doi: 10.5216/rpt.v49i3.63783

ORIGINAL ARTICLE

PARASITE DETECTION IN SAND FROM BAYS ON THE NORTH COAST OF SÃO PAULO STATE, BRAZIL

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ABSTRACT

Soil contamination by protozoan parasites and soil-transmitted helminths (STH) is common in beach sand due to a number of factors such as pets, pluvial water, garbage, etc. These pathogens may cause many diseases in humans and animals and become a public health problem. Thus, systematic evaluation and inspection are necessary to develop control strategies regarding public contamination. For this purpose, our aims were to evaluate the parasitic profile of sandy soils on an urban beach and an untouched beach and correlate this with environmental and seasonal characteristics in Ubatuba, Brazil, in two seasons (winter and summer). 132 soil samples were collected for parasite analysis utilizing Rugai's method and the sedimentation adapted method. Our results showed positivity in 62% of the samples for at least one parasite in the urban beach and no parasitic structures on the untouched beach. The positivity was higher in summer (85%) than in winter (51.7%). All samples were positive for both, helminths and protozoa. Seasonal influence was noted regarding the presence of STH while for protozoa this influence was not observed. The parasitic structures most found were larvae of hookworms (35%) and *Toxocara* eggs (31.7%). We also noted the presence of *Strongyloides* sp, *Ascaris lumbricoides*, coccidia, *Dipylidium caninum, Entamoeba hystolitica/dispar, Endolimax nana, Entamoeba coli, Giardia* sp, *Toxascaris leonina, Trichuris* sp and *Dibothricephalus latus*. There was a positive correlation between temperature and the presence of STH in the sand samples. Most of the collection sites on the urban beach presented dogs or canine traces and garbage in both seasons. There was an association between the presence of dogs or their traces and parasitic structures. In conclusion, seasonality, urbanization and the presence of pets on beaches can potentially favor environmental contamination by parasites increasing the risk of transmission of zoonotic and parasitic diseases.

KEY WORDS: Soil-transmitted helminths; protozoa; environmental contamination; sand; beaches.

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Received for publication: 5/6/2020. Reviewed: 19/8/2020. Accepted: 4/9/2020.

INTRODUCTION

Soil-transmitted helminth (STH) infections are the most prevalent neglected tropical diseases (NTDs) and affect more than 1 billion people causing high morbidity rates (Salam & Azam, 2017). These parasites affect animal and human populations mainly in the emerging countries where health infrastructure is fragile (Mascarini-Serra, 2011; Parija et al., 2017; Salam & Azam, 2017). Besides STH, some protozoans also cause intestinal infection and may use the soil to develop their life cycles, contaminating the soil while waiting for a new host (Campbell et al., 2016). Among the STHs, it is possible to highlight the role of *Strongyloides stercoralis, Toxocara, Ascaris lumbricoides, Trichuris trichiura*, hookworms, coccidia and some species of *Giardia*, as the intestinal parasites of greater clinical and veterinary importance (Campbell et al., 2016; CDC, 2017; Robertson & Thompson, 2002; Santamaría & Toranzos, 2003).

Infections caused by these parasites are related to the soil characteristics where the pathogen is located and also to the environmental conditions associated with their development. STH and protozoa can develop easily in soils of coastal areas due to environmental conditions such as temperature, moisture, pH and the local geomorphological structure (Chieffi, 2015; Rocha et al., 2011), increasing the risk of human and pet infection. However, the presence of infected zoonotic and human hosts is fundamental for soil contamination by the parasites. These hosts release evolutionary forms (eggs, larvae, cysts or oocysts) into the feces and these mature in the soil, which may result in an increase in parasite prevalence in the environment (Chieffi, 2015; Parija et al., 2017).

Several studies have quantified STH and protozoa soil contamination in Brazil and other countries and their results show values ranging from 20 to 90% positivity. The high prevalence is usually justified by the presence of zoonotic and human hosts, lack of sanitation and health surveillance (Cassenote et al., 2011; de Souza et al., 2007; Marques et al., 2012; Vaz Nery et al., 2019).

In Brazil, the sanitary conditions of beaches is monitored by evaluating soil quality, based on Resolution 274/00 of the National Environment Council (CONAMA). This resolution contains the criteria that regulate the use of the beaches according to the environmental agencies assessing the parasitological and microbiological conditions of the place. However, the presence of zoonotic hosts is not mentioned in the resolution, which is the major factor of soil contamination. Therefore, studies aiming at detecting and qualifying the contamination of sandy soils may contribute to determining risk factors regarding parasitic diseases and consequently assist in drafting environmental laws and implementing initiatives to control and prevent such diseases. The purpose of this study was to evaluate the parasitic profile of sandy soils on an urban beach and an untouched beach and correlate this with environmental and seasonal characteristics in Ubatuba, São Paulo State, Brazil, in two seasons (winter and summer).

MATERIAL AND METHODS

Study Area

The study was conducted in Ubatuba (north coast of São Paulo State – (Latitude: 23º 26' 02"S, Longitude: 45º 04' 16"W). This city is on the border of Rio de Janeiro State and is located 226 km from the state capital. Ubatuba has more than 84 beaches along 100 km of coastline. The beaches present crystal clear water, with varied landscapes, some untouched and others urban, with infrastructure (Rocha et al., 2010). The beaches evaluated in our study were Enseada beach (urban) and Fora beach (untouched). Enseada beach is urban, located in the southern region of Ubatuba and is approximately 9.3 km from the commercial center. It is one of the main hotel and gastronomic centers of this city. The sea is calm and suitable for swimming and water sports. This beach is 1.5 km long and can be reached by the Rio-Santos freeway. Fora beach can be reached from Enseada beach by trail or boat and presents rough seas with large waves, rocks and submerged pockets. Its extension is about 200m and it is 8.9 km from the commercial center of the city (STU, 2011).

Sand Sample Collection and Environmental Analysis

The sand sample collection and environmental analysis occurred in two seasons: in winter (July) and summer (January), after high tide on both beaches. To mark the points of collection, we conducted transects so that the points were 50 yards away from each other and as close as possible to the "boardwalk". We took photographs of each collection point to ensure that the collection in both seasons was in the same place.

From each point, two aliquots were collected; one on the surface and another 15 centimeters below the surface, approximately 200g of sand each. All samples were placed separately in plastic bags, duly listed and forwarded to the Microscopy Laboratory (LAMIC) Faculty of Integrated Sciences Pontal (Federal University of Uberlândia - UFU-FACIP) to be analyzed for the presence of parasitic structures up to 72 hours post -collection.

Altogether 132 samples were collected in the morning, 66 in winter and 66 in summer. Of the 132 samples collected, 120 (60 in winter and 60 in summer) were on the Enseada beach and 12 (six in winter and six in summer) on Fora beach due to the size of each beach. The presence or absence of garbage, pets and / or tracks (footprints, feces, etc.) were also noted within a radius of 25 meters from each collection point. The sand temperature of all collection points was measured (surface and 15 centimeters underground) using an analog thermometer.

Laboratory Analysis

To investigate the presence of parasitic structures, two parasitological methods were utilized: Rugai, for the detection of nematodes larvae (Rugai et al., 1954) and Hoffman, Pons and Janer sedimentation (Hoffman et al., 1934) adapted for detection of helminth eggs, protozoan oocysts and/or protozoan cysts. The Rugai method is based on the hydric thermotropism of nematode larvae. For this purpose, the samples (50 grams) were separated wrapped in gauze and placed in a sedimentation glass with water at 45°C for 1 hour. After this period the pellet was extracted with a pipette, placed on slides and stained with Lugol iodine solution (5%). The slides were examined utilizing a binocular optical microscope objective lenses in the 4× and 10× magnification. Three slides for each sample were prepared and examined by two different observers. Samples that contained larvae characteristics of pathogenic nematodes were considered positive.

The sedimentation method adapted was performed as follows: sand samples (100 grams) were transferred to nylon sieves under a sedimentation glass and washed with 200 mL of mineral water for filtering. Next, the sand samples with water were stirred with a glass rod and left to rest for 24 hours. Aliquots of the sediment surface layer were withdrawn with a Pasteur pipette and deposited in a test tube, shaken and subsequently centrifuged at 1,500 rpm for 10 minutes. The sediment formed on the rim of the sedimentation glass above the sand was collected with a pipette and transferred to a slide and subsequently stained with Lugol iodine solution (5%). The slides were examined under a binocular optical microscope objective lenses in the 10× and 40× magnifications. Three slides were prepared for each sample and examined by two different observers. Samples containing any parasitic structure were considered positive.

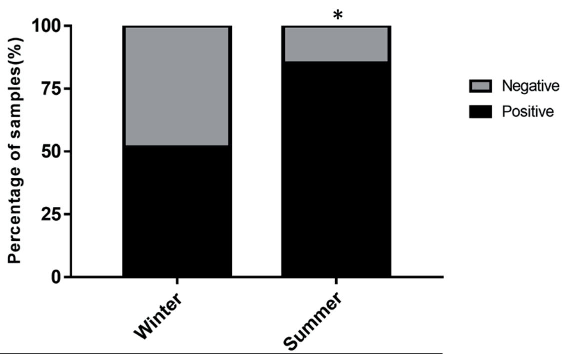
Statistics

Descriptive statistics were performed using the StatPlus® 2009 program. Multifactorial analysis were performed by Chi-square (X^2) and the Spearman method was applied to study the correlations (r). Binary logistic regression analysis was used to measure the level of significance between variables. Tables and graphs were created in the Microsoft Excel® 2010 and Statistica® 8.0 programs. Data were grouped according to the environmental temperature and the presence or absence of variables such as: garbage, dogs or dog traces.

RESULTS

The presence of parasite structures occurred only on the urban beach being higher in the summer

62% (82) of all samples collected proved positive. The presence of bathers was noted in both seasons only on the urban beach which presented a larger number of bathers in summer compared to winter. The samples (12) from the untouched beach showed no parasitic structures after parasitological analysis, however, the samples (120) from the urban beach presented 67.5% (81) parasitic structures. Those collected in winter showed 51.7% (31) positive samples and those collected in summer 85% (50) (p=0,45) (Figure 1).



Structure	Parasite	Fr (%)	Fr (%)
Larvae	Hookworm	8 (13.3)	36 (60.0)
	Strongyloides sp	6 (10.0)	26 (45.3)
	Toxocara sp	11 (18.3)	27 (45.0)
	Ascaris lumbricoide	9 (15.0)	22 (36.7)
	Hookworm	5 (8.3)	27 (45.0)
Eggs	*Thricuris* sp	0 (0.0)	1 (1.7)
	Dipylidium caninum	1 (1.7)	8 (13.3)
	Toxascaris leonina	1 (1.7)	3 (5.0)
	Dibothricephalus latus	0 (0.0)	1 (1.7)
Oocyst	Coccidia	9 (15.0)	7 (11.7)
Cyst	*Entamoeba coli*	2 (3.3)	3 (5.0)
	Endolimax nana	3 (5.0)	3 (5.0)
	Entamoeba hystolitica/dispar	3 (5.0)	4 (6.7)
	Giardia sp	2 (3.3)	3 (5.0)

Figure 1. Prevalence of parasites and evolutionary forms according to season in sand samples collected in winter and summer on Enseada beach in Ubatuba, São Paulo State, Brazil. N = 60 per station. *p=0,042.
Fr: Frequency.

STH structures were present in the majority of the sand samples

The 120 samples collected on the urban beach, yielded STH in 62.5% (85) of the samples and protozoa in 26.7% (32). Of the samples collected in the summer (60) 85% (51) contained STH and 26.7% (16) protozoa (p=0,036). More than one parasite structure was noted in some samples. In the winter samples (60), 40% (24) contained STH and 26.7% (16) protozoa (Figure 2). Importantly, the percentage of positive samples with protozoa was similar in the different periods: winter and summer (p=0,042).

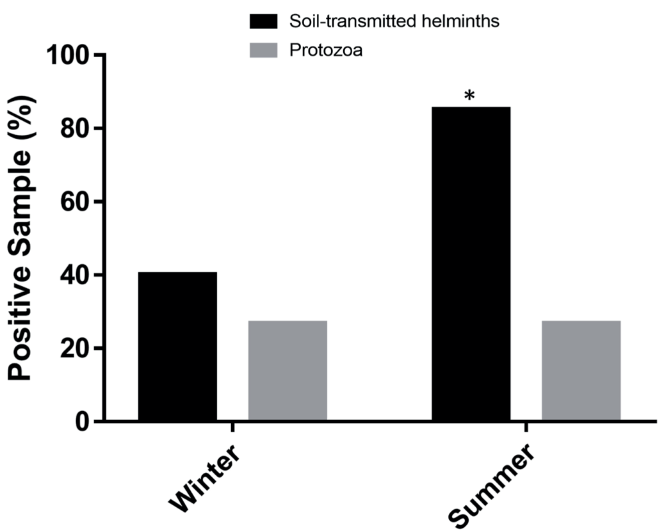


Figure 2. Prevalence of soil-transmitted helminths and protozoa in winter and summer on Enseada beach in Ubatuba - São Paulo state - Brazil. N = 60 per station. *p=0,036.

The parasitic structures most frequently noted in the sand samples were hookworm larvae [35% (42)], followed by *Toxocara* sp eggs [31.7% (38)], *Strongyloides* sp larvae and Hookworm eggs [26.7 % (32)], *Ascaris lumbricoides* eggs [25.8% (31)], coccidia oocysts [13.3% (16)], *Dipylidium caninum* eggs [7.5% (9)], *Entamoeba histolytica / dispar* cysts [5.8% (7)], *Endolimax nana* cysts [5% (6)], *Entamoeba coli* and *Giardia* sp cysts [4.2% (5)], *Toxascaris leonina* eggs [3,3 % (4)], *Trichuris* sp eggs [0.7% (1)] and *Dibothricephalus latus* eggs [0.7% (1)] (Table).

Table. Parasitic structures in sand samples from Enseada beach in Ubatuba - Sao Paulo state – Brazil.

Structures	Parasites	(n=120) % (fr*)
Larvae	Hookworm	35.0 (42)
	Strongyloides sp	26.7 (32)
Eggs	*Toxocara* sp	31.7 (38)
	Ascaris lumbricoide	25.8 (31)
	Hookworm	26.7 (32)
	Trichuris sp	0.8 (1)
	Dipylidium caninum	7.5 (9)
	Toxascaris leonina	3.3 (4)
	Dibothricephalus latus	0.8 (1)
Oocyst	Coccidia	13.3 (16)
Cyst	*Entamoeba coli*	4.2 (5)
	Endolimax nana	5.0 (6)
	Entamoeba histolytica/dispar	5.8 (7)
	Giardia sp	4.2 (5)

*Fr: Frequency.

Within the samples collected in winter (60), the parasitic structures most frequently observed were *Toxocara* sp eggs [18.3% (11)], followed by *Ascaris lumbricoides* eggs and coccidia oocysts [15% (9)], hookworms larvae [13.3% (8)], *Strongyloides* sp larvae [10% (6)], hookworm eggs [8.3% (5)], *Endolimax nana* and *Entamoeba hystolitica / dispar* cysts [5% (3)], *Giardia* sp and *Entamoeba coli* cysts [3.3% (2)], and *Dipilydium caninum* and *Toxascaris leonina* eggs [1.7% (1)]. *Trichuris* sp and *Dibothricephalus latus* eggs were not noted this time of year. In the summer samples (60), on the other hand, hookworms larvae were noted in 60% (36) of the samples. *Toxocara* sp eggs and hookworm eggs were present in 45% (27) of the samples, followed by *Strongyloides* sp larvae [43.3% (26)], *Ascaris lumbricoides* eggs [36.7% (22)], *Dipylidium caninum* eggs [13.3% (8)], coccidia oocysts [11.7% (7)], *Entamoeba hystolitica / dispar* cysts [6.7% (4)], *Entamoeba coli* cysts, *Endolimax nana* cysts, *Giardia* sp cysts and *Toxascaris leonina* eggs [5% (3)], and *Trichuris* sp eggs and *Dibothricephalus latus* eggs [1.7% (1)] (Figure 1). It is worth mentioning that all larvae recovered by Rugai's method were observed in the sedimentation method adapted. There were no differences regarding the presence of parasite structures and the origin of the samples (surface or underground).

Of the 120 urban beach samples, 36.7% (44) collected on the surface were positive and 30.8% (37) collected at a depth of 15 cm were positive. Most of the samples with parasitic structures (81) were collected on the surface (53.7%). The winter samples (60) showed 25% (15) positivity on the surface and 26.7% (16) underground while the summer samples (60) showed 48.3% (29) positivity on the surface and 36.7% (22) underground. No significant association was found between the positive samples and the collection sites (surface or underground) or period (winter or summer) ($X^2 = 0.34$ and $p > 0.05$).

There was a connection between temperature and presence of STH structures. In summer the average soil temperature on the urban beach was 26.3°C and 22.5°C on the untouched beach, while in winter the average temperature measured on the urban beach was 17°C and 13.5°C on the untouched beach. In the winter, the average soil temperature on the surface of the urban beach was 17.1°C and 16.8°C underground. In the summer the average temperature was higher: 27.2°C on surface and 25.4°C underground.

All of the samples (100%) with temperatures between 21 and 23°C showed some STH. Higher temperatures, from 24 to 32°C exhibited STH in 80% to 88% of the samples. Soils with lower temperatures, measured only in winter, presented fewer samples positive for STH, 36-41% (Figure 3). There was no correlation between the presence of protozoa and temperature ($r=0.12$ and $p=0.68$).

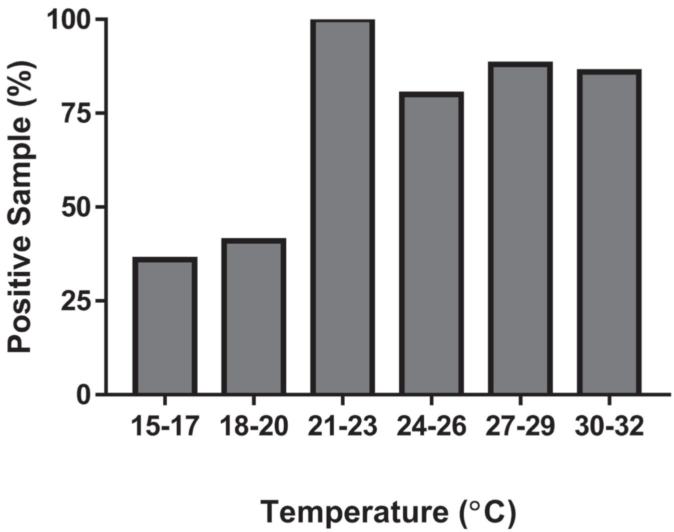
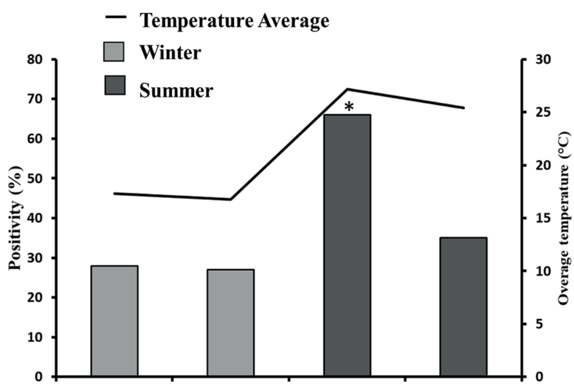


Figure 3. Percentage of samples positive for soil-transmitted helminths in relation to temperature variation recorded in winter and summer in the sandy soil of Enseada beach in Ubatuba - São Paulo state - Brazil.

Regarding the parasitic structures observed, the presence of 33.3% (10) STH eggs was noted in the winter samples (30) from the surface, as well as 20% (6) of STH larvae, 23,3% (7) of protozoan cysts and 16.7% (5) of protozoa oocysts. At the same time of the year, the underground samples (30) presented 43.3% (13) samples with STH eggs, 13.3% (4) with STH larvae, 20% (6) with protozoan cysts and 13.3% (4) with protozoa oocysts. The 30 surface samples collected in January 2012 (summer) showed STH eggs in 83.3% (25), STH larvae in 90% (27), cysts in 23.3% (7) and protozoa oocysts in 23.3% (7). Underground (30 samples), presented STH eggs in 60% (18) of the samples, STH larvae in 43.3% (13) and protozoan cysts in 13.3% (4) of the samples. No protozoan oocysts were detected. These data demonstrate the correlation between temperature and STH observation, because the higher the average temperature of the soil, the higher the positivity of the samples, particularly STH larvae ($p = 0.00006$) ($r = 0.4$) (Figure 4).



Evolutionary Forms	Surface	Depth	Surface	Depth
Larvae	6	4	27	13
Eggs	10	13	25	18
Cysts	7	6	7	4
Oocysts	5	4	7	0

Figure 4. Relation between the presence of parasites and their evolutionary forms in sand samples and average soil temperatures in winter and summer on Enseada beach in Ubatuba - São Paulo state – Brazil. *p = 0.00006.

Utilizing binary logistic regression, a highly significant association was noted between the presence of hookworm larvae and soil temperatures recorded in winter ($p=0.003$), this association proved marginally significant regarding the presence of *Strongyloides* sp larvae in the same season ($p=0.080$). In the summer there was a marginally significant association between the presence of *Strongyloides* sp larvae and soil temperatures ($p=0.080$), also significance for hookworm eggs ($p = 0.064$).

There was an association between the presence of dogs or canine traces in positive samples in winter. The environmental analysis revealed that 80% had garbage and 73.3% presented dogs or canine traces in a 25m radius around each collection point in winter. The analysis performed in summer showed garbage spots in 90% while 76.7% had dogs or canine traces in a 25m radius around each collection point. The statistical analysis showed an association between the presence of dogs or canine traces in positive samples in winter ($X^2 = 0.034$ and $p<0.05$), whereas in the summer this association was not noted ($X^2=0.44$ and $p>0.05$). No associations were observed between the presence of garbage and the presence of parasitic structures in both collection periods ($X^2=0.46$ in July 2011, $X^2=0.90$ in January 2012 and $p>0.05$). It is noteworthy that there were signs indicating that pets were not allowed on the beach. The untouched beach did not present any of these variables (garbage, pet and canine traces) at any of the collection times.

DISCUSSION

The results obtained in our study demonstrated a high prevalence (67.5%) and diversity of parasitic structures in sandy soil samples from the Ubatuba (SP) Enseada beach. However, studies reveal variations in parasite positivity in beach sand in different locations in Brazil. Sand samples collected in Santos (São Paulo State, Brazil) showed a positivity of 18.3% for parasites (Rocha et al., 2011). The prevalence of 29.4% of STH in sand samples in Salvador (Bahia state-Brazil) (Santos et al., 2006) has been reported. Soil samples from a beach in Porto Alegre (Rio Grande do Sul State, Brazil) showed 13.3% positivity for helminths (Matesco et al., 2007).

Despite reports in the literature regarding beach sand studies, these usually focus on other types of sandy soil. 83.7% positivity for helminth eggs and larvae was noted in soil samples from playgrounds in São Paulo (São Paulo State, Brazil) (Mello et al., 2011). Soil samples from public parks in Seropédica city (Rio de Janeiro State, Brazil) presented a positivity of 22.4% for STH eggs and larvae (Mandarino-Pereira et al., 2010).

In this study, we transected the portion of sand closest to the boardwalk with a view to collecting samples above the high tide line, since some parasitic structures exhibit impaired development due to high concentration of sodium chloride and these structures may be transported by seawater. Moreover, this method allowed collecting samples near beach tents, where sunbathers tend to stay. We are aware that beach sand studies generally demarcate random points or equidistant quadrants selecting random points within these (Nooraldeen, 2015; Rocha et al., 2011).

Positive samples for STH (62.5%) and protozoa (26.7%) were detected. Most research on environmental contamination by human or zoonotic parasites focuses on the presence of STH, however, does not prioritize the presence of protozoa.

Seasonality was a key factor regarding the presence of parasites. Significantly larger number of samples with parasitic structures were noted in January 2012 (85%) compared to July 2011 (51.7%). This difference is related to temperature and possibly other factors such as humidity and higher frequency of bathers in the summer. In July 2011 the prevalence of STH (40%) was lower than in January 2012 (85%) and 100% of the samples showed that temperatures 21-23°C presented some STH structures with the highest prevalence of parasites occurring in soil samples collected when the average temperature of the soil ranged from 23 to 25°C (Rocha et al., 2011). Furthermore, the STH eggs and larvae presence was higher from January to May and lower in July (Rocha et al., 2011).

Higher temperatures, from 24 to 32°C exhibited STH structures in 80% to 88% of the samples analyzed. A correlation was noted between temperatures and the presence of STH structures in sandy soils in São Paulo city (Brazil), where the higher the temperature the greater the positivity (Mello et al., 2011). The relationship between parasitic development and the presence of STH with temperatures and seasonality was noted in Maringá (Paraná State, Brazil) and determined the close relationship between the presence of STH larvae and temperatures, showing that the higher the average temperature of the soil, the greater the prevalence of these larvae (Tiyo et al., 2008). Nematode larvae present motility which allows them to settle in environments favorable for their development regarding temperature, humidity and oxygenation. The development of *Strongyloides* sp hookworm larvae occurs more readily at temperatures of 21-27ºC. Considering temperatures higher than 27°C were recorded, the same being noted in the sand samples, the absence of parasitic forms was probably due to this.

Although differences were noted between the surface and underground in relation to temperature and positivity in samples, such inequality was not significant. Other studies also used collection methods for sand on the surface and underground, and similarly no significant differences regarding these collection sites were reported (de Souza et al., 2007; Rocha et al., 2011; Silva et al., 2009)

There was no significant difference in the prevalence of protozoa (cysts or oocysts) in different sampling periods (winter and summer). A slight variation in the prevalence of protozoa was detected in the samples with a higher prevalence in May (Rocha et al., 2011). The sedimentation technique was applied and adapted. There were significant differences in the presence of STH among samples from July 2011 (winter) and January 2012 (summer), in which, *Toxocara* eggs (18.3%) and hookworm larvae (60%) were the most prevalent, respectively. Rocha et al. (2011) noted a high prevalence of both parasites in soil samples from Santos (São Paulo State, Brazil), where hookworm larvae accounted for 90% and *Toxocara* eggs for about 57% of the positive samples (Rocha et al., 2011). Other studies have also reported high positivity for at least one of these two parasitic structures (Cassenote et al., 2011; Mandarino-Pereira et al., 2010; Mello et al., 2011; Nooraldeen, 2015).

Hookworm and *Toxocara* are zoonotic parasites, etiologic agents of larva migrans, in which eggs are eliminated from the feces of dogs and cats. *Toxocara* eggs can survive for months in moist land, taking several weeks to mature. After contact with the soil, the *Toxocara* eggs are not yet in the infectious period, in other words not yet embryonated with second stage larvae, requiring appropriate temperature and humidity. Ideally, 85% of the eggs become infective after two to six weeks. The maturation ends at temperatures below 10ºC and the eggs become unviable at temperatures below 15ºC. On the other hand, hookworm eggs develop in environments with high concentrations of oxygen and favorable humidity and temperature. A suitable temperature for the development and hatching of larvae, is 23 to 30°C. Moreover, they are sensitive to freezing and low temperatures, becoming unfeasible in these conditions (Overgaauw, 1997; Fedorov, 1982). Such considerations are relevant to this study as in both collection periods the temperature was convenient for the development of the parasite, leading to soil contamination.

Enseada beach presented a considerable number of dogs or canines' traces such as faeces and paw prints in various spots during the collection periods. Castro et al. (2005) noted the prevalence of STH on beaches in the city of Praia Grande (State of São Paulo, Brazil) in samples of dog feces present along the seafront presenting *Toxocara* sp (45.9%) and *Toxocara canis* (1.2%) (Castro et al., 2005). As observed in the present study, the prevalence of parasites in soil samples is associated with the presence of zoonotic hosts like dogs and cats (Mandarino-Pereira et al., 2010; Mello et al., 2011; Rocha et al., 2011).

The presence of zoonotic hosts is the main cause of environmental contamination of beaches considering that *Toxocara* eggs and hookworm require predominantly dogs and/or cats for their development. These data are important since these parasites are the etiological agents of cutaneous and visceral larva migrans, currently neglected diseases (Werneck et al., 2011).

The other STH structures most frequently detected were *Strongyloides* sp larvae (26.7%) and *Ascaris lumbricoides* eggs (25.8%). The presence of both was noted in soil samples from Maringa (Parana state - Brazil), with 4% presenting *Strongyloides* sp larvae and/or *Ascaris* spp eggs. This difference may be related to geographical and environmental characteristics of the sites evaluated (Mandarino-Pereira et al., 2010).

As previously noted, there are few studies which use methods for recovery of protozoa in soil samples. This study, however, noted a diversity of these parasites, in which the most prevalent in the samples were coccidia oocysts (13.3%). Reportedly 16% of soil samples collected in Juiz de Fora city (Minas Gerais State, Brazil) showed coccidia. Similarly, the most frequently noted protozoa in soil samples analyzed in Santos city (São Paulo State - Brazil) were coccidia oocysts (3.3%) (Rocha et al., 2011). The presence of these parasites may be associated with the fact that there are several species of coccidia parasites that are zoonotic and can be easily found infecting domestic animals such as dogs, frequently present on Enseada beach.

The prevalence of other protozoan cysts was 5.8% for *Entamoeba hystolitica/dispar*, 5% for *Endolimax nana,* 4.2% for *Giardia* sp and also 4.2% for *Entamaoeba coli* on Enseada beach. Despite the scarcity of studies reporting the presence of protozoa in soil, there is a report regarding the presence of *Entamoeba* sp cysts in 0.7% of samples collected during a year on beaches in Santos (São Paulo State, Brazil) (Rocha et al., 2011).

Anthropogenic influence and the presence of garbage may have caused the prevalence of parasites on Enseada beach, since on Fora beach (untouched) the presence of garbage and zoonotic hosts such as dogs and cats were not noted, and therefore, no parasitic structures were noted in both collection periods. On beaches visited by people and local fauna, these may favor the mixture of fecal matter with sand, making visualization and removal difficult. Biotic and abiotic factors play an important role in the biological aspects of parasites present in sand (larvae maturation), requiring cleaning up feces from beaches either by pet owners or the local administration. Awareness programs are also advisable regarding the zoonotic potential of helminths transmitted by soil, and the importance of hygiene in beach areas frequented by the population. The results obtained in this work may encourage other researchers to carry out studies on other beaches frequented by people and their pets.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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