

**ORIGINAL ARTICLE**


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**PARASITES WITH ZONOTIC POTENTIAL IN DOG  
FECES AND SAND COLLECTED IN PUBLIC SQUARES  
OF BLUMENAU, SANTA CATARINA, BRAZIL**

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**ABSTRACT**

The presence of parasitized animals in streets, squares, parks, and other public places contributes to environmental contamination by infective forms of zoonotic parasites. Thus, this study aimed to evaluate the frequency of eggs, cysts, oocysts and/or larvae of parasites in samples of sand and feces present in nine public squares in the city of Blumenau, Santa Catarina, Brazil, through the four seasons of the year. Samples were collected between March 2021 and April 2022, totaling 223 dog feces samples and 106 sand samples. The dog feces samples were analyzed by the Willis method and by the Faust method, and the sand samples were analyzed by the Hoffman method and by the method of Flotation in Saturated Sodium Chloride Solution. All analyzed squares had at least one positive sample. Of the 223 stool samples collected from March 2021 to April 2022, 31 (13.9%) were positive for at least one parasite. Of the registered positive samples, *Ancylostoma* spp. was the most frequent parasite, observed in 26 samples (57.8%); followed by *Entamoeba* sp., identified in 11 samples. As for the contamination of the sandboxes, of the 106 samples collected, 11 (10.4%) were positive for some parasite. These results highlight the importance of improving public spaces, and school educational campaigns highlighting the importance of hygiene and reducing contamination by infective parasites, thus contributing to improving public health in the city of Blumenau, SC.

**KEY WORDS:** Zoonoses; sand contamination; hookworm; public spaces.

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## INTRODUCTION

Dogs and cats, despite being companions to humans and contributing to their emotional, physical, and social well-being, may often serve as definitive hosts for various zoonotic parasites (Sandhu & Singh, 2014). The presence of parasitized dogs and cats in streets, squares, parks, and other public places contributes to environmental contamination by infectious forms of zoonotic parasites. Although there are records of parasitic forms worldwide, these organisms are mostly found in tropical and subtropical regions, where socioeconomic challenges are present (Jafari et al., 2012; Abe et al., 2019).

According to the Ministry of Health, a significant portion of the Brazilian population has inadequate basic sanitation, and it is in a situation of social vulnerability (Castro et al., 2023). Despite Santa Catarina having a established waste collection system and achieving coverage rates close to 90%, (Santa Catarina, 2018) and the water supply network having above-average coverage compared to the national average, the sanitary sewage system is one of the worst in the country (Instituto Trata Brasil, 2021; Brasil, 2022).

Concerning parasite contamination in public areas of the southern region of Brazil, a study involving sand samples collected over one year in the municipality of Prudentópolis in the State of Paraná, Brazil revealed a contamination rate of 55% (132 samples), and the identification of parasitic forms including *Ascaris* sp., *Ancylostoma* sp., *Entamoeba* sp., and *Trichuris* sp. (Sanchez et al., 2021). In the far western region of Santa Catarina, another study conducted over six months found a contamination rate of 3.4% (2 samples).

Social vulnerability and the inadequacy of basic sanitation networks influence the contamination of animals. These animals end up contributing to the contamination of these environments, and studies worldwide have revealed significant environmental contamination rates in public and urban recreational areas (Traversa et al., 2014). Parasitic organisms with zoonotic potential have been found in research conducted in the soil of public squares, daycare centers, and schools, posing serious risks to human health (Mello et al., 2022).

Considering this issue, this study aimed to assess the frequency of eggs, cysts, oocysts, and/or larvae of parasites in the samples of sand and feces found in seven public squares and two public dog and cat parks in the city of Blumenau, Santa Catarina, Brazil, throughout the four seasons of the year. These locations were chosen to analyze the risk of contamination for both animals and humans who visit these spaces. The study of public parks designated for animals is also necessary because there is a risk of contamination for the animals that frequent these spaces, as well as for the children and the adults who accompany them. Furthermore, the spread of parasites in animals can increase the risk of transmission in other public squares and, concurrently, to humans. Therefore, expanding the research to these spaces, enables a more detailed analysis of contamination and parasite transmission.

## MATERIAL AND METHODS

### *Collection Points for Dog Feces and Sand Samples*

The collection of dog feces and sand samples was conducted in Blumenau, Santa Catarina, Brazil. The municipality features rugged terrain with significant differences in altitude and slopes, situated at an elevation of twenty-one meters above sea level. The climate of the region is classified as humid subtropical, characterized by hot and rainy summers throughout all seasons. The municipality covers a total area of 518.619 km<sup>2</sup> (IBGE, 2019), with a population of 361,261 inhabitants, of which 95.5% live in urban areas and 4.5% in rural areas (IBGE, 2010).

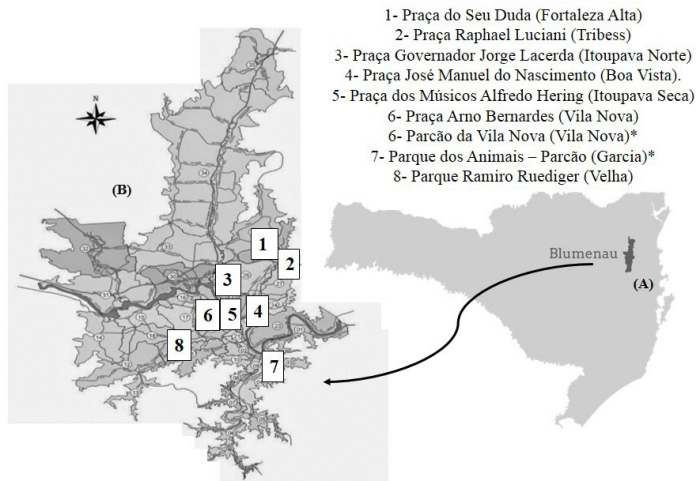
Different recreational spaces were visited throughout the year 2020 to understand the characteristics of each location and to select the squares that participated in the sample collections. For this purpose, the following characteristics were assessed in each visited square: the presence of sandboxes/sand areas, whether enclosed or not, usage by residents, the presence of animals in the area, primary activities conducted in the squares, the presence of trash bins, and the maintenance (cleanliness) of the place.

Out of the twenty-two places visited, nine squares were selected, with the primary criterion for their selection being the presence of sandboxes/sand areas, whether enclosed or not. Another factor taken into consideration was the preference for squares with higher foot traffic, as parasite infections may be more prevalent in such areas.

The selected squares and their respective neighborhoods of location were Arno Bernardes Square (Vila Nova), Musicians Square - Alfredo Hering (Itoupava Seca), Mrs. Duda's Square (Fortaleza Alta), Raphael Luciani Square (Tribess), Ramiro Ruediger Park (Velha), Jorge Lacerda Governor's Square (Itoupava Norte) and José Manuel do Nascimento Square (Boa Vista). In addition to these squares, two spaces designed for animal recreation were visited, the Vila Nova Park (Vila Nova) and the Animal's Park – Parcão (Garcia), as shown in Figure.

### *Collection of Dog Feces and Sand Samples*

The samplings were carried out between March 2021 and April 2022, encompassing all four seasons of the year (spring, summer, autumn, and winter). Collections were conducted monthly, with all squares visited on the same day and during the same period to minimize potential disturbances, such as precipitation and/or environmental cleaning.



*Figure.* The State of Santa Catarina (A), with emphasis on the municipality of Blumenau and the location of the selected squares for the collection of dog feces and sand (B). The points marked with \* indicate the public spaces designated for animal recreation. Adapted from the Municipality of Blumenau, SC.

For the collection of dog feces samples, sterile plastic containers were used and labeled with the location name and collection date. All feces found in the squares were collected, except for those that were excessively dry. The sand collection was performed within the sandboxes, with five sampling points established along the sand area (four at the corners and one in the center). At each point, approximately 50 grams of sand were collected from  $10 \times 10$  cm squares with a depth of 5 cm, resulting in 250 grams per square. These sand samples were stored in properly labeled sterile plastic bags.

After collection, all samples were stored in styrofoam coolers with ice packs and transported to the Parasitology Laboratory at Fundação Universidade Regional de Blumenau for subsequent analysis. In the laboratory, the dog feces samples were supplemented with a preservative solution of sodium acetate and acid formalin (SAF) and stored in a refrigerator until the time of analysis.

### *Analysis of Dog Feces and Sand Samples*

The dog feces samples were analyzed using the Willis method and the Faust method - zinc sulfate centrifugal flotation (Carli, 2011).

For the Willis method, approximately two grams of feces were used, which were homogenized with NaCl solution, filtered through a sieve and gauze, and added to a Borrel flask, filling the flask and placing a cover glass at the mouth of the flask. After 15 minutes, the cover glass was examined

under an Olympus CBA Micronal optical microscope at 40× magnification. The optical microscope was used to measure the findings on the slide, which aided in parasite identification. Additionally, a zig-zag scan of the slide was performed during observation, manipulating the stage to ensure visualization of all the different fields of the slide.

For the Faust methodology, approximately two grams of feces were employed. The feces were homogenized with distilled water, filtered through a sieve, and gauze folded into a new beaker. Then, the contents were transferred to a 15 mL Falcon-type tube and centrifuged for one minute at 600 rpm. The supernatant was discarded, and the remaining material was homogenized with 33% Zinc Sulfate and centrifuged again for another minute at 600 g. Using a flamed platinum loop, the surface film was removed and placed on a slide. The slide was examined with a drop of Lugol's solution and a cover glass under an optical microscope at 10× and 40× magnification (Beck et al., 2005).

The sand samples were analyzed using the Hoffman method (1934) and the Flotation in Saturated Sodium Chloride Solution method adapted from Ribeiro (2016). For the Hoffman methodology, approximately two grams of sand were used. They were transferred to a disposable plastic cup with distilled water, homogenized with a glass rod, and filtered through a sieve and gauze. Subsequently, they were transferred to a cup for spontaneous sedimentation, and the cup's volume (100 mL) was filled with water. The samples were left to settle for an hour, and the sediment was collected with a pipette to create a slide, with a drop of sediment and a drop of Lugol's solution, covered with a cover glass. The analysis was performed using an optical microscope with a 10× and 40× objectives.

For the Flotation with NaCl method, approximately two grams of sand were employed. They were transferred to a Falcon tube with 10 mL of saturated NaCl solution, homogenized ten times, filling the entire container, and a cover glass was placed at the mouth of the tube. After 10 minutes, the cover glass was examined with a drop of Lugol's solution and a cover glass under an optical microscope with a 10× and 40× objectives.

Parasitological atlas books in veterinary medicine by Foreyt (2005), Fortes (2004), and Hendri and Robson (2011) were used as morphological references. Additionally, a micrometer was used in the ocular to carry out precise measurements of the findings, thus aiding in parasite identification.

### *Statistical Analysis*

After reading the slides, the data were tabulated in Microsoft Office Excel® software (Microsoft Corp. Redmond, WA), and the results were expressed using descriptive analysis (mean) and percentage (%) of positive/negative samples. The contamination rate was calculated due to the difference in the number of samples collected in different public spaces.

To compare the contamination rate of parasites found in the squares and in the samples of feces and sand, the chi-square independence test was used. The analysis was performed in GraphPad Prism (8.0), with  $p \leq 0.05$  considered as the reference for assigning statistical significance.

## RESULTS

### *Fecal samples*

At the end of the sampling period, 223 dog feces samples were collected, and all the analyzed squares had at least one positive sample for the presence of parasites. It is important to highlight that, as the amounts of canine feces collected in the squares were different, a contamination rate was calculated, that is, the data were transformed into a percentage to facilitate comparison between the different squares.

Of the feces samples collected between March 2021 and April 2022 (Table 1), 30 (13.5%) tested positive for the presence of at least one parasite, with the highest incidence recorded in Ramiro Ruediger Park (33.3%) and Musician's Square - Alfredo Hering with 22.7%. Meanwhile, among the animal parks, Vila Nova Park had a contamination rate of 31.6%.

*Table 1.* Analysis of feces collected in public places in Blumenau-SC, from March 2021 to April 2022.

Squares/Parks	Collected Samples	Positive Samples	Rate of Contamination
Arno Bernardes Square	18	3	16.6%
Square of Musicians - Alfredo Hering	22	5	22.7%
Mrs. Duda's Square	17	3	17.6%
Raphael Luciani Square	32	3	9.3%
Ramiro Ruediger Park	12	4	33.3%
Gov. Jorge Lacerda's Square	13	2	15.3%
José Manuel do Nascimento Square	58	9	15.5%
Vila Nova Park	38	12	31.5%
Animal's Park (Parcão)	13	1	7.6%
Total	223	30	13.4%

In the recorded positive samples, *Ancylostoma* spp. was the most prevalent parasite in them, identified in 26 samples (57.8%). *Entamoeba* sp. was the second most found genus, identified in 11 samples (24.4%). In addition to these parasites, *Toxocara* sp. and *Trichuris* sp. were also identified in the samples.

Regarding the different techniques used, the Faust technique was able to recover the highest number of eggs and larvae for the parasites *Ancylostoma* sp. (20 positive samples), *Entamoeba* sp. (10 positive samples), and *Toxocara* sp. (5 positive samples), followed by the Willis-Mollay technique, which recovered 13, 4, and 3 positive samples, respectively. The seasons changes of the year affect the dynamics of parasites in fecal samples. Therefore, we analyzed the contamination rate of parasites in the samples collected according to each season of the year. Consequently, it was observed that the highest contamination rate was recorded in the autumn, with 31.8% out of the 44 samples collected. In the summer, we obtained a contamination rate of 20.4% out of the 49 samples collected, followed by spring with 17.0% out of the 47 samples collected, and in winter, 16.7% out of the 84 samples collected.

### *Sand samples*

Regarding the contamination of sandboxes, a total of 106 samples were collected, revealing a contamination rate of 11 (10.4%) (Table 2). It was observed that Raphael Luciani Square had the highest contamination rate (18.2%). Gov. Jorge Lacerda Square and Arno Bernardes Square, both with 16.7%, shared the second position in the ranking of playground sands with the highest prevalence of parasites. As for parks designated for animals, only Vila Nova Park registered 8.3% of contaminated samples.

Regarding the parasites identified according to the techniques used for this matrix, it was observed that the technique of flotation in saturated sodium chloride (NaCl) solution allowed for the recovery of a greater number of parasites (4 positive samples) compared to the Hoffman technique (2 positive samples).

Finally, when we analyzed the contamination rate of the sand collected from public squares, it was observed that, like the fecal samples, autumn was the period during which a greater number of parasites could be recovered. During this season, the contamination rate was 16.6%, while in winter, summer, and spring, the rates were 14.8%, 7.4%, and 3.7%, respectively.

A significant difference was recorded when comparing the values obtained from the analysis of dog feces and public square sand ( $p = 0.0045$ ), as this result was expected since the contamination of sand, as well as soil, is primarily derived from feces of contaminated animals.

*Table 2.* Analysis of sand samples collected in public places in Blumenau-SC, from March 2021 to April 2022.

Squares/Parks	Collected Samples	Positive Samples	Rate of Contamination
Arno Bernardes Square	12	2	16.6%
Musician's Square - Alfredo Hering	12	1	8.3%
Mrs. Duda's Square	12	1	8.3%
Raphael Luciani Square	11	2	18.1%
Ramiro Ruediger Park	12	1	8.3%
Gov. Jorge Lacerda Square	12	2	16.6%
José Manuel do Nascimento Square	12	1	8.3%
Vila Nova Park	12	1	8.3%
Animal's Park (Parcão)	11	0	-
Total	106	11	10.3%

## DISCUSSION

The frequency of positive samples, both in feces (13.4%) and sand (10.4%) with the presence of parasites found in this study, indicates a significant public health issue. These results suggest that the public spaces analyzed in this study represent a contamination risk for people who frequently visit these areas.

Similar studies have found higher contamination rates, for example, Capuano and Rocha (2006) identified zoonotic parasite forms in 56.8% (188/331) of fecal samples collected from public locations in the city of Ribeirão Preto in the State of São Paulo, and Bricarello et al. (2018) found an occurrence of 47.7% (71/149) of contaminated feces with parasites in samples collected from the south of Florianópolis in the State of Santa Catarina.

In fecal analyses, despite having the smallest number of samples collected (12), Ramiro Ruediger Park had the highest contamination rate of samples (33.3%). This result is extremely relevant considering the location is a prominent leisure spot in the city (Blumenau, 2021). Apart from Ramiro Ruediger Park, Musician's Square - Alfredo Hering and José Manuel do Nascimento's Square had 22.7% and 15.5% positive samples for the presence of parasites, respectively. Both places are public spaces with a high flow of people and animals, increasing the chances of contamination by animal feces.



Among the parks designated for animals, Vila Nova Park showed contamination in 31.6% of the samples analyzed (38). In contrast, Animal's Park (Parcão) had the lowest contamination rate at 7.7% (13). Animal's park is becoming increasingly common in urban areas, where small spaces are reserved for off-leash dog interactions. This is already a reality in many States, including Rio de Janeiro, São Paulo, Recife, Fortaleza, Salvador, and Santa Catarina (Maciel, 2017). Thus, the importance of monitoring these shared spaces is emphasized.

The most frequent parasite in this study was *Ancylostoma* spp., found in 26 samples (57.8%). A similar result was found in the study by Ferraz et al. (2022) in Pelotas in the State of Rio Grande do Sul (RS), where the authors found 52.1% of samples contaminated by the parasite. Scaini et al. (2003), analyzing environmental contamination by helminth eggs and larvae in Balneário Cassino (RS), found a predominance of this same parasite, present in 71.3% of the samples. Other studies, such as those by Matesco et al. (2006) in Porto Alegre (RS), and Ferraz et al. (2018) on the beaches of Pelotas also reported a higher frequency of *Ancylostoma* spp. For this parasite, humans are accidental hosts. The infective larvae penetrate through the skin but cannot complete their biological cycle and migrate within the skin, causing map-like lesions called Cutaneous Larva Migrans (Junior et al., 2015). Even though they do not reach vital organs, the larvae primarily penetrate regions of the lower limbs or butt, causing irritability, itching, and even secondary infections in human hosts (Alipour & Goldust, 2015).

*Entamoeba* sp. was the second most frequently found genus, identified in 11 samples (24.4%), a percentage higher than that found in the study by Sanches et al. (2021), which reported 11%. *Toxocara* sp. was identified in 13.3% of the samples (6 samples), with a percentage value like the one mentioned in the study conducted in the municipality of Pelotas, which reported 9%. Eggs of *Trichuris* sp. and Nematoda were also observed in a lower percentage, both at 2.2%.

This parasite is the causative agent of toxocariasis, primarily in puppies, as in maturity, dogs develop strong immunity against ascarids (Capuano & Rocha, 2006). Since adult dogs are more common in the streets, it justifies the lower percentage of *Toxocara* spp. found. In humans, it is responsible for the parasitic zoonosis visceral larva migrans (VLM) and ocular larva migrans (OLM), which occur due to the ingestion of embryonated eggs and the migration of larvae through various organs (Capuano & Rocha, 2006).

In the diagnosis of *Ancylostoma* sp., a difference was noticed between the two techniques used, with the Faust technique demonstrating greater diagnostic efficiency, as well as in the recognition of *Entamoeba* sp. and *Toxocara*. At the same time, the limited number of *Trichuris* sp. records does not allow for a proper analysis of this variable.

The Willis-Mollay method, based on the principle of spontaneous flotation, is primarily indicated for the detection of light eggs like Ancylostomatids. On the other hand, the Faust method, based on the principles of flotation and centrifugation, is more suitable for the detection of cysts, oocysts of protozoa, and light eggs (SBP, 2020). For this study, the Faust technique showed less debris, and it was more effective in recovering parasites when compared to the Willis-Mollay method. Therefore, it is recommended to use both methods, thus enabling a greater number of parasites to be recovered.

Regarding the contamination of sandboxes, out of the 106 samples collected, 11 (10.4%) tested positive for various parasites, including eggs, larvae, and cysts. Raphael Luciani Square had the highest contamination rate at 18.2%. Gov. Jorge Lacerda and Arno Bernardes squares, both with a 16.7% contamination rate, shared the second position in the ranking of the most contaminated playground sands.

The study by Leon et al. (2019) assessed soil in public areas in Laguna dos Patos, Pelotas and found a contamination rate of 8.3% (10/120) in the collected samples. Moreover, longer-term studies, such as Mota et al. (2018) in Uberlândia in the State of Minas Gerais and Sanches et al. (2021) in Prudentópolis in the State of Paraná, observed parasitic positivity in 9% and 55% of the total sand samples, respectively. According to Ferreira et al. (2022), recreational areas are conducive to soil contamination by parasites since there is a high circulation of stray animals and people accompanied by their pets, creating a reservoir of parasites exposed to favorable abiotic transmission factors.

In the calculation of the contamination rate in the squares, the positive fecal and sand samples collected at each location during the study period were combined. In the analysis, out of the total 329 samples collected, 53 (16.1%) were contaminated, with Parcão from Vila Nova and Praça José Manuel do Nascimento being the locations responsible for the highest number of positive samples, 13 and 10, respectively.

In the local contamination analysis, Vila Nova Park and Ramiro Ruediger Park appeared as the locations with the highest contamination rates, 26% and 20.8%, respectively. Animal's Park (Parcão) was the location with the lowest number of infected collections, with a rate of 4.17%. Furthermore, there was a contamination rate of 17.7% in samples from Musician's Square - Alfredo Hering, 16.7% in Arno Bernardes' Square, 16% in Governor Jorge Lacerda's Square, 14.3% in José Manuel do Nascimento Square, 13.8% in Mrs. Duda's Square, and 11.6% in Raphael Luciani Square. It is worth noting that even with a greater number of negative samples during the study year, these results indicate significant contamination risk for the general population frequenting these areas.

It is important to emphasize that the collections were carried out in public squares, and open-air environments without protection against climatic factors like temperature, rain, and wind. Therefore, environmental changes may have influenced the sample analysis over the year, potentially resulting in a different number of positive samples.

In this study, the months that comprised autumn showed the highest contamination rates for both fecal and sand samples, which aligns with the findings of Sanches and colleagues (2021) in Prudentópolis. However, this contradicts what has been reported in other similar studies available in literature, highlighting the importance of tailoring each study to its specific region.

As Blumenau has a temperate climate (Blumenau, 2023) with relative changes in temperature and humidity between seasons, autumn is a period of milder temperatures (Climate Data, 2023) that helps preserve the samples in the environment in which they are subsequently collected (Brener, 2008). Additionally, there is less rainfall in autumn compared to summer and spring (Climate Data, 2023), which typically results in lower parasite contamination as heavy rains can wash away parasites from the soil (Brener, 2008). This can explain why autumn had the highest rate of positive samples.

In conclusion, seasonality is important not only for evaluating soil contamination but also for understanding the primary parasites that can be found at each time of the year and the best conditions for these parasites to infect users of public squares and their respective animals (Matesco, 2006).

This study demonstrated contamination by infective forms of parasites in public squares in the municipality of Blumenau. This raises an alert regarding the importance of pet owners taking care of animal health, as well as the habit of cleaning up after their pets defecate. Furthermore, it is of utmost importance for the government to seek the development of actions aimed at combating this contamination in public squares, protecting the visiting population from the risk of parasitic infections.

These results emphasize the need for improved hygiene measures, treatment for both humans and animals, and awareness of the public health issues that parasites cause in the municipality of Blumenau to reduce the risks of contamination.

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## CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest to disclose.

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