Potential zoonotic parasites in dog and cat feces from three beaches and surrounding areas of Greater Florianópolis, Santa Catarina, Brazil

ABSTRACT
In Brazil, dogs and cats have free access in public areas in some cities, such as squares, parks and beaches, despite restrictive laws for their permanence in these places. Animals infected with intestinal parasites contaminate the environment through their feces. The present study aimed to verify the occurrence of helminth eggs, oocysts and protozoan cysts in stool samples from three beaches in Greater Florianópolis, Santa Catarina, Brazil, from March 2017 to April 2018. Stool samples were processed by the Willis-Mollay flotation technique and the Hoffman sedimentation technique. The percentage of 47.65% of positive samples for helminths or protozoa was found on the southern beaches of Florianópolis island, with an occurrence of 52.78% at Morro das Pedras Beach and 42.86% at Campeche Beach. At Pinheira beach, in Palhoça, 56.66% of the samples were contaminated with one or more zoonotic parasites. In this study, the most prevalent parasites on the three beaches analyzed were hookworms and *Trichuris vulpis*, followed by *Giardia* spp. and *Cystoisospora* spp. Samples containing *Toxocara* spp. eggs were collected at Pinheira beach. It is concluded that the contamination of the beaches in the present study constitutes a public health problem, considering the possibility of zoonoses transmission. The importance of implementing sanitary programs for the control of parasites in dogs and cats is emphasized, as well as awareness and education programs, in order to reduce environmental contamination in public places.


RESUMO
No Brasil, cães e gatos têm livre acesso em áreas públicas em algumas cidades, como praças, parques e praias, apesar de leis restritivas para sua permanência nestes locais. Animais infectados por parasitos intestinais contaminam o meio ambiente por meio de suas fezes. O presente estudo teve como objetivo verificar a ocorrência de ovos de helmintos, oocistos e cistos de protozoários em amostras de fezes de três praias da Grande Florianópolis, Santa Catarina, Brasil, no período de março de 2017 a abril de 2018. As amostras de fezes foram processadas pela técnica de flutuação de Willis-Mollay e pela técnica de sedimentação de Hoffman. O percentual de 47,65% de amostras positivas para helmintos ou protozoários foi encontrado nas praias do sul da ilha de Florianópolis, na praia do Morro das Pedras a ocorrência foi de 52,78% e na do Campeche 42,86%. Na praia da Pinheira, em Palhoça, 56,66% das amostras estavam contaminadas com um ou mais parasitos zoonóticos. Os parasitos de maior ocorrência nas três praias analisadas foram os ancilostomídeos e *Trichuris vulpis*, seguidos de *Giardia* spp. e *Cystoisospora* spp. Amostras contendo ovos de *Toxocara* spp. foram colhidas na praia da Pinheira. Conclui-se que a contaminação das praias do presente estudo constitui um problema de saúde pública, tendo em vista à possibilidade de transmissão de zoonoses. Deve ser ressaltada a importância da implantação de programas sanitários para o controle de parasitas de cães e gatos e programas de conscientização e educação, a fim de reduzir a contaminação ambiental em locais públicos.


Introduction

Based on data released by the Brazilian Institute of Geography and Statistics (Instituto Brasileiro de Geografia e Estatística, 2013) and updated by the commercial intelligence of Pet Brazil Institute, it was estimated that in 2018 in Brazil, the population of dogs was 54.2 million and cats 23.9 million, with a probable increase of 5% per year (Instituto Pet Brasil, 2019). This means that 44.3% of households have at least one dog and 17.7% have at least one cat. Gradually, dogs and cats have become part of the family, contributing to the welfare of their owners.

The World Organization for Animal Health - OIE, estimates that, in Brazil alone, there are more than 30 million abandoned animals, including 10 million cats and 20 million dogs. Dogs and cats can contribute to the perpetuation of the biological cycle of several parasites. Stray dogs are highly exposed to a variety of zoonotic parasites, and they can become important reservoirs for human infection, particularly in poor urban and rural environments (Otranto et al., 2017). When infected, they eliminate helminth eggs, protozoan cysts and oocysts into the environment through their feces. Dogs and cats are definitive hosts for several endoparasites with zoonotic importance, such as helminths (Ancylostoma spp., Uncinaria spp., Toxocara spp., Trichuris spp.) and protozoa (Giardia spp., Cryptosporidium spp.) (Dantas-Torres & Otranto, 2014; Bricarello et al., 2018; Otranto et al., 2017).

The feces of dogs and cats in public places are possible sources of environmental contamination, representing a high risk of infection for humans mainly due to the inadequate deworming routine of the animals, and incorrect disposal of the waste of companion animals; they are recognized as public health problems, especially in developing countries and in communities that are socioeconomically unfortunate (Lavallén et al., 2011; Diakou et al., 2019; Otranto et al., 2017; Traversa, 2012). Humans get exposed to these parasites by direct contact with animals or through water, contaminated food, contaminated hands and soil (Overgaauw & Nederland, 1997; Roddie et al., 2008; Rostami et al., 2019). Dogs and cats are hosts of hookworms that can cause zoonotic disease, such as cutaneous larva migrans, and has been associated with Ancylostoma caninum, A. braziliense, and Uncinaria stenocephala, which are all hookworms of dogs and cats (Centers for Disease Control and Prevention, 2020). Ancylostoma braziliense is most often implicated in dermatological lesions, and Ancylostoma caninum has been associated with eosinophilic enteritis and suggested as a possible cause of diffuse unilateral subacute neuroretinitis in humans (Bowman et al., 2010; Traversa, 2012). Another important zoonosis is visceral larva migrans syndrome, caused by the migration of Toxocara spp. larvae, especially T. canis, through the liver, lungs and brain (Centers for Disease Control and Prevention, 2019a; Rostami et al., 2019). And when it reaches the eyeball, it is called the larva migrans ocular (Rubinsky-Elefant & Ferreira, 2012). Sakano et al. (1980) and Mirdha et al. (1998) reported occurrence of visceral larva migrans caused by T. vulpis, besides ulcers and intestinal infections caused by the adult parasite. Giardia lamblia, also known as G. duodenalis, is a parasite cosmopolitan intestinal system that can cause intestinal malabsorption syndrome, diarrhea in humans and animals (Bartelt & Sartor, 2015; Bouzid et al., 2015).

In Brazil, the beach is a public place with free movement of people and without access control for animals, although there are laws in many cities prohibiting staying in these places. Stray dogs are highly exposed to a variety of zoonotic parasites, and they can become important reservoirs for human infection, particularly in poor urban and rural environments (Otranto et al., 2017). Complementary law no. 94, 2001, municipality of Florianópolis (2001) and the Municipal Law no. 193 of Palhoça (1994) states that the presence of dogs, cats or other animals on beaches is expressly prohibited, accompanied or not by their owners. However, no type of inspection by city officials or environmental agencies is carried out for this purpose. On the three beaches of Greater Florianópolis, it is common to see animals circulating on the beach and surrounding areas, a fact that can pose a serious risk to human and animal health, due to the transmission of several zoonoses. It must be emphasized that in all beaches there were signs prohibiting the presence of dogs on the beach.
This study aimed at verifying the occurrence of intestinal parasites with zoonotic potential in fecal samples collected on three beaches of Greater Florianópolis, Santa Catarina state (SC), Brazil, in order to contribute as a source of reference, so that public health authorities can take prophylactic measures to minimize the occurrence of parasitic zoonoses.

**Material and Methods**

**Study locations and subjects**

This study involved fecal samples from dogs and/or cats, collected from Campeche Beach and Morro das Pedras, located in the south of the island of Florianópolis, and Pinheira Beach in Palhoça, SC, Brazil (Figure 1). The extension to Campeche Beach and Morro das Pedras totals 7.500m and there are no physical barriers dividing these beaches. The study period for the collection and analysis was March 2017 to March 2018. On the southern beaches of the island of Florianópolis, the collection started at Morro das Pedras Beach, near the lifeguard tower, and extended to Rio da Noca at Campeche Beach.

Pinheira Beach is subdivided into Praia de Baixo, Mar Aberto and Ponta do Papagaio, representing 6.200m total area. There are no physical barriers dividing Praia de Baixo and Mar Aberto, but Ponta do Papagaio is separated by a river. The collections were carried out at Praia de Baixo and Mar Aberto, totaling an area of 4.700m. The study period for the collection and feces analysis was April 2017 to April 2018.

**Collection of fecal samples**

The collections of fecal samples were performed on the beach sand, as well as in surrounding areas, such as squares, parks, walkways and sidewalks giving access to the beaches. Collections were carried out once a month on each beach, avoiding rainy days.

The collections were performed preferably in the morning. However, when no samples were found in the morning, a new collection was performed in late afternoon on the same day, with only fresh, non-dried samples collected.

About 50g of fecal samples were collected and stored in individual plastic bags, identified by sample number, collection date and location. Subsequently, those were placed inside a thermal bag containing recyclable ice for transportation to the laboratory where tests were conducted. Each sample was transferred to a plastic container (100ml), which received the same identifications used in the plastic bag; then preservative solution SAF (1,5% sodium acetate, 2% acetic acid, 4% formaldehyde and dissolved in distilled water) was added in the proportion of one part of feces to three of the preservative (De Carli, 2011). The analyses were carried out at the Laboratory of Animal Parasitology, Department of Animal Science and Rural Development,
Center for Agrarian Sciences (CCA), Federal University of Santa Catarina (UFSC) in Florianópolis, SC, Brazil.

Parasitological techniques

For the analysis of fecal samples, Willis-Mollay flotation techniques (Monteiro, 2018) and Hoffmann sedimentation techniques (Hoffmann, 1987) were used. A drop of Lugol was added to the samples to stain the material and facilitate identification of possible protozoan cysts. When necessary, ocular morphometry under optical microscope of parasitic structures was used for identification and differential diagnosis of helminth eggs, oocysts and protozoan cysts.

Study trial and statistical analysis

This article was designed according to an epidemiological study model, analytic ecological type. This type of study aims to examine the existence of associations between the occurrence of a certain disease or health-related condition with a certain ecological factor (such as geographical location, for example). The estimate of the occurrence of zoonotic parasites in pet feces found on beaches in Greater Florianópolis provides relevant information on the risks of infection of these respective zoonotic diseases in these places. For the fecal sample collection, convenience sampling was applied.

When analyzing studies that apply experimental designs, similar to the present study, it was verified that there is still no standardization as to the lowest experimental n capable of representing, in a sensitive way, the risk estimates related to infection of the zoonoses of interest. Studies were carried out with experimental n ranging from 18 up to 137 for each studied geographic location (Bricarello et al., 2018; Ferraz et al., 2018, 2019; Santarém et al., 2012). Considering these pieces of information, the present research was carried out with 150 fecal samples representing Pinheiro Beach and 149 fecal samples representing the beaches Morro das Pedras and Campeche. Data were worked through descriptive statistics, in order to make the information presentation clearer and explanatory.

Results

Positive samples for parasites (52.17%) with zoonotic potential were found in all beaches of the present study (Table 1). The collected fecal samples at Campeche Beach and Morro das Pedras (south of Florianópolis island) and at Pinheira Beach/Palhoça were found on the shore and in access areas to the beaches and in places where people move freely and without footwear.

Of 149 fecal samples collected from the two beaches on the south of the island of Florianópolis, 71 (47.65%) were contaminated by helminths or protozoa (Table 1). The most prevalent parasites were hookworms (42.28%) and *Trichurus vulpis* (13.42%), followed by *Giardia* spp. (2.02%) and *Cystoisospora* spp. (0.67%).

Of 150 fecal samples collected at Pinheiro Beach in Palhoça, 85 (56.66%) were contaminated by helminths or protozoa (Table 1). The most common parasites were hookworms (56.66%) and *Trichurus vulpis* (8%), followed by *Giardia* spp. (2.67%) and *Toxocara* spp. (1.33%).

Discussion

In the present study, the occurrence of parasites of zoonotic importance was 47.65% on the Campeche and Morro das Pedras beaches, and 56.66% on Pinheiro beach in Palhoça, during a 12-month period. The highest occurrence in the samples was hookworms, which indicates a high risk for humans to contract cutaneous larva migrans, in contact with the beach sand. Hookworm-related cutaneous larva migrans (CLM) is a neglected tropical skin disease associated with significant clinical pathology, as eosinophilic enteritis and pneumonitis, localized myositis, folliculitis, erythema multiforme, or ophthalmological manifestations (Bowman et al., 2010; Traversa, 2012). There is little knowledge about prevalence and risk factors of CLM in endemic regions (Reichert et al., 2016).

Similarly, a study in the 2016/2017 summer season on the beaches of southern Florianópolis, SC, showed even higher occurrence of parasites from dogs and cats, where Campeche Beach presented 72.22% of fecal samples contaminated with hookworm predominance (Bricarello et al., 2018). The occurrence of this helminth was predominant at the Morro das Pedras, Campeche and Pinheiro beaches, the same way it was observed in other studies carried out on

<table>
<thead>
<tr>
<th>Beaches of Greater Florianópolis, SC*, Brazil</th>
<th>Nº of samples</th>
<th>Nº of samples positive</th>
<th>Occurrence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>South of Island</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Campeche Beach</td>
<td>77</td>
<td>33</td>
<td>42.86</td>
</tr>
<tr>
<td>Morro das Pedras Beach</td>
<td>72</td>
<td>38</td>
<td>52.78</td>
</tr>
<tr>
<td></td>
<td>149</td>
<td>71</td>
<td>47.65</td>
</tr>
<tr>
<td>Palhoça</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pinheiro Beach</td>
<td>150</td>
<td>85</td>
<td>56.66</td>
</tr>
<tr>
<td>Total</td>
<td>299</td>
<td>156</td>
<td>52.17</td>
</tr>
</tbody>
</table>

*SC = Santa Catarina, State.
beaches in Brazil (Blazius et al., 2005; Coronato et al., 2012; Ferraz et al., 2018; Pedrosa et al., 2014; Santos et al., 2006). The lack of current data on the transmission of zoonotic parasites on Brazilian beaches reveals negligence regarding the prevention of infections caused by these agents. Despite the current legislation prohibiting the presence of pets on the beach, a large number of stray dogs were found and animal feces were present on all beaches and surrounding areas during the study period. While there are zoonosis control centers in Florianópolis and Palhoça, dogs and cats are not periodically treated against intestinal helminths in public campaigns.

Hookworm eggs require an environment that protects them from desiccation to evolve into infective larvae of the third stage and the beach sand can perfectly fulfill this role and can survive 3 to 4 weeks (Centers for Disease Control and Prevention, 2019b). The presence of dogs and cats may also play an important role on soil contamination by agents of larva migrans and the sandy environments are favorable for the propagation of hookworm larvae of animals (Santarém et al., 2011). Cassenote et al. (2011) observed that the number of dogs in parks had an impact on soil contamination in public spaces and has been recognized as a risk factor.

The second most prevalent parasite was Trichuris vulpis. It was present in 8% of the contaminated samples at Pinheira Beach and in 13.42% of the positive samples in the south of Florianópolis Island. Other authors in similar studies also verified the presence of this helmint in fecal samples (Bricarello et al., 2018; Ferraz et al., 2019). It is a parasite of zoonotic importance, with a direct life cycle and infection occurs through the ingestion of embryonic eggs contained in the soil (Di Cesare et al., 2012; Jia-Chi et al., 2016). Despite few cases reported in humans in the past with Trichuris vulpis, it is considered a parasite of zoonotic importance as a causative agent of visceral larva migrans (Sakano et al., 1980) and intestinal infections in humans (Dunn et al., 2002). However, it could be that T. vulpis was occasionally incriminated as a cause of zoonosis, although it has not been convincingly proven as a cause of zoonosis (Traversa, 2011).

Toxocara spp. was another helmint with zoonotic potential found at Pinheira Beach. Ferraz et al. (2019) on the shore at São Lourenço do Sul Beach, Rio Grande do Sul (RS), reported this parasite in fecal samples; Bricarello et al. (2018) verified similar results on the southern beaches on the island of Florianópolis. The presence of Toxocara spp. eggs indicates risk of human contamination for visceral larva migrans syndrome, by ingestion of larvae eggs of this parasite (Overgaauw & Van Knapen, 2013; Ferraz et al., 2019).

In the present study, the presence of the protozoan Giardia spp. was verified, with a frequency of 2.67% at Pinheira Beach, and 2.02% at the southern beaches of the island of Florianópolis. Similar results were found by Bricarello et al. (2018) in Florianópolis. Cardoso et al. (2018), Pelotas, RS, reported greater prevalence at Laranjal Beach. The presence of Giardia spp. was relevant due to the zoonotic potential of this protozoan, since dogs may be parasitized by Giardia strains, which are potentially infectious to humans (Ferreira et al., 2013; Reboredo-Fernández et al., 2017; Rodrigues et al., 2014).

Specific legislation to regulate the permanence of animals on the beach must be discussed publicly, but the risks to human health must be prioritized. Aspects such as stray animal population monitoring and environmental sanity is extremely relevant regarding the one health approach of the population. Cutaneous larva migrans is the most common dermatological condition that affects population in Florianópolis, SC (Florianópolis, 2014). The imported cases are often reported after exposure to beaches in regions where Ancylostoma caninum is commonly found in its definitive hosts (Santarém et al., 2011).

The biggest problem of environmental contamination seems to be the permanence of fecal matter on the beaches and public areas and the incorrect disposal of dog and cat waste. The urban cleaning company in the municipality of Florianópolis, which belongs to the Municipal Government, advises on the correct disposal of domestic animal waste and it alerts on the occurrence of zoonoses, the risks of environmental contamination and the transmission of diseases to humans (Florianópolis, 2020). The visited beaches do not have adequate dumps for the collection of animal waste and there is no government plan for the final disposal; only guidance for disposal in domestic toilets. In Californian beaches, where dogs are allowed, a study determined that feces from dogs, which are not collected, are likely to contribute significantly to fecal contamination (Oates et al., 2017).

In countries like Australia, where there are reserved beach areas for dogs, a recent article has shown that the management of domestic dogs in natural areas is controversial, requiring more effective policies and management, due to high fecal contamination in those places (Schneider et al., 2019). In some areas, dog access is regulated or restricted to minimize harmful and often substantial interactions with wildlife (Wierzbowska et al., 2016).
Research shows that most citizens are unaware of the health risks related to canine feces left in public soils (Lima et al., 2010; Moreira et al., 2013; Pereira et al., 2016; Simonato et al., 2019). The dissemination of information on topics, such as disease transmission mechanisms, as well as prevention of zoonoses, is of great importance so that the population become aware. The importance of regular anthelmintic treatment or support to some pet health program must be urged for the public, promoted by government policies. In addition, guidance from veterinarians, health and environmental professionals is essential to minimize the transmission risks of parasitic zoonoses, in the context of One Health.

The One Health concept helps us understand the interactions among animals, humans and the environment, and how these interactions affect the occurrence of parasitic diseases (Schurer et al., 2016).

**Conclusion**

According to results of the present work, it was concluded that the contamination of the Campeche, Morro das Pedras and Pinheira beaches, Greater Florianópolis, SC, Brazil constitutes a public health problem, and reinforces the importance of implementing a parasite-control program for the population of dogs and cats to reduce the risks of parasitic infection on beaches. In addition, there is the need for development of health education programs, clarifying to the population the risks of transmission of these zoonoses.

**Conflict of Interest**

The authors declared no potential conflicts of interest with respect to the research, authorship, and publication of this article.

**Ethics Statement**

This article does not contain any studies with human participants or animals.

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