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Rapid on-site diagnosis of canine giardiosis: time *versus* performance

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Abstract

Background: Infections by protozoans of the genus *Giardia* are a common cause of diarrhea in dogs. Canine giardiosis constitutes a disease with a zoonotic potential; however, it is often underestimated due to its challenging diagnosis. The objective of the study was to assess the diagnostic performance of an immunochromatographic strip test (Speed™ *Giardia*, Virbac, France) comparing it with microscopy (zinc sulfate flotation) by utilizing the combination of an enzyme immunoassay (ProSpecT™ *Giardia* EZ Microplate Assay, Oxoid Ltd., UK) and the PCR as the gold standard. A positive result in both ELISA and PCR was set as the gold standard.

Methods: Initially, fecal samples from dogs with clinical signs compatible with giardiosis were tested with the Speed™ *Giardia* test and separated into two groups of 50 samples each: group A (positive) and group B (negative). Thereafter, all samples were examined by zinc sulfate centrifugal flotation technique and assayed by the ProSpecT™ *Giardia* Microplate Assay and PCR. The performance of the Speed™ *Giardia* and zinc sulfate centrifugal flotation tests were calculated estimating sensitivity, specificity, and positive and negative likelihood ratio; the chi-square and McNemar tests were used for the comparison of the two methods.

Results: *Giardia* cysts were not detected by microscopy in 16 out of the 50 samples (32%) of group A and in none of group B samples. Eight out of 50 samples in group B (16%) were tested positive both with the ProSpecT™ *Giardia* Microplate Assay and PCR. Fecal examination with the Speed™ *Giardia* test was more sensitive (86.2%) than the parasitological method (58.6%, $P < 0.001$) while the specificity of both methods was 100%.

Conclusions: The Speed™ *Giardia* test is an easy-to-perform diagnostic method for the detection of *Giardia* spp., which can increase laboratory efficiency by reducing time and cost and decrease underdiagnosis of *Giardia* spp. infections. This immunochromatographic strip test may be routinely exploited when a rapid and reliable diagnosis is required, other diagnostic techniques are unavailable and microscopy expertise is inefficient. In negative dogs with compatible clinical signs of giardiosis, it is recommended either to repeat the exam or proceed with further ELISA and PCR testing.

Keywords: *Giardia* spp., Dogs, Diagnosis, Speed™ *Giardia*, Microscopy, PCR

Background

Giardia spp. are flagellated protozoans that colonize the duodenum of many mammals, including dogs and humans [1]. Among the several species of *Giardia*, only *Giardia duodenalis* (syn. *G. lamblia*, *G. intestinalis*) has been recovered from both the aforementioned mammals, hence being regarded as potentially zoonotic [2–5]. The transmission chain includes the defecation of *Giardia* spp. cysts, which are infective immediately after excretion, the contamination of feeds and water and the infection *via* the fecal-oral route [6]. *Giardia* spp. infections

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may impair dogs' health and welfare resulting in a wide spectrum of clinical manifestations, such as diarrhea, bloating, abdominal discomfort, weight loss, malabsorption, growth retardation and sometimes even death, especially in puppies [7–9]. The occurrence of disease depends on many factors, such as the *Giardia* strain virulence, the parasite burden, the age and immunity of the host, whereas many animals remain sub-clinically infected and act as reservoirs of the parasite.

Giardia duodenalis contains at least eight distinct genetic assemblages (A-H) as demonstrated by molecular typing methods [10, 11]. Assemblages A and B have been reported in many mammals, including humans [12–15] and dogs [16, 17] and have a zoonotic potential which renders them of great public health concern [9, 18–20]. Currently, there is cumulative evidence that dogs act as a source of contamination for humans and pose a risk especially for pet owners and shelter staff [21, 22]. Also, giardiosis outbreaks due to contaminated drinking water and food have been reported [23–26].

Giardiosis is a common parasitosis even in the well-cared dog populations [27]. Relatively high prevalence of canine *Giardia* infection has been reported in many European countries by employing microscopy (28.5% for Belgium, 27.5% for France, 25.9% for Italy, 25.1% for Spain, 24.6% for the Netherlands, 23.8% for Germany, and 14.6% for the UK) [28]. In a recent large-scale study conducted in Greece, *Giardia* spp. were detected by microscopy following zinc sulfate flotation in 9.5% of the sampled dogs with no apparent clinical signs [29]. Studies employing immunological and molecular assays have reported much higher prevalence than microscopy [28], indicating that *Giardia* spp. infections may remain underdiagnosed when microscopy is the selected exam. In any case, the infection level is considerably higher in young animals [30], while it is up to 100% in dogs living in kennels or shelters [31], due to overcrowding and inadequate hygiene conditions [32].

The well-documented pathogenicity of *Giardia* spp. both for humans and animals, coupled with the high prevalence of giardiosis underpin the demand for universally accepted diagnostic tests and protocols for the early and accurate diagnosis of this disease. Therefore, the development of valid and cost-effective assays is essential for the surveillance of the disease and the evidence-based planning for its control (preventive measures and treatment) [33].

Nowadays, a variety of diagnostic methods for canine giardiosis are available. Among them, microscopy following zinc sulfate flotation, and immunoassay methods such as the direct fluorescent antibody (DFA) tests, which detect intact parasites [34], and the immunofluorescence antibody (IFA) microscopy, which detects epitopes of cysts [35], are commonly used. Other immunoassays include the enzyme-linked immunosorbent assays (ELISA) and the immunochromatographic lateral-flow tests, also known as rapid diagnostic tests (RDT), and detect soluble coproantigens of the parasite [36–39]. RDT are qualitative, commercially available enzyme immunoassays, which have become popular diagnostic tools for practitioners [36, 38]. Finally, molecular techniques such as the polymerase chain reaction (PCR) have also been developed for the detection of *Giardia* spp. [6, 40, 41]. All the aforementioned methods have both advantages and limitations and the selection of the suitable diagnostic tests in practice is mostly dependent on their performance, the availability of laboratory infrastructures and equipment, the personnel expertise, as well as their quickness and cost-effectiveness [42, 43].

The aim of this study was to evaluate the performance of the Speed^{TM} *Giardia* test (Virbac, Carros, France), a rapid immunochromatographic lateral-flow test for the detection of *Giardia* spp. in canine fecal samples and to compare it with microscopy, using the combination of enzyme immunoassay ProSpecT^{TM} *Giardia* Microplate Assay (Oxoid Ltd., Hampshire, UK) and PCR as gold standard.

Methods

Dog population and fecal sample collection

A total of 100 dogs with diarrhea (the main clinical sign of giardiosis) were included in the study. Canine fecal samples were collected from local animal shelters and veterinary clinics in northern Greece, between February and June 2018. Sex was almost evenly distributed (52 male and 48 female dogs) while all dogs were older than 6 months. None of the examined animals received any antiparasitic treatment at least 3 months prior to inclusion. From each individual dog, a fecal sample was collected either immediately after spontaneous elimination or fresh from kennel grounds avoiding contamination. Samples were placed individually in plastic containers, labelled with consecutive numbers, stored at 2–6 °C, transferred to the Laboratory of Parasitology and Parasitic Diseases of the School of Veterinary Medicine, Thessaloniki, Greece, and processed within 1 day.

Rapid diagnostic test (RDT)

Initially, the Speed^{TM} *Giardia* assay was performed according to the manufacturer's instructions. In brief, one spoonful of each labelled fecal sample was added to the buffer diluent in a corresponding vial, which was closed and shaken to homogenize. The solution was allowed to sediment for 3 min. The strip was gently plunged into the solution in the direction indicated by the arrow and allowed to stand for 1 min, without immersing the central reactive zone in the solution. Thereafter, it was removed and placed on a flat, horizontal surface. The liquid was left to migrate and the results were read

after 5 min. The test was valid when a blue control band appeared. The test was considered as positive when a red band appeared at the *Giardia* test window along with the blue control band. Any red colouration of the test band regardless of colour gradation was interpreted as a positive result. Two groups of animals emerged, each consisting of 50 dogs. Group A consisted of 50 *Giardia*-positive dogs and group B consisted of 50 *Giardia*-negative dogs.

Microscopy following zinc sulfate flotation

All fecal samples were examined by qualitative flotation with zinc sulphate ($ZnSO_4$ 33.2%, specific weight 1.3) [44–46]. In detail, 1 g of feces was diluted with water, passed through a sieve (No. 150) into a centrifuge tube and centrifuged at 200× *g* for 3 min. The supernatant was discarded and zinc sulphate solution was added to the sediment, which was then completely diluted. Zinc sulphate solution was added to the tube so as to form a crescent and a coverslip was placed on top of it. Following centrifugation at 150× *g* for 1 min, the coverslip was carefully removed and placed on a microscope slide. Microscopic examination was carried out by the same experienced parasitologist. Identification of *Giardia* spp. cysts was based on morphological characteristics [45, 47]. A dog was considered positive if at least one cyst was observed.

ELISA

A copro-antigen ELISA was performed using the ProSpecT™ *Giardia* EZ Microplate Assay (Oxoid Ltd.) for all the samples according to the manufacturer's instructions. This immunoassay uses a monoclonal antibody for the qualitative detection of *Giardia* specific antigen 65 (GSA 65) in aqueous extracts of fecal specimens.

DNA extraction

Genomic DNA was extracted directly from all preserved *Giardia*-positive and *Giardia*-negative fecal samples using QIAmp® Fast DNA Stool Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol for isolating DNA for pathogen detection. To maximize cyst lysis, an initial step of three freeze-thaw cycles (heating at 80 °C water bath for 5 min, followed by freezing at -20 °C for 5 min) was incorporated in the protocol as proposed by Tan et al. [1]. The extracted DNA was eluted in 50 µl of elution buffer, and all the eluates were stored at -20 °C until further molecular analyses.

PCR amplification of the *18S* rRNA gene

A region of the *18S* ribosomal RNA gene was amplified by using a forward primer RH11 (5′-CAT CCG GTC GAT CCT GCC-3′) and a reverse primer RH4 (5′-AGT CGA ACC CTG ATT CTC CGC CAG G-3′) as described by Hopkins et al. [40]. The predictive amplification fragment was 292 bp. All PCRs were performed in a total volume of 25 µl containing 4 µl DEPC (diethyl pyrocarbonate) water, 2 µl of each primer RH11/RH4 (50 µmol/l), 2.5 µl One *Taq* high GC enhancer, 12.5 µl One *Taq* 2× master mix with GC Buffer (M0483S; New England BioLabs Inc., Hitchin, UK) and 2 µl DNA template. All reaction components were assembled on ice. The thermocycler program consisted of an initial denaturation of 96 °C for 4 min, followed by a set of 35 cycles, each consisting of 20 s at 96 °C, 20 s for annealing at 59 °C, 30 s at 72 °C, followed by a final extension step at 72 °C for 7 min. Along with the samples a negative control (doubled distilled water) and a positive control (genomic DNA from a fecal sample positive in all other three tests) were tested for each reaction. All amplification products were submitted to 1.5% ethidium bromide-stained agarose gel electrophoresis. The obtained gel images were recorded with a CCD camera under UV light and visualized with the MiniBis Pro gel documentation system (DNR BioImaging systems, Neve Yamin, Israel).

Statistical analyses

Initially, data were recorded in a specially designed Microsoft Excel spreadsheet. In the subsequent statistical analyses, accuracy [(true positive + true negative)/(true positive + true negative + false positive + false negative)], sensitivity [Sn, true positive/(true positive + false negative)], specificity [Sp, true negative/(true negative + false positive)], positive likelihood ratio [LR+, Sn/(1−Sp)] and negative likelihood ratio [LR−, (1−Sn)/Sp] were calculated from the 2 × 2 contingency tables of the studied methods using chi-square test in SPSS 23. McNemar test was used for the comparison between Speed™ *Giardia* test and microscopy. A positive result in both ELISA and PCR was chosen as the gold standard.

Results

Out of the 50 samples of group A (positive with the Speed™ *Giardia* test, Fig. 1a), microscopy confirmed 34 (68%) positive for *Giardia* spp. (Fig. 2). In the remaining 16 (32%) samples of group A *Giardia* cysts were not detected during microscopy. On the contrary, results from the microscopic examination of the 50 samples of group B (negative with Speed™ *Giardia* test, Fig. 1b) were in agreement (100%) with the Speed™ *Giardia* test results. All samples in group A were ELISA-positive, while 15 out of 50 samples (30%) in group B were also ELISA-positive. Eight out of the 15 ELISA-positive samples were also PCR-positive, whereas the remaining 7 were PCR-negative (Table 1).

PCR amplifications of the *18S* rRNA gene of *Giardia* spp. were consistent with the expected size (292 bp), without non-specific bands. Samples from group A were all PCR-positive (Fig. 3a). Twenty-two out of the 50 samples from group B (44%) tested positive by PCR (Fig. 3b). Fourteen out of these 22 samples were ELISA-negative (Table 1).

Sensitivity, specificity, accuracy, positive and negative likelihood ratios for the four diagnostic tests are summarized in Table 2. Fecal examination with the Speed^{TM} *Giardia* assay was more sensitive (86.2%) than microscopy (58.6%, McNemar test, $P < 0.001$) while the specificity of both methods was 100%.

Discussion

The objective of this study was to evaluate the diagnostic performance (sensitivity and specificity) of the Speed^{TM} *Giardia* method by comparing it with microscopy using the combination of ELISA and PCR.

Speed^{TM} *Giardia* assay is a commercially available RDT. This test allows the detection of specific soluble antigens of *Giardia* spp. in preserved canine fecal samples [36, 39]. Up to date, a number of commercial RDT have been developed for the detection of *G. duodenalis* coproantigens in dogs [34, 37] and they have become increasingly popular [38]. Overall, several studies have evaluated the methods for diagnosing canine *Giardia* spp. infections [48–54], but, none of them so far has assessed simultaneously the four tests employed here and compared the Speed^{TM} *Giardia* test with microscopy. In the present study, a positive ELISA and PCR was defined as the gold standard. To set the gold standard, the combination of the two methods was preferred due to the high sensitivity and specificity of both methods and the lack of a validated and universally accepted gold standard for the diagnosis of giardiosis [38, 52, 54]. In detail and according to the international literature, PCR assays exhibit high sensitivity and specificity [52, 55–57], whereas the reported sensitivities and specificities of commercially available ELISA range from 94 to 97% and from 99 to 100%, respectively [37, 58, 59]. ELISA use antibodies for the qualitative detection of *Giardia*-specific antigens in fecal specimens [36, 60]. The ProSpecT^{TM} *Giardia* EZ Microplate Assay was selected as being one of the most reliable ELISA [61, 62]. It is a fast assay, as it requires the fewest washing steps, it provides a more efficient interpretation of the results and also has a very high specificity and positive predictive values (98–100%) as well as the

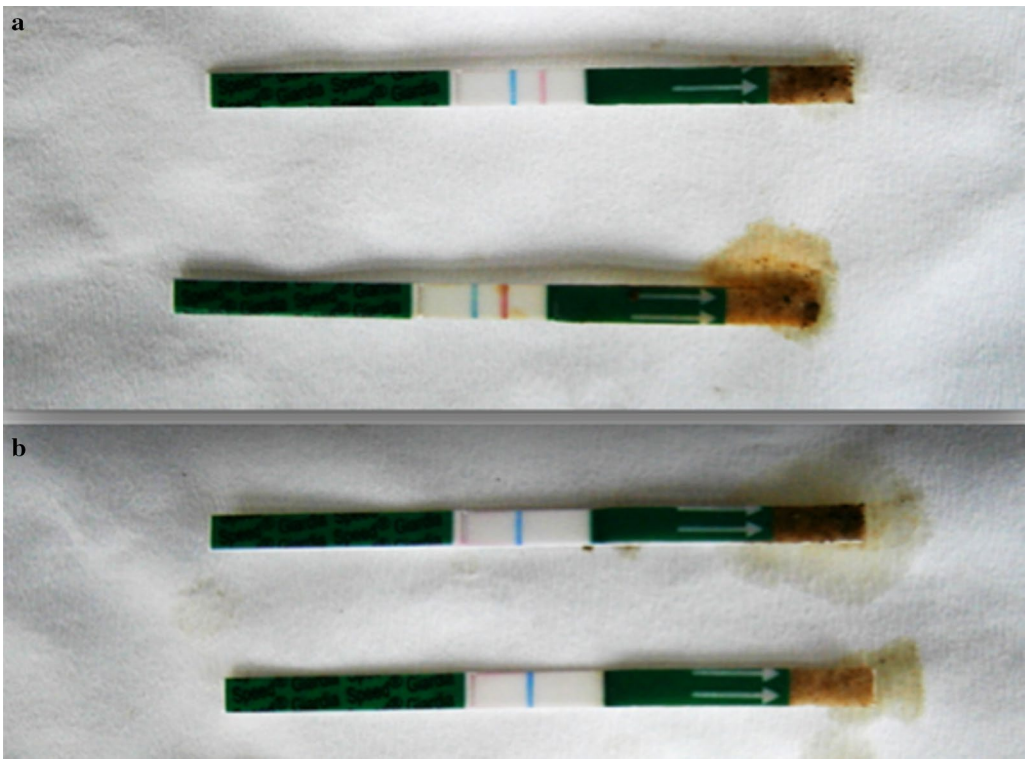

Fig. 1 **a** Samples testing positive with the Speed^{TM} *Giardia* test (blue line: control band, red line: positive samples). **b** Samples testing negative with the Speed^{TM} *Giardia* test (blue line: control band, no other line was detected: negative samples)

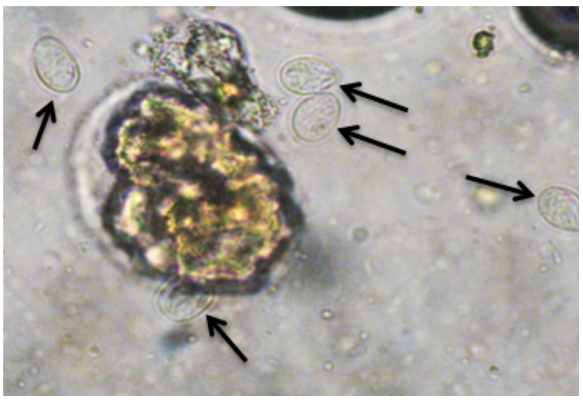

Fig. 2 *Giardia* spp. cysts (arrow) as seen with microscopy (400× magnification) following the zinc sulfate flotation technique

Table 1 2 × 2 contingency tables of the four techniques used for the diagnosis of *Giardia* spp. infection

	ELISA and PCR combined		Total
	Negative	Positive	
Speed™ *Giardia*			
Negative	42	8	50
Positive	0	50	50
Microscopy			
Negative	42	24	66
Positive	0	34	34
ELISA			
Negative	35	0	35
Positive	7	58	65
PCR			
Negative	28	0	28
Positive	14	58	72
Total	42	58	100

highest sensitivity (96–98%) among the other commercially available ELISA [61, 63].

The Speed™ *Giardia* test displayed higher sensitivity (86.2%) compared to microscopy (58.6%, $P < 0.001$), being in accordance with numerous other studies, which have demonstrated that various different commercial RDT were more sensitive than microscopy [38, 50–52, 54, 64–66]. It is indeed expected that tests based on antigen detection, such as the RDT, to be more sensitive than microscopy and to at least approach the PCR results, as highlighted by McGlade et al. [55]. Although microscopy following zinc sulfate flotation for the recovery of *Giardia* spp. cysts is a commonly used method for the diagnosis of giardiosis, it has low sensitivity, as is the case of the present study (58.6%). This can be attributed to the intermittency of excretion of this protozoon in canine feces [67, 68]. Given this excretion pattern of *Giardia* cysts, a single coprological examination could partially explain the occasional inexistence of cysts during microscopy in the present study, as the fecal samples were collected and examined only once. Moreover, a low concentration of shedding cysts (not detectable with microscopy), and/or collapsed cysts (cysts with different density which are not able to float and be identified) may be associated with the low sensitivity recorded for microscopy [69]. Based on the above, it is evident that a single negative result may not definitely determine the presence or absence of *Giardia* spp. in examined dogs and therefore re-examination of fecal samples is necessary to increase the sensitivity of the method [48, 70, 71]. The demand for repeated testing renders microscopy a time-consuming and labor-strenuous method. However, poor performance of microscopy has been reported even in cases where consecutive samples were pooled and tested [66]. This can be justified due to the fact that even at the peak of cyst excretion, the accurate identification of *Giardia* cysts still requires personnel with a high level of expertise. *Giardia* cysts can be easily misdiagnosed because of their small size (8–12 µm × 7–10 µm) and their resemblance to plant remnants, yeasts and debris, which are common in fecal samples [50]. Consequently, the Speed™ *Giardia* test provides an alternative assay to overcome diagnostic challenges in clinical practice. This RDT detects excreted antigens and thus cyst identification is no longer required, overcoming the major drawback of microscopy [72]. According to relevant research findings, it is recommended to use centrifugal fecal flotation in conjunction with an immunoassay for increasing the sensitivity of diagnosing *Giardia* spp. infections in veterinary practices [68, 69].

Nevertheless, in our study eight out of the 50 samples (16%) of group B (negative by the Speed™ *Giardia* assay) were tested positive both with the ProSpecT™ *Giardia* EZ Microplate Assay and PCR. This lower sensitivity of the RDT (86.2%) compared to that of the two methods combined is an expected finding, as the combination of the two methods was considered the gold standard in this experimental design. This is in accordance with other studies where RDT false negative results were observed in cases of low parasitic burdens [73–75]. Low parasite load results in diminished coproantigen production and possible failure of the RDT to detect it [66, 72]. Conclusively, the sensitivity of this method implies that almost one out of six infected by *Giardia* spp. diarrheic dogs might test negative with the Speed™ *Giardia* assay. To overcome it, it could be suggested for the practitioners either to repeat the test (after 48 h) or to test a pool sample from feces collected for two consecutive days

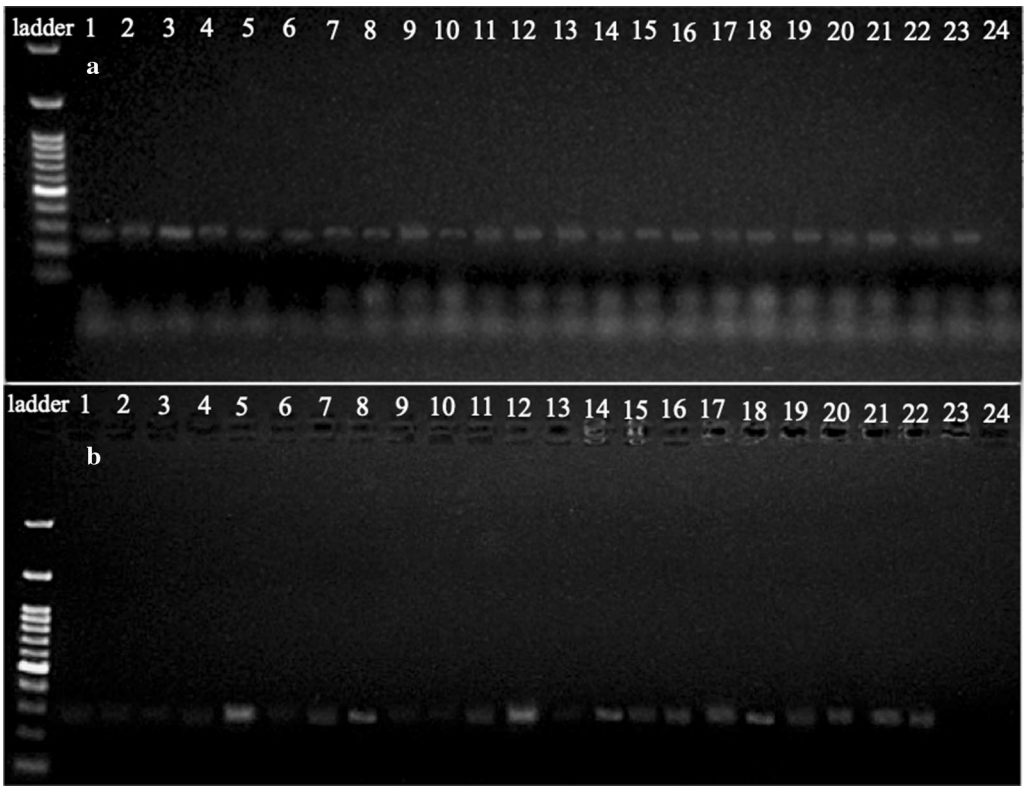

Fig. 3 Positive and negative samples with PCR from the examined groups of dogs. PCR products analysed by electrophoresis on 1.5% agarose gel, stained with ethidium bromide and visualized on a UV transilluminator. The band of 292 bp corresponds to the amplified product of the *18S* rRNA gene. **a** Lane L: DNA ladder; Lanes 1–23: positive samples from group A; Lane 24: control. **b** Lane L: DNA ladder; Lanes 5, 7, 8, 11, 12, 14–22: positive samples from group B; Lanes 1–4, 6, 9, 10, 13, 23: negative samples from group B; Lane 24: control

Table 2 Sensitivity, specificity, accuracy, positive likelihood ratio and negative likelihood ratio of the four studied methods. The combination of ELISA and PCR was used as reference method

Method	Accuracy (%)	Sn (%)	Sp (%)	LR+	LR−
Speed™ *Giardia*	92.0	86.2	100.0	Infinity	0.1
Microscopy	76.0	58.6	100.0	Infinity	0.4
ELISA	93.0	100.0	83.3	6.0	0.00
PCR	86.0	100.0	66.7	3.0	0.00

Abbreviations: Sn, sensitivity; Sp, specificity; LR+, positive likelihood ratio; LR−, negative likelihood ratio

in clinically suspected animals with a negative Speed™ *Giardia* test result and, furthermore, in the case of dogs from the same breeding unit, it is recommended to conduct tests on several animals [63].

All samples of the group A (confirmed positive by the Speed™ *Giardia* test) were tested positive with both the ProSpecT *Giardia* EZ Microplate Assay and PCR, resulting in 100% PPV. The specificity for *Giardia* spp. is also optimal, reaching 100% for RDT, as it has been extensively reported in the literature [64, 74–81] and confirmed by our study.

In the routine diagnostic practice, the veterinarian may face other possible combinations of contradictory results, as was the case in the present study. More precisely, seven specimens of group B were found positive according to ELISA, while at the same time they were negative by PCR. This suggests either an ELISA false positive or a PCR false negative result. In the first assumption, ELISA may indeed give a false positive result in a limited number of cases (2%), due to 98–100% specificity and positive predictive values (98–100%), as registered by the manufacturer.

In the case of the second assumption, PCR false negative results may arise because the DNA yields from feces remain poor [82, 83]. *Giardia* cysts wall is difficult to disrupt and this may lead to insufficient DNA yields, whereas at the same time stool specimens commonly contain compounds such as DNases, proteases, bile salts, and polysaccharides that might cause DNA degradation and inhibition of enzymatic reactions [56, 84]. In this

study the QIAmp® Fast DNA Stool Mini Kit was used for DNA isolation, which provides high inhibitor removal efficiency. Additionally, a pre-treatment of the cysts with three freeze-thaw cycles and extension of the incubation time were incorporated in the protocol to maximize cyst lysis, which according to [85] renders the QIAmp® Fast DNA Stool Mini Kit more sensitive than other conventional extraction methods, i.e. the phenol-chloroform protocol. Another possible reason of failure to amplify *Giardia* spp. is the inhomogeneous distribution of the cysts within a sample [86], or other minor factors, which may be the case in our study. According to Rochelle et al. [87] PCR amplification of the *Giardia 18S* rRNA gene may result false negative due to the unusually high GC content of its sequence. However, the *18S* rRNA gene was selected as a target sequence in our study, because it is represented by high copy numbers (approximately 60 to 130 copies of *G. duodenalis* per nucleus, arranged in tandem repeats) and thus it is considered of higher sensitivity [88, 89]. Furthermore, to overcome the aforementioned GC limitation, the One *Taq*® 2× master mix with GC Buffer (M0483S; New England BioLabs Inc., Hitchin, UK) was used. This optimized blend of *Taq* and Deep Vent™ DNA polymerases has higher fidelity than solely *Taq* and provides robust amplification of GC rich templates.

Another combination of contradictory results that arose was that 14 samples negative according to ELISA resulted positive to PCR. This implies either an ELISA false negative result or a PCR false positive one.

The ProSpecT *Giardia* EZ Microplate Assay has certain performance limitations and a false negative result is likely to occur when the antigen level in the sample is below the detection level of the assay [75]. Lower antigen levels may arise due to a low parasite load at the start of the infection and also in cases of some immunocompetent infected animals, which manage to maintain it very low, thus not detectable. Finally, since the ProSpecT™ *Giardia* EZ Microplate Assay is an immunoassay commonly used for humans, the lower sensitivity of this assay in veterinary medicine could be attributed to genetic heterogeneity between *Giardia* spp. isolates of human and canine origin [90].

On the other hand, false positive PCR findings may occur due to excessive PCR cycling resulting in amplification of similar to the target sequence DNA, low specificity of the primers or through the inclusion of contaminated DNA within the reaction, either at the stage of DNA extraction or at the set-up process [91]. In this study a region of the *18S* rRNA was amplified by using a valid protocol, as described by Hopkins et al. [40], with well-tested cycling conditions and primers. Furthermore, all precautions regarding avoidance of contamination were taken, as proved by the inclusion of the negative control template which was similarly subjected to DNA extraction. Indeed, gel electrophoresis revealed that no contaminated nucleic acid was introduced in the master mix during specimen processing. All the above support the hypothesis that these fourteen samples were most likely ELISA false negative samples. In any case, a positive PCR test cannot discriminate living and dead protozoa, as genetic material is present in both cases. Consequently, although a positive PCR result indicates the detection of the pathogen, it cannot differentiate between its incidental presence and active infection with clinical manifestations. It is therefore evident that PCR results should be interpreted in conjunction with the case history and clinical evidence of giardiosis.

Conclusions

Veterinary practitioners must be aware of canine giardiosis in order to take into appropriate account the impact of this underestimated protozoan infection in the canine population as well as its possible zoonotic implication. The present study contributes to the understanding of the complex diagnosis of canine giardiosis. To the best of our knowledge, this is the first attempt to evaluate the performance of the Speed™ *Giardia* test, which is a very commonly used diagnostic approach in veterinary practice. The sensitivity of the test was sufficient while specificity was excellent for *Giardia* spp. This diagnostic tool supports valid sample testing that is more rapid, easy to use and interpret and affordable. In conclusion, the Speed™ *Giardia* test can be a valuable tool in veterinary settings with a high caseload where rapid diagnosis is required as well as in smaller practices where other techniques are often not available or there is limited training in fecal flotation interpretation. Our findings highlight the need to further improve the quality of current diagnostic methods in terms of sensitivity. This may elucidate most of the diagnostic challenges and assist towards reliable surveys and effective treatment of giardiosis under the umbrella of one health leading to protection of animal and public health.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s13071-020-04422-6.

Additional file 1: Table S1. Excel dataset for 100 samples.

Abbreviations

DEPC: Diethyl pyrocarbonate; DFA: direct fluorescent antibody; ELISA: enzyme-linked immunosorbent assay; GSA 65: *Giardia* Specific antigen 65; IFA: immunofluorescence antibody; LR+: positive likelihood ratio; LR−: negative likelihood ratio; PCR: polymerase chain reaction; RDT: rapid diagnostic test; Sn: sensitivity; Sp: specificity; *18S* rRNA: *18S* ribosomal RNA.

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Authors' contributions
IS performed the study design, parasitological, immunological and molecular methods and preparation of the manuscript. AIG performed the study design, statistical analyses and preparation of the manuscript. ANM conducted the molecular analysis. AA carried out the parasitological and molecular studies. KVA carried out the parasitological and immunological studies. SL and EP developed the study design and critical reviewed the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The dataset supporting the conclusions of this study are included within the article and its Additional file 1.

Ethics approval and consent to participate
The study was conducted in compliance with the national animal welfare regulations, i.e. the Presidential Decree 56/13 "Bringing Greek legislation into line with Directive 2010/63/EC of the European Parliament and of the Council of 22nd September 2010 (L 276/33/20.10.2010) regarding the protection of animals used for experimental and other scientific purposes". The applied diagnostic veterinary procedures are not within the context of relevant EU legislation for animal experimentations (Directive 86/609/EC) and may be performed in order to diagnose animal diseases and improve animal welfare. No suffering was caused during sample collection. Consent was ensured by owners or registered veterinarians (private, public or municipality, depending per case).

Consent for publication
Not applicable.

Competing interests
SL is an employee of Virbac. IS, AIG, ANM, AA, KVA and EP declare that they have have no competing interests.

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