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Identification of enteroparasites in recreation areas of elementary schools in Northern Espírito Santo, Brazil

Identificación de enteroparásitos en áreas de recreación de escuelas primarias en el norte de Espírito Santo, Brasil

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ABSTRACT

Objective Since sand is an important child means of contagion by enteroparasites, this study aimed to conduct a parasitological evaluation in recreation areas of elementary schools and early childhood education centers, in São Mateus-ES.

Material and Methods Sand samples were collected in seven municipal elementary schools and in a municipal kindergarten and nursery center. For the recovery of helminth eggs and protozoan cysts, flotation and spontaneous sedimentation techniques were used.

Results It was found that 77 % of the analyzed institutions were positive for helminth eggs and larvae. Among the eggs and larvae identified, the main were *Ascaris lumbricoides* and *Toxocara canis*. Pearson's chi-square test identified the best results when using the spontaneous sedimentation method.

Conclusions Considering that individuals, especially children living in poor sanitation conditions, are more prone to enteroparasites, studies point to the need of the application of preventive and health education measures for the population enrolled in municipal schools in São Mateus.

Key Words: Soil pollutants; parasites; child health (*source: MeSH, NLM*).

RESUMEN

Objetivo Dado que la arena es un medio importante de contagio infantil por enteroparásitos, este estudio tuvo como objetivo realizar una evaluación parasitológica en áreas recreativas de escuelas primarias y centros de educación infantil, en São Mateus-ES.

Materiales y Métodos Se tomaron muestras de arena en siete escuelas primarias municipales, en un jardín de infantes y en una guardería municipal. Para la recuperación de huevos de helmintos y quistes de protozoos, se usaron las técnicas de flotación y de sedimentación espontánea.

Resultados Se encontró que el 77 % de las instituciones analizadas dieron positivo para huevos y larvas de helmintos. Entre los huevos y larvas identificados los principales fueron *Ascaris lumbricoides* y *Toxocara canis*. La prueba de ji-cuadrado de Pearson identifica los mejores resultados cuando se usa el método de sedimentación espontánea.

Conclusión Teniendo en cuenta que las personas, especialmente los niños que viven en malas condiciones de saneamiento, son más propensas a enteropatías, los estudios apuntan a la necesidad de la aplicación de medidas preventivas y de educación sanitaria para la población inscrita en las escuelas municipales de São Mateus.

Palabras Clave: Contaminantes del suelo; parásitos; salud del niño (*fuentes: DeCS, BIREME*).

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Enteric protozoan and helminth infections are among the most frequent health problems worldwide (1,2). Even with scientific and technological advances over the years, intestinal parasitism remains a serious public health problem, especially in underdeveloped or developing countries (3-5).

Soil-transmitted parasitic infections are frequent and usually related to the deficiency in sanitation and hygiene conditions (6,7).

In America, it is estimated that 20 % to 30 % of the population are infected by enteroparasites (3,8,9). Worldwide, more than 1.5 billion people are affected by soil-transmitted helminths and 50 % of this total are children of school-age (6,9,10).

The high prevalence in children is due to their greater exposure, especially to sandboxes in public squares, schools and day care institutions (6,10-12). In this context, and considering the greater female participation in the labor market, day care became the first external environment that children attend (13-15).

In such environments, normally open, contamination frequently occurs, since they are characterized by free access to cats, dogs and other animals, disseminators of parasitic diseases (16). These animals contaminate the soil, eliminating a large number of parasite eggs per gram of faeces, which remain viable for long periods in the external environment, exposing the human population at risk of infection and development of diseases (14,15).

When contaminated, sand enables the transmission of parasitic diseases such as *ascariasis*, *toxocariasis*, *taeniasis*, hookworm infections and especially visceral larva *migrans* and cutaneous larva *migrans* (6,7) and, among the helminths with a higher zoonotic potential, the etiological agents of cutaneous larva *migrans* (*Ancylostoma braziliense* and *A. caninum*) and visceral larva *migrans* (*Toxocara canis* and *T. cati*) are among the most important (10,17,18).

Since sand is an important child means of contagion by enteroparasites, this study aimed to conduct a parasitological evaluation in sand samples from recreation areas of elementary and kindergarten schools in São Mateus, Espírito Santo, Brazil.

MATERIAL AND METHODS

Study area

The municipality of São Mateus belongs to the northern region of the State of Espírito Santo and is the second largest land area in the state, with 2 343 km². Its economy is diversified, based mainly on trade and petroleum production (19).

In the educational parameter, it has municipal elementary schools, as well as primary and high schools (20).

Sample Collection and Procedure

As a sample, about 20 % of the schools, distributed among municipal elementary schools and early childhood education centers, participated in this study.

Sand samples were collected during six months. A 1-m² quadrant was established from 10 m to 10 m of sand range in the recreation areas, where four samples were collected, one at each vertex of the quadrant. In recreation areas whose perimeter of the sand range was lower than 10m, a single quadrant was drawn in the center, where the samples were collected.

In each quadrant, approximately 80 g of sand were collected in each of the four vertices with the aid of a plastic container. Each sample included about 320g of sand, which was wrapped in a clean plastic bag and labeled. This procedure was repeated in each educational establishment under study.

After collection, the samples were transported to the Clinical Analysis Laboratory of the Federal University of Espírito Santo, São Mateus campus, with the aid of a cooler, where they were kept under refrigeration until the time of analysis.

Sample Analysis

The samples were subjected to spontaneous sedimentation and flotation techniques for the recovery of protozoan cysts, larvae and helminth eggs (21,22).

In the 18 quadrants analyzed (three quadrants in School 1, one quadrant in School 2, two quadrants in School 3, one quadrant in School 4, three quadrants in School 5, two quadrants in School 6, three quadrants in School 7 and 3 quadrants in School 8), 54 slides were examined by the spontaneous sedimentation method and 54 slides by the flotation method. Each of the slides was examined under a light microscope, in triplicate.

For the spontaneous sedimentation technique (21), about 40 g of sand, which is equivalent to half of the sample collected, was placed in a beaker with 150 mL of distilled water. It was stirred with the aid of a glass rod.

The suspension was then filtered into a 200-mL glass conical flask, through a metal or nylon gauze with about 80 meshes/cm² to 100 meshes/cm², or surgical gauze folded in four. The retained debris was washed with 20 mL of water, constantly stirred with a glass rod, and the washing liquid was collected in the same flask. Finally, the flask was completed with water and sedimentation was expected between 2 hours and 24 hours (21), for the analysis of the slides in triplicate.

For the flotation method (22), the remaining portion of the sample, which equals about 40 g sand, was used. This portion was placed in a Borel vial, with a saturated sugar solution, and stirred with the aid of a glass rod. The volume of the vial was completed with saturated solution and, subsequently, two slides were gently placed in the edge of the flask, in contact with the solution, leaving them at rest for five minutes.

After this time, the slides were quickly removed, turning the wet side up, taken to the microscope and examined with 10x and 40x objective lenses, with the use of optional cover slip (23). Analyses were also performed in triplicate.

Statistical Analysis

Descriptive and univariate analyses were performed between the independent variables “periphery/center school” and “type of public (Kindergarten/Elementary)”, and the dependent variable “presence of parasitosis”, using Pearson’s chi-square test, considering “p” < 0.20 as significant. Subsequently, for each dependent variable, successive multivariate analyses were performed by Poisson regression in such a way that, after each multivariate step, the variables with “p” values higher than 0.05 were removed. The same statistical analysis was performed to test significant differences between the two parasitological techniques used in the study.

RESULTS

It was found that 77 % of public educational institutions analyzed in São Mateus tested positive for parasitic forms. A total of four helminth species was found in the sands of recreation areas (Table 1). Considering the observed species, it was found that *T. canis* was the most frequent (66.7 %), followed by *A. lumbricoides* (27.8 %) and *H. nana* (5.5 %).

Table 1. Parasitic forms in sand samples of recreation areas from educational institutions of São Mateus, Espírito Santo, Brazil

Educational institutions	Species
School 1	<i>T. canis</i>
	<i>A. lumbricoides</i>
	<i>S. stercoralis</i>
School 2	<i>A. lumbricoides</i>
	<i>T. canis</i>
School 3	<i>T. canis</i>
	<i>A. lumbricoides</i>
	<i>H. nana</i>
School 4	Negative
School 5	<i>T. canis</i>
School 6	Negative
School 7	<i>T. canis</i>
School 8	<i>T. canis</i>

Considering the geographic location of the educational institutions (periphery or center schools) and positive parasitic forms, the statistical test showed no significant difference among locations.

On the other hand, comparing the two parasitological techniques used in this study, it was observed that the spontaneous sedimentation method showed the best results, since a greater number of positive samples was detected by this method (Table 2), with a significant statistical difference ($p=0.011/IC\ 0.02-0.63$).

Table 2. Positive parasitic forms in sand samples of recreation areas from educational institutions of São Mateus, Espírito Santo, Brazil. Parasitological sedimentation and flotation techniques

Educational institutions	Spontaneous sedimentation	Flotation technique
School 1	<i>T. canis</i>	<i>T. canis</i>
	<i>A. lumbricoides</i>	<i>S. stercoralis</i> larvae
School 2	<i>A. lumbricoides</i>	<i>A. lumbricoides</i>
	<i>T. canis</i>	-
School 3	<i>T. canis</i>	-
	<i>A. lumbricoides</i>	-
	<i>H. nana</i>	-
School 4	-	-
School 5	<i>T. canis</i>	<i>T. canis</i>
School 6	-	-
School 7	<i>T. canis</i>	-
School 8	<i>T. canis</i>	-

DISCUSSION

In this study, a high percentage of positive samples (75 %) was observed for enteroparasites in sands from recreational areas of educational institutions in the city, also reported in studies conducted in the municipalities of Santa Maria (24); Uruguaiana (11,25); Rio Grande do Sul state, who found 93.3 %, 100 % and 100 % positive samples, respectively.

Moreover, it is noteworthy that the parasitic species found in São Mateus were also observed in studies conducted by other researchers in different cities of Brazil. In one of them it was observed the presence of *Strongyloides* sp. larvae, besides helminth eggs from the families Ancylostomidae and Taeniidae in Uberlândia-MG (6). In another study, it was found *Toxocara* spp. eggs, hookworms, *Dipylidium caninum*, *Ascaris* spp. and *Trichuris* spp. in Uruguaiana-RS [11]. *Toxocara* spp. eggs were also reported in public squares in Lavras-MG (17).

On the other hand, the presence of *H. nana* eggs, as occurred in São Mateus, Espírito Santo, was not reported in the municipalities of Uberlândia, MG, Uruguaiana, RS and Lavras, MG (6,11,17). However, it was observed in a study conducted in recreation areas of the East Zone of São Paulo-SP (26). In this context, the researchers state that the presence of zoonotic enteroparasites, such as *H.*

nana and *T. canis*, points to the poor sanitary conditions in these recreation areas, and factors as improper garbage disposal and sewage exposure can be attractive to dogs, cats and other synanthropic animals, like rats, hosts of diseases transmissible to humans (10,26,27).

In addition, it was found a higher frequency of association between eggs of *A. lumbricoides* and *Toxocara* spp., in Várzea Paulista, São Paulo, whose positivity rates in the studied child education institutions reached 100 % (28). This association may also be a result of poor sanitary conditions of recreation areas. In São Mateus, ES, this association was present in 50 % of these institutions.

There were no statistically significant differences regarding the location of the participating schools (center or periphery), when Pearson's chi-square test was used, followed by multivariate analysis. Poor habits of hygiene in the studied age group, lack of guidance from the school and parents, low investment in health, prevention and infrastructure, can lead to an increase in the prevalence of parasitic diseases, as well as recurrence in treated individuals (29).

The use of at least two laboratory techniques is suggested to get better responses in parasitological analyses (30). In the analysis of sand samples from recreation areas in São Mateus, the flotation method was used, besides spontaneous sedimentation. Some authors (31), compared the efficiency of the methods used in their studies, and found that 32.2 % of the eggs, cysts and larvae of helminths were recovered by the spontaneous sedimentation method, and the index was 27.7 % with the flotation method. In São Mateus, it did not differ, significantly reflecting the best use of parasitological techniques, when using the spontaneous sedimentation method. It was observed that this method was able to detect 78.95 % of parasitic forms, compared with 21.05 % of the flotation method.

Considering the topic of parasitic infections as a public health problem, the Ministry of Health issued the National Plan for Monitoring and Control of parasitic infections in 2005, in order to define control strategies, through information on prevalence, morbidity and mortality caused by or associated to parasitic infections. Other objectives were established, such as knowing the etiologic agents, regulating, coordinating and evaluating strategic actions of prevention and control, identifying key risk factors and developing continuing education activities for healthcare professionals (32).

In view of the ideals of improvements and preventions for this problem, it was reported that the movement of animals, dogs and cats, in these recreation sites should be restricted (17). However, the frequency of helminth larvae observed in this environment suggests that these measures are probably not adopted.

According to Araújo et al. (6), the origin of sand and the frequency of exchange at each institution may also be important risk factors for contamination. Establishments that sell, store and distribute sand cannot effectively control sanitation, not avoiding animal access.

Also, it was reported that, among the preventive measures against infection by soil-transmitted helminths, only the sand exchange in these environments as a way to control contamination by helminth eggs from dogs and cats, is not as efficient as the results achieved covering sandboxes with linings during the night (33). In addition, they reported that the implementation of a zoonotic control can also assist in this control of parasitosis (6).

Given the severity of the problems caused by a parasitic infection and the observed results, it is expected that the municipality of São Mateus adopt measures to ensure the quality of sand used in recreation areas, thus avoiding the transmission of diseases to this portion of the population ♣

Conflict of interest: None.

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