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THE PRESENCE OF PARASITIC STRUCTURES IN SANDY SOIL SAMPLES FROM BEACHES LOCATED ALONG THE NORTH COAST OF RIO GRANDE DO SUL, BRAZIL

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Abstract

Beaches are a source of parasite contamination because protozoan cysts and helminth eggs and larvae can remain viable in sandy soils for months. Parasitic infections are mainly related to hygiene conditions, health, and environmental education, and can affect the life quality of the population. Therefore, this study aimed to assess the presence of parasitic structures in five beaches located along the north coast of Rio Grande do Sul, Brazil. Eighty samples were collected in August and September 2020 at the beaches of Torres, Tramandaí, Capão da Canoa, Arroio Teixeira, and Arroio do Sal and processed with the spontaneous sedimentation method for microscopic analyses. The results showed that 32% (26/80) of samples were positive for the presence of parasitic structures, and there was a significantly higher frequency of positive samples in September than in August. Torres was the beach with the highest frequency of positive samples, while Arroio Teixeira and Arroio do Sal presented the lowest frequency of contamination. Nematode larvae, including hookworm larvae, were the most prevalent parasitic structures in the samples. Hence, the sandy soil from beaches must be monitored to develop programs and strategies for improving infrastructures and basic sanitation conditions, thus ensuring the health of the population.

Keywords: Beaches. Environmental contamination. Geohelminth. Helminths. Sand.

1. Introduction

Soil-transmitted helminth infections are usually neglected and affect mainly low-income people living in poor basic sanitary conditions. The World Health Organization (WHO 2020) estimates that more than 1.5 billion people, or 24% of the global population, are infected with soil-transmitted helminths. Infections are extensively distributed in tropical and subtropical areas, with the highest numbers in sub-Saharan Africa, the Americas, China, and East Asia.

The biological characteristics of parasites in the gastrointestinal tract, the ability to invade and migrate, their nutrient and blood consumption, and host conditions such as nutrition, immune competence, and associated diseases are related to several clinical manifestations (Silva et al. 2019). These effects include diarrhea, abdominal pain, malnutrition, general malaise, weakness, and impaired growth and physical development (Neves 2016; WHO 2020).

Geohelminths are parasites that need soil to complete their life cycle. Initially, the soil receives (by fecal contamination) non-infectious parasitic stages. Then, the soil provides conditions to develop the infectious stage until the parasite may contact a susceptible individual orally or by skin penetration (Bethony et al. 2006). Helminths of the *Ancylostoma*, *Dipylidium*, and *Toxocara* genera are examples of

parasites transmitted by animal feces. Their eggs and larvae may remain in the environment for a long time and consequently affect human health (Labruna et al. 2006; Marques et al. 2012). In this context, several non-domiciliated or semi-domiciliated dogs and cats in cities and the inadequate protection of leisure areas contribute to the high prevalence of parasites in the feces of these animals, increasing the risk of infection for the population (Araújo et al. 2021).

Sandy soils are an important source of human infection by parasites because of their geological characteristics. They are made of sand particles with diameters ranging from 0.02 to 2 mm and can retain water (Rocha et al. 2011). Thus, this type of soil becomes the ideal environment for egg survival and larvae development, given its increased humidity and oxygenation conditions (Rocha et al. 2011; Etewa et al. 2016).

According to the Brazilian Environmental Council (CONAMA) - Resolution 274/2000, human health and welfare might be affected by bathing conditions on beaches, considering these sites may represent a source of contamination for the population through water and sand. Environmental organs should also evaluate the parasitological and microbiological conditions of sand for further standards (Brasil 2000). However, the resolution does not mention the presence of zoonotic hosts, which is the main factor of soil contamination. Thus, studies investigating the occurrence of parasites on beaches are essential.

Several studies have demonstrated the parasitic contamination of sandy soils of Brazilian beaches, where the frequencies of positive samples varied from 12.5 to 62% (Santiago and Gagliani 2011; Crause et al. 2018; Jacinto et al. 2020; Ramos et al. 2020). The presence of parasites in the soil is usually attributed to zoonotic and human hosts and the lack of basic sanitation. Therefore, considering the scarcity of data on the parasitological contamination of sandy soils of beaches in the southern region of Brazil, the present study aimed to assess the occurrence of parasitic structures in Tramandaí, Torres, Capão da Canoa, Arroio Teixeira, and Arroio do Sal.

2. Material and Methods

The study was conducted in the coastal region of the state of Rio Grande do Sul, southern Brazil. The climate of the region is humid subtropical, the mean annual temperature varies from 14 to 22°C, and the mean rainfall oscillates between 1,250 and 2,000 mm (Nery 2005). Sandy soil samples were collected in the beaches of Tramandaí, Capão da Canoa, Arroio do Sal, Arroio Teixeira, and Torres (Fig. 1). These beaches were chosen because of their high population density and search by tourists and bathers, especially during the summer.

Sandy soil was collected in August and September 2020. Each sampling site was photographed to ensure that the collection in different months would occur in the same place. Eighty sand samples were collected: 50 samples in August (10 samples per beach) and 30 in September (6 samples per beach). In both months, all samples from different beaches were collected on the same day, in the first week of each month. The distance between sampling sites was 500 m. The samples were collected at least 15 m away from streams to prevent contamination by other agents in water/surface runoff discharges. Each sample consisted of approximately 250 g of sandy soil collected by scraping the soil surface with a metal spoon at 5 to 10 cm of depth at each site (Marques et al. 2012). The samples were identified and placed in thermal boxes to be transported to the Laboratory of Parasitology of the *Centro Universitário CNEC de Bento Gonçalves*.

The spontaneous sedimentation method was performed up to 72 h after the collection to investigate the presence of parasitic structures in the samples. No preservative solution was used in the samples because the period between sample collection and analysis was less than 72 h (Rocha et al. 2011). For processing, 50 g of sand was mixed with 20 mL of water. Then, the sample was filtered with surgical gauze and transferred to a sedimentation vessel (250-mL capacity). The volume of the vessel was completed with water, and the sediments were allowed to settle for 24 h. Then, the sediment was collected with a Pasteur pipette, transferred to two glass slides (duplicate analysis), stained with 5% Lugol iodine solution, and covered with a coverslip. The slides were analyzed with an optical microscope at 10× and 40× magnifications (Olympus CX31) (Rosa et al. 2018). Samples containing any parasitic structure were considered positive. Descriptive statistics were performed with the GraphPad Prism 8.0.1 software for

Windows. The chi-square and Fisher's exact tests were used to assess the association between positive samples and sampling sites, as well as positive samples and sampling periods. Values of $p<0.05$ were considered significant.

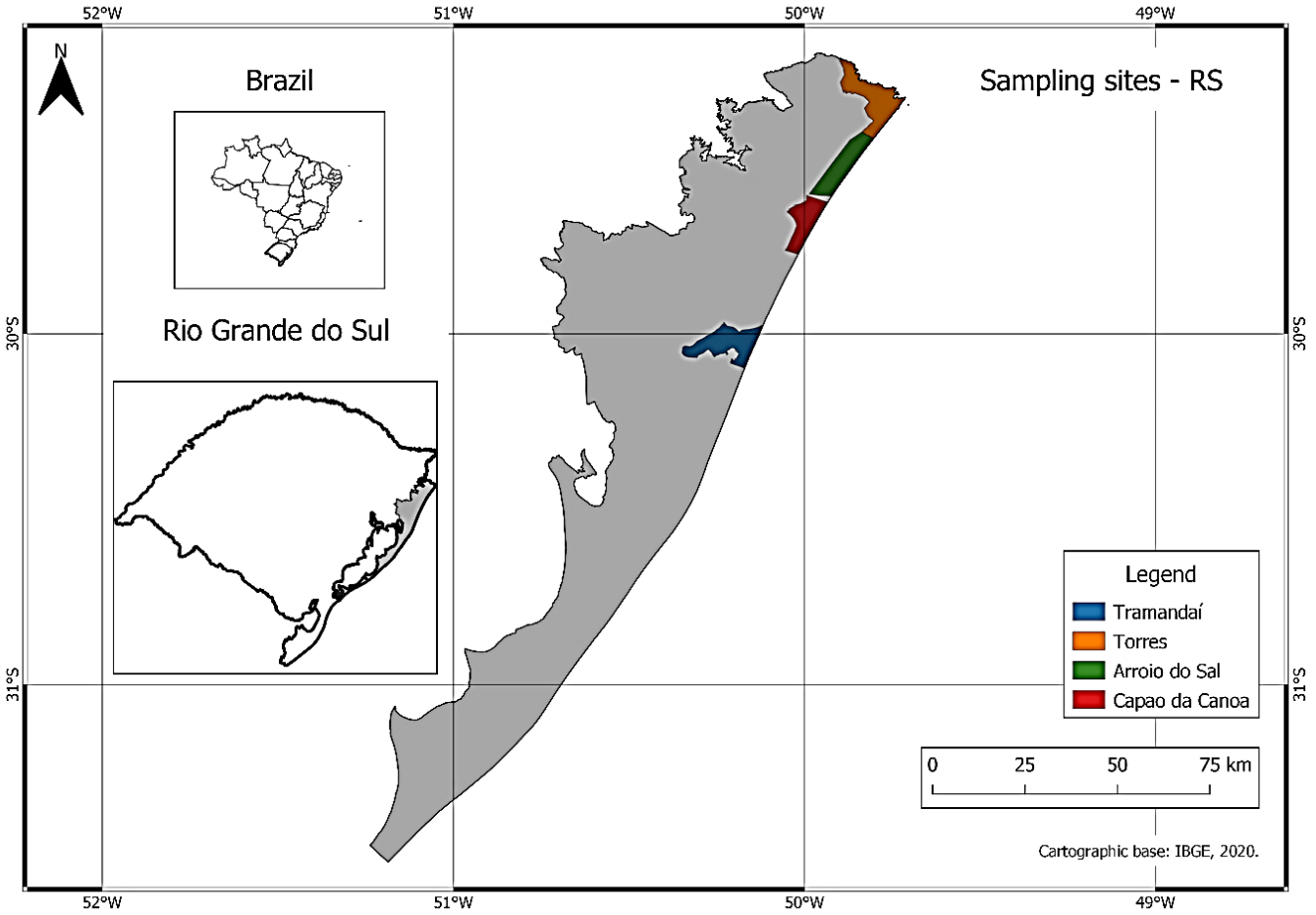

Figure 1. Location of sampling sites in the coastal region of Rio Grande do Sul, southern Brazil. Arroio Teixeira beach is part of the city of Arroio do Sal.

3. Results

Overall, 32% (26/80) of the samples were positive for the presence of parasitic structures. Torres showed the highest frequency of positive samples, corresponding to 43.8% (7/16). Conversely, Arroio Teixeira and Arroio do Sal presented the lowest frequency of contamination, both with 15% (4/16) of positivity (Table 1). Nonetheless, there was no significant association between positive samples and collection sites ($p = 0.74$).

Table 1. Frequency of parasitic structures in sandy soil samples collected at five beaches located in the coastal region of Rio Grande do Sul, Brazil.

Beach	Number of samples analyzed	Number of positive samples	Frequency (%)
Arroio do Sal	16	4	25%
Arroio Teixeira	16	4	25%
Capão da Canoa	16	6	37.5%
Torres	16	7	43.8%
Tramandaí	16	5	31.3%
TOTAL	80	26	32%

Regarding the sampling period, there was a significantly higher parasitological contamination in September, when 87% (22/30) of the samples presented parasitic structures than in August, when only 8% (4/50) of the samples were positive ($p<0.001$) (Table 2).

The parasitic structures most frequently found in the sand samples were nematode larvae. Among these, 10 samples allowed a specific identification of hookworm larvae. Other parasites observed included *Entamoeba* sp. cysts, hookworm eggs, and *Echinococcus granulosus* eggs (Table 3). It is worth noting that some samples presented more than one type of parasitic structure.

Table 2. Number of positive samples for parasitic structures according to the sampling period.

Sampling period	Number of samples analyzed	Number of negative samples and frequency (%)	Number of positive samples and frequency (%)
August	50	46 (92%)	4 (8%)*
September	30	6 (13%)	22 (87%)*
TOTAL	80	54	26

* Statistically different ($p<0.001$).

Table 3. Parasitic structures found in sandy soil samples collected at five beaches located in the coastal region of Rio Grande do Sul, Brazil.

Structures	N=26	Frequency (%)	Beaches
Unidentified nematode larvae	19	73%	All
Hookworm larvae	10	38%	Arroio do Sal, Capão da Canoa, Tramandaí and Torres
Hookworm eggs	4	15%	Arroio do Sal, Arroio Teixeira, Capão da Canoa and Tramandaí
Echinococcus granulosus egg	2	8%	Arroio do Sal and Capão da Canoa
Entamoeba sp. cyst	1	4%	Tramandaí

4. Discussion

The present study showed a 32% overall prevalence of parasites in the sandy soil of beaches. Other studies conducted in Brazil observed similar results. Araújo et al. (2020) found parasites in 14.3% of sand samples collected in São Luís, MA, Brazil. In another study, 32.7% of sand samples collected in São Vicente, SP, Brazil were positive (Santiago and Gagliani 2011). Likewise, there were parasites in 13.89% of 144 sand samples collected in Conceição da Barra, ES, Brazil (Crause et al. 2018).

The higher parasitological contamination observed in September can be explained because social distancing measures were more restricting before and during August due to the Covid-19 pandemic. Another factor that may have contributed to the higher contamination in September is that more people (tourists and residents) and their pets accessed the beaches during this month because of the higher temperatures and less restricting Covid-19-related measures.

Although Torres presented the highest frequency of positive samples, there were also parasitic structures in the other beaches studied. Capão da Canoa and Tramandaí are among the most populated cities on the coast of Rio Grande do Sul. According to data from the Brazilian Institute of Geography and Statistics (IBGE 2021), Tramandaí has 53,507 inhabitants and Capão da Canoa has 55,009. In contrast, Torres is the fifth most populated city in the region with 39,381 inhabitants. The higher population density, the search by tourists and bathers, and the access of animals (mainly dogs) to the beaches could explain the presence of parasitic structures in the samples analyzed.

Previous studies (Santiago and Gagliani 2011; Neto et al. 2017; Crause et al. 2018) have also frequently found hookworms (larvae and eggs). Moreover, this parasite was the most common/prevalent in other studies, found in 82% (32/39) of the samples collected at three beaches located in Fortaleza, CE, Brazil (Pedrosa et al. 2014) and in 35% (42/120) of the samples collected at Enseada beach, SP, Brazil (Ramos et al. 2020). Moreover, review studies on the parasitological contamination of Brazilian soils have also shown hookworm as the most prevalent parasite (Dalzochio et al. 2020; Araújo et al. 2021).

Hookworm is a zoonotic parasite whose eggs are eliminated from the feces of dogs, cats, and other animals. Thus, sandy soils from beaches, as well as other public places with free animal access, such as squares, parks, and peridomiciliar areas, are subjected to parasitic contamination (Rocha et al. 2019). The factors related to the development of hookworm eggs in the soil include permeability, aeration, granularity, organic matter richness, humidity, and temperature (Neves 2016; Ramos et al. 2020).

Humans may be contaminated by hookworms orally and by percutaneous penetration. The oral route is especially important for young children, who acquire parasites by accidentally ingesting soil and/or

contaminated toys, considering their direct contact with sand, precarious hygiene habitats, and some cases of geophagy (Sousa et al. 2014; Martins and Alves 2018). However, when percutaneous penetration occurs, the parasite triggers an inflammatory response and causes cutaneous larva migrans (CLM). Considering that larvae cannot complete their biological cycle, they migrate under the skin surface for weeks and cause erythematous, serpiginous, pruritic, and skin eruptions (Maciel et al. 2016; Rocha et al. 2019). In Brazil, CLM is caused mainly by *Ancylostoma braziliense* and *Ancylostoma caninum* larvae, especially in coastal regions with tropical and subtropical climates (Maciel et al. 2016).

Other parasitic structures found in the present study include *E. granulosus* eggs and *Entamoeba* sp. cysts. No previous studies reported the presence of *E. granulosus* eggs in the sand. This parasite causes cystic echinococcosis in humans when they ingest food contaminated by eggs or parasite larvae in the offal of livestock (Tamarozzi et al. 2020; CDC 2019). Dogs are the definitive hosts of *E. granulosus* and release eggs in their feces, leading to environmental contamination (CDC 2019). Regarding the presence of *Entamoeba* sp. cysts, Ramos et al. (2020) also found a lower prevalence of protozoa in the sand of bays in São Paulo, SP, Brazil, than helminths. In turn, Junior et al. (2021) and Sousa et al. (2014) reported a prevalence of protozoa in beaches in the Brazilian states of Pará and Paraíba, including *Entamoeba* sp. Among the species from the *Entamoeba* genus, only *Entamoeba histolytica* is pathogenic, causing intestinal infection and liver abscesses in more severe cases. The infection occurs by the oral ingestion of cysts via contaminated food or water (Sellau et al. 2021).

5. Conclusions

The high frequency of parasitic structures found in the present study confirms the importance of monitoring the parasitological contamination of the sandy soils of beaches. Thus, programs to improve infrastructures and basic sanitary conditions should be developed. Furthermore, the proper collection of solid waste, reduction of sewage discharge into the sea, and treatment of population and animals are required to minimize the occurrence of soil-transmitted helminthiasis.

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Ethics Approval: Not applicable.

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