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Short communication

Prevalence of parasites in soil and dog feces according to diagnostic tests

Arisa Mandarino-Pereira[a], Fábio Silva de Souza[b], Carlos Wilson G. Lopes[c], Maria Julia S. Pereira[c,*]

[a] Curso de Pós-graduação em Ciências Veterinárias, Universidade Federal Rural do Rio de Janeiro, Departamento de Parasitologia Animal, BR 465, km 7, Seropédica, RJ, CEP 23.890-000, Brazil
[b] Departamento de Epidemiologia e Saúde Publica, Universidade Federal Rural do Rio de Janeiro, BR 465, km 7, Seropédica, Rio de Janeiro, CEP 23890-000, Brazil
[c] Departamento de Parasitologia Animal, Universidade Federal Rural do Rio de Janeiro, BR 465, km 7, Seropédica, Rio de Janeiro, CEP 23890-000, Brazil

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ABSTRACT

The objective of this study was to determine the prevalence of parasites in soil and dog feces according to diagnostic tests. We studied soil from 25 public squares in Seropédica, Brazil. Five samples of soil were collected from each square. Eighty-one fresh fecal samples from dogs were analyzed. The technique described by Dunsmore et al. and an adaptation of the Rugai et al. method were used to recover parasites in soil, and the Willis, Hoffman and Centrifugal-Flotation techniques were used to detect parasites in feces. The χ^2 and Fischer's exact tests were used to analyze the statistical significance of the results. Seven squares were found to be contaminated, and the most prevalent parasites were *Ancylostoma* spp. (13.6%) and *Toxocara* spp. (4.0%). The Dunsmore et al. technique and the adaptation of the Rugai et al. method did not differ in the detection of *Toxocara* spp. ($p = 0.21$), *Trichuris* spp. ($p = 0.25$), *Ascaris* spp. ($p = 0.49$) and *Strongyloides* spp. ($p = 0.49$) in soil. However, the two methods differed in the detection of *Ancylostoma* spp. eggs ($p = 0.029$) and larvae ($p = 0.001$). According to granulometric analysis, the soil samples consisted mainly of sand (from 96.6% to 82.8%). Parasites were detected in 75 fecal samples, the most frequent being *Ancylostoma* spp. (80.1%), *Toxocara* spp. (11.1%) and *Cryptosporidium* spp. (7.4%). There was no difference between the Willis and Centrifugal-Flotation techniques in the detection of *Ancylostoma* spp., and both techniques were better than the Hoffman technique for detecting this parasite in feces. The Hoffman and Centrifugal-Flotation techniques were different ($p = 0.03$) in *Toxocara* spp. detection. No difference was observed among these three for *Cryptosporidium* spp. detection. The prevalences of zoonotic parasites in both dog feces and soil have implications for the spread of human disease in these areas.

1. Introduction

Although most gastrointestinal parasites are found worldwide, they are more prevalent in tropical and subtropical regions where populations experience poor socioeconomic conditions (Katagiri and Oliveira-Sequeira, 2007). Some of these parasites live in the soil during their development and for protection until they infect their next host. The main source of soil contamination with helminthes and protozoa is infected dog and cat feces (Corrêa and Moreira, 1996). These animals can be reservoirs for gastrointestinal parasites that occasionally cause infection in humans. Among these intestinal parasites, *Toxocara* spp., *Ancylostoma* spp. and *Cryptosporidium parvum* have

* Corresponding author. Tel.: +55 21 2682 1617; fax: +55 21 2682 1617.
E-mail address: m.salim@ufrrj.br (M.J.S. Pereira).

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received particular attention because they are zoonoses (Robertson and Thompson, 2002; Acha and Szyfres, 2003).

Analyses of fecal samples found in public places can predict levels of soil contamination. However, investigation of the soil itself determines the real risk of zoonosis caused by direct contact with contaminated soil (Araújo et al., 1999). To determine the burdens of zoonotic parasites in soil is necessary to identify places in which infective forms can be found. In addition, the assessment of soil contamination requires reliable techniques for separating parasites from soil particles to facilitate identification. Many techniques have been described (Dada, 1979; Quinn et al., 1980; Kazacos, 1983; Dunsmore et al., 1984; O'Lorcain, 1994; Carvalho et al., 2005), and they vary in the materials used and the percentage of parasites recovered.

When studying the prevalence of soil contamination by zoonotic parasites, some variables must be taken into consideration. The soil texture is one of those important variables once that interactions between the soil structure and the flotation solutions can interfere with parasite recovery (Nunes et al., 1994). Thus, preliminary granulometric analysis is essential to determine the soil composition and select the best method to investigate the prevalence of zoonotic parasites in soil in a determined geographical area. Nevertheless, the relationship between soil texture and the presence of *Toxocara* spp. eggs is not direct. Samples with similar grain size composition can vary in the number of eggs present due to other factors such as intensity of contamination, action of earthworms (Mizgajska, 1997), wind and rainfall (Nunes et al., 1994).

During the course of sampling and laboratory analyses, many factors influence the results of soil examinations. These include sample site selection, the number and volume of samples, depth of sampling, season of examination, method of egg recovery, preservation of samples and laboratory skills (Mizgajska, 2001).

The objective of this study was to determine, using diagnostic tests, the prevalence of parasites in soil and fresh dog feces samples from public squares in the municipality of Seropédica in the State of Rio de Janeiro, Brazil.

2. Materials and methods

The municipality of Seropédica has 25 public squares that were visited between April 28th and August 3rd 2006. Each public square was visited once during the study. Five samples of approximately 250 g each were collected from different points of each square. An eight-centimeter PVC pipe was used to collect the soil samples because *Toxocara* spp. eggs are more abundant in the top 0 ± 8 cm than in deeper layers (O'Lorcain, 1994).

To recover parasites, the soil samples were examined by Dunsmore et al. modified technique (Dunsmore et al., 1984) and by Adaptation of Rugai's et al. method (Carvalho et al., 2005). The Dunsmore technique is a Centrifugal-Flotation technique that originally consists in the analyses of 25 g of soil. According to Kazacos (1983) the possibility to observation eggs of parasites increases when the amount of soil increases. These authors mentioned that 30 g of soil is the maximum quantity of soil that can be efficiently processed. In this way we used 30 g of soil in our study. In the laboratory, the soil was processed using a version of technique describe by Dunsmore et al. (1984) as follows:

(1) In a beaker of 100 mL, 30 g of soil were soaked overnight a 50 mL of distillated water and three drops of Tween 80
(2) The mixture were homogenized using an electric mixer (Multimixer and Creamer, Tattile®) for 10 min and rested for 5 min.
(3) Two centrifuge tubes of 15 mL were filled with the mixture and centrifugated for 10 min/2000 rpm. The supernatants were discarded and $NaNO_3$ ($d = 1{,}22$) were added until half of tube and the sediment were suspended.
(4) The tubes topped with $NaNO_3$ and a slide placed in the menisc for 25 min. To each tube we used three slides. Then the slides were observed in microscope.

The Adaptation of Rugai's et al. method (Carvalho et al., 2005) is a spontaneous sedimentation method. First of all we had folded the gaze in eight then made bundles containing 100 g of soil. The Bundles of gaze were plunged in water in 45 °C in sediment chalices of 125 mL. After 1 h the bundles were discarded and the sediment rested overnight. If necessary the sediment was washed and rested for more 2 h. The supernatants were discarded carefully and the sediments centrifuged for 2 min in 2000 rpm. After that we put one aliquot of sediment and a drop of lugol in a slide to be observed in microscope.

After separating the soil to the parasitological techniques, the remaining soil collected in the five points of each square was mixed in a plastic bag to obtain a homogeneous sample soil. Now from 125 samples of soil we obtained 25 homogeneous samples soil. Approximately 500 g of homogeneous samples soil from each square were select and sent to Embrapa Soils, which is a unit of the Brazilian Agricultural Research Corporation. In this laboratory the granulometric analyses of the samples was done and the soil classified into sand, silt and clay according to their composition (Embrapa, 1997).

To predict levels of soil contamination, all fresh dog feces found during the visits were collected and analyzed using the Willis, Hoffman and the Centrifugal-Flotation techniques. The fecal samples were divided in three aliquots: 2 g to Willis technique, 2 g to Centrifugal-Flotation technique and 10 g to Hoffman technique.

Although Willis technique was done according to Willis (1921) we used saturated solution of sugar ($d = 1{,}22$) in the present study. The same solution was used to the Centrifugal-Flotation technique that was done according to Sloss et al. (1999). The Hoffman technique was done according to Hoffman et al. (1934) description however in our study we used 10 g of feces and sedimentation chalices of 125 mL to do the technique.

Feces that were not fresh enough to be examined (old feces) were counted only. Were considered old feces those that in a macroscopic view were dry, crumble or mixed with soil or vegetation.