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Parasitoses of clinical interest in sediment of rio: an approach to Public Health

Parasitoses de interesse clínico em sedimento de rio: uma abordagem na Saúde Pública

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ABSTRACT
Lack of basic sanitation facilitates the spread of parasitic diseases that impact human health, with the sediment being able to house these microorganisms. The present study aims to evaluate the presence of pathogenic parasites to humans in riverbank sediments, addressing the risks of parasitic infections in public health. Eighty samples of sediments from the Paranhana and Caí rivers were evaluated using the HPJ technique. Fifty-three positive samples (66.2%) were obtained with different parasites, *Ancylostoma* sp., *Strongyloides* sp., *Endolimax nana*, *Ascaris lumbricoides*, *Toxocara canis*, *Giardia lamblia, Entamoeba coli*, *Entamoeba histolytica/dispar*, *Trichiuris trichiura* and *Taenia* sp. Places with greater urbanization presented 60% of positive samples and a greater number of species. Riverbank sediments indicated to be an appropriate means for the maintenance of infectious forms of parasites. Adequate sanitation is necessary in order to minimize environmental contamination as well as the population's health risk.

Keywords: Environment. Health. Parasites.

RESUMO
Falta de saneamento básico facilita a disseminação de doenças parasitárias que impactam à saúde humana, sendo o sedimento capaz de albergar esses microrganismos. Objetivo do presente estudo foi avaliar a presença de parasitas patogênicos ao ser humano em sedimentos de borda de rio, abordando os riscos de infecções parasitárias na questão de saúde pública. Avaliaram-se pela técnica de HPJ 80 amostras de sedimentos dos rios Paranhana e Caí. Obtiveram-se 53 amostras positivas (66,2%) com diferentes parasitas, *Ancylostoma* sp., *Strongyloides* sp., *Endolimax nana*, *Ascaris lumbricoides*, *Toxocara canis*, *Giardia lamblia*, *Entamoeba coli*, *Entamoeba histolytica/dispar*, *Trichiuris trichiura* e *Taenia* sp. Locais com maior urbanização apresentaram 60% de amostras positivas e maior número de espécies. O sedimento de borda de rio indicou ser um meio apropriado para a manutenção das formas infectantes de parasitas. Faz-se necessário um saneamento adequado, a fim de minimizar a contaminação ambiental bem como o risco à saúde da população.

Palavras-chave: Meio ambiente. Parasitas. Saúde.

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INTRODUCTION

Demographic expansions of urban and rural areas have triggered population agglomerations in peripheries characterized by lack of basic sanitation and by the dissemination of parasites. Parasite-caused diseases have drastically affected human health and caused two to three million deaths per year worldwide.1,2,3 According to the World Health Organization (WHO), there are some 2.4 billion people without any type of adequate sanitation.4

Approximately 100 million Brazilians do not have any access to the sewerage system and 35 million are without treated water. Less than half the population (46%) has treated sewerage system. The south of Brazil features only 44.5% of treated sewerage and approximately 89.7% of the population has treated drinking water. One hundred percent coverage is still a long way off.[5] Consequently, the above data are the cause of growth in parasite infections by hydric transmission and an increase in public expenditure.

Several parasite-caused infections are frequent, with special reference to intestine parasites such as *Ascaris lumbricoides,* hookworms (the species *Necatur americanus* and *Ancylostoma duodenale*), *Strongyloides* sp. and *Trichiuris trichiura* are the most common with high pathogenic potential in children, pregnant women and immuno-impaired people,[6,4,7] with significant impacts on human health. Since most laboratory analyses come from clinical fecal samples, analyses from environmental samples are still lacking.[7,6]

Parasites´ environmental monitoring is highly relevant to pinpoint risk areas for population health. Soil use and occupation in urban or rural areas affect the dissemination of several parasites since non-treated sewerage is discarded *in natura*, disperses into water bodies and affects soil and surface sediments and geo-hydric interactions in the environment.[8,2]

The sediment is the soil´s degraded compound produced by the processes rainfall, biotic and abiotic factors deposited on the soil´s upper surface which is greatly important in the assessment of the environment. Sediments at the river banks are capable of retaining water, hosting pathogens, retaining sanitary residues and effluents due to appropriate features such as pH, humidity, temperature and nutrients for the microorganisms. Consequently, the matrix may be characterized as host for parasites in the infecting and non-infecting stages, with survival conditions and maintenance of viability till they meet their hosts. [10,9]

The analysis of sediments on the river banks at different places is relevant to identify clinically interesting pathogenic parasites especially in countries with sanitary deficits and vulnerable populations. Current study evaluates the presence of pathogenic parasites, harmful to humans, in sediments of rivers, with special reference to their distribution in different types of soil usages and occupations and their importance to public health.

2 METHODOLOGY

2.1 SITE AND CHARACTERISTICS OF THE AREA UNDER ANALYSIS

The sediment of the banks of the water body of the river Paranhana in the hydrographic basin of the Rio dos Sinos (HBRS) and of river Caí in the hydrographic basic of the river Caí (HBRC), in south Brazil, is analyzed.[11,12] The two rivers were chosen due to their importance for the region´s water supply and to erosions, degradation and different types of soil occupation on their banks.

The mouth of river Paranhana lies in the Rio dos Sinos, the main river of the basin in the Vale dos Sinos region which is well-known for its lack of basic sanitation. In fact, it ranks fourth in the list of the most polluted rivers in Brazil. The estuary of the river Caí lies in the Lake Guaíba, ranking eighth in pollution.[12,13]

The investigated extension of the river Paranhana is 87.5 km, whilst that of the river Caí is 98.5 km, each passing respectively through five and six municipalities. Ten sites were marked at different places (rural and urban) by mapping the soil´s usage and occupation (Figure 1). Soil in the region of the riv-

er Paranhana is made up of sedimentary rocks and sandstone on the river´s banks, with heavy slopes. Although Permanent Preservation Areas (PPAs) are extant, anthropic processes are rife owing to increase in urban pollution. The soil of the river Caí region is composed of constant deposits of sand, marked by humid soil, especially on the edges of the water body, with erosion, greater industrial wastes and heavy anthropic activities. Flooding is common in the two regions[11,12].

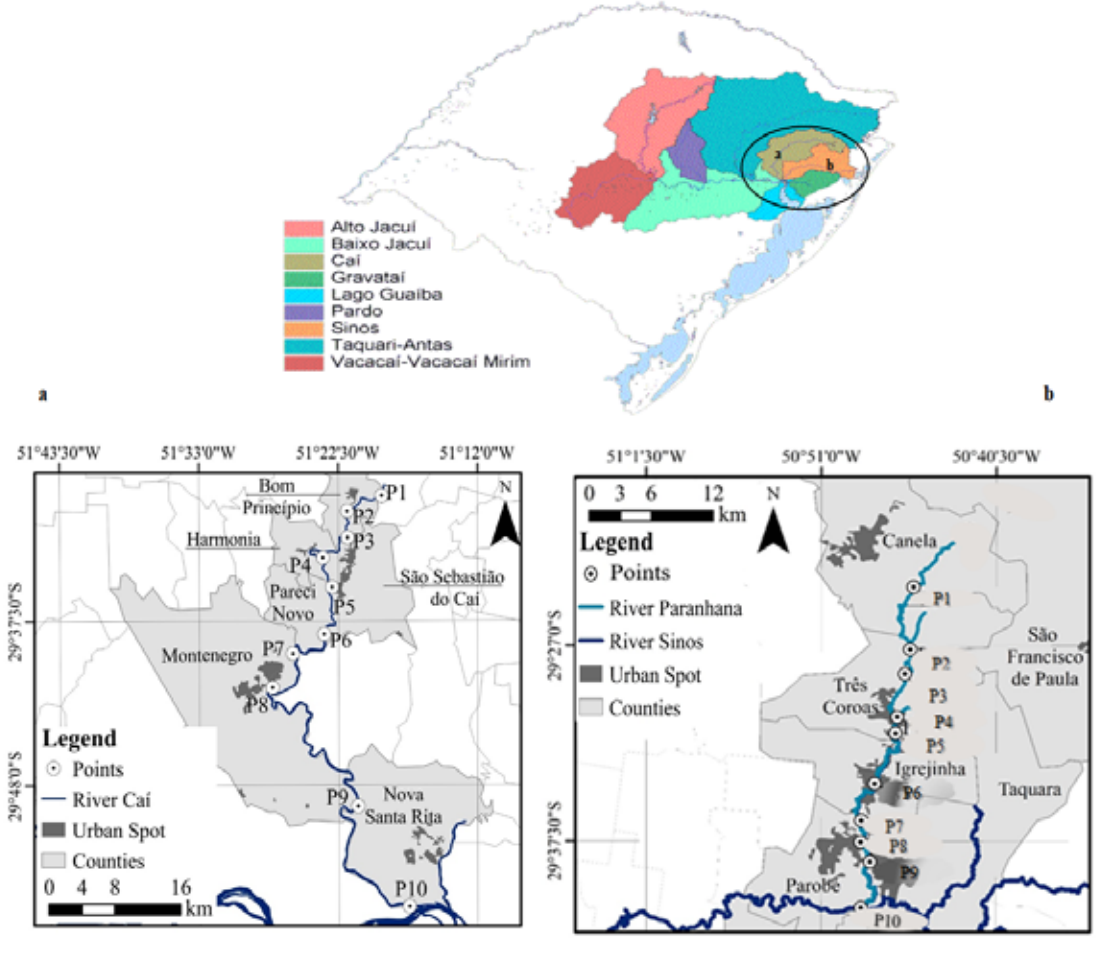


Figure 1. (a): HBRC (river Caí); (b): HBRS (river Paranhana) – Collecting sites and urban spots in the contiguous basins (Roberta Plangg Riegel) – Adapted by Tatiana Heck / (Pró-Guaíba - http://www.proguaiba.rs.gov.br/bacias.htm).

2.2 SAMPLING OF THE SEDIMENT ON THE RIVER BANKS

Samples from surface sediments from the river Paranhana were retrieved in May, August and November 2015; similarly, samples were retrieved in June and September 2016 and December and March 2017 from the river Caí. Four collections were undertaken with 40 samples from each river. The approximately 50 kg samples were collected by a spatula and stored in sterile 50 ml falcon tubes, refrigerated at 4°C for better stability of the material till their arrival in the laboratory and processed in up to 24 h after retrieval. Collection method followed Pritsch & Frighetto (2016)[2].

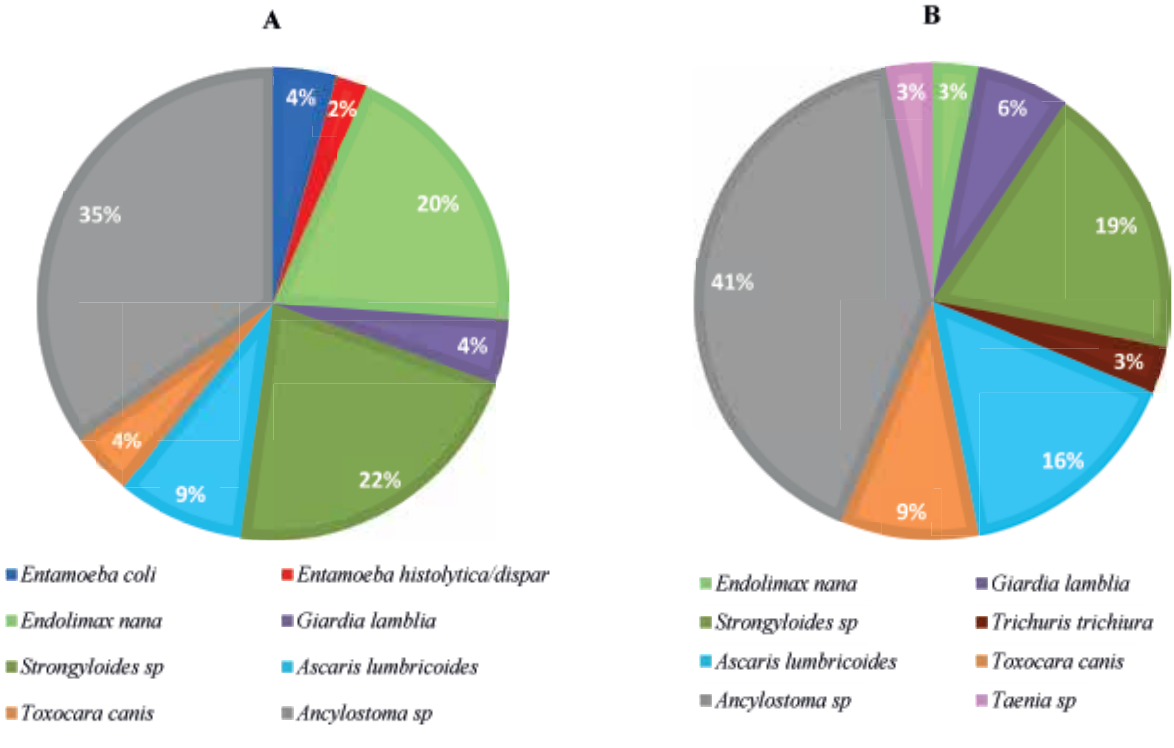
Parasites in the sediment were evaluated by spontaneous sedimentation technique, following Hoffmann, Pons & Janer (HPJ)[14]. Further, approxi-

mately 8 g of the sediment diluted in 125 ml of water were used in each sample. They were filtered by parasite filter and then sedimented for 2 h. A drop of the sedimented compound was extracted and a drop of lugol was added under coverslips and slides for analysis by optic microscope.

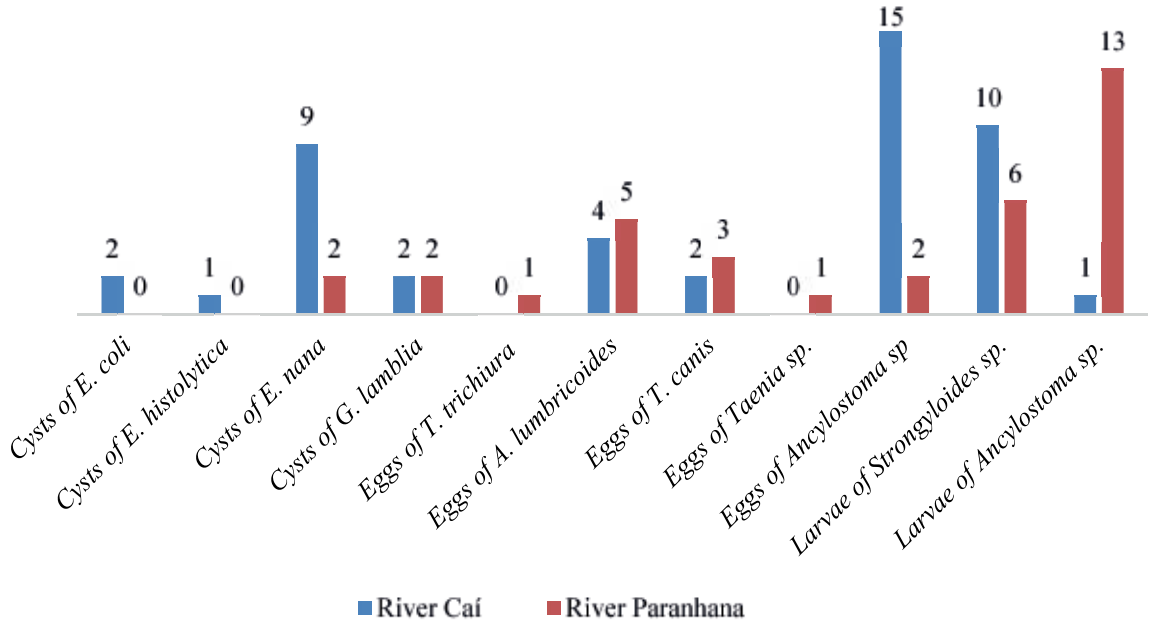
Parasites were magnified 100 times (10x objective) on the slides, sorted and analyzed in duplicate to confirm results. If the existence of parasites was confirmed, they were magnified 400 times (40x objective). Duplicate negative samples were evaluated again for confirmation, characterizing triplicate. The analysis of morphological characteristics for eggs, cysts and larvae identified parasites, coupled to size, color, shape and other features. Comparison was undertaken by the Handbook of Parasitology, Morphology and Biology of Sanitary Interesting Parasites and by electronic handbooks, such as WHO standard protocols, following previous studies[2,3,15]. Eighty samples were retrieved and analyzed.

3 RESULTS

Fifty-three (66.2%) out of the 80 samples revealed the presence of parasites. The river Caí had 65% of positive samples for parasites, with 67.5% for the river Paranhana. The ten different species were *Ancylostoma* sp.; *Strongyloides* sp.; *Endolimax nana*; *Ascaris lumbricoides*; *Toxocara canis*; *Giardia lamblia*; *Entamoeba coli*; *Entamoeba histolytica/dispar*, *Trichiuris trichiura*, and *Taenia* sp. When results were obtained for each water course, *E. coli* and *E. histolytica/dispar* were detected only in river Caí and *T. trichiura* and *Taenia* sp was detected only in the river Paranhana (Graph 1). Graph 2 shows the parasites detected in the rivers Caí and Paranhana. Maps of soil usage and occupation of the two rivers showed that 60% of parasites lie in urban spots in which the highest species rates were also found (Chart 1).



Graph 1. Parasites in samples of sediment on the banks of rivers (A) Caí and (B) Paranhana.



Graph 2. Absolute numbers of parasite types on the banks of the water bodies under analysis.

Chart 1. Parasites in the rural and urban areas of the two rivers

River Caí		River Paranhana	
Rural area	**Urban spot**	**Rural area**	**Urban spot**
Cysts of *E. nana*	Cysts of *E. coli*	Cysts of *E. nana*	Cysts of *E. nana*
Eggs of *T. canis*	Cysts of *E. histolytica/díspar*	Eggs of *A. lumbricoides*	Cysts of *G. lamblia*
Eggs of *Ancylostoma* sp.	Cysts of *E. nana*	Eggs of *T. canis*	Eggs of *T. canis*
	Cysts of *G. lamblia*	Larvae of *Strongyloides* sp.	Eggs of *A. lumbricoides*
	Eggs of *A. lumbricoides*		Eggs of *T. trichiura*
	Eggs and larvae of *Ancylostoma* sp.		Eggs of *Taenia* sp.
			Eggs and larvae of *Ancylostoma* sp.
			Larvae *Strongyloides* sp.

DISCUSSION

Parasite diseases in humans are a serious health problem. They have been widely neglected even though they have caused liabilities such as sick leave, expenditure in medicines and hospitalization. The environment has a relevant role in the parasites´ life cycle, providing nutrition and development of young and adult larvae, and the maintenance of infectant types. Different pathologies, such as iron deficiency, pneumonia, growth delays, neuropathies and Loeffler syndrome, may be listed, with long term treatment.[16,17,10,18,19,20]

Current analysis revealed 23% of parasite cysts in the bank sediment of the two rivers, featuring *E. nana* (14%) and *E. coli* (3%) commensal cysts,

which may be ingested with water and with incorrectly washed vegetables. Parasite cysts indicate fecal wastes and, consequently, poor hygiene conditions. Cysts of *E. histolytica/dispar* (1%), identical under the microscope, and *G. lamblia* (5%), were detected only in the river Caí. The latter may cause prolonged periods of diarrhea, bellyaches, weight loss and anemia and may be disseminated by the blood current and reach the liver, lungs and brain.[20,1,7]

A study by Oliveira Filho et al. (2011)[21] revealed parasites in 67.8% of samples from the littoral sand collected in the northeastern region of Brazil (state of Paraíba), of which the most frequent were *E. nana* (28,1%), *E. coli* (25%) and *G. lamblia* (6,3%). Eighty samples were evaluated in the same state, of which 83.7% were positive for different parasites, featuring cysts of G. lamblia (52.5%), E. coli (10.9%), E. nana (8.7%) and E. histolytica/dispar (2.2%). Although results of current study come from another type of soil, with chemical and physical differences, both revealed fecal contamination by commensal parasites of the human intestine tract and by infectious parasites. A study by Pritsch & Frighetto (2016)[2] in the sands of beaches in the state of Santa Catarina, south Brazil, detected 40% of positive samples with *A. lumbricoides* eggs and ancylostomata larvae, indicating the need of prevention measures and improvement in basic sanitation.

Parasite eggs in current study comprises *Ancylostoma* sp (37%); *A. lumbricoides* (12%); *T. canis* (6%) and *T. trichiura* and *Taenia* sp (1% each), detecting contamination at the river edge in the two water bodies. Both are constantly used for recreation, fishing and primary contact by the population. Although *T. trichiura* and *Taenia* sp may be found only in the river Paranhana with low frequency rates, sanitation precariousness and risks in being infected by trichuriasis which causes malnutrition, growth delay, anemia and tapeworm with irreversible sequences (cysticercosis and neurocysticercosis) cannot be minimized.[22,18]

Research by Saito & Rodrigues (2012)[10], in Juiz de Fora MG Brazil, made a relationship between soil and epidemiological studies. Although the authors obtained negative results from people under analysis, results of soil surface samples close to the vale banks, in the open, revealed eggs of *A. lumbricoides (*96%) and T. trichiura (3%), similar to results in current study. According to the same authors[10], negative results of clinical samples showed that hosts were temporarily free from parasites even though the environment was still the transmission route to reinfection which is characteristic in the matrix surface soil/sediment. In fact, it is capable of hosting pathogens and has the role of being their intermediate host.

Research works by Figueiredo et al. (2012)[23]; Moura et al. (2013)[24] and Sprenger et al. (2014)[25] in Brazil have shown predominance of ancylostomata, similar to current study. Similar to the above-mentioned studies, current study detected other parasites, such as *A. lumbricoides* and *Strongyloides* sp. The soil´s features, or rather, its physical and chemical factors, heat and high humidity, enhance the develop of larvae till infecting larval stage. It is actually an excellent medium to vehicle different species of intestine parasites. Ancylostomata and *Strongyloides* sp larvae are commonest in the environment, with great importance for community health, dissemination form, circulation of animals and their presence in water bodies.[10] On the other hand, different types of soil may cause variations in parasite egg distribution. Por example, sandy soils have larger particles and enhance a more uniform distribution. However, more clayey soils have smaller particles and adsorb egg, with agglutination, and have more spatial distribution in aggregates besides differences in pH, humidity and temperature.[26,10]

Current research detected 6% of egg of *T. canis,* a parasite which causes gastro-intestinal infections in dogs, toxocariasis in humans, and Visceral Larva Migrans (VLM), Neurological Larva Migrans (NLM) and Ocular Larva Migrans (OLM) syndromes. Otero et al. (2014)[27] evaluated the soil of public parks in the metropolitan area of Lisbon, Portugal, and exposed feces on the same sites and detected an 85% prevalence of *Toxocara sp.* in the soil of children´s playgrounds and 50% in urban parks and open gardens, whereas samples of human feces were reported in 14.3% of children´s playgrounds and in 16.7% of urban parks and gardens. Although there is a difference between species detected and percentages between the studies, contamination risks on public sites, such as parks,

and the exposure to zoonosis in animals and humans are evident. Transmission infection sites and their disposal in the environment are very similar to those described in current research

Samples with *Ancylostoma* sp. and Strongyloides sp. larvae corroborate results by other research works. They refer to larvae as the principal form in the environment since they develop their infectious stage in the soil, with greater risks for the population.[28,22] Pedrosa et al. (2014) [28] reported 39 positive samples (72.2%) out of 54, with *T. trichiura* eggs and *Ancylostoma* sp and *Strongyloides* sp larvae. The migration of larvae towards the soil surface may be affected by heat and humidity, facilitating contagion in the summer. During this period, heavy or light rains are common and they increase the larval movement in the soil/sediment towards the surface.

The rivers Caí and Paranhana are heavily used by industrial complexes, in crop irrigation, stockbreeding, recreation, production of electricity and public treated water supply.[11,12] Several issues related to pollution, effluent discharge and washing of animals have to be coped with. They may be the cause of pollution in the sediment of the river banks and, consequently, the contamination of water courses. In fact, the matrix water and soil are associated with the adhesion-desorption of particle material and pathogens in the sediment of the river edge, percolating and returning to the water body.

Surface sediment features soil erosion which is capable of hosting microorganisms and retains the waste of chemical compounds and fecal material.[9,29] Although current research deals with river bank sediments, the matrix has the necessary characteristics to concentrate and retain parasites even on surface matrix. Due to its locality on river banks, the sediment is more liable to environmental changes, such as humidity, difference in temperature and flooding. These factors interfere in the characteristics of the soil/sediment, making it liable to best and favorable host conditions for the survival of parasite microorganisms. Graciliano Neto et al. (2017)[3] researched sando on wet and dry beaches at different depths and found that deeper and wetter sand could host more larvae and protozoon cysts. Vasconcelos et al. (2016)[17] underscore that temperature (22ºC - 32ºC), relative air humidity (between 60% and 86%), good oxygenation and sandy or clayey soils have better conditions for the evolution and viability of parasites with a phase in their life cycle in the soil. They are the best characteristics in the sites under analysis.

Results from the sediments of the two river banks under analysis are very similar, although *A. lumbricoides* is in the river Paranhana (16%) and in the river Caí (9%); and *E. nana* (3%) in the river Paranhana and (20%) in the river Caí (Graph 1). Although *A. lumbricoides* is pathogenic, causing pneumonia, ascariasis and Loeffler´s syndrome, the cysts of *E. nana*, albeit commensal, are a risk to health if ingested in their mature phase (Chart 1), underscoring the urban spots of soil occupation (Figure 1). The two rivers show human contamination, although they show evolution forms of different parasite species. The river Caí is particularly vulnerable in the urban spot at Site 8, whilst the river Paranhana has the greatest number of larvae at Sites 6,8 and 9, perhaps related to larger population agglomerations and soil occupation revealed by urban spots with an increase in environmental contamination, premises close to the river banks and raw sewerage discharged in the environment perceived on the edge sediment.

The lack of adequate basic sanitation structure and more populous areas in precarious conditions, without drinkable water, worsens health conditions. In fact, eggs, cysts and larvae may be ingested in contaminated water or food.

In spite of complication and harm that the types of parasitosis may cause, they are classified as neglectable diseases due to the low priority given in several countries, although they are a relevant issue to public health.[24]

Matos & Cruz (2012)[30] analyzed enteroparasitosis in patients living in rural and urban areas and detected 39.7% contaminated patients in urban area and a slight increase (42.1%) in urban patients. The study underscores *E. histolytica/dispar* and *Strongyloides* sp. in people living in the rural areas and *A. lumbricoides* and *G. lamblia* in urban residents. Current study shows higher percentage (60%) of parasites in sites with urban spots (Chart 1) when compared to

that in the rural area (40%), demonstrating relevant contamination not only for human health but also for the environment.

CONCLUSION

As several studies have underscored, sediment on the river bank represents an appropriate means for the conservation of parasites and contributes towards their proliferation and dissemination within the environment and contamination risks for different living beings and local populations that depend on and use water of the rivers Caí and Paranhana.

The two basins are greatly relevant for water supply to several towns and to the environment. The need for further studies on pathogenic microorganisms is evident. Since studies on parasites in HBRS and HBRC are rare, investigation on microorganisms in these water sources should be emphasized, with monitoring to minimize impact on the environment and human health.

People lack information on infections by water-borne diseases, such as parasitosis, since environmental matrixes water and sediment exercise constant geo-hydric interactions. Actions against parasites involve hygiene care which is a simple and effective process to decrease transmission and pathologies and to contribute towards an improvement in life quality and decrease in public expenditure. Areas which are susceptible to inappropriate soil use should be managed to minimize parasite illnesses and their study should foreground health and the environment. Sediment analysis of river banks in different areas should be highlighted since it is a matrix which hosts clinically interesting parasites. Actually basic sanitation which prevents the transmission of parasitosis that compromises human health is rare.

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