ and SNAP examinations for the detection of positive samples has minimised false negatives, as it has been shown that the combined sensitivity of the two methods (Se: 0·98; Mekaru *et al.* 2007) is similar to that for direct immunofluorescence (Se: 0·91; Geurden *et al.* 2008), which has been adopted as the gold standard in some studies (Rimhanen-Finne *et al.* 2007, Rishniw *et al.* 2010). Another limitation resides in the fact that we used spare samples from routine coproanalysis. Although this has allowed us to select a higher number of positive samples, it has lead to a partial lack of blinding to the previous results. However, blindness for the results of all coproantigenic detection tests was maintained.

We conclude that the Single and Triple Rapid kits exhibit an excellent PPV value at all prevalence rates but an excellent NPV only at low prevalence rates (15 and 7%). Narrowing the prior probability of giardiasis based solely on clinical history and physical examination is not possible. However, based on the published studies, it is reasonable to assume that in most clinical settings in the UK, the prevalence is most likely to be low (<15%) (Guest *et al.* 2007, Batchelor *et al.* 2008, Epe *et al.* 2010). Therefore, we propose that the Single and Triple Rapid kits may be helpful in the in-house diagnosis of *Giardia* species infection in the U.K. However, in high prevalence settings or where clinical suspicion is high, and a negative Single or Triple rapid result is obtained, a *Giardia* species infection cannot be ruled out as these tests, particularly the Single Rapid, exhibited lower sensitivity when compared to the combined results of the $ZnSO_4$ and SNAP tests.

Conflict of interest

None of the authors of this article has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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