Helminth eggs with zoonotic potential in the vicinity of public schools in southern Brazil

Ovos de helmintos com potencial zoonótico nas proximidades de escolas públicas no extremo sul do Brasil

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Abstract
The aim of this study was to evaluate environmental contamination by helminth eggs with zoonotic potential that were found in dog feces in the vicinity of elementary schools. Seventy-nine samples of dog feces were collected from 28 municipal schools located in five neighborhoods in Pelotas, Rio Grande do Sul (RS), Brazil. The samples were processed using the Willis-Mollay technique and analyzed using an optical microscope (40X), to identify any parasite eggs present. All neighborhoods were positive and 74.7% of the samples exhibited one or more helminth genera. The agent with the highest prevalence was *Ancylostoma* spp. (93.2%), followed by *Trichuris* spp. (18.6%), *Toxocara* spp. (11.9%) and *Toxascaris* (1.7%). These data show that there is a need for greater care towards controlling these helminths with zoonotic potential, including responsible pet ownership and daily activities to clean and collect dog feces in the vicinity of schools, because these are places where children play and study.

Keywords: Environmental contamination, larva migrans, hookworms, *Toxocara* spp.

Resumo
O objetivo deste estudo foi avaliar a contaminação ambiental por ovos de helmintos com potencial zoonótico, diagnosticados em fezes caninas depositadas nas proximidades de escolas primárias. Setenta e nove amostras de fezes foram coletadas em 28 escolas municipais localizadas em cinco bairros da cidade de Pelotas, Rio Grande do Sul (RS), Brasil. As amostras foram processadas pela técnica de Willis-Mollay e analisadas em microscópio óptico (40X), para identificar quaisquer parasitas presentes. Todos os bairros foram positivos e 74.7% das amostras apresentaram um ou mais gêneros de helmintos. O agente com maior prevalência foi *Ancylostoma* spp. (93,2%), seguido por *Trichuris* spp. (18,6%), *Toxocara* spp. (11,9%) e *Toxascaris* (1,7%). Essas informações mostram que há necessidade de maior cuidado no controle desses helmintos com potencial zoonótico, incluindo a responsabilidade dos animais e atividades diárias de limpeza e colheita de fezes de cães nas proximidades das escolas, porque esses são locais onde as crianças brincam e estudam.

Palavras-chave: Contaminação ambiental, larva migrans, ancilostomídeos, *Toxocara* spp.
Close relationships between humans and domestic animals such as dogs and cats do not just become established in households. These animals also frequent public spaces that are used for leisure activities, especially by children, who thus are exposed to the risk of infection caused by some helminth species (Carvalho & Rocha, 2011).

Dogs and cats are the domestic animals that maintain closest the contact with humans. Consequently, sanitary control regarding these animals is essential for human health. Among the diseases that affect dogs and cats, gastrointestinal helminthiases require attention within veterinary practice and public health promotion (Acha & Szyfres, 2003; Hotez et al., 2008; Peruc et al., 2009). These parasitoses may lead to anemia, diarrhea, weight loss, enteritis and even dogs’ and cats’ death (Robertson & Thompson, 2002). Humans may also contract them when they have direct contact with these animals, drink/eat water/food contaminated by parasite eggs or are affected by active larval penetration through the skin. Cutaneous larva migrans (CLM) and visceral larva migrans (VLM) are among the infections that are transmitted to humans by dogs and cats (Santarém et al., 2004; Mendonça et al., 2013; Moura et al., 2013).

Since these parasitoses can have an impact on both human and animal health, it is fundamental to monitor areas where there are many domestic, semi-domestic and stray dogs, so as to evaluate the risks that they pose to the community. Therefore, the aim of this study was to evaluate environmental contamination by zoonotic agents in dog feces collected in the vicinity of elementary schools.

Samples of dog feces were collected within a radius of 50 meters from the entrance gate of every municipal elementary school located in five neighborhoods in Pelotas, Rio Grande do Sul (RS), Brazil. They were stored in plastic bags and kept under refrigeration at 4°C until the time of processing at the Laboratory of Parasitic Diseases (LADOPAR), which belongs to the School of Veterinary Sciences at the Federal University of Pelotas (UFPel). Parasite eggs were evaluated using the modified Willis technique (Willis, 1921) and their morphological characteristics were identified using an optical microscope (40X objective lens).

The results were expressed as descriptive statistics and were then tabulated and analyzed using the Microsoft Excel® software. The chi-square test (χ² test) was used to ascertain whether any statistically significant differences were present among the variables (feces, neighborhoods and distances between schools). P-values ≤ 0.05 were considered significant.

Twenty-eight (84.8%) out of the 33 schools that were investigated between March and September 2017 presented dog feces in their vicinity. Most of these schools (23) were located on the outskirts of the city, while five were downtown. Data provided by the Municipal Department of Education and Sports (SMED) showed that the schools under investigation represented 80.5% of the schools located in the urban area and had 20,812 students enrolled (Table 1).

| Neighborhood | % of schools visited | % of students enrolled |
|--------------|----------------------|------------------------|
| Areal        | 77.8% (7/9)          | 84.3% (5012/5945)      |
| Centro       | 85.7% (6/7)          | 96.7% (5426/5613)      |
| Fragata      | 100% (7/7)           | 100% (4238/4238)       |
| Laranjal     | 100% (3/3)           | 100% (1913/1913)       |
| Três Vendas  | 66.7% (10/15)        | 52.4% (4223/8053)      |
| Total        | 80.5% (33/41)        | 80.8% (20812/25762)    |

Seventy-nine samples of feces were collected in the vicinity of the schools investigated; 74.7% of these samples exhibited one or more helminth genera. The lowest
prevalence of contamination (60.0%) was found downtown, while the highest (80.0%) was found in a suburb called Areal. However, there were no significant differences among the neighborhoods regarding the frequency of findings of feces with parasites in the vicinity of the schools under investigation (p ≥ 0.05) (Table 2).

Table 2. Number of samples of helminth-positive feces in the vicinity of schools and their neighborhoods in Pelotas, RS, Brazil.

| Neighborhood | No. of schools | No. of stool samples collected | No. of helminth-positive stool samples | % of positive samples |
|--------------|---------------|-------------------------------|--------------------------------------|----------------------|
| Areal        | 7             | 20                            | 16                                   | 80.0                 |
| Centro       | 5             | 10                            | 6                                    | 60.0                 |
| Fragata      | 5             | 10                            | 7                                    | 70.0                 |
| Laranjal     | 2             | 9                             | 7                                    | 77.8                 |
| Três Vendas  | 9             | 30                            | 23                                   | 76.7                 |
| Total        | 28            | 79                            | 59                                   | 74.7                 |

Lower prevalences of contamination were reported by La Torre et al. (2018) in samples of dog feces in Rome and Padua, Italy (9.7%), and by Medina-Pinto et al. (2018) in samples of dog feces collected from public parks in Yucatán, Mexico, where the positivity rate was only 11%. In RS, Lopes et al. (2014) found positivity similar to what is reported in the present study, i.e. contamination by parasite eggs in 71.6% of feces samples collected from public squares in municipalities located in the south of RS. In the same state, Scaini et al. (2003) observed 86.1% positivity in feces samples collected from streets in Cassino, a neighborhood in the municipality of Rio Grande, RS, while Villela et al. (2009) found 54.3% positivity in feces deposited on beaches located in Laranjal, in Pelotas, RS. The latter corroborate the data collected in the present study, since Laranjal was also evaluated in this study and exhibited contamination in 77.8% of the feces samples (Table 2).

Table 3. Occurrence of monoparasitism and polyparasitism caused by helminths in samples of dog feces collected in the vicinity of schools in Pelotas, RS, Brazil.

| Parasite(s)                      | Absolute frequency (%) | Relative frequency (%) |
|----------------------------------|------------------------|------------------------|
| Ancylostoma spp.                 | 41                     | 69.5                   |
| Trichuris spp.                   | 3                      | 5.1                    |
| Toxocara spp.                    | 1                      | 1.7                    |
| Ancylostoma spp. + Trichuris spp.| 8                      | 13.6                   |
| Ancylostoma spp. + Toxocara spp.| 5                      | 8.5                    |
| Ancylostoma spp. + Trichuris spp. + Toxascaris spp. | 1 | 1.7 |

In 76.3% (45/59) of the samples, one parasite genus was detected, while in 23.7% (14/59) of them, polyparasitism was observed (Table 3).

Regarding the method used in the present investigation, the Willis technique was chosen because it presents good results in environmental contamination studies, showing significant difference when compared to the Faust, Sedimentation and direct examination technique (Táparo et al., 2006). Moreover, such technique has lower material costs, shorter execution time and only requires the use of supersaturated solution (Novaes & Martins, 2015). However, it should be noted that other authors have detected different results. According to Santana et al. (2015), the centrifugal flotation technique showed higher sensitivity towards detecting Ancylostoma spp. and Toxocara spp.
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in dog feces. Therefore, the use of only one technique may not reveal the total parasitism present in the samples, which can be considered one of the limitations of this research.

Ancylostoma spp. was the most prevalent parasite, since it was found in 93.2% (55/59) of the positive samples (p < 0.0001); followed by Trichuris spp., in 18.6% (11/59); Toxocara spp., in 11.9% (7/59); and Toxascaris spp., in 1.7% (1/59). Similar distribution was reported by Scaini et al. (2003) in Cassino, a neighborhood and beach in Rio Grande, RS, where the percentage prevalences were 71.3%, 32.5%, and 9.3%, and by Villela et al. (2009) on the seashore in Laranjal, a neighborhood in Pelotas, RS, where they found 90%, 14.7% and 6.3%, in the cases of Ancylostoma, Trichuris and Toxocara, respectively. Other studies carried out in different Brazilian regions also found that Ancylostoma spp. was the helminth with the highest prevalence (Blazius et al., 2005; Labruna et al., 2006; Katagiri & Oliveira-Sequeira, 2008; Oliveira et al., 2009; Prates et al., 2009; Klimpel et al., 2010; Ferreira et al., 2013, 2016). Thus, precautions need to be taken since cutaneous larva migrans may be acquired in the vicinity of schools, given that the larvae of both Ancylostoma braziliense and Ancylostoma caninum have the capacity to penetrate through the skin (Bowman et al., 2010). Moreover, this is currently considered to be a neglected parasitic skin disease, and cutaneous larva migrans is one of the most common skin diseases reported in travelers returning from tropical regions (Leung et al., 2017; Reichert et al., 2018).

The possibility of Toxocara transmission, together with favorable environmental conditions, is a serious risk to public health (Capuano & Rocha, 2006; Santos et al. 2017). The nematode Toxocara spp. is usually identified as the causative agent of visceral larva migrans and ocular larva migrans (Sviben et al., 2009), and it still may result in central nervous system alterations (Hotez, 2014). A study carried out by Schoenardie et al. (2013) in the same region found that the seroprevalence of T. canis in children in Pelotas, RS, was 50.6%. These authors correlated their finding with the high rate of occurrence of stray dogs in this city (Schoenardie et al., 2013). Although no investigation of seroprevalence has been conducted among schoolchildren from the schools covered in this study, it is possible that a significant proportion of these individuals will be positive for anti-Toxocara canis antibodies, since parasite eggs have been identified in animal feces in the vicinity of the schools.

Concerning Trichuris spp. eggs, they may have belonged to the species Trichuris vulpis, which is a nematode that occurs in dogs, since dogs were often seen in the vicinity of these schools, even following children to the school gates. It should be highlighted that this finding requires attention because the zoonotic potential of T. vulpis has already been proven and its importance has been acknowledged, because this helminth may cause damage to digestive system (Traversa, 2011; Márquez-Navarro et al., 2012; Mohd-Shaharuddin et al., 2019).

Education for the population regarding hygiene measures and attention to the health of animals that are kept by the population not only enables changes in concepts and habits relating to zoonoses but also encourages responsible pet ownership. The importance of sanitary care for pets and responsible pet rearing in households needs to be emphasized, since lack of knowledge among the population regarding the risks involved in these diseases is one of the main factors that trigger these diseases (Peruca et al., 2009). From this, an educational folder about "environmental contamination by parasites and the importance of responsible pet ownership" was distributed to schools students.

There was high occurrence of helminth eggs with zoonotic potential in dog feces found in the vicinity of elementary schools in Pelotas, RS, Brazil. The results showed the role played by dogs in environmental contamination and emphasized the need for practices that prevent and control parasite infections. Such practices need to be implemented in association with responsible pet ownership and control over stray dogs, so as to decrease contamination in these areas and, consequently, decrease the population’s exposure to these infections.
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