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ORIGINAL ARTICLE

EPIDEMIOLOGICAL PROFILE OF INTESTINAL PARASITES IN CHILDREN UNDER FIVE YEARS OF AGE IN MAPUTO, MOZAMBIQUE

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ABSTRACT

Intestinal parasites are one of the most sought-after causes in pediatrics. Its occurrence is one of the indicators of socioeconomic status that may be associated with several sociodemographic factors. It is estimated that approximately 50% of the vulnerable population is parasitized with one or more types of parasites, such as children. Considering that this population is essential for human development, the goal of this work was to evaluate the epidemiological profile of intestinal parasites in children up to five years of age in Maputo, Mozambique. To this end, a cross-sectional descriptive study was done with 70 children, through the application of a questionnaire with content related to sociodemographic conditions, clinical profile and identification of the patient's parasitosis. A stool sample was also requested for parasitological analysis by Ritchie concentration, Ziehl-Neelsen and rapid test techniques. From the 70 children, 36 (51.4%) were infected with at least one intestinal parasite, with *Ascaris lumbricoides* and *Trichuris trichiura* being the most prevalent. Furthermore, it was possible to observe a direct correlation between basic sanitation and intestinal parasites. Fever symptoms were also correlated with the presence of intestinal parasitism. Although the government of Mozambique has adopted since 2009 a policy of massive drug administration to deworm children, the high rate of intestinal parasites remains present in the population. Therefore, preventive measures must be taken to contribute to the reduction of this rate of intestinal parasites in this population.

KEY WORDS: Public health; Mozambique; kids; intestinal parasites.

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INTRODUCTION

According to the National Institute of Statistics of Mozambique, in 2017, Maputo City is estimated to have a total population of 1,080,277 inhabitants, of which 229,842 are in the 0 to 10 age group (21.3%) (INE, 2017). Chamanculo is an urban area of Maputo City, densely populated, with poor sanitary conditions and a high burden of disease. In this region there is the General Hospital of Chamanculo (HGC), it is a reference hospital that serves patients from all over Maputo province, with a great demand for the treatment of diseases and illnesses, including intestinal parasites (Lage, 2020).

Intestinal parasites are one of the most sought-after causes in pediatrics. This infection is caused by intestinal parasites, a wide group of organisms and with greater representation the protozoa (*Entamoeba histolytica, Dientamoeba fragilis, Giardia duodenalis* and *Cryptosporidium parvum*) and the helminths (*Ascaris lumbricoides, Trichuris trichiura, Ancylostoma duodenale* and *Taenia solium*) (Auler et al., 2018). Among the various forms of transmission of the disease, exposure via the fecal-oral route to water and/or food contaminated by the parasite should be highlighted (Maldotti & Dalzochio, 2021). The prevalence of this set of parasitic diseases varies, being influenced by different factors such as geographic zone, sanitary and climatic conditions (Taghipour et al., 2020).

Africa is a region with a high prevalence of intestinal parasitic diseases. It is estimated that approximately 50% of the vulnerable population (children, elderly, poor people) is infected with one or more types of parasites (Chelkeba et al., 2020; Ahmed et al., 2023). According to data from the World Health Organization (WHO), Mozambique is an endemic city for five different neglected tropical diseases, including parasitic diseases, which they are controlled and eliminated through public policies on preventive chemotherapy aimed at vulnerable people, especially children.

This high prevalence of the disease in children is a consequence of behavioral, biological and environmental factors, which they are exposed to, as well as financial factors, such as lack of investment (Kantzanou et al., 2021). The financial disproportion is partly attributed to the fact that, with the advent of the vaccine era, these parasitic diseases have become increasingly neglected and, until recently, silently ravaged the most impoverished countries (Ola-Fadunsin et al., 2020). In addition, in endemic regions, the population with the highest prevalence rates is 14 years old, a population that constitutes an important group in the country development (Beavogui et al., 2021).

Considering that almost all children living below the poverty level are infected with one or more parasites and that this population is essential for human development, studies focusing on this population become increasingly urgent and justifiable (Fauziah et al., 2022). Therefore, the present study aimed to evaluate the profile of intestinal parasites in children under five years of age in Maputo, Mozambique, using three different methods, Ritchie concentration, Ziehl-Neelsen staining and rapid test.

MATERIAL AND METHODS

Ethical approval

This study was approved by the Mozambican health ethics committee with reference, 91/CNBS/2014 dated December 15, 2015. A free and informed consent form (TCLE) was explained and given to all volunteers who agreed to participate in the study.

Study area

The study was carried out at the Hospital Geral de Chamanculo (HGC), located in the city of Maputo, in the south of Mozambique at the geographical coordinates 25° 58' S 32° 35' E. This city has an area of 346.77 km^2 and estimated population of 1,088,449 inhabitants, corresponding to 3,628.2 inhabitants/ km^2. It is characterized by a dry tropical climate with a hot period of the year between November and April and a cold period between May and October. The period of greatest precipitation occurs in the warmer months, between November and March.

Study population

The population of this study consists of children up to five years of age who sought pediatric services at the HGC from October, 2015 to April, 2016. To be included in this study, children should have at least one of the symptoms of fever, cough, stomach pain, weight loss, diarrhea, constipation, vomiting or who sought the hospital for vaccination. In addition, for all children included in this study, the person responsible for the child signed and agreed to the consent form.

Obtaining data

For data collection, children were recruited after doctor appointments or appointments at the HGC. For those who agreed to participate in the study, a questionnaire was applied to the person responsible for the child, addressing content related to sociodemographic conditions, clinical profile and identification of the patient's parasitism, with the help of a hospital health professional. A sample of the child's feces was also requested, which should be delivered the next day to the hospital laboratory.

The first part of the questionnaire had six questions related to: (I) gender; (II) neighborhood of residence; (III) age; (IV) caregiver education; (V) access to potable water; and (VI) access to sewage treatment. The second part had three questions related to the clinical profile of the child (characteristics of feces,

symptoms and treatment of parasites). Finally, the third part of the questionnaire, completed by the technician in charge, had questions related to the three methods of detecting parasites: (I) Ritchie concentration; (II) Ziehl-Neelsen; and (III) rapid test (Immunocard STAT® Crypto/Giardia, Meridian Bioscience, 750830).

Parasitological analysis

The laboratory diagnosis of fecal samples was done at the Laboratory of Parasitology of the National Institute of Health in Maputo, Mozambique, within 24 hours after obtaining the sample, using the concentration technique of Ritchie (Ritchie, 1948), Ziehl-Neelsen (Githui et al., 1993) modified for diagnosis of helminths and protozoa, and the Immunocard STAT® Crypto/ Giardia rapid test (Meridian Bioscience, 750830).

Ritchie test

The Ritchie test was performed according to Ritchie (1948), the test used for the identification of the parasites *Schistosoma mansoni, Ascaris lumbricoides, Trichuris trichiura, Entamoeba histolytica, Dientamoeba fragilis, Giardia duodenalis, Cryptosporidium parvum* and among other protozoa, without stain. Briefly, a total of 2 g of feces from each child was resuspended in 10 mL of water at 45 °C. The samples were homogenized and filtered using a folded gauze with the help of a funnel. Then the samples were centrifuged at 2,500 rpm for 5 minutes and the supernatant material removed. Later, the sediment was resuspended with 100 µL of neutral detergent and 10 mL of water at 45 °C, homogenized and centrifuged again under the same conditions. After centrifugation, a drop of the sediment obtained was deposited on a microscope slide and 2 drops of Lugol were added to the sample. Thereafter, the prepared slide was observed under an optical microscope with a 10 and 40× objective lens to verify the presence of parasites.

Ziehl-Neelsen test

After concentration of the sample by the Ritchie method, the Ziehl-Neelsen stain assay was performed according to Githui et al. (1993), with modifications for diagnosing protozoa such as *Cryptosporidium* spp. Briefly, after collecting the samples, 10 g were diluted in water for 15 minutes, and centrifuged at 2,500 rpm for 5 minutes, the supernatant was discarded. Then, the obtained sediments were used in the preparation of smears on the microscopy slides and stained with the Ziehl-Neelsen method. Then, each slide was examined under a 40× objective lens to verify the presence of parasite eggs.

Rapid test

The Immunocard STAT® Crypto/ Giardia Rapid Test (Meridian Bioscience, 750830) was used to detect and qualitatively distinguish *Cryptosporidium* spp. and *Giardia duodenalis* antigens in stool samples from volunteers. Briefly, the collected samples were prepared in a test tube where treatment buffer and antibodies from the kit were added and homogenized with the aid of a vortex. Then, 60 µL of the material was transferred to the rapid test device containing a capture reagent (an avidin derivative) for *Giardia*, a capture antibody for *Cryptosporidium* and a control antibody. After 10 minutes the qualitative result is obtained, results after 15 minutes of incubation on the plate were considered invalid.

Statistical analysis

The software R (version 4.2.1) was used for all statistical analysis in this study. The Shapiro-Wilk test was used to verify data normality. For parametric data, the t test (Student) was used for independent samples, while the U test (Mann-Whitney) was used to compare the differences between two groups with non-parametric data distribution. For categorical data analysis, the chi-square (X^2) or Fisher test was used. In this study, p value < 0.05 was determined to have statistically significant differences.

RESULTS

During this study period, 70 stool samples were collected for laboratory tests from children aged younger than five years, and after the laboratory tests, 36 samples (51.4%) were positive. Based on this result, the volunteers' data were divided into two groups: parasitized, for those who tested positive for at least one intestinal parasite, and non-parasitized, for those who tested negative for intestinal parasitism.

In this study, the average age of children was 28.5 months and 45.7% (n= 32) from them were male. A higher prevalence of intestinal parasites was observed in female children (61.1%). Regarding the person responsible for the child, 42.8% (n= 30) had an elementary school degree. As for the distribution of the volunteers' place of residence, a total of 17 different neighborhoods in Maputo were evaluated, from which 50% (n= 35) were from Chamanculo, where the General Hospital of Chamanculo is located (Table 1). For all sociodemographic variables, none showed a significant difference when comparing the parasitized and non-parasitized groups ($p > 0.05$).

Table 1. Sociodemographic characteristics of participants.

Variable	TOTAL (n= 70)		Parasitized (n= 36)		Non-parasitized (n= 34)	
	n	%	n	%	n	%
Gender						
Male	32	45.7	14	38.9	18	52.9
Female	38	54.3	22	61.1	16	47.1
Age (months)						
≤ 20	22	31.5	8	22.2	14	41.2
> 20 - ≤ 40	34	48.5	17	47.2	17	50.0
> 40 - ≤ 60	14	20.0	11	30.6	3	8.8
Guardian's schooling						
Elementary	30	42.8	11	30.5	19	55.9
Primary	19	27.2	12	33.4	7	20.6
Basic	21	30.0	13	36.1	8	23.5
Place of residence						
25 de Julho	3	4.3	1	2.8	2	5.9
Aeroporto	2	2.9	1	2.8	1	2.9
Alto-maé	1	1.4	1	2.8	0	0.0
Benfica	3	4.3	1	2.8	2	5.9
Chamanculo	35	50.0	20	55.5	15	44.2
CMC	2	2.9	0	0.0	2	5.9
Hulene	1	1.4	0	0.0	1	2.9
Jardim	1	1.4	0	0.0	1	2.9
Luis Cabral	5	7.2	2	5.5	3	8.9
Maguanine	1	1.4	1	2.8	0	0.0
Malanga	8	11.4	6	16.6	2	5.9
Matola Gare	1	1.4	1	2.8	0	0.0
Ndlavela	1	1.4	1	2.8	0	0.0
Patrice Lumumba	1	1.4	0	0.0	1	2.9
São Damaso	2	2.9	1	2.8	1	2.9
T-3	1	1.4	0	0.0	1	2.9
Zona Verde	2	2.9	0	0.0	2	5.9

Table 2 shows the characterization of the children's feces, symptoms and health profile. Among two children studied, one was infected with at least one intestinal parasite, exposing the high prevalence of intestinal parasitosis in children in Maputo. Furthermore, most parasitized children (48.5%) were aged between 20 and 40 months old, a stage of life with great development. Both groups had the soft state as the main characteristic of the stool sample (61.4%).

Table 2. Stool characteristics, symptoms and basic sanitation of children.

Variable	Total (n= 70)		Parasitized (n= 36)		Non- parasitized (n= 34)		p-value
	n	%	n	%	n	%	
Stool characteristics							
Soft	43	61.4	22	61.2	21	61.8	0.42
Semi-solid	11	15.7	6	16.6	5	14.7	0.46
Solid	16	22.9	8	22.2	8	23.5	0.41
Symptoms							
Fever	22	31.4	14	38.9	8	23.5	< 0.05
Diarrhea	11	15.7	4	11.1	7	20.5	0.23
Abdominal pain	16	22.9	8	22.2	8	23.5	0.41
Cough	14	20.0	6	16.7	8	23.5	0.36
Others	7	10.0	4	11.1	3	8.9	0.48
Access to sewage treatment							
Yes	25	35.7	6	16.7	19	55.9	< 0.01
No	45	64.3	30	83.3	15	44.1	< 0.01
Access to potable water							
Yes	30	42.6	12	33.4	18	52.9	0.04
No	40	57.4	24	66.6	16	47.1	0.03

As for the children´s clinical symptoms, in the parasitized group, the main symptom was fever (38.9%) followed by abdominal pain (22.2%), while in the non-parasitized group, the main symptoms were distributed more homogeneously between fever, abdominal pain, cough (23.5% each) and diarrhea (20.5%). When comparing the two groups, a significant increase ($p < 0.05$) was observed in fever symptoms in parasitized children. In addition, a direct correlation was established between the symptom of fever and the presence of intestinal parasites.

Another important factor evaluated in this study was the children's basic sanitation. It was possible to observe that among those parasitized, 57.4% did not have access to potable water and 83.3% did not have adequate sewage treatment in their homes. As with fever, significant differences ($p< 0.05$) were found between the presence of intestinal parasitism in children with access to potable water and/ or sewage treatment and children without these accesses. Both basic sanitation factors were relevant for the presence/ absence of intestinal parasites since children who had no access to potable water or sewage treatment had significantly more intestinal parasites ($p< 0.05$). Furthermore, the lack of potable water and the absence of sewage treatment caused an even greater increase in these parasites ($p< 0.05$).

Regarding parasite identification, the Ritchie concentration technique was able to identify a greater variety of parasites, however, it was unable to identify *Cryptosporidium* spp. In addition, a low efficiency in the detection of *Giardia duodenalis* was observed, since from the eight children infected with *Giardia*, only one (12.5%) infected child was identified by this technique. When comparing the ability of the Ziehl-Neelsen and rapid tests to identify *Cryptosporidium* spp., the rapid test identified 100% of infected children, while the Ziehl-Neelsen test identified 87.5%. In addition to the identification of *Cryptosporidium* spp., the rapid test allows the identification of *Giardia duodenalis* (Table 3).

Table 3. Identification number of parasites in stool samples from children younger than 5 years old by 3 different methods.

Methods	Parasites				
	Helminths				Protozoa
	A. lumbricoides	*T. trichiura*	*S. stercoralis*	*G. duodenalis*	*Cryptosporidium* spp.
Ritchie test	16	27	1	1	-
Ziehl-Neelsen	-	-	-	-	7
Rapid test*	-	-	-	8	8

*Immunocard STAT® Crypto/ Giardia (Meridian Bioscience, 750830).

About those parasitized (n= 36), through tests used for parasitological identification, the following parasites were identified: (i) *Ascaris lumbricoides* (n= 16; 44.5%); (ii) *Trichuris trichiura* (n= 27; 75%); (iii) *Cryptosporidium* spp. (n= 8; 22.2%); (iv) *Giardia duodenalis* (n= 8; 22.2%); and (v) *Strongyloides stercoralis* (n= 1; 2.8%). No significant differences were observed between the tests for parasite identification ($p > 0.05$). This result is due to the particularity of each method, making it difficult to compare them. Although no significant differences were observed, the difference in parasite identification in the samples is notorious, with the Ritchie test having the greatest capacity to identify different parasites and the rapid test being the most sensitive in identification, among the tests evaluated in this study.

DISCUSSION

The occurrence of infections by intestinal parasites is one of the indicators of socioeconomic status that may be associated with several sociodemographic factors, such as lack of basic sanitation, contaminated water and food consumption, in addition to biological factors such as age and type of parasite (Chelkeba et al., 2020). In this study, it was possible to observe a direct correlation between basic sanitation and the incidence of intestinal parasites in children. In addition, from the data obtained in this study, it was possible to observe that the symptom of fever, together with the presence of soft stools, are potential indicators of intestinal parasites.

The high prevalence of these parasites found in this study (51.4%) corroborate the findings of other authors. In Ethiopia, a cross-sectional study on intestinal parasites in primary school-aged children showed a high prevalence for the disease, where 235 (57.9%) children were positive for one or more intestinal parasites (Sitotaw et al., 2019). However, this prevalence of intestinal parasites is influenced by several factors previously presented (Chelkeba et al., 2020), ranging from 5% (Belyhun et al., 2010) to 85% (Nyantekyi et al., 2010). Although it was carried out in Ethiopia at the same time, another study evaluating intestinal parasites in children under the age of five showed a low prevalence, where only 15.5% of children were parasitized (Gebretsadik et al., 2018).

In Mozambique, intestinal parasites occur throughout the national territory, especially in children. Although, since 2009, the government of Mozambique has adopted a policy of massive drug administration to deworm children and populations at risk in the country, however, the high rate of intestinal parasites remains present in the population (Mozambique, 2013). Because they cause malabsorption, diarrhea and malnutrition, parasitized children may have delayed physical and cognitive development, directly reflecting on school performance and the development of the country (Auler et al., 2018).

In this study, most of the participants live in neighborhoods with low human development, a factor that further aggravated the presence of intestinal parasitism. It is known that one of the main forms of transmission of these diseases is through the oral route, through the ingestion of contaminated water and food (Oyegue-Liabagui et al., 2020). This study showed that, regardless of the gender and the age of the child (up to 60 months old), the lack of basic sanitation, such as public drinking water supply and sewage system, is a crucial point that can increase the incidence of parasites in the region.

Although there are many studies on intestinal parasites in children (Chelkeba et al., 2020), few studies explore the symptoms caused by the parasite in the child's body. In this study, the main symptoms resulted by the infection caused by intestinal parasites were explored and it was observed that fever, diarrhea and stomach pain are commonly present in these parasitized children. As well as malnutrition, triggered by some parasites, some chronic symptoms developed by the infection can compromise the health and physical development of the child (Yoseph & Beyene, 2020). Therefore, it is necessary to correctly identify the parasite so that it is possible to carry out an efficient treatment of the child (Vivancos et al., 2018).

As noted, the Immunocard STAT® Crypto/ *Giardia* rapid test (Meridian Bioscience, 750830) showed greater sensitivity for identifying *Cryptosporidium* spp. when compared to the Ziehl-Neelsen test. This finding was like the study published by Zaglool et al. (2013), where it was observed that from the 15 positive controls, the rapid test detected 13 (86.7%), while the Ziehl-Neelsen detected 11 (73.3%) positive cases. Furthermore, it was shown that the rapid test did not detect any positive cases in 40 negative controls, while the Ziehl-Neelsen detected 2 (5%) positive cases (Zaglool et al., 2013).

Although they are accurate tests, the rapid and Ziehl-Neelsen tests are limited to *Cryptosporidium* spp. parasites and *Giardia duodenalis*. On the other hand, the Ritchie concentration technique proved capable of identifying a greater variety of parasites, including *Ascaris lumbricoides* and *Trichuris trichiura*, parasites most frequently present in parasitized children. However, due to the inability to identify *Cryptosporidium* spp. and, in addition to the low efficiency in detecting *Giardia duodenalis*, it is necessary to use complementary tests when identifying the parasite.

Children are the main carriers and source of continuous transmission of intestinal parasitism. Therefore, children should be the main target of interventions to control the disease. The observed high prevalence of parasitic diseases among children in Maputo, added to the lack of basic sanitation, must be considered for future intervention measures, since these parasites contribute to the poor child development. As a result, it generates problems such as malabsorption, malnutrition, anemia and, consequently, reduced physical capacities. It is known that children in many developing countries are the largest proportion of the age pyramid, which when affected, can further

aggravate the city's poverty situation. Even with the adoption of a policy of massive administration of medication to deworm children by the Mozambican government, rates of intestinal parasites have remained high in the population. For this reason, measures to reduce intestinal parasites should focus on reducing exposure, especially with children. In addition to these measures are improvements in the domestic water supply with the introduction of a piped sewage system. Also, the use of rapid tests can be useful in screening children to ensure a quick and accurate parasite identification result and, consequently, parasite control. Thus, it is expected that these measures will have a greater impact on reducing the rates of parasitic infection in this population.

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CONFLICT OF INTEREST

All authors declare that they have no commercial or associative interest that represents a conflict of interest in relation to the submitted work.

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