

# Evaluation of Different Parasitological Techniques for Diagnosing Intestinal Parasites in Dogs

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## Abstract

Intestinal nematodes are among the most common parasites of dogs, being *Toxocara* spp. and *Ancylostoma* spp. threats to public health due to their zoonotic potential. In this study, three parasitological diagnostic techniques for detecting the eggs/oocysts of canine intestinal parasites were evaluated. Fecal samples (n = 285) were collected from the environment (*i.e.* public streets, squares and homes) in Garanhuns, Pernambuco, Brazil. Samples were analyzed using the Willis-Mollay simple flotation technique, spontaneous sedimentation (Hoffman, Pons and Janer) and modified centrifugal flotation. Among the samples analyzed, 56.49% (161/285) showed eggs and/or oocysts of intestinal parasites. In particular, 44.21% (126/285) were detected by the Willis technique, 45.14% (121/268) by centrifugal flotation and 31.57% (90/285) by the Hoffmann technique. The kappa index indicated good concordance between the centrifugal flotation and Willis techniques ( $\kappa = 0.625$ ), whereas fair concordance was observed between Hoffmann and Willis ( $\kappa = 0.480$ ), and between Hoffman and centrifugal flotation ( $\kappa = 0.433$ ). In conclusion, the centrifugal flotation technique showed high sensitivity towards detecting *Ancylostoma* spp. and *Toxocara* spp. (the most common parasites in this study) and could be used in association with the Willis technique to obtain safe diagnoses within routine laboratory analysis.

## Keywords

Willis-Mollay, Modified Centrifugal Flotation, Hoffman, Dogs, Zoonotic Helminths

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## 1. Introduction

Parasitic diseases are among the most important zoonoses throughout the world and are considered to be a serious threat to socioeconomic equilibrium, particularly in developing countries [1]. For example, parasites that affect dogs, such as the helminths *Toxocara* spp. and *Ancylostoma* spp., which are the causative agents of visceral *larva migrans* (VLM) and cutaneous *larva migrans* (CLM), respectively, present great sanitary importance [2] [3].

Despite the wide range of therapeutic and prophylactic measures currently available, eggs and oocysts of parasites are commonly detected in the feces of dogs, and their elimination in the environment facilitates transmission to other hosts, including humans [4] [5]. Most of these intestinal parasites (e.g. *Toxocara* spp., *Ancylostoma* spp. and *Cystoisospora canis*) present cosmopolitan distribution, and they are most prevalent in tropical areas with inadequate sanitary conditions [6] [7]. Currently, with increasing dog ownership, the number of people at risk of acquiring parasitic zoonoses is also increasing [2]. However, human infection is not restricted only to the household environment, but comes especially through public places such as squares and streets [8]-[12].

Parasitological diagnostic methods are useful for detecting intestinal parasites through searching for different parasitic forms (e.g. eggs, larvae, oocysts or trophozoites) that are eliminated in feces. Despite improvements in immunological techniques and the advent of molecular tests, parasitological methods are still considered important, particularly because of their simplicity and low cost [13]-[15]. However, since the diagnosis is based on viewing eggs or oocysts, confirmation may in some cases be difficult [6]. In addition, choosing the most suitable method for use within the routine of the diagnostic laboratory may represent an obstacle for efficient diagnosis. Therefore, the aim of this study was to evaluate three different techniques (Willis-Mollay flotation technique; spontaneous sedimentation of Hoffman, Pons and Janer; modified centrifugal flotation) for detecting eggs/oocysts of intestinal parasites in dogs.

## 2. Materials and Methods

### 2.1. Sample Collection

From August 2011 to July 2012, canine fecal samples (n = 285) were collected directly from the environment (*i.e.* public streets, squares and backyards) in the city of Garanhuns, state of Pernambuco, Brazil. The samples collected were put into plastic vials, identified and stored in isothermal boxes at 4°C until laboratory processing.

### 2.2. Laboratorial Procedures

All samples were analyzed using three different techniques for detecting eggs and oocysts: 1) Willis-Mollay flotation technique [16]; 2) spontaneous sedimentation of Hoffman, Pons and Janer [17]; 3) modified centrifugal flotation [18]. From here on, and throughout the text, the methods will be referred to as the Willis, Hoffman and centrifugal flotation techniques.

After doing the preparations for each technique, the fecal material was transferred to slides and a drop of Lugol solution (2%) was added. Finally the slides were covered with cover slips for observations to be made under a microscope at different magnifications (10× and 40×). The eggs and oocysts were identified as previously described [19] [20].

### 2.3. Data Analysis

Kappa concordance analysis was used to compare the results. The Kappa values were interpreted in accordance with [21]. The sensitivity, specificity, positive predictive value (+PV), negative predictive value (-PV) and accuracy were determined taking the Willis technique to be the gold standard [22]. The Bioestat 5.0 and Epi-Info software was used to calculate all parameters.

## 3. Results

Out of 285 samples analyzed, eggs/oocysts of intestinal parasites were detected in 56.49% (161/285): 44.21% (126/285) detected through the Willis technique, 45.14% (121/268) through centrifugal flotation and 31.57% (90/285) through the Hoffman technique. The overall results regarding infections and coinfections detected using each technique are reported in **Table 1**.

Interestingly, *Ancylostoma* spp. and *Toxocara* spp. were the parasites most frequently detected, independently of the method used. The average numbers of eggs/oocysts detected by means of all the techniques are reported in **Table 2**.

**Table 1.** Infections and co-infections detected in each technique of intestinal parasites in canine fecal samples in the municipality of Garanhuns, PE.

Techniques	Parasites	Positivity (%/n)
Willis-Mollay floating	<i>Ancylostoma</i> spp.	76.20% (96/126)
	<i>Toxocara</i> spp.	4.80% (06/126)
	<i>Dipylidium</i> spp.	1.70% (02/126)
	<i>Ancylostoma</i> spp. + <i>Toxocara</i> spp.	8.73% (11/126)
	<i>Dipylidium</i> spp. + <i>Toxocara</i> spp.	2.55% (03/126)
	<i>Ancylostoma</i> spp. + <i>Dipylidium</i> spp.	2.55% (03/126)
	<i>Ancylostoma</i> spp. + <i>Isospora</i> spp.	1.70% (02/126)
	<i>Toxocara</i> spp. + <i>Isospora</i> spp. + <i>Dipylidium</i> spp. + <i>Ancylostoma</i> spp. + <i>Trichuris</i> spp.	0.85% (1/126)
	<i>Ancylostoma</i> spp. + <i>Trichuris</i> spp. + <i>Giardia</i> spp. + <i>Isospora</i> spp.	0.85% (1/126)
Modified centrifugal flotation	<i>Ancylostoma</i> spp.	80.16% (97/121)
	<i>Toxocara</i> spp.	5.77% (07/121)
	<i>Dipylidium</i> spp.	0.83% (01/121)
	<i>Ancylostoma</i> spp. + <i>Toxocara</i> spp.	9.09% (11/121)
	<i>Ancylostoma</i> spp. + <i>Dipylidium</i> spp.	0.83% (01/121)
	<i>Ancylostoma</i> spp. + <i>Strongyloides</i> spp.	
	<i>Toxocara</i> spp. + <i>Isospora</i> spp.	0.83% (01/121)
<i>Ancylostoma</i> spp + <i>Isospora</i> spp.	0.83% (01/121)	
Spontaneous sedimentation of Hoffman, Pons and Janer	<i>Ancylostoma</i> spp.	75.56% (68/90)
	<i>Toxocara</i> spp.	8.89% (08/90)
	<i>Dipylidium</i> spp.	2.22% (02/90)
	<i>Ancylostoma</i> spp. + <i>Toxocara</i> spp.	8.89% (08/90)
	<i>Ancylostoma</i> spp. + <i>Dipylidium</i> spp.	2.22% (02/90)
	<i>Ancylostoma</i> spp. + <i>Trichuris</i> spp	1.11% (01/90)
	<i>Ancylostoma</i> spp. + <i>Toxocara</i> spp. + <i>Trichuris</i> spp.	1.11% (01/90)

**Table 2.** Absolute and relative frequency, number mean of eggs and standard deviation of intestinal parasites detected in fecal samples from dogs in the municipality of Garanhuns, PE.

	Willis-Mollay floating					Modified centrifugal flotation					Hoffman, Pons and Janer				
	AF (n)	Mn	Mx	$\bar{x} \pm SD$	RF (%)	AF (n)	Mn	Mx	$\bar{x} \pm SD$	RF (%)	AF (n)	Mn	Mx	$\bar{x} \pm SD$	RF (%)
<i>Ancylostoma</i> spp.	1595	0	113	5.96 ± 0.9	88.7	2171	0	200	8.101 ± 1.4	79.1	392	0	45	1.38 ± 0.2	63.7
<i>Toxocara</i> spp.	196	0	55	0.7 ± 0.3	10.9	541	0	336	2.02 ± 1.3	19.7	220	0	72	0.77 ± 0.3	35.7
<i>Trichuris</i> spp.	06	0	06	0.02 ± 0.01	0.33	06	0	06	0.02 ± 0.02	0.22	03	0	02	0.01 ± 0.007	0.4
<i>Strongyloides</i> spp.	-	-	-	-	-	24	0	17	0.08 ± 0.06	0.88	-	-	-	-	-
Total (n)	1791	-	-	-	100	2742	-	-	-	100	617	-	-	-	100

From the kappa analysis, the following results were obtained: Willis versus centrifugal flotation ( $\kappa = 0.625$ ;  $P < 0.001$ ): good concordance; Willis versus Hoffman, ( $\kappa = 0.480$ ;  $P < 0.001$ ): fair concordance; centrifugal flotation versus Hoffman ( $\kappa = 0.433$ ;  $P < 0.001$ ): fair concordance. The sensitivity, specificity, +PV, -PV and accuracy values are shown in **Table 3**.

#### 4. Discussion

In this study, three different parasitological diagnosis techniques (Willis, Hoffman and centrifugal flotation techniques) were evaluated. The results demonstrated different performances between the methods evaluated, especially considering the percentages of positive of 44.21% (126/285), 45.14% (121/268) and 31.57% (90/285) for the Willis, Hoffman and centrifugal flotation techniques respectively. Although minimal, the differences reported here might be related to specific characteristics presented by each method.

*Ancylostoma* spp. eggs were the most frequently detected, followed those of *Toxocara* spp. and *Trichuris* spp. These results are similar to those obtained in a previous study in the state of Santa Catarina in which *Dipylidium caninum*, *Toxocara canis* and *Trichuris vulpis* were the most prevalent species [23].

The highest relative frequency was detected for *Ancylostoma* spp. (63.74% to 88.76%), in all the techniques used. On the other hand, *Toxocara* spp. was the most common parasite detected using the Hoffman technique (35.77%), in contrast with the Willis and centrifugal flotation techniques, which both presented relative frequencies lower than 20%. Differently from the other parasites, the relative frequencies of eggs of *Trichuris* spp. and *Strongyloides* spp. were lower than 1%. The latter was detected only by means of centrifugal flotation. Interestingly, the high number of eggs detected in some samples (e.g. cases in which *Toxocara* spp. and *Ancylostoma* spp. presented 336 and 200 eggs, respectively, detected by means of centrifugal flotation) revealed that these samples presented severe parasitism and reinforces the importance of using these techniques in diagnosing parasitic helminths.

Considering the Willis technique to be the gold standard, sensitivity and specificity of 79% and 85%, respectively, were achieved in diagnosing *Ancylostoma* spp., which was classified as “good” according to the kappa index (0.625). Conversely, the Hoffman technique presented a similar specificity value (89%) but lower sensitivity (54%).

The centrifugal flotation features concentration of eggs through several washes, thereby facilitating their identification. In this method, high sensitivity (80%) and specificity (98%) for detection of *Toxocara* spp. was observed. On the other hand, the Hoffman technique showed sensitivity and specificity of 67% and 100%, respectively. For both of these tests, the kappa value of 0.48 was considered to be “fair”, thus indicating the need to perform tests in parallel in order to improve the diagnosing of toxocarids.

In general, the Willis and centrifugal-flotation techniques showed good reproducibility; in addition both techniques detected higher numbers of eggs of *Ancylostoma* spp. and *Toxocara* spp. The differences in sensitivity and specificity between the techniques derive from the individual characteristics of each of them, which give rise to differences in sensitivity and/or specificity in relation to certain parasites [24]. In this context, it is recommended that examination of feces using more than one technique. Use of a single method may not reveal the real situation of parasitism, especially considering that in many cases, animals show mixed infections.

**Table 3.** Evaluation of the techniques of Modified Centrifugal-flotation and Spontaneous sedimentation of Hoffman, Pons and Janer in relation to the Willis-Mollay Floating technique (gold standard) for the diagnosis of intestinal parasites in canine fecal samples in the municipality of Garanhuns, PE.

	Modified centrifugal flotation			Spontaneous sedimentation of Hoffman, Pons and Janer		
	<i>Ancylostoma</i> spp.	<i>Toxocara</i> spp.	<i>Trichuris</i> spp.	<i>Ancylostoma</i> spp.	<i>Toxocara</i> spp.	<i>Trichuris</i> spp.
Sensitivity	79.00%	80.00%	000.00%	54.00%	67.00%	000.00%
Specificity	85.00%	98.00%	100.00%	89.00%	100.00%	100.00%
Predictive value (+)	80.00%	73.00%	000.00%	76.00%	100.00%	000.00%
Predictive value (-)	85.00%	98.00%	100.00%	75.00%	97.00%	100.00%
Accuracy	83.00%	96.00%	99.00%	75.00%	97.00%	99.00%

## 5. Conclusion

In conclusion, in order to obtain a reliable diagnosis, the centrifugal flotation method, which showed higher sensitivity for detecting *Ancylostoma* spp. and *Toxocara* spp. (the most common parasites in this study), could be used in association with the Willis technique within routine diagnostic laboratory analysis.

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