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Comparative study of five techniques for the diagnosis of canine gastrointestinal parasites

Estudo comparativo de cinco técnicas para o diagnóstico de parasitos gastrointestinais caninos

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Abstract

Differences in the efficacy of diagnostic techniques employed in the parasitological examination of feces are a limiting factor of this laboratory procedure in the field of Veterinary Parasitology. To verify advances in this type of examination in dogs, we conducted a study using a new technique (*TFGIII/Dog*). Fifty naturally infected dogs were housed in individual stalls, and their feces were evaluated comparatively using this technique and four other conventional techniques. The *TFGIII/Dog* showed high levels of sensitivity and efficiency, surpassing the diagnostic accuracy of the other techniques with a *kappa* concordance index of 0.739 (Substantial), as opposed to 0.546 (Moderate), 0.485 (Moderate), 0.467 (Moderate), and 0.325 (Fair) of the Spontaneous-Sedimentation, Centrifugal-Flotation in Saturated Zinc Sulfate Solution, Centrifugal-Flotation in Saturated Sugar Solution, and Spontaneous-Flotation in Saturated Sodium Chloride Solution techniques, respectively. The combination of positive results of all techniques comprises eight genera of parasites, with *Ancylostoma* spp. predominating among helminths, and *Cystoisospora* spp. among protozoa. The *TFGIII/Dog* technique showed better diagnostic performance, and can therefore be considered an important tool for optimizing the results of laboratory routines and for the control of canine gastrointestinal parasites.

Keywords: Parasitological techniques, dog, helminths, protozoa, *TFGIII/Dog*.

Resumo

As diferenças na eficácia de técnicas de diagnóstico empregadas no exame parasitológico das fezes é um fator limitante desse procedimento de laboratório no campo da Medicina Veterinária. Com o objetivo de confirmar avanços desse tipo de examinação em cães, a abordagem desse trabalho foi apresentar um estudo com o uso de uma nova técnica (*TFGIII/Dog*). Cinquenta cães naturalmente infectados foram alojados em baias individuais, e suas fezes foram avaliadas comparativamente, usando-se a nova técnica e outras quatro técnicas convencionais. O *TFGIII/Dog* apresentou altos níveis de sensibilidade e eficiência, superando o diagnóstico de outras técnicas com um índice de concordância kappa de 0,739 (Substancial), em oposição a 0,546 (Moderado), 0,485 (Moderado), 0,467 (Moderado) e 0,325 (Pobre) de Sedimentação-Espontânea, Centrífugo-Flutuação em Solução Saturada de Sulfato de Zinco, Centrífugo-Flutuação em Solução Saturada de Açúcar, e Flutuação-Espontânea em Solução Saturada de Cloreto de Sódio, respectivamente. A combinação de resultados positivos das técnicas mostrou oito gêneros de parasitos, com *Ancylostoma* spp. predominando entre helmintos, e *Cystoisospora* spp. entre os protozoários. A técnica de *TFGIII/Dog* apresentou melhor desempenho diagnóstico e, portanto, pode ser considerada uma importante ferramenta para otimizar os resultados de rotinas de laboratório e o controle de parasitos gastrintestinais de cães.

Palavras-chave: Técnicas parasitológicas, cães, helmintos, protozoários, *TFGIII/Dog*.

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Introduction

Environmental changes caused by humans and their interaction with domestic animals, particularly with dogs, have favored the occurrence of various parasitic diseases with zoonotic potential (BRESCIANI et al., 2008; COELHO et al., 2013). This scenario has led to a high worldwide prevalence of canine gastrointestinal parasites, especially in the tropical regions of underdeveloped and developing countries (CHOMEL, 2008; COELHO et al., 2013).

Numerous studies on dog feces describe discrepancies in the diagnostic efficacy of different parasitological techniques and/or commercial kits (TÁPARO et al., 2006; COELHO et al., 2013). In view of this finding, some researchers recommend the collection of triplicate fecal samples, on alternate days, in combination with the high parasite concentration technique for this same diagnostic modality (GOMES et al., 2004; CARVALHO et al., 2012).

In view of the presented, and unlike Coelho and co-workers (COELHO et al., 2013), and aiming to contribute to scientific and technological advances in the diagnosis of canine gastrointestinal parasites, the current study involved the statistical evaluation of a new parasitological technique (*TFGII/Dog*), with the implementation of three laboratory processing approaches, based on laboratory principles developed specifically by Falcão et al. for the diagnosis of human intestinal parasites (FALCÃO et al., 2010).

Materials and Methods

Fifty naturally infected dogs used in this study were obtained from the Zoonosis Control Center of the municipality of Andradina, State of São Paulo, Brazil (20.896 South, 51.37944 West, 405m altitude), where they were housed in individual stalls, monitored, watered and fed (dog food). For the *TFGII/Dog* technique, the feces were identified and analyzed in the laboratory according to the operational protocol described by Falcão et al. in humans (FALCÃO et al., 2011). In addition, fecal samples were collected for laboratory processing by other conventional techniques, such as: spontaneous-flotation using saturated sodium chloride solution with a specific density of 1.20 g/mL (WILLIS, 1921); centrifugal-flotation in a saturated solution of zinc sulfate with a density of 1.18 g/mL (FAUST et al., 1938); centrifugal-flotation in saturated sugar solution with a density 1.18 g/mL (SHEATHER, 1923); and spontaneous-sedimentation (HOFFMAN et al., 1934; LUTZ, 1919). These techniques are referred in this paper as *SF-Sodium Chloride*, *CF-Zinc Sulfate*, *CF-Sugar* and *S-Sedimentation*, respectively. This study, which is in line with the precepts of the Ethics Committee on Animal

Use (CEUA) of UNESP at Jaboticabal, SP, Brazil, was approved on 22 Mar 2011 under Protocol no. 004201/11.

The results of this study were applied by means of comparisons of positive data from fecal samples obtained by parasitological techniques. To this end, the following statistical parameters were determined: sensitivity (FLEISS et al., 2003) - detection of true positives animal, specificity (GALEN & GAMBINO, 1975) - detection of true negative animal, and Kappa (k) concordance - agreement degree between qualitative data (positive and negative) obtained by different techniques - and its classification: Almost Perfect (0.81 to 1.00), Substantial (0.61 to 0.80), Moderate (0.41 to 0.60), Fair (0.21 to 0.40) and Slight (0 to 0.20).

Results

In the statistical performance assessment of the five different parasitological techniques, the of *TFGII/Dog* and *S-Sedimentation* presented the same diagnostic positivity for 40 dogs (80%), as shown in Table 1. These techniques were followed by the *CF-Zinc Sulfate* and *CF-Sugar* techniques, which showed 39 positive dogs (78%), and the *SF-Sodium Chloride* technique, with 37 positive dogs (74%). However, the type of infection detected, as single, double, triple, quadruple or quintuple, was higher with the use of the parasitological technique of *TFGII/Dog*.

Eight genera, five of helminths and three of protozoa, were identified by the five parasite detection techniques (Table 2). In the 250 clinical analyses of the feces of the 50 dogs, the *TFGII/Dog* was the only one able to detect eight different parasitic genera. It is therefore comparable to the *Gold Standard* (positive results from the combination of all the techniques), since it showed the highest accuracy in the identification of 90 positive analyses. In contrast, based on the same number of clinical analyses (250), seven genera of parasites and 83 positive analyses were identified by the *S-Sedimentation*; followed by seven genera and 80 positive analyses by the *CF-Zinc Sulfate*; six genera and 79 positive analyses by the *CF-Sugar*; and, lastly, six genera of parasites and 69 positive analyses by the *SF-Sodium Chloride* technique (Table 1).

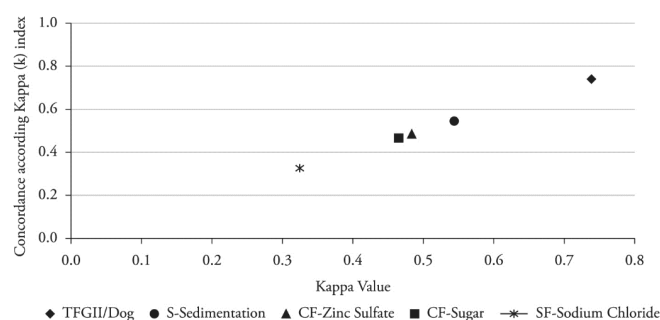
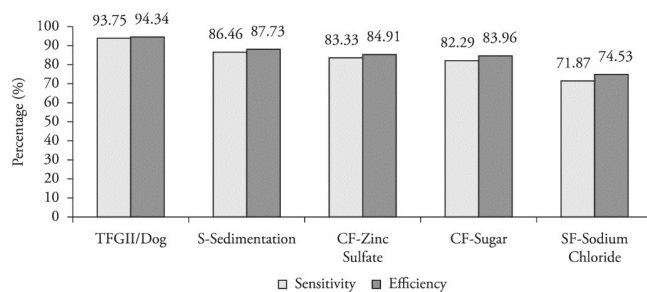
In accordance with the *Gold Standard*, among the group of helminths, *Ancylostoma* spp. predominated in the infections of 35 (70%) dogs (Table 2). In the detection of this parasite genus, the *S-Sedimentation* excelled over the other techniques, revealing infection in 34 (68%) dogs, especially when compared to the 33 (66%) dogs detected by the *TFGII/Dog*, 31 (62%) dogs by the *CF-Zinc Sulfate* and *SF-Sodium Chloride*, and 29 (58%) dogs by the *CF-Sugar* technique. However, in the identification of *Dipylidium caninum* eggs and/or egg packets and *Trichuris*

Table 1. Positivity and type of infection found in the feces of fifty dogs by five different parasitological techniques.

Technique	Positivity	Type of infection					Total
		Single	Double	Triple	Quadruple	Quintuple	
<i>TFGII/Dog</i>	40 (80%)	7	19	12	1	1	90
<i>S-Sedimentation</i>	40 (80%)	13	14	11	1	1	83
<i>CF-Zinc Sulfate</i>	39 (78%)	15	12	11	2	0	80
<i>CF-Sugar</i>	39 (78%)	12	15	11	1	0	79
<i>SF-Sodium Chloride</i>	37 (74%)	11	21	4	1	0	69

Table 2. Gastrointestinal parasites detected by five parasitological techniques in the feces of fifty dogs by five parasitological techniques and the *Gold Standard* (positive results from the combination of all the techniques).

	Parasites	TFGII/Dog	S-Sedimentation	CF-Zinc Sulfate	CF-Sugar	SF-Sodium Chloride	Gold Standard
Helminths	<i>Ancylostoma</i> spp.	33 (66%)	34 (68%)	31 (62%)	29 (58%)	31 (62%)	35 (70%)
	<i>Dipylidium. caninum</i>	18 (36%)	14 (28%)	13 (26%)	14 (28%)	14 (28%)	20 (40%)
	<i>Taenia</i> spp.	1 (2%)	-----	-----	-----	1 (2%)	1 (2%)
	<i>Toxocara</i> spp.	7 (14%)	7 (14%)	5 (10%)	7 (14%)	5 (10%)	8 (16%)
	<i>Trichuris</i> spp.	2 (4%)	1 (2%)	1 (2%)	-----	1 (2%)	2 (4%)
Protozoa	<i>Cystoisospora</i> spp.	23 (46%)	21 (42%)	24 (48%)	23 (46%)	17 (34%)	24 (48%)
	<i>Cryptosporidium</i> spp.	1 (2%)	1 (2%)	1 (2%)	1 (2%)	-----	1 (2%)
	<i>Giardia</i> spp.	5 (10%)	5 (10%)	5 (10%)	5 (10%)	-----	5 (10%)
Total		90	83	80	79	69	96

**Figure 1.** Sensitivity and Efficiency of the five parasitological techniques applied to the feces of fifty dogs.**Figure 2.** Kappa (k) concordance index obtained by the five parasitological techniques, in the study of feces of fifty dogs.

spp. eggs, the *TFGII/Dog* proved more accurate than the other conventional techniques.

Furthermore, as can be seen in Table 2, despite only one case (dog) of parasitism, *Taenia* spp. eggs were identified by the *TFGII/Dog* and by *SF-Sodium Chloride*, but not by the other techniques, thus putting the former on a par with the *Gold Standard*. In the diagnosis of *Toxocara* spp., in turn, the *TFGII/Dog*, *S-Sedimentation* and *CF-Sugar* predominated, producing identical results.

Among the protozoan groups, the genus *Cystoisospora* spp. predominated, infecting 24 (48%) dogs, according to the *Gold Standard* (Table 2). The *CF-Zinc Sulfate* showed the best performance in identifying this unicellular parasite, identifying 24 (48%) infected dogs, followed by 23 (46%) dogs by the *TFGII/Dog* and

CF-Sugar, 21 (42%) dogs by the *S-Sedimentation*, and 17 (34%) dogs by the *SF-Sodium Chloride* technique.

The genus *Cryptosporidium* spp. was detected in a single animal (2%) by *S-Sedimentation*, *CF-Zinc Sulfate*, *CF-Sugar* and the by *TFGII/Dog*, which presented the same result as the *Gold Standard*. Each of these same techniques was also able to identify the parasite *Giardia* spp. in five (10%) dogs, which also puts them on a par with the *Gold Standard* (Table 2).

In terms of the diagnostic performance of the techniques, the *TFGII/Dog* showed 93.75% sensitivity and 94.34% efficiency, compared to 86.46% sensitivity and 87.73% efficiency of *S-Sedimentation*, 83.33% sensitivity and 84.91% efficiency of *CF-Zinc Sulfate*, 82.29% sensitivity and 83.96% efficiency of *CF-Sugar*, and 71.87% sensitivity and 74.53% efficiency of *SF-Sodium Chloride* (Figure 1).

Lastly, as can be seen in Figure 2, the kappa (k) index ranked the *TFGII/Dog* as Substantial (0.739); *S-Sedimentation* (0.546), *CF-Zinc Sulfate* (0.485) and *CF-Sugar* (0.467) techniques as Moderate; and the *SF-Sodium Chloride* technique as Fair (0.325).

Discussion

According to Table 1, the total infection rate (positivity and type of infection found in 50 dogs) observed by means of the *TFGII/Dog* was higher than that identified by the *S-Sedimentation*, *CF-Zinc Sulfate*, *CF-Sugar* and *SF-Sodium Chloride* techniques. This finding can be explained by the intermittent elimination of the evolutionary forms of each parasitic group, notably enteric protozoa and helminths (CARVALHO et al., 2012; COELHO et al., 2013), indicating that the *TFGII/Dog* is more efficient, and particularly in cases of multiple parasitism. Two procedures contributed to the high diagnostic efficacy of the *TFGII/Dog* technique: a) the collection of fecal samples on three alternate days, and their one-time processing in the laboratory (CARVALHO et al., 2012; COELHO et al., 2013); and, b) the application of the three laboratory principles of parasitic concentration, such as, centrifugal-sedimentation, spontaneous-flotation and spontaneous-sedimentation (HOFFMAN et al., 1934; FAUST et al., 1938; GOMES et al., 2004). In these two procedures (a and b), the sample collection and laboratory processing procedures are exclusive to the *TFGII/Dog* (FALCÃO et al., 2010).

The large number of protozoan cysts and oocysts identified can be explained by the fact that techniques employed in this study take advantage of the selective principles of flotation and sedimentation, which strongly concentrate the structures of these parasitic genera. The fact that we used naturally infected stray dogs that were captured and housed, the low immunity of these animals may have favored the regular elimination of protozoan cysts and oocysts, which, in natural conditions, are expelled intermittently and irregularly in the feces (TÁPARO et al., 2006; BRESCIANI et al., 2008; COELHO et al., 2013).

Although the *SF-Sodium Chloride* presents a specific flotation principle of parasitic concentration, above all for *Ancylostomatidae* eggs, the saturated sodium chloride solution (1.20 g/mL) used in this technique causes the lysis of most intestinal protozoa, especially when using a hypertonic medium. Nevertheless, our experience has shown that the genus *Cystoisospora* spp. is tolerant to this condition (Table 2).

On a par with the *Gold Standard*, the *TFGII/Dog* was the only one that was highly efficient in detecting the eight genera of canine gastrointestinal parasites. Thus, this study led to the conclusion that this technique can be widely employed in the qualitative diagnosis of canine gastrointestinal protozoa and helminths, with high performance and is practical, in particular because the kit is completely disposable, thereby reducing the work in the laboratory. It is also cost-effective, with cost equivalent to other conventional techniques and commercial kits (according to the patent applicant - University of Campinas/Inova Innovation Agency). Despite its qualities, the operating protocol of *TFGII/Dog* must be followed carefully to avoid some sort of limitation to this technique. Lastly, there is no doubt that the gains in sensitivity and efficiency provided by this technique should improve the laboratory diagnosis and control of canine parasitic infections.

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