Risk factors and prevalence of enteroparasitic diseases in Shellfish Pickers from a lake area in the Northeast of Brazil

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ABSTRACT

Introduction: Intestinal parasitosis are a public health problem worldwide. There are several risk factors and a high association with some specific labor activities. Objective: The present study assessed the risk factors and prevalence of enteroparasitic diseases in shellfish pickers from one district of Maceió, Alagoas state, Brazil. Methods: Cross-sectional study of 41 female shellfish pickers including parasitological tests in fecal samples and a questionnaire with objective and subjective questions. Sand samples from their working environment were also analyzed. Results: At least one species of parasite was found in 19.51% of the fecal samples. Pathogenic species of Giardia lamblia, Trichuris trichiura, Schistosoma mansoni, Ascaris lumbricoides, Enterobius vermicularis, from the Ancylostomatidae family, and non-pathogenic species of Entamoeba coli were found. Polyparasitism was diagnosed in 37.5% of the positive samples. A total of 57.14% of sand samples contained hookworm larvae. Regarding the risk factor, low educational level was statistically associated to the presence of parasites (p<0.05). Conclusion: Greater investment in basic education is needed to increase the knowledge about preventive measures against parasitic diseases and the promotion food-handling courses in order to change existing inadequate habits in the community. Basic sanitation is also essential in preventing environmental contamination.

Keywords: risk factors; educational status; parasitic diseases; basic sanitation; environmental pollution.

INTRODUCTION

Intestinal parasitosis are a public health problem worldwide and present high morbidity indices in developing countries, where population growth is not accompanied by better living conditions⁴. The parasitosis can affect nutritional balance, interfering in nutrient absorption, inducing intestinal bleeding, reducing food ingestion and, in cases of overpopulation, may lead to death⁵. The warm environments of tropical countries, associated with malnutrition, lack of health care, poor sanitary conditions, inadequate personal hygiene, housing and peridomestic environments are associated with higher exposure of the population to infection⁶.
Brazil has 12% of the world's fresh water, including 8.2 billion meters of water distributed into rivers, lakes, dams and reservoirs, in addition to environmental and climatic conditions favorable to making it one of the leading fishing producers in the world. The shellfish picking is a manual fishing technique in Brazil, exerted mainly by women called “marisqueiras”, who harvest shrimp, “sururu”, oyster, soft crab, and crab. Shellfish pickers work both for subsistence and commercial purposes, being responsible for their own equipment and all the production stages, from preparing the materials for shellfish harvesting to selling of the final product. These stages are conducted at home, and in peridomestic and extradomestic environments.

Some fishing communities in the Brazilian northeast have no access to public supply of water, sewage and garbage collection, what favors fish contamination. The lack of basic sanitation increases the risk of parasitic infections such as Schistosomiasis among fishermen. In Alagoas, Northeast of Brazil, fishing is an important economic activity. Among the fishing areas, the Mundaú lake is highlighted, with 23 km² of area, vast mangrove and several species of fishes, crustaceans and mollusk, located among three different municipalities: Maceió, Santa Luzia do Norte and Coqueiro Seco. The Pontal da Barra district, located in Maceió, Alagoas, harbors a community of shellfish pickers on the banks of Lake Mundaú, contributing to the community economy.

Shellfish picking is a form of subsistence for socioeconomically disadvantaged groups, which normally manipulate the shellfish without adequate equipment in areas with no basic sanitation increasing the risk of contamination for themselves and the food. Given the importance of such activity, the aim of the present study was to determine the prevalence of enteroparasites and identify the risk factors that are related to its transmission in of Vieira Lima fishing community, localized at Pontal da Barra, Maceió, Alagoas state, Brazil.

METHODS

Ethical considerations
The research was submitted to the Brazilian national database for registering of research involving humans, and approved according to protocol number 1.147.774 by the Research Ethics Committee of the Alagoas State University of Health Science (CAAE 46490015.0.0000.5011). The informed consent was obtained from all the participants.

The study was performed between December 2015 and June 2016. From 82 “marisqueiras” registered in the fishing community and invited to give their informed consent, 41 accepted.

Questionnaire
Each shellfish picker was interviewed, with the aid of a standard questionnaire previously structured by the authors. The questionnaire had objective and subjective questions related to the following variables: age, education, residential address, marital status, financial incoming, presence of sanitary installations, source and type of consumed water, consume of crude vegetables, preparation of crude vegetables, habit of hand-washing before eating and after toilet use, and destination of residential garbage. The same person throughout the entire experiment interviewed all the shellfish pickers.

Analysis of feces samples
One fecal sample was obtained from each shellfish picker and conditioned in individual flasks with formaldehyde (10%, v:v). The parasitological evaluations were performed in the Laboratory of Infectious and Parasitic Diseases (LaDIP) of the Alagoas State University of Health Science, as well as in the Laboratory of Parasitic Diseases of the Federal University of Alagoas – Viçosa. The methods adopted were: Hoffman, Pons e Janer to assess helminthes and protozoa, and FLOTAC double technique to identify helminthes. Two slides from each sample/technique were evaluated under bright-field microscopy (100 and 400× magnification).

Sand collection and analysis
Seven sand samples were collected from two areas of shellfish harvesting and manipulation. The samples were collected 5cm deep from the land surface and 1m distant from each other. The sand samples were inserted in flasks with lids, without preservative, and evaluated in the same day. The methods adopted were: Hoffman, Pons & Janer and Baermann-Moraes. Two slides from each sample/technique were evaluated under bright-field microscopy (100 and 400× magnification).

Statistics
Risk factors were identified via interviews consisting of multiple-choice questions, considering the economic situation, education, eating and hygiene habits and basic sanitation. The chi-squared test ($X^2$) was used to determine the relation between the variables (risk factors) and prevalence of parasitic infections in the study population. $p<0.05$ was considered significant.

RESULTS
The socioeconomic profile, environmental characteristics and hygienic sanitary habits of the 41 shellfish pickers interviewed are shown in the Tables 1 and 2.

The majority of the shellfish pickers aged between 30-59 years old (85.35%), whereas 14.63% were ≥60 years old. Most of the shellfish pickers also lived in the same fishing community neighborhood (85.35%), whereas 14.63% were from adjacent neighborhoods.
### Table 1: Characterization and socioeconomic profile of shellfish pickers from a lake area in the Northeast of Brazil, Maceió, Alagoas, Brazil, 2015-2016 (n=41).

| Parasitological test | Positive | Negative | p* |
|----------------------|----------|----------|----|
|                      | n | %   | n | %   |
| Age (years)          |          |        |    |        |    |
| 30-59                | 8 | 100 | 27 | 81.81 | 0.454 |
| ≥60                  | 0 | 0   | 6  | 18.18 |        |
| Education            |          |        |    |        |    |
| Illiterate           | 3 | 37.5| 1  | 3.03  |        |
| 1 to 4 years*        | 3 | 37.5| 9  | 27.27 |        |
| 5 to 8 years**       | 2 | 25  | 16 | 48.48 | 0.031**|
| Complete High school education | 0 | 0 | 5 | 15.15 |   |
| Incomplete high school education | 0 | 0 | 2 | 6.06 |   |
| Residence            |          |        |    |        |    |
| Pontal da Barra      | 8 | 100 | 27 | 81.81 | 0.454 |
| Other                | 0 | 0   | 6  | 18.18 |        |
| Marital status       |          |        |    |        |    |
| Single               | 4 | 50  | 8  | 24.24 |        |
| Married              | 4 | 50  | 13 | 39.39 | 0.208 |
| Divorced             | 0 | 0   | 7  | 21.21 |        |
| Widow                | 0 | 0   | 5  | 15.15 |        |
| Income (minimum wage)|          |        |    |        |    |
| ≤1                   | 7 | 87.5| 26 | 78.78 | 0.951 |
| >1 to 3              | 1 | 12.5| 7  | 21.21 |        |

*Chi-square test, **p<0.05.

### Table 2: Environmental characteristics and hygienic sanitary habits of shellfish pickers from a lake area in the Northeast of Brazil. Maceió, Alagoas, Brazil, 2015-2016 (n=41).

| Parasitological test | Positive | Negative | p* |
|----------------------|----------|----------|----|
|                      | n | %   | n | %   |
| Waste disposal       |          |        |    |        |    |
| Septic tank          | 7 | 87.5| 31 | 93.93 | 0.897 |
| Lake                 | 1 | 12.5| 2  | 6.06  |        |
| Water supply         |          |        |    |        |    |
| Public network       | 8 | 100 | 31 | 93.93 | 0.84  |
| Well                 | 0 | 0   | 2  | 6.06  |        |
| Type of drinking water |       |        |    |        |    |
| Unfiltered           | 3 | 37.5| 9  | 27.27 | 0.687 |
| Filtered             | 2 | 25  | 6  | 18.18 |        |
| Mineral              | 3 | 37.5| 18 | 54.54 |        |
| Raw vegetable consumption |   |        |    |        |    |
| Yes                  | 7 | 87.5| 24 | 72.72 | 0.678 |
| No                   | 1 | 12.5| 9  | 27.27 |        |
| Vegetable preparation |         |        |    |        |    |
| Untreated water      | 2 | 25  | 10 | 30.30 | 0.714 |
| Boiled/filtered water | 0 | 0  | 2  | 6.06  |        |
| Water and hypochlorite | 6 | 75  | 21 | 63.63 |        |
| Food preparation     |          |        |    |        |    |
| Study Participant    | 7 | 87.5| 28 | 84.84 | 0.713 |
| Other                | 1 | 12.5| 5  | 15.15 |        |
| Washes hands before eating |   |        |    |        |    |
| Yes                  | 7 | 87.5| 31 | 93.93 | 0.897 |
| Sometimes            | 1 | 12.5| 2  | 6.06  |        |
| Washes hands after using the bathroom |     |        |    |        |    |
| Yes                  | 7 | 87.5| 33 | 100   | 0.436 |
| Sometimes            | 1 | 12.5| 0  | 0     |        |
| Trash destination    |          |        |    |        |    |
| Collected            | 8 | 100 | 33 | 100   | NS**  |
| Other                | 0 | 0   | 0  | 0     |        |

*Chi-square test, **NS: non-significant.
It was observed that shellfish pickers with positive sample had at maximum five years of education. The monthly income declared by 82.92% of the shellfish pickers was below one minimum wage, which increased to 87.5% when only shellfish pickers with positive samples were considered.

The presence of septic tanks in the house was reported by 92.68% of the shellfish pickers interviewed, whereas 7.4% said that dispose the waste in the lake. Most of the houses utilized public service as the main source of water, but 4.8% had artesian wells. Furthermore, the majority of the interviewed shellfish pickers reported the habit of washing hands before the meal and after the toilet use (95.12% and 97.5%, respectively).

The majority of the shellfish pickers (70.7%) affirmed that had a previous positive parasitic diagnosis, 26.82% referred to negative previous parasitic diagnosis and 2.4% never did the evaluation.

The parasitological examination of 41 female shellfish pickers showed that eight (19.51%) had been infected by enteroparasites, with three (7.35%) presenting poly parasitism and five (62.5%) monoparasitism (Table 3). Poly parasitism occurred with \textit{Schistosoma mansoni} and \textit{Ascaris lumbricoides} (1 occurrence), \textit{Giardia lamblia} and \textit{Enterobius vermicularis} (1 occurrence) and \textit{Enterobius vermicularis} and \textit{Ancylostomatidae} (1 occurrence). Table 4 shows the percentage of parasites found.

Regarding the sand samples, 4/7 (57.14%) were positive for hookworm \textit{Ancylostomatidae} larvae.

All the shellfish pickers received the laboratory results. Positive cases were oriented about parasitic prevention and referred to the appropriate health units for treatment.

**DISCUSSION**

The manual fishing, especially in urban areas, is commonly a casualty of environmental problems originated from irregular urban growing\(^1\). In recent years, environmental problems, such as contaminated water, air, soil, domestic and work environments, have had a significant impact on human health\(^1\). Moreover, considering the three cities shored by the Mundaú Lake, the percentage of houses with basic sanitation is low. The biggest city shored by Mundaú Lake has only 19% of the houses with basic sanitation and, consequently, waste can be disposed in the Mundaú Lake and urban rivers\(^1\). The present study stresses this problem, as 7.4% of the shellfish pickers waste directly in the Mundaú Lake and the remaining population interviewed have septic tanks, which means that there is no basic sanitation in the community.

The contamination of the environment leads to a potential transmission of important parasitic diseases. Similar to the finding in the present study, with shellfish pickers being positive for schistosomiasis, autochthonous cases of \textit{Schistosomiasis mansoni} were previously diagnosed in fishermen’s families of Alagoas\(^1\). The fishing environment gathers propitious factors for parasitic infection\(^2\). The transmission of Schistosomiasis is associated with local risk factors, presumably resulting from human fecal contamination, the limited movement of snails and the frequency of contact with water near home\(^3\).

Alagoas state is among the nine Brazilian states with higher Schistosomiasis prevalence, including its capital Maceió, considered an endemic area. Between 2015 and 2016, 170 Schistosomiasis cases were diagnosed in Alagoas, 36 from Maceió (21 autochthonous and 15 undetermined)\(^2\). It is of note that shellfish pickers are

| Table 3: Monoparasitism and poly parasitism in shellfish pickers from a lake area in the Northeast of Brazil. Maceió, Alagoas, Brazil, 2015-2016 (n=41). |
|---------------------------------------------------------------|
| **Case** | **Protozoans** | **Helminthes** | **Positive Cases** |
| 1 | - | \textit{Ancylostomatidae} eggs | monoparasitism |
| 2 | - | \textit{Trichurus trichiura} eggs | monoparasitism |
| 3 | - | \textit{Schistosoma mansoni} eggs | poly parasitism |
| 4 | \textit{Giardia lamblia} cysts | \textit{Enterobius vermicularis} eggs | poly parasitism |
| 5 | - | \textit{Enterobius vermicularis} eggs | poly parasitism |
| 6 | \textit{Entamoeba coli} cysts | - | monoparasitism |
| 7 | \textit{Entamoeba coli} cysts | - | monoparasitism |
| 8 | \textit{Entamoeba coli} cysts | - | monoparasitism |

| Table 4: Percentage of enteroparasites diagnosed in shellfish pickers from a lake area in the Northeast of Brazil. Maceió, Alagoas, Brazil, 2015-2016 (n=41). |
|---------------------------------------------------------------|
| **Positive Cases** | **N** | **%** |
| **Protozoans** | | |
| \textit{Giardia lamblia} | 1 | 12.5% |
| \textit{Entamoeba coli} | 3 | 37.5% |
| **Helminths** | | |
| \textit{Schistosoma mansoni} | 1 | 12.5% |
| \textit{Ascaris lumbricoides} | 1 | 12.5% |
| \textit{Enterobius vermicularis} | 2 | 25.0% |
| \textit{Ancylostomatidae} | 2 | 25.0% |
| \textit{Trichurus trichiura} | 1 | 12.5% |
commonly exposed to infection sources because their work depends on water contact\textsuperscript{11}. Schistosoma mansoni eggs were found in 12.5\% of the samples. This percentage would increase if a more sensitive method to diagnosis this specie was used, such as Kato-Katz.

Previous studies conducted in the Brazilian northeast identify that the shellfish pickers handle the fishing inappropriately, with adapted tools, at open areas, in the front or backyard of their own homes. They also lay the shellfish above plastic bags, on the floor, and even on wood tables, commonly with pets around. All together shown the lack of knowledge about good handling practices\textsuperscript{24,22,21}. A similar work environment was observed in this research and, as the majority of the participants’ houses (92.68\%) have septic tanks and, likely, the infection occurred while the shellfish pickers worked, what is corroborated by the results of sand analysis.

Other pathogenic parasites diagnosed in the samples were: Giardia lamblia cysts, Trichuris trichiura, Ascaris lumbricoides and Enterobius vermicularis eggs. All these parasites can be transmitted by fecal-oral rout or through direct contact with the host and/or contaminated water and food. Giardia lamblia is one of the protozoa that have been highlighted by World Health Organization as important foodborne pathogen\textsuperscript{25}. Therefore, the comprehension about the parasite cycle and hygiene habits are fundamental to avoiding that the shellfish pickers can act as an infection source to their own family and shellfish costumers.

The E. vermicularis eggs that were found, without the need of the parasitic specific Graham test, might indicate that the real prevalence is higher than our present results. Furthermore, it is noted that an adult was positive for this specific parasite, considering that children are more frequently affected. Previous parasitologic studies with adults food handlers and fishermen also reported cases of E. vermicularis\textsuperscript{26-27}. Adequate hygienic sanitary habits are more important than basic sanitation to prevent the aforementioned parasite transmission, considering that it is commonly diagnosed in people with close proximity and transmitted by direct contact or through contaminated objects.

Three of the four positive sand areas were adjacent and located in a region where shellfish are handled and sold, contributing to food contamination and affecting more individuals. The larvae found in the sand were from the Ancylostomatidae family, and eggs of this parasite were found in the parasitological examination of feces, which increase the hypothesis that the shellfish pickers may have become infected at their work environment\textsuperscript{28}.

The extreme precariousness of artisanal fishing increases the likelihood of fishers suffering accidents and contracting diseases due to the significant physical effort required, climatic variations and contact with pathological agents in an environment with not enough basic sanitation\textsuperscript{29-31}. Thus, the age is a restraint factor for shellfish pickers, reducing their working period, which could explain the negative results in all “marisqueiras” aging above 60 years old.

The low education level, including analphabetsim, was statistically significant as a risk factor among the shellfish pickers. Higher prevalence of intestinal parasites is found in lower socioeconomic classes with less education\textsuperscript{32}. Education level is an important factor in understanding diseases, forms of transmission and prevention\textsuperscript{33-34}. Other studies also found a relationship between low education levels and transmission of schistosomiasis\textsuperscript{35,36} and other enteroparasites\textsuperscript{37}.

Despite importance of artisanal fishing communities in the Brazilian fish production, these people are generally among the poorest groups in the population, and this may be due to their dependence on exploiting a limited natural resource and the inherent unpredictability of fishing\textsuperscript{38,39}. In our study, the monthly family income of 33 shellfish pickers (80.48\%) was below one minimum wage. Similar percentages were found in a parasitological study in the suburb of Manaus, where 90\% of the people earned less than one minimum wage\textsuperscript{31}. Such a low income precludes investing in personal protective equipment (PPE), important to minimize risks and infection during work-related activities\textsuperscript{40}.

Considering that low education was the risk factor related to parasitic infection, a higher investment of the government in adult education is necessary, in order to facilitate the population knowledge about preventive measures for parasitic diseases. Some of the diagnosed parasitic infections could be controlled if the shellfish pickers had access to courses focused in personal hygiene, food handling and environment contamination, with the main goal of changing the established habits and further protecting the shellfish pickers and costumers health. Furthermore, actions related to better basic sanitation and adequate wasting are fundamental.

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