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ORIGINAL ARTICLE

HIGH PREVALENCE OF PROTOZOAN INFECTIONS: A PERMANENT CYCLE IN A BRAZILIAN SEMI-ARID PERIPHERIC AREA

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ABSTRACT

In regions where sanitary conditions are poor, prophylactic administration of antiparasitic drugs is common and affects the prevalence of intestinal parasites, in spite of the environmental maintenance of their life cycle. The purpose of this study was to evaluate the prevalence of parasitic infections, patient lifestyles, health conditions and environmental sources of contamination. One hundred seventy two children and adolescents, 5 to 15 years of age of both sexes were analyzed. Data were obtained through epidemiological questionnaires, parasitological examinations using Paratest®, IgG testing for *Entamoeba histolytica*, and analysis of sources of contamination in living areas. The study detected a prevalence of 45.9% (79/172) parasitic infections, of which 62.0% (49/79) *Entamoeba histolytica/E. dispar* complex; 46.8% (37/79) *Entamoeba coli*; 27.8% (22/79) *Giardia intestinalis*; 12.7% (10/79) *Endolimax nana*; 10.1% (8/79) *Hymenolepis nana*; 8.9% (7/79) *Ascaris lumbricoides* and 2.5% (2/79) *Trichuris trichiura*. Out of the 49 samples positive for *Entamoeba histolytica* complex, there was only one case of antibodies to *E. histolytica*. The children's life habits demonstrated inadequate food hygiene practices. These insufficient sanitary parameters revealed a general lack of information in face of an alarming situation regarding sewage and other sources of environmental contamination in 20% of the areas. The high prevalence of protozoan infections despite chemoprophylaxis, with environmental sources of contamination/risk, inadequate health conditions and the general apathy of the population suggest the ineffectiveness of current preventive practices. Thus, it is advisable that control actions include protozoa as therapeutic targets and, above all, health education as a routine practice to prevent the long-term continuance of this vicious cycle.

KEY WORDS: Parasitic infections; chemoprophylaxis; environmental sources.

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INTRODUCTION

The interruption of the vicious cycle involving parasitic infection-diarrhea-malnutrition is vital to protect children against these diseases, whether involving weight and height, intellectual and cognitive functions, or even related school performance (Casavechia et al., 2016). Despite countless changes in the quality of life of the Brazilian population and a remarkable reduction in the incidence of infectious diseases, parasitic infections continue endemic in many regions (Oliveira et al., 2020).

The occurrence of diarrhea resulting from parasitic reinfection and/or persistence entails nutritional damage (Rasella et al., 2013). To reduce the morbidity of helminth infections the World Health Organization (WHO) recommends mass prophylaxis (Ezeamama et al., 2018). However, upon removal of helminthiasis, changes can occur in the epidemiological profile and in the spectrum of sensitivity to antihelmintics (Casavechia et al., 2016, Oliveira et al., 2020). Widespread use of chemoprophylaxis in populations of children is worrying since there remains a high probability of selecting for even more pathogenic parasites and consequently resistance to medication (Nery et al., 2018).

Indiscriminate administration of chemotherapy, without previous parasitological screening can provide an ideal microenvironment for the establishment of more serious infections, with fatal outcomes. The interaction between helminths and protozoa and the host's microbiota requires greater investigation. *Ascaris lumbricoides* infection is believed to modulate immunity against *Giardia intestinalis*, and in children from endemic areas, this can play an important role in susceptibility to infection after chemotherapy (Hagel et al., 2011). Studies have shown that the association of *Entamoeba histolytica* with enteric bacteria can modulate amoebic virulence factors (Oliveira et al., 2020).

Entamoeba species harbored by the human digestive tract have different degrees of pathogenicity and impact on public health. Although some species are considered commensal and non-pathogenic, *E. histolytica* can cause serious, life-threatening, and invasive infections such as amoebic dysentery and liver abscess (Calegar et al., 2021). *E. histolytica* produces tetra-nucleated cysts, which are indistinguishable from those produced by *E. dispar.* The similarity of the cysts led to the adoption of the nomenclature *E. histolytica/E. dispar* complex, which also includes *Entamoeba moshkovskii,* another tetra-nucleated cyst-producing species. However, few studies in Brazil have truly investigated the prevalence of *E. histolytica* (Santos et al., 2014).

The need for preventive education practices is evident in the presence of intestinal parasites in the population and indicates the lack of epidemiological studies.

The absence of permanent educational projects with effective participation of the families, together with precarious basic sanitation are the principal reasons for failures in programs aimed to control intestinal parasites (Casavechia et al., 2016). According to Hotez et al. (2009), education is an effective strategy, yet little used. In this context, our study proposed to evaluate the prevalence of parasitic infections, include socioeconomic and hygiene-sanitary criteria, and to encourage health professionals to implement effective preventive measures against parasites, while encouraging bonding with the community.

MATERIAL AND METHODS

Study population

This cross-sectional study was conducted in a basic health unit in the municipality of Paulo Afonso, State of Bahia, Northeast Brazil, from February 2015 to March 2017. The region was selected due to its low socioeconomic and educational standards, its geographical position, and its health infrastructure. Paulo Afonso presents an estimated population of 108,396 (IBGE, 2010), with 22,070 children enrolled in schools in the region, 1,715 in the municipal school assigned to the Primary Health Care Unit (UBS) under study. Paulo Afonso is a town planned by CHESF (Companhia Hidro-Eletrica do São Francisco), it is on an artificial island with good sanitary conditions, but there are peripheral areas with poor infrastructure affecting the health of the population. Therefore, 172 children and adolescents were surveyed, aged 5 to 15 years, of both sexes. For the sample calculation, an estimated 30% prevalence of parasitic infections was considered, a sample error of 5%, and a 95% confidence level. This prevalence was obtained from a preliminary study conducted in the region (Lander et al., 2012). Children and adolescents were asked to come to the basic health unit spontaneously. Only subjects whose parent/legal guardian signed a consent form participated in this study, in accordance to the Ethics Committee approval (number 43337115.0.0000.5196). During the study, 238 individuals were approached; however, only 172 fulfilled the research inclusion criteria: age between 5 and 15 years; enrolled/attending public schools, written authorization from legal guardians, interview with a semi-structured questionnaire, and providing biological samples

Parasitological analysis

The parents/legal guardians of the 172 children and adolescents received previous instruction about collection and conservation of fecal samples. One fecal sample was collected with a parasitological collector with a dry preservative. These samples were transferred to the Paratest™/ Diagnostek

Scientific Products Kits, where subsequently three readings of each sample were performed using optical microscopy.

Serological analysis

Blood samples of the 49 children and adolescents with results positive to *E. histolytica* complex were collected in a sterile vacuum tube without anticoagulant. ELISA kit for IgG *E. histolytica* testing was used following the manufacturer's guidelines (Ridascreen®).

Socioeconomic survey

Families who agreed to participate answered an epidemiological questionnaire to ascertain the profile of the study population with regard to sex, age, level of education, household income, type of housing, number of people who lived in the same house, and the existence of treated water, sewage system, and domestic animals.

Soil analysis

Soil analysis was performed in the living areas. Five areas were chosen and mapped for analysis. Five collection spots were defined in each area, at least 2 meters apart. Each collection point yielded 100g soil sample: topsoil, 5 and 10 cm deep. Dry areas and those exposed to direct sunlight were avoided. The superficial, intermediate, and deep aliquots were joined in accordance to their depths. The samples were then homogenized, weighed, and standardized at 100g. Distilled water was added and sediments were filtered with the aid of gauze folded four times (Nunes et al., 2000). After 24 hours, the supernatant was discarded. The sediments were then analyzed after applying the Hoffmann et al. (1944), Faust et al. (1939) and Rugai et al. (1954) methods.

Health education activities and treatment of infected individuals

Health education activities for the population under study were performed by distributing leaflets and guidelines on the transmission mechanisms and preventive measures of the main intestinal parasites. The doctor and nurse at the basic health unit confidentially informed the parents/ legal guardians of the children and adolescents about their parasitological results. Positive cases were instructed regarding hygiene habits and treated accordingly.

Statistical analysis

The database was analyzed using IBM® SPSS Statistics®. In this program, analysis of prevalence was performed using case frequencies within the studied population. Pearson's chi-square test was also applied to obtain the levels of significance between each variable and the outcome (positive parasitological exam result), with a 95% confidence interval (p-value <0.05).

RESULTS

The study found a prevalence of 45.9% (79/172) individuals infected with enteroparasites. The main parasites observed were 62.0% (49/79) *E. histolytica/E. dispar/ E. moshkovskii* complex, 46.8% (37/79) *E. coli*, 27.8% (22/79) *G. intestinalis*, 12.7% (10/79) *E. nana*, 10.1% (8/79) *H. nana*, 8.9% (7/79) *A. lumbricoides* and 2.5% (2/79) *T. trichiura* (Table 1).

Table 1. Parasitological stool analysis of 172 children and adolescents in Northeast Brazil.

Intestinal parasite	Number of parasitized individuals	
	Sample number (n)	Prevalence (%)
Entamoeba dispar/ E. moshkovskii	48	60.7
Entamoeba coli	37	46.8
Giardia intestinalis	22	27.8
Endolimax nana	10	12.6
Hymenolepis nana	8	10.1
Ascaris lumbricoides	7	8.8
Trichuris trichiura	2	2.5

Infection by *E. histolytica* requires that the specific IgG concentration present a dosage above 1.1 µg/mL, established by the kit manufacturer. In this study, only one sample exceeded cut-off with an IgG concentration of 1.60µg/mL. Therefore, enabling the determination of a prevalence of 60.8% (48/79) for *E. dispar/E. moshkovskii* and 1.3% (1/79) for *E. histolytica.*

In the analysis according to sex, a higher prevalence of enteroparasites was detected in females when compared to males, with a significant difference (p= 0.026). However, there was not difference according to age group, p>0.05 (Table 2). A low level of maternal education was associated with parasitic infections (p= 0.042). The monthly income most noted for the studied families was below the minimum wage. Governmental financial aid social programs were observed in 40% of the population.

Table 2. Positivity of parasitological stool test results, according to sex and age of 172 children and adolescents in Northeast Brazil.

Studied variables	Prevalence (%)	Positive parasitological test		p-value
		Sample number (n)	%	
Sex				
Female	45.3	43	54.4	0.02*
Male	54.7	36	45.6	
Age				
4-7	31	22	28.2	0.3
8-11	46.2	36	46.2	0.5
12-15	22.8	20	25.6	0.4

Chi-square test; Fisher's exact test. *: statistically significant association (p-value <0.05).

However, the number of people in the household was not associated with parasitic infections, p= 0.082 (Table 3).

Table 3. Socioeconomic conditions versus positive parasitological results of 172 children and adolescents in Northeast Brazil.

Studied variables	Prevalence (%)	Positive parasitological test		p-value
		Sample number (n)	%	
Maternal education level				
Incomplete high school	89.9	75	94.9	0.04
Incomplete or complete graduation	10.1	4	5.1	
Monthly income				
<1 minimum wage	89.9	65	87.8	0.5
> 1 minimum wage	10.1	9	12.2	
Responsible for supporting the family				
Parents	59.7	48	60.8	0.8
Others	40.3	31	39.2	
Residents/ household				
1-4 people	44.8	37	52.9	0.08
> 4 people	55.2	33	47.1	

Others: stepfather; grandparents; governmental financial aid social program or external assistance. Chi-square test; Fisher's exact test

The lifestyle of the entoparasite-infected individuals revealed that 79.7% (63/79) used untreated water. Although there was no statistical difference, fruit and vegetables were washed by 97.5% (77/79). However, 77.2% (61/79) presented inadequate cleaning methods, using only water, p> 0.05 (Table 4).

Table 4. Prevalence of parasitic infections, according to hygiene practices of 172 children and adolescents in Northeast Brazil.

Studied variables	Prevalence (%)	Positive parasitological test		p-value
		Sample number (n)	%	
Water consumption directly from the tap				
Yes	75.5	63	79.7	0.2
No	24.5	16	20.3	
Washing of fruit and vegetables				
Yes	95	77	97.5	0.2
No	5	2	2.5	
Washing food only with water				
Yes	78.6	61	77.2	0.4
No	21.4	18	22.8	

Chi-square test; Fisher's exact test

Basic sanitation conditions were inferred from information in the semi-structured questionnaire. The results demonstrated 97.5% (76/79) of the population had piped water, 78.6% (58/79) of the residences had sewage collection, and 95.6% (74/79) regular trash collection. However, these variables were not associated with parasitic infections, p> 0.05. The quest for sources of infection in the soil in the five areas of the community detected 20% (9/45) contamination with enteroparasite evolutive forms. Eggs and larvae were identified, such as the families Rhabdiasidae and Ancylostomatidae (Table 5).

Table 5. Soil analysis through parasitological techniques in living areas of five children and adolescents in Northeast Brazil.

Mapped areas	Soil sample	Parasitological techniques		
		Hoffmann et al. (1944)	Faust et al. (1939)	Rugai et al. (1954)
1	Superficial	Family larvae Rhabdiasidae	Family larvae Rhabdiasidae	Negative
	5 cm	Negative	Family larvae Rhabdiasidae	Negative
	10 cm	Family larvae Rhabdiasidae	Family larvae Rhabdiasidae	Negative
2	Superficial	Negative	Family larvae Rhabdiasidae	Negative
	5 cm	Negative	Family larvae Rhabdiasidae	Negative
	10 cm	Negative	Negative	Negative
3	Superficial	Negative	Family Ancylostomatidae	Negative
	5 cm	Negative	Family larvae Ancylostomatidae	Negative
	10 cm	Negative	Negative	Negative
4	Superficial	Negative	Negative	Negative
	5 cm	Negative	Negative	Negative
	10 cm	Negative	Negative	Negative
5	Superficial	Negative	Negative	Negative
	5 cm	Negative	Negative	Negative
	10 cm	Negative	Negative	Negative

DISCUSSION

Intestinal protozoan infections are closely related to inadequate sanitation and environmental contamination with fecal matter (Calegar et al., 2021). In this study, a parasitic prevalence of 45.9% was detected in children and adolescents, predominantly protozoan infections. According to Casavechia et al. (2016), treatment with broad-spectrum antihelmintics might modify this pattern of parasitic infection. In communities in the State of Sergipe – Brazil using an annual chemoprophylaxis, Oliveira et al. (2020) detected a marked reduction in the prevalence of *A. lumbricoides*, Ancylostomatidae family and *T. trichiura*. However, there was an increase in the prevalence of intestinal protozoa, specifically *E. histolytica/E. dispar*, *Blastocystis hominis*, and *G. intestinalis.*

We suggest that the prevalence of protozoan infections is the result of the treatment for parasitic infections using a single dose of albendazole. The dramatic reduction in the helminthic infections and an increase in intestinal protozoan infections, indicates an alteration in the epidemiological profile presumably caused by inadequate chemoprophylaxis administration with albendazole or mebendazole (Ignacio et al., 2017). According to Nery et al. (2018) indiscriminate chemotherapy temporarily protects children and adolescents from parasitic infections, but presents limited protection against protozoans and certain helminths. Sinniah et al. (2014) found that in populations treated with antihelmintics, reinfections generally occur after four months, demonstrating the ineffectiveness of this measure when taken in isolation.

In this study, the commensal parasites *E. dispar/ E. moshkovskii, E. coli* and *E. nana* were found most frequently. The presence of nonpathogenic protozoa suggests contaminated food, water and poor fecal-oral hygiene (Nery et al., 2018). The differentiation between *E. dispar/ E. moshkovskii* was not performed because of the need to apply molecular diagnosis. Molecular epidemiological studies showed that *E. dispar* is the species most commonly found among the tetra-nucleated cysts (Santos et al., 2014, Calegar et al., 2021). The prognosis for patients with nonpathogenic protozoa is excellent, as most patients are asymptomatic (Calegar et al., 2021). According to Haidar & De Jesus (2021), treatment is not indicated for *E. coli* infection, supportive care should be provided for patients, and proper hygiene should be maintained. If the patient is symptomatic and no other organism or causes are detected, treatment may be indicated.

The detection of *E. histolytica* infection is alarming for the region. Santos et al. (2014) detected the infection in 8.3% (2/24) while researching coproantigens in a settlement located in the municipality of Ilhéus in southern Bahia. The severity of an asymptomatic *E. histolytica* infection consists in treatment applied immediately after differential diagnosis. The presence of anti-amoebic antibodies in serum samples of non-diarrheal patients is imperative to

begin treatment (Rivera et al., 2012). Therefore, the adoption of differentiation methods to detect *Entamoeba* spp. is extremely important to reduce possible false negatives, allow early treatment, and avoid future complications.

Regardless of age, there was a higher prevalence of intestinal parasitic infections in females than in males, mainly poly-parasitism. Bosqui et al. (2015) noted predominant parasitism in the female public, despite most studies indicating that males present a risk factor for parasitic diseases. Campbell et al. (2017) detected greater infection rates by *Necator americanus* in females as compared to males. This is laughingly attributed to the direct contact with contaminated soil frequently observed in females, however further investigations are needed. The absence of risk in relation to age group demonstrates the importance of health education for school-age children, since this public is more vulnerable to infection. Abe et al. (2019) when comparing rates of parasitic infection in schoolchildren, also suggested that all ages and both sexes are susceptible to infection.

The socioeconomic conditions and poor hygiene connected to eating habits revealed in the epidemiological survey were favorable to the maintenance of this vicious cycle. The socioeconomic aspects of the studied population revealed significant vulnerability regarding the risk of contracting some type of intestinal parasitosis, highlighting low family income and poor maternal instruction. Governmental financial aid social programs were cited as a source of home income, and despite not having statistical significance; this reveals the socioeconomic vulnerability of the public under study. These programs are an effective strategy to reduce the impacts of child poverty in developing countries, such as mortality attributed to malnutrition and diarrhea (Rasella et al., 2013).

Despite having no significant association with the outcomes, it is important to note that the majority of respondents reported drinking tap water, an alarming finding since public water distribution systems function as an important vehicle for the spread of diseases, especially protozoan infections. Corroborating these findings, Belo et al. (2012) demonstrated that the use of water filters in homes is associated with a reduction in intestinal parasites. Contamination risk in relation to washing fruit and vegetables was noted, despite the majority of participants claiming to wash their food before consumption but only using water. This is an inadequate practice, which contributed to the high prevalence of parasitized individuals, proving the community needs guidance concerning effective preventive measures.

The semi-structured questionnaire revealed that 78.6% of the homes have sewage disposal, 97.5% reported piped water, and 95.6% reported garbage collection. However, according to SNIS (2014), the municipality presents a sewage collection rate of 13.4%. Such incongruities concerning real health conditions in the region demonstrate the population's level of knowledge about basic sanitation. This aversion to health issues exposes general inertia

regarding the inadequate living conditions in the region and engenders negative repercussions during inspections involving the activities of public agencies. Such misinformation with a consequent apathy in the local population allows the continuance of parasitic infectious cycles (Oliveira et al., 2020).

Parasitic contamination of the peridomestic soil with larvae of the families Rhabdiasidae and Ancylostomatidae was also observed. Abe et al. (2019) found that in areas with poor hygiene conditions, children prefer to defecate in the open, usually in areas close to houses, dumps, and shrubs. This and bad habits, as well as inadequate hygiene help perpetuate the parasitic life cycle. Araujo et al. (2021) concluded that soil contamination by parasites is directly linked to the presence of animals in these environments, due to their feces and favorable environmental conditions. Melo et al. (2020) observed potential risk of zoonoses transmission through soil in a population in the Northeast, especially during the dry season. Abe et al. (2019) found that integrated control approaches, such as preventive chemotherapy, health education campaigns, improvement in basic sanitation and access to clean and safe water can eliminate neglected tropical diseases.

The high prevalence of protozoan infections from environmental sources of contamination, inadequate sanitary conditions, and apathy in the local population suggest ineffective preventive practices, even with chemoprophylaxis. This overall environment may well entail more pathogenic parasitic populations and bring severe implications regarding cognitive and corporal development in the local youth, as well as negatively affect the spectrum of drug sensitivity. When considering the high morbidity and mortality associated with *E. histolytica*, differential diagnosis is essential for early treatment of this infection. The absence of hygienic food preparation practices and low income combined with the lack of community education are the main factors in the spread of enteroparasites. This scenario, associated with the lack of preventive measures, including correct food hygiene and water treatment favors the perpetuation of the parasitic cycle. Continued awareness of health professionals and the local population can ensure educational and preventive actions, and it is believed that this is the path to a definitive solution for this ancient yet constant public health concern.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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