





Diagnostic of gastrointestinal helminths in sands and canine feces from public locations in Alegre city, Espírito Santo - Brazil

Diagnóstico de helmintos gastrointestinais em areias e fezes caninas de locais públicos no município de Alegre, Espírito Santo - Brasil

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Abstract

The aim of this experiment was to identify gastrointestinal helminths, also comparing different diagnosis parasitology technique in dog feces and sands samples from public locations in Alegre-ES. It was collected faecal samples from dogs and sands from public locations, such as parks, flower beds, sidewalks. These samples were sent to parasitology laboratory of the Veterinary Hospital of Centro de Ciências Agrárias e Engenharias of the Universidade Federal do Espírito Santo, where these samples were analyzed by Willis-Mollay and Simple Centrifugal-Flotation technique and the sands samples were analyzed by Baermann modified and Silva and others technique. A total of 92 faecal samples were analyzed, of which 60.7% were positive for *Ancylostoma* spp., 24% for *Toxocara canis*, 8.7% for *Dipylidium caninum* and 1% for *Taenia* sp. In soil and sand samples hookworms larvae were found in 33.3%. Simple Centrifugal-Flotation technique were more appropriated for parasites detection in dog feces, and Baermann technique was more appropriated to identify larvae in sands.

Keywords: coproparasitological techniques, dogs, helminths, soil contamination, zoonoses.

Resumo

Este trabalho teve como objetivo diagnosticar a frequência de helmintos gastrintestinais comparando diferentes técnicas usadas no diagnóstico de estruturas parasitárias presentes nas fezes de cães e no solo de locais públicos, no município de Alegre-ES. Foram coletadas amostras de fezes e areias em locais públicos como praças, canteiros, calçadas e essas foram encaminhadas para o laboratório de Parasitologia do Hospital Veterinário do Centro de Ciências Agrárias e Engenharias da Universidade Federal do Espírito Santo, onde as amostras de fezes foram analisadas pela técnica de Willis-Mollay e Centrífugo-Flutuação Simples e as de areia analisadas pela técnica de Baermann modificada e pela técnica de Silva e colaboradores. Foram analisadas 92 amostras de fezes e foram positivas 60,7% para *Ancylostoma* spp., 24% *Toxocara canis*, 8,7% *Dipylidium caninum*. e 1% *Taenia* sp. Nas amostras de solo e areia as larvas de ancilostomatídeos foram encontradas em 33,3%. Foi observado ainda que a técnica de Centrífugo-Flutuação Simples foi mais apropriada para a detecção das formas parasitárias nas fezes de cães e que a técnica de Baermann foi mais apropriada no diagnóstico das larvas encontradas na areia.

Palavras-chave: técnicas coproparasitológicas, cães, helmintos, contaminação solo, zoonoses.

Introduction

Agglomeration of canine population in urban areas associated with an increasing number of non-domiciled dogs has an important epidemiological role in soil contamination in public parks and squares and in infections spread by various parasite genus (Cassenote et al., 2011; Silva Mello et al., 2011).

The eggs, oocysts and larvae of the parasites are eliminated by animal feces, contaminating the environment, and most cycles are dependent on external environment conditions for their development. Several of these agents have zoonotic potential, with eggs and larvae potentially



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reaching humans, such as *Toxocara canis* and *T. cati*, causing visceral larva migrans (VLM) and *Ancylostoma braziliense* and *A. caninum*, leading to cutaneous larva migrans (CLM) (Scaini et al., 2003; Rocha et al., 2011; Guimarães et al., 2005).

Child population is the most exposed due to greater contact with the soil, curiosity and discovery behavior, nutritional deficiencies or even appetite disturbances that make contact the soil with the mouth (Capuano & Rocha, 2006). Therefore, checking the presence of eggs, oocysts and larvae in contaminated soil plays an important role and occurs through techniques that detect them in soil particles, facilitating decision making for control measures (Oliveira et al., 2009).

The objective of this work was to evaluate the frequency of gastrointestinal helminths collected in public places, comparing different techniques used for parasitic structures diagnosis in dog feces and soil in public places of Alegre-ES.

Material and methods

Primarily it was selected locations in the city for faecal and sand samples collection, with dog feces occurring in open areas prioritizing the locations where there were stray animals in contact with the population.

Stool and sand samples were collected once a month, in the afternoon, choosing preferably fresh feces and also the nearby sand where the feces were found. Collection was done with a deep iron shovel of two centimeters and packed in plastic bags, later stored refrigerated and sent to Parasitology laboratory of the Veterinary Hospital of the Centro de Ciências Agrárias e Engenharias of the Universidade Federal do Espírito Santo.

Dog faecal samples sent to the laboratory were submitted to Willis-Molay (Willis, 1921) and Simple Centrifugal-Flotation techniques (Sloss et al., 1999). Sand samples were analysed by the techniques of Silva et al. (1991) and Baermann (Ueno & Gonçalves, 1998). Once these samples arrived at the laboratory they were transferred to small plastic boxes without lid to promote oxygenation and placed in a BOD greenhouse at 26 °C to perform analysis by Silva et al. (1991), undergoing 24 hours incubation for both technique applied.

The resulting eggs were identified based on the morphology described by Foreyt (2005) and Bowman (2010), and *Dipylidium caninum* diagnosis was target to proglottids observation in feces or by visualization of the ovigerous capsule in confirmed tests.

For statistical analysis, descriptive statistics were used to calculate parasites frequency and chisquare test (X^2) in the Epi info program (2006), using Yates correction and Fisher exact to evaluate diagnostic techniques.

Results and discussion

In a total of 92 dog feces samples, 56 (60.7%), 23 (24%), 8 (8.7%) and 1 (1%), respectively, were positive for *Ancylostoma* spp., *Toxocara canis*, *Dipylidium caninum* and *Taenia* spp., and also, from the total faecal samples analysed 25% presented mixes infection for more than one helminth type. Table 1 shows the values found individually at each analyzed location.

Table 1. Number of samples collected and percentage of larvae found in soils and sands in the analyzed municipality of Alegre-ES.

Locations	Number of samples	<i>Ancylostoma</i> spp.	
		Baermann	Silva
Square	18	16.7	27.8
Sidewalk	21	4.7	4.7
Park	2	100	-
Flowerbed	23	43.5	26
School	12	-	-
kindergarten	1	-	-

Diagnosis of the structures obtained different results, e.g. 35 samples were positive for hookworm and 10 for toxocarids by Willis-Mollay technique, while by Simple Centrifugal-Flotation (SCF) it was obtained 49 and 21 positive samples, respectively. However, when analyzing the results for *D. caninum* and *Taenia* spp. by willis-mollay technique, positive samples were higher, once this technique diagnosed 8 samples for *D. caninum*, and one for *Taenia* sp.

SCF technique showed greater recovery of *Ancylostoma* spp. and *Toxocara* spp. when compared to Willis-Mollay ($X^2=45.02$ $p<0.001$ for *Ancylostoma* sp. and $X^2= 24.62$ and $p<0.001$ for *Toxocara*), hookworms were the most prevalent in both samples and techniques, followed by *T. canis*. Táparo et al. (2006) had already compared other techniques, reporting that Willis-Mollay technique for ancylostomatides and toxocarides diagnosis were more appropriate when compared to Faust, direct examination and sedimentation techniques.

Sprenger et al. (2014) collected feces and also soil contamination material to perform diagnosis by Faust, Lutz and Baermann methodology, describing a total of 36% positive samples for helminths and 65.2% areas classified as contaminated in one or more samples. The most identified helminth eggs were *Ancylostoma* sp. 14.5%, followed by *Toxocara* sp. 9.6% and Strongylida order, 2.3% similar to the present study in the main helminths found.

Regarding statistical analysis of larval recovery techniques, the proportion of positive tests for Baermann was 25.64%, while for Silva et al. (2011) the proportion was 14.10%, demonstrating advantage of Baermann technique. ($X^2= 7.52$, with $p <0.01$). From the 78 sand and soil samples collected, 26 were positive for *Ancylostoma* spp. larvae, identified by the presence of free-living nematode larvae.

Souza et al. (2008) studies with helminth eggs and larvae in day care centers sandboxes from municipal schools and public squares in Cuiabá - MT using Baermann technique (Ueno & Gonçalves, 1998) for larval recovery and Hoffman et al. (1934) technique for helminth egg recovery, it was observed seven positive samples for helminth larvae. Baermann technique detected five larvae and Hoffmann two larvae, concluding that Baermann technique (Ueno & Gonçalves, 1998) was more efficient for larval recovery, as shown in this present work.

Mota et al. (2018) in their evaluation studies in samples from parks, schools and clubs in Uberlândia- MG, employing different techniques compared to this present study, reported eggs of *Ancylostoma* sp., 11.90% by Hoffmann technique and 4.76% by Ritchie; Ascarididae eggs identification 5.43% and 12.5% respectively; and *Strongyloides* larvae 19.7% and 21.3%, respectively.

Several authors found higher hookworms prevalence in distinct country regions, following by *Toxocara* genus (Oliveira et al., 2009; Silva et al., 1991; Sloss et al., 1999; Alves et al., 2005; Sousa et al., 2010; Táparo et al., 2006) as in other countries such as Portugal (Lebre, 2011), Nepal (Satyal et al., 2013) and Malaysia (Mahdy et al., 2012). Infection in non domiciled dog or in constant contact with public places was also cited with highest prevalence by other authors (Oliveira et al., 2009; Alves et al., 2005; Satyal et al., 2013).

Lower occurrence of *D. caninum* is also cited by more authors (Oliveira et al., 2009; Lebre, 2011; Mahdy et al., 2012) and its diagnosis is underestimated. This can be explained by difficult detection of ovigerous capsules in the stool (Gennari et al., 1999) or by inadequate exam method applied (Alves et al., 2005). It is noteworthy that in the present study the samples were considered positive by proglottids observation in feces or in the taken exams.

Regarding the execution of the techniques, no execution difficulties were found for stool samples, considering that Willis-Mollay and SCF use the principles of floatation based on saturated sugar solution, the difference is in the concentration and suspension of the sediment than in SCF, there is centrifugation and consequently at runtime, where SCF requires more time to perform. The techniques are simple and involve the use of low cost materials and widely available, so they can be used in the laboratory routine (Matesco et al., 2011).

The high prevalence of parasites in dogs in municipality underscores hygienic-sanitary control problems, especially because they are zoonotic forms. High occurrences of hookworms and ascarids found in faecal waste from public places are due to the fact that non domiciled animals are more exposed to contaminated environment. Táparo et al. (2006) and Dryden et al. (2005) described that for the most accurate canine helminths diagnosis it is recommended to combine different coproparasitological techniques, such as sedimentation and flotation, to diagnose light and heavy eggs, different larval forms, and even to decrease the numbers of false negative results.

The results of this work carried out in schools, kindergartens and other public environments were satisfactory, since in kindergartens no positive results were found. The school that was identified with contamination, was carried out socio-educational actions, aiming to indicate prophylaxis and prevention to prevent soil contamination, in order to make students and staff aware of the importance of implementing hygiene practice to prevent future infections.

Soil analysis is an important criterion to be evaluated as well, considering that these samples are directly related to animal circulation and the presence of feces in public places.

Furthermore, there is a constant need to analyze dog and sand/soil feces from municipalities public environments, considering the possible occurrence of zoonotic parasite agents, and also validation of techniques in laboratory routines aiming to improve diagnosis.

Conclusion

In the present study, the highest frequency of gastrointestinal helminths was hookworm. The simple flotation centrifuge technique was more suitable for egg diagnosis, while Baermann's technique was more suitable for larval diagnosis.

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