PARASITOLOGICAL INDICATORS OF CONTAMINATION AT SAND OF BEACHES AND MONITORING BY TRADITIONAL METHODS AND IMMUNOENZYMATIC ASSAY

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ABSTRACT

Intestinal helminths are among the pathogens most often found in humans and can result in serious pathological, such as visceral *larva migrans* syndrome. Domestic animals serve as the natural hosts, but under certain conditions humans can acquire infection, developing an atypical cycle. Thus, the contamination for animal droppings in public spaces, such as beaches, poses risk factor in the transmission, increasing the incidence of these zoonoses. In this context, informations about environmental health is necessary because the monitoring would be the first step for treating unhealthy environments. Therefore, the study monitored the sand of beaches of two islands within Guanabara Bay, Rio de Janeiro state, was carried out by traditional methods (Lutz and Baermann) and the ELISA immunological test, in order to determine the level and the influence of seasonality on this contamination. Among the genera detected, *Ascaris* sp. and *Ancylostoma* sp. occurred with greatest frequency, functioning as important biological markers of environmental contamination. The highest frequency of parasite structures occurred in the summer, meaning a greater risk of disease transmission. The results evidence the high level of environmental contamination and the seasonal variations of this contamination. Additionally, the data obtained from ELISA confirm the sensitivity of this technique to detect cysts and oocysts of protozoa in the sand samples analyzed.

***Keywords**:* environmental contamination, sand, parasitological techniques

RESUMO

Helmintos intestinais estão entre os patógenos mais frequentemente encontrados em seres humanos e podem resultar em patologias graves, como a síndrome de *larva migrans* visceral. Os animais domésticos servem como hospedeiros naturais e, em certas condições, os seres humanos podem ser afetados, desenvolvendo um ciclo atípico. Por isso, a contaminação por fezes de animais em espaços públicos, como praias, representa risco na transmissão, aumentando a incidência de possíveis zoonoses. Neste contexto, informações sobre a salubridade ambiental se faz necessária visto que o monitoramento é a primeira etapa para o

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tratamento de ambientes insalubres. Portanto, o estudo monitorou a areia de praia de duas ilhas na Baía de Guanabara, Rio de Janeiro, por métodos tradicionais (Lutz e Baermann) e por teste imunológico de ELISA, com o objetivo de determinar o nível e a sazonalidade desta contaminação. Entre os gêneros detectados, *Ascaris* sp. e *Ancylostoma* sp. ocorreram em maior frequência, funcionando como importantes marcadores biológicos de contaminação ambiental. A maior frequência de parasitos ocorreu no verão, o que significa maior risco de transmissão de doenças. Os resultados evidenciam o elevado nível de contaminação ambiental e as variações sazonais desta contaminação. Além disso, os dados de ELISA confirmam a sensibilidade desta técnica para detectar cistos e oocistos de protozoários nas amostras de areia analisadas.

Palavras-chave: contaminação ambiental, areia, técnicas parasitológicas

INTRODUCTION

Intestinal helminthoses are among the most commonly found infections in humans, sometimes resulting in serious pathologies. According to the World Health Organization (WHO), zoonotic parasitic diseases pose a significant public health problem throughout the world, and their presence is often associated with the level of economic development and behavioral habits (ROCHA et al., 2011). Domestic animals are the natural hosts of many of the helminths causing these diseases, but under determined conditions humans can be infected, developing an atypical cycle. In this context, the contamination by animal droppings in public places, such as the sand at beaches and parks and soil around houses, is an important risk factor for the transmission of these agents, especially in places with precarious sanitation. That fact is especially important because of the increasing urban sprawl in Brazil, with large concentrations of people on the peripheries of large cities in places that often have poor sanitation services, favoring the environmental contamination process (SILVA et al, 1991; OGE & OGE, 2000; SILVA et al., 2009).

Silva et al. (2009) described the involvement of *Ancylostoma* sp., *Toxocara canis*, *Trichuris* sp. and *Ascaris* sp. eggs as contaminating agents in the sand at beaches in northeastern Brazil, highlighting the risk to the local people of acquiring infections such as visceral *larva migrans*. Additionally, Oliveira-Filho et al. (2011) observed, from a parasitological study of beaches in the northeastern state of Paraíba, the presence of larval stages of *Strongyloides stercoralis*, cysts of *Giardia lamblia* and eggs of *Taenia* sp. These findings indicate the role of contaminated beach sand in spreading zoonotic diseases, because many of these agents use beaches as places for development to enable infection of natural and accidental hosts, including humans.

According to the current legislation and regulations in many Brazilian cities, the sanitary conditions of recreational areas of primary contact are based only on bacteriological bioindicators, especially determination of the total and fecal coliforms (MENDES et al., 1993; BOUKAI, 2005), without considering the risks associated with contamination by geohelminths and protozoa. However, in recent years Sato et al. (2005) and Sotero-Martins et al. (2013) have indicated the importance of assessing the sanitary quality of sand, especially to detect zoonotic parasites, to provide information for the formulation of new regulations or improvement of existing ones (the main one being Resolution 274/2000 from the National Environmental Council – CONAMA (CONAMA, 2000). Mentz et al. (2004) and Matesco et al. (2006) identified the presence of various parasite

species, among them *Strongyloides stercoralis, T. canis* and *A. brasiliensis,* in sand samples, indicating the importance of beaches in the transmission of diseases to humans. Despite these studies, little is known about the epidemiological aspects of parasite contamination of sand in recreational areas and soil around houses in the state of Rio de Janeiro. This is worrying because the primary contact of humans engaged in sports and other recreational activities poses a high risk of infection.

Therefore, we carried out the study reported here to obtain updated data on the sanitary conditions at beaches of two islands located in Guanabara Bay, aimed at standardizing methods to detect infective parasite structures, eggs, larvae, cysts and oocysts in sand, because of absence of environmental information to enable development of actions to control the diseases caused by these agents.

MATERIALS AND METHODS

Sand samples were collected from four beaches in Guanabara Bay, Rio de Janeiro state: Bica and Ponta do Tubiacanga, both on Ilha do Governador (IG), and José Bonifácio and Tamoios on Ilha de Paquetá (IP). A total of 129 sand samples were collected in the period from 2008 to 2011.

All the samples were first decontaminated in an autoclave (121 ºC/1 atm) for 15 minutes or by continuous exposure to UV radiation in a laminar flow cabinet for 40 minutes.

The samples were taken from the topmost layer with a scoop (volume of 100 mL). All the sand collected from each point was placed in a plastic bag, with final mass of 270 g (standard deviation of 3.77 g). The bags were then put in a cooler chest with ice to keep them at about 4 °C until reaching the laboratory.

The technique used to detect eggs, cysts and larvae in the dried sand samples was that described by Lutz (1919), due to its simplicity and low cost. The Baermann-Moraes technique (1917) was also used to detect nematode larvae. Both techniques were adapted for better utilization of the samples. Three slides were prepared for each sample and were examined under a light microscope at magnifications of 100 and 400X, as described by Amaral (2012). For the adapted Lutz technique, 100 g of sand was used. The samples were homogenized and then transferred to glass vials containing 100 mL of commercial neutral detergent diluted to 0.5%. The samples were then vigorously shaken for 5 minutes and left at rest until total decantation of the substrate. The supernatant was filtered through four sheets of gauze (a single sheet folded twice) to a sedimentation beaker, after which the volume was completed to 250 mL with distilled water. This suspension was left at rest for 2 hours. For the adapted Baermann-Moraes technique, the sample was homogenized and then 100-gram aliquots were placed on folded gauze sheets over a polypropylene sieve inside a sedimentation beaker. After that, 180 mL of distilled water, heated to 45 °C, was added to each beaker to cover the material, and the beakers were left at rest for 2 hours.

The sediment was divided into two aliquots of 1.5 mL. One was preserved in SAF for subsequent microscopic examination and the other was frozen without preservatives for analysis by ELISA, with the intention of establishing a protocol to enhance the detection of *G. lamblia* and *E. histolytica*/*dispar* cysts and *Cryptosporidium* sp. oocysts with commercial kits developed for the study of parasite antigens.

The technique was carried out according to the manufacturer's instructions, except for omission of the dilution of the samples, since the kit used

was developed for examining fecal samples. The ELISA plates, including positive and negative controls, were read with a reader with wavelength according to the technical standards of the manufacturer (450/650 nm). The interpretation of the visual results was only to confirm the expected reading.

The cutoff points were established according to the accompanying instructions for study of parasite antigens in fecal samples. The cutoff value for *Giardia* sp. and *Cryptosporidium* sp. was an absorbance reading above 0.08, while for *Entamoeba* sp. the figure was 0.15.

RESULTS AND DISCUSSION

Of the 129 dried sand samples analyzed by both the traditional microscopic techniques (adapted Lutz and adapted Baermann-Moraes), the highest absolute positive frequency was for *Ascaris* sp. eggs, of which 7 samples (13.7%) were positive by the Lutz technique and 3 (5.8%) by the Baermann-Moraes technique. The two techniques returned the same results for *Ancylostoma* sp., with only 1 positive sample in each case (Figure 1).

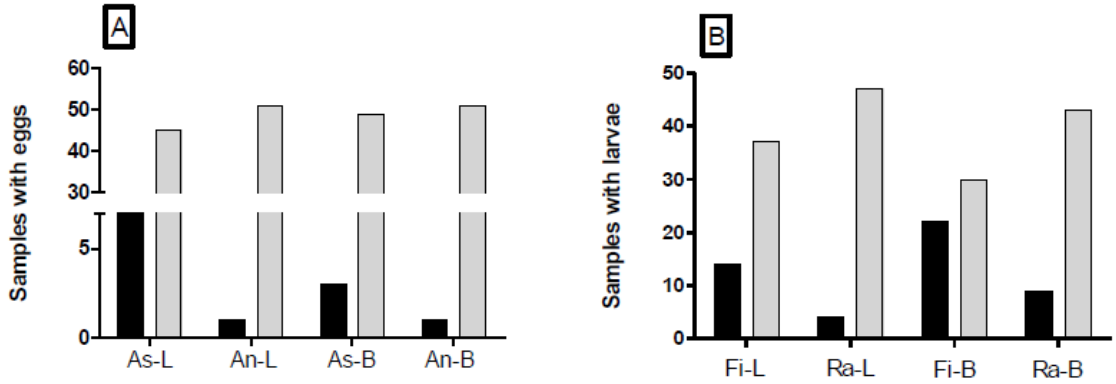

Figure 1: Comparison of the absolute frequency of eggs and larvae of *Ascaris* sp. and *Ancylostoma* sp. in samples collected in 2010 and 2011 by the techniques of Lutz - Adapted (L) and Baermann-Moraes - Adapted (B). A – number of samples with eggs; B – number of samples with larvae; As - *Ascaris* sp.; An – *Ancylostoma* sp.; Fi – Filariod; Ra – Rabditoid; ■ positive; □ negative. Break interval: 15 - 20.

With respect to samples positive for larvae, the Baermann-Moraes technique was more sensitive, identifying the presence of larvae in 61% of the samples (31), of which 22 were positive for filaroid larvae and 9 were positive for rabditoid larvae. In contrast, with the Lutz technique it was only possible to detect larvae en 18 samples (14 filaroids and 4 rabditoids), as shown in Figure 1.

It also observed variations in the absolute frequency of positive samples for helminths and protozoa in function of season of the year. The highest positive frequency was in winter, with 39 samples positive for infective parasite structures, followed by autumn with 38 positive samples. It also observed positive samples in the summer and spring, with 33 and 29 positive samples, respectively (Figure 2).

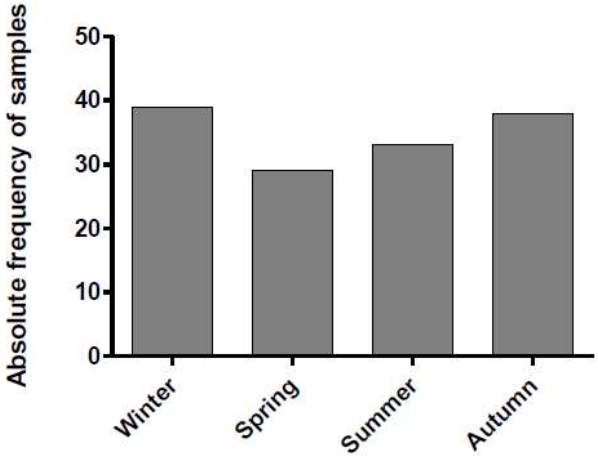

Figure 2: Absolute frequency of samples found to be positive for parasite structures (helminths/protozoa) according to season of the year, in samples collected at four beaches in the period 2008-2011.

The immunoenzymatic assay (ELISA) was more sensitive and effective in detecting infective protozoan cysts of public health interest compared to the traditional fecal analysis methods, suggesting its application to monitor beach sand. Of the 129 samples from the four beaches studied, it was possible to detect *Giardia* sp. cysts in 3.1% (4) and *Cryptosporidium* sp. oocysts in 82.9% (107) by the ELISA technique, while with the traditional microscopic techniques it was not possible to observe any *Giardia* sp. cysts or *Cryptosporidium* sp. oocysts. None of the three techniques enabled detecting samples positive for *Entamoeba* sp. cysts.

In recent years, epidemiological studies to monitor sanitary conditions at beaches have indicated the importance of beach sand as a source for transmission of diseases, especially those caused by gastrointestinal helminths (SANTOS & DE SOUZA, 2006). Due to these studies, it is possible to determine the level of insalubrity of the places and the habits and behaviors of the population studied, helping to explain the increased rates of certain infections. According to Peruca et al. (2009), the increased prevalence of certain syndromes caused by parasites, such as cutaneous and visceral larva migrans, is directly related to the level of environmental contamination, especially by dog and cat droppings, since these animals, when infected, eliminate parasite structures that can infect humans. Therefore, pet droppings in areas where pedestrians pass or people play, particularly beaches and sandboxes, are sources that spread diseases.

The results of this study demonstrated environmental contamination of sand at beaches within Guanabara Bay, indicating the possibility of catching parasitic diseases from these places. Among the genera found, *Ascaris* sp. and *Ancylostom*a sp. occurred with the greatest frequency, indicating the environmental contamination was caused by feces. This type of monitoring can therefore be an important way to detect biological contamination (SANTARÉM et al., 1998). Our results are in agreement with those reported by Rocha et al. (2011), who described high frequency of Ancylostomidae larvae and *Ascaris lumbricoides* eggs during an environmental analysis of four beaches in Santos, São Paulo state.

Additionally, variations in detection of parasites and parasite structures in the samples were strongly influenced by the technique employed (MELLO, 2010). From the results obtained, we suggest using the adapted Lutz technique for recovery of eggs from sand and the adapted Baermann-Moraes technique for

recovery of larvae. Our results corroborate those of other authors in which points out that the Lutz technique is more effective for recovery of helminth eggs in general, among them eggs of Ancylostomidae and Ascarididea, providing significantly better results compared to other techniques (SILVA et al., 1991). That fact can be explained because the great majority of eggs of gastrointestinal helminths are heavy, favoring their detection by sedimentation.

When evaluating the influence of seasonality on the absolute frequency of samples positive for geohelminths, the results demonstrated that the prevalence frequency of samples positive was observed in winter and autumn, indicating this is the season of highest risk of human infection by parasites. This is due to the development cycle of these helminths, part of whose ontogenic development occurs in the environment, causing this development to be strongly influenced by climatic factors like temperature and humidity (COSTA-CRUZ et al., 1994). That situation has previously been mentioned by other authors, who have indicated that the pattern of contamination by geohelminth eggs and larvae is directly influenced by abiotic factors, suggesting the existence of periods with higher frequency of viable eggs and larvae in the environment (SMITH, 1998; CÁRERES et al., 2004). Hence, our results indicate that the highest contamination rate occurred in the period when people are most present in these settings, favoring disease transmission.

The results obtained with ELISA confirm the greater sensitivity of this technique for detection of cysts and oocysts from protozoa in the sand samples analyzed in comparison with the traditional parasitological diagnostic methods. These results are in agreement with those of Da Silva et al. (2012), who found greater efficacy of immunoenzymatic methods to detect parasites, especially protozoa, in soil samples from the state of Rio de Janeiro.

This greater sensitivity of immunoenzymatic methods can be explained by the fact that they can identify surface antigens regardless of the integrity level of the cysts and oocysts. In contrast, in traditional techniques based on microscopic examination, the integrity of protozoan cysts and oocysts is an essential factor for diagnosis. Another important aspect related to the sensitivity of these techniques is their ability to detect small quantities of antigens, enabling diagnosis even when the parasite load in the sample is low. This fact was previously confirmed by Machado et al. (MACHADO et al., 2001), who used ELISA to identify the presence of *Giardia lamblia* in children's stools, while traditional coprological techniques failed to detect any presence.

CONCLUSIONS

The results presented here demonstrate the importance of monitoring stage as the first step of treatment due to lack of information about these contaminants in the sand used for recreation, indicating winter as the season with the highest presence of parasite structures. These results likely derive from weather conditions such as rainfall and wind movement, which according to Araújo et al. (2008), would be decisive for the development and spread of helminth eggs. Additionally, the presence of *Ascaris* sp. and *Ancylostoma* sp. eggs in the samples studied is an important biological marker of contamination by human and animal feces, indicating possible transmission of other infections agents. Finally, the results obtained by applying ELISA confirm the higher sensitivity of this technique in detecting cysts and oocysts from protozoa in the sand samples analyzed, suggesting its use for parasitological monitoring of beach sand.

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REFERENCES

AMARAL, L. S. Monitoramento de parasito e coliformes como parâmetro de avaliação sanitária de areia e água de praias da Baía de Guanabara/ RJ. [Dissertação] Rio de Janeiro: Programa de Saúde Pública e Meio Ambiente. Escola Nacional de Saúde Pública Sergio Arouca, Fundação Oswaldo Cruz, 2012.ARAÚJO, N. S.; RODRIGUES, C.; CURY, M. Helminthes in sandboxes of day care centers of a city in Southeastern Brazil. Rev Saúde Pública 2008; 42:150-153.

BAERMANN, G. A simple method for the detection of Ankylostomum (nematode) larvae in soil tests. Mededelingen uit het Geneeskundig Laboratorium te Weltevreden 1917; 41-47.

BOUKAI, N. Qualidade sanitária da areia das praias no município do Rio de Janeiro: diagnóstico e estratégia para monitoramento e controle [dissertação]. Rio de Janeiro: Faculdade de Engenharia, Universidade do Estado do Rio de Janeiro; 2005.

CÁCERES, A. P. S. G.; GONÇALVES, F. A.; CAZORLA, I. M.; CARVALHO, S.M.S. Contaminação do solo por helmintos de importância médica na Praia do Sul (Milionários), Ilhéus, BA. NewsLab, 2004; 67: 146-155.

COSTA-CRUZ, J. M.; NUNES, R. S.; BUSO, A. G. Presença de ovos de Toxocara sp. em praças públicas da cidade de Uberlândia, Minas Gerais, Brasil. *Revista do Instituto de Medicina Tropical de São Paulo* 1994; 36: 39-42.

DA SILVA, B. A.; ANTUNES, U. C.; LAURENTINO, S.V.; NASCIMENTO, A. D.; BANDEIRA, V. M.; MONTEIRO, F. A. B.; PEREIRA, B. M. Comparative study of parasitological techniques and ELISA for analysis environmental samples, RJ, Brazil. *Revista Ibero-Latinoamicana de Parasitologia* 2012; 71: 90-96.

LUTZ, A. O *Shistosoma mansoni* e a shistosomatose segundo observações feitas no Brasil. *Memórias do Instituto Oswaldo Cruz*, 1919; 11: 121-155.

MACHADO, R. L. D.; FIGUEIREDO, M. C.; FRADE, A.F.; KUDÓ, M.E.; FILHO, M.G.S.; PÓVOA, M.M. Comparação de quatro métodos laboratoriais para diagnóstico da *Giardia lamblia* em fezes de crianças residentes em Belém, Pará. *Revista da Sociedade Brasileira de Medicina Tropical* 2001; 34: 91-93.

MATESCO, V. C.; MENTZ, M. B.; ROTT, M. B.; SILVEIRA, C. O. Contaminação sazonal por ovos de helmintos na praia de Ipanema, em Porto Alegre, Rio Grande do Sul, Brasil. *Revista de Patologia Tropical* 2006; 35: 135-141.

MELLO, C. B. S. Avaliação parasitológica e contaminação sazonal de areias de parques públicos na região da zona leste da cidade de São Paulo [dissertação]. São Paulo: Departamento de Saúde Ambiental, Universidade de São Paulo; 2010.

MENDES, B.; NASCIMENTO, M. J.; OLIVEIRA, J. S. Preliminary characterization and proposal of microbiological quality standard of sand beaches. *Water Science Technology* 1993; 27(3-4):453-6.

MENTZ, M. B.; ROTT, M. B.; JACOBSEN, S. I. V.; BALDO, G.; RODRIGUES-JUNIOR, V. Frequência de

ovos de Toxocara spp. em três parques públicos da cidade de Porto Alegre, Rio Grande do Sul, Brasil. *Revista de Patologia Tropical* 2004; 33: 105-112.

OGE, H.; OGE, S. Quantitative comparison of various methods for detecting eggs of Toxocara canis in samples of sand. *Veterinary Parasitology* 2000; 92: 75-79.

OLIVEIRA-FILHO, A. A.; FERNANDES, H. M.; ALCÂNTARA, N. D.; ASSIS, T. J.; FREITAS, F. I. Frequência de enteroparasitas nas areias das praias da Paraíba. *Revista de Biologia e Farmácia* 2011; 06: 108-113.

PEREUCA, L. C. B.; LAGONI, H.; LUCHEIS, A. B. Larva migrans visceral e cutânea como zoonozes: Revisão de Literatura. *Veterinária e Zootecnia* 2009;16: 601-616.

CONAMA - Conselho Nacional do Meio Ambiente. Resolução nº. 274 de 29 de novembro de 2000. Revisão dos critérios de Balneabilidade em Águas Costeiras.

ROCHA, S.; PINTO, R. M. F.; FLORIANO, A. P.; TEIXEIRA, L. H.; BASSILI, B.; MARTINEZ, A.; COSTA, S.O.P.; CASEIRO, M. M. Environmental analyses of the parasitic profile found in the Sandy soil from the Santos municipality beaches, SP, Brazil. *Revista do Instituto de Medicina Tropical de São Paulo* 2011; 53: 277-281.

SANTARÉM, V. A.; SARTOR, I. F.; BERGAMO, F. M. M. Contaminação, por ovos de *Toxocara* spp. de parques e praças públicas de Botucatu, São Paulo, Brasil. *Revista da Sociedade Brasileira de Medicina Tropical* 1998; 31: 529-532.

SANTOS, A. B.; DE SOUZA, T. P. Contaminação das praias por parasitos caninos de importância zoonótica na orla da parte alta da cidade de Salvador - Ba. *Revista de Ciências Médicas e Biológicas* 2006; 5: 40-47.

SATO, M. I. Z.; BARI, M.; LAMPARELLI, C. C.; TRUZZI, A. C.; COELHO, M. C.; HACHICH, E. Sanitary quality of sands from marine recreational beaches of São Paulo, *Brazilian Journal of Microbiology* 2005; 36: 321-326.

SILVA, J. P.; MARZOCHI, M. C. A.; SANTOS, E. C. L. Avaliação da contaminação experimental de areias de praias por enteroparasitas. Pesquisa de ovos de Helmintos. *Cadernos de Saúde Pública* 1991; 7:90-99.

SILVA, P.F.; CAVALCANTI, I. M. D.; IRMÃO, J. I.; ROCHA, F.J. Common beach sand contamination due to enteroparasites on the southern coast of Pernambuco state, Brazil. *Revista do Instituto de Medicina Tropical de São Paulo* 2009; 51: 217-218.

SMITH, H. V. Detection of parasites in the environmental. *Parasitology* 1998; 117: 113-141.

SOTERO-MARTINS, A.; DUARTE, N. A.; CARVAJAL, E.; SARQUIS, M. I. M.; FERNANDES, O. C. C. Controle da Qualidade Microbiológica e Parasitária em Áreas de Recreação. *Revista Eletrônica Gestão & Saúde* 2013; 4: 1075-1092.