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Original Article

Intestinal parasitic infections in children under five in the Central Hospital of Nampula, Northern Mozambique

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Abstract
Introduction: Intestinal parasites are known to cause infection in humans worldwide, with higher prevalence in low- and middle- incoming countries. Children are greatly affected leading to malnutrition and subsequently to physical and cognitive development impairment. Despite the scale and importance of this issue, there are few studies conducted in Mozambique concerning parasitic intestinal infections in hospitalized children. To our knowledge this is the first published report with data on this subject from Northern Mozambique.
Methodology: A cross-sectional study was conducted in 2012 and 2013 in 831 children, attending the Central Hospital of Nampula in Northern Mozambique. One single stool sample was obtained from each child. Socio-demographic and clinical data were also obtained. Parasitological analysis of feces was performed through direct examination and Ritchie concentration technique and *Giardia duodenalis* antigen detection by rapid immunochromatographic test. Modified Ziehl-Neelsen staining was used for coccidia detection.
Results: The global prevalence of pathogenic intestinal parasites was 31.6%. *G. duodenalis* (23.9%) was by far the most prevalent parasite followed by *Strongyloides stercoralis* (4.1%) and *Cryptosporidium* sp. (3.4%). Intestinal parasites were more frequent in older children (p = 0.005; aOR = 1.025).
Conclusions: This work is one of the few published studies reporting intestinal parasites infection in hospitalized children. The percentage of children affected with *G. duodenalis* is higher than found in other studies in the African region. This highlights the need of particular attention being given to this intestinal protozoan and its resistance to water treatment, as well as to environmental health and personal hygiene.

Key words: Intestinal parasites; hospital; children; Northern Mozambique.

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Introduction

Intestinal parasitic infections, including helminths and protozoa, are an important cause of morbidity and mortality in middle- and low-income countries [1,2]. Although the death rate associated with these infections is low, they affect more than 2 billion people worldwide, resulting in symptomatic illness or chronic diarrhea, malnutrition and impaired mental development, especially in children [3,4]. According to the World Health Organization (WHO), over 270 million pre-schools and over 600 millions of school children live in areas where parasites are intensively transmitted [5,6].

Ascaris lumbricoides is the most frequent helminth, followed by *Trichuris trichiura* and hookworm, respectively infecting 819 464,600 and 438,900 millions of people globally [4,7]. *G. duodenalis* and the pathogenic *Entamoeba* spp are the two most prevalent intestinal protozoa, the first affecting 200 and the last 500 millions people [4,8]. Other intestinal parasites such as *Cryptosporidium* sp., *Cystoisospora belli*, and *Strongyloides stercoralis* are commonly found in immunocompromised hosts [4,8]. Symptoms and treatment of the intestinal parasitic infections previously cited have been described elsewhere [9-17].

Intestinal parasites transmission occurs trough fecal contamination of soil, water, food and person to person contamination [1], being common in countries with inadequate water supplies, poor sanitation, insecure hygiene conditions and people living in precarious conditions in crowded scenarios.

The major diseases affecting Mozambican children under five years of age are in generally attributed to preventable causes and include malaria, acute respiratory infections, diarrhea, measles, anemia, meningitis, tuberculosis, malnutrition, HIV/AIDS as also intestinal parasites infections. This situation is similar to that described for the African Region [18].

In Northern Mozambique, Nampula district is dominated by malaria, diarrhea, sexually transmitted infections and AIDS, which together account for almost all cases of diseases reported in the district [19].

Worldwide, most data on the prevalence of intestinal parasites infections come from studies conducted at the community level with asymptomatic individuals, rather than in the hospital setting. There are very few Mozambican studies published reporting the prevalence of these parasites in this context [20,21]. Furthermore, most hospitals in low- and middle-income countries do not have clinical microbiology laboratories, so the causes of childhood diarrhea still remain unknown in many regions [22, 23], including those caused by intestinal parasites.

The goal of the present study was to assess the frequency of intestinal parasitic infections and the associated risk factors in children under-five attending Central Hospital of Nampula (CHN), in Northern Mozambique.

Methodology

Study design

This study is a cross-sectional observational study, which included 831 children 59 months of age or younger, from the Diarrhea (ED) and Malnutrition Wards (EM), and Malnutrition and HIV Outpatient Clinic (CH) of the Central Hospital of Nampula (CHN). CHN has 529 beds and 8 main wards: Medicine, Surgery, Pediatrics, Gynecology and Obstetrics, Orthopedics, Urgency and Reanimation, Ophthalmology, Physical Medicine and Rehabilitation. This hospital receives patients from all the province and also from the Northern districts of the Zambezia province, located in the south of Nampula. Besides the CHN there are three distrital hospitals in Namula province: the Distrital Hospital of Marrere, Namapa and Nacala. Inclusion criteria were for children of 59 months of age or younger, who had not yet been recruited previously and had obtained informed consent from the caregiver. A stool sample was taken from each participant during the rainy season and the dry season of 2012 (February-April and June-September) and 2013 (January-March and June-August).

Ethical considerations

Only the children whose parents or caregivers signed an informed consent were included in the study. The present study was approved by the Ethics Council of the Institute of Hygiene and Tropical Medicine (IHMT), as well as by the National Bioethics Committee for Health of the Ministry of Health of Mozambique.

Study area

The study was carried out in Nampula, which is the capital city of Nampula Province in Northern Mozambique and the most populous province of the country with an estimated population of 6 102,867, in 2017 according with data from the National Statistical Institute [24].

Sample size and sampling method

In order to estimate the sample size an unknown prevalence of intestinal parasites was assumed. In addition, a variation in the frequency of parasites of 10% between the two seasons was considered, being 50% for the rainy season and 40% for dry season. Sample size was estimated using the Epitools software with a 95% confidence interval and a test power of 80%. This tool provided a minimal sample size of 408 children per season.

A convenience sample was obtained by recruiting children admitted as inpatient or outpatient at the Diarrhea and Malnutrition Wards or at the HIV and Malnutrition pediatric clinic. This non-probabilistic sampling method was performed not only due to the greater operational ease, but also due to the impossibility of access to a complete listing of all the children attending the Hospital.

Sociodemographic and clinical data

Sociodemographic and clinical data were collected through a questionnaire applied to the legal representatives of the children included in the study and completed with the medical information contained in the clinical records.

Parasitological analysis

This procedure was performed at the CHN Laboratory and described below. Every result was communicated to the pediatricians and registered in the clinical processes of each child.

Microscopic examination

Stool specimens were collected to sterile containers provided to parents or caregivers, after prior explanation of the collection procedure, without the use of any external stimulus.

Fresh feces were processed according to standard coprological techniques (direct examination and concentration Ritchie method) for microscopic observation, stained with Lugol solution. Microscopic observation of the fresh stools was also performed directly on the slide. Modified Ziehl-Neelsen staining was used for *Cryptosporidium* sp. and other coccidia detection.

Giardia duodenalis antigen detection

Every stool sample was analyzed by rapid diagnostic tests (RDT) for *G. duodenalis* antigen detection (RIDA®QUICK Giardia, R-Biopharm (Darmstadt, Germany) and CERTEST Giardia from Biotec (Zaragoza, Spain)), according to the manufacturer's instructions.

Table 1. Demographic and socioeconomic characteristics.

	n (%)
Gender (n = 831)	
Male	460 (55.4)
Female	371 (44.6)
Age (months) (n = 831)	
< 12	278 (33.5)
12 - 24	320 (38.5)
24 - 36	128 (15.4)
36 - 48	71 (8.5)
48 - 60	34 (4.1)
Breastfeeding (at data collection) (n = 804)	
No breastfeeding	420 (52.2)
Non-exclusive	361 (44.9)
Exclusive	23 (2.9)
Mother´s education (n = 774)	
No education	168 (21.7)
Primary education	323 (41.7)
Secondary education	279 (36.0)
Higher education	4 (0.5)
Housing (n = 831)	
Natural clay walls and dry grass roof	442 (53.2)
Baked or cemented clay walls and metal cover roof	343 (41.3)
Others	46 (5.5)
Density of people in housing * (n = 828)	
< 3	707 (85.4)
≥ 3	121 (14.6)
Drinking water source (n = 820)	
Tap	507 (61.8)
Well water	129 (15.5)
Public fountain	80 (9.6)
Mineral	59 (7.1)
Other sources	45 (5.5)
Water treatment (n = 760)	
Yes	376 (49.5)
No	384 (50.5)
Sanitation (n = 824)	
Toilet	34 (4.1)
Latrine	704 (85.4)
Open defecation	86 (10.4)
Waste disposal (n = 831)	
Container	392 (47.2)
Open air	439 (52.8)

* defined as number of people per division.

Statistical analysis

Data was analyzed through the Statistical Package for Social Sciences - SPSS v25. For the characterization of the variables under study, counts (absolute frequencies, n) and percentages (relative frequencies, %) were used. Intervals with 95% confidence (95% CI) were calculated for the percentages using the software *EpiTools epidemiological calculators* (http://epitools.ausvet.com.au/content.php?page=CIProportion). To evaluate the associations between variables a multivariate logistic regression analysis was performed for the dependent variables, which are conceptually related with intestinal parasitic infections. With this analysis, the values of p, as well as the Odds Ratio (aOR) values and their respective 95% confidence intervals, were considered adjusted. Adjustment of the models was evaluated by the Hosmer and Lemeshow test.

Results

Demographic and socioeconomic characteristics

Overall, 831 children attending the CHN were enrolled in the study. The majority of the children were hospitalized at the diarrhea ward (n = 335) and at the malnutrition ward (n = 274) and the remaining attended the malnutrition (n = 104) and HIV outpatient clinics (n = 118). The participants were 59 months of age or younger, with a mean age of 20.1 months. Children of 24 months of age or less accounted for 72.0% (598/831) of the study population. Moreover, only a small number of children were exclusively being breastfed at the moment of data collection (2.9%, 23/804). A significant proportion of the mothers were illiterate (21.7%, 168/774). Only a small proportion of the children drink mineral water (7.1%, 59/820) and water treatment was only reported by half of the caregivers (49.5%, 376/760). Access to toilet was limited to a reduced number of children (4.1%, 34/824). Other demographic and socioeconomic characteristics of the participants are described in Table 1.

Intestinal parasites infection

Of the enrolled children, 263/831 (31.6%) were infected with at least one intestinal parasite. Simple infections were found in 27.7% (230/831) of the participants and protozoans were the predominant pathogenic intestinal parasites detected (26.5%, 220/831) (Table 2). The patients have experienced vomit (23.7%), fever (34.9%), abdominal pain (43.9%), lack of appetite (50.8%) and cough (52.1%).

G. duodenalis was identified in 23.9% (199/831) stool samples, followed by *S. stercoralis* in 4.1% (34/831) and *Cryptosporidium* sp. in 3.4% (28/831). One third of the children infected with *Cryptosporidium* sp. were also infected with HIV and more than 75% were malnourished (data not shown). Even considering microscopy alone, *G. duodenalis* counts to 13.1% of the intestinal infections found (109/831). Intestinal parasites with lower frequencies are mentioned in Table 3.

Statistical associations

Table 4 shows a significant association between children age and global intestinal parasitic infection (p=0.005; aOR = 1.025; 95% CI: 1.008-1.042), demonstrating an increase of 2.5% chance of infection with any intestinal parasite for each month added to age. No other significant association was verified for the studied variables.

Discussion

Intestinal parasites are known to be an important public health problem and one of the leading causes of death among children in low- and middle-income countries [1,2]. Besides, individuals living in endemic areas are repeatedly infected from early age [1,25]. This type of infections may also be used as socioeconomic development indicators. Nevertheless, current data on intestinal infections concerning hospitalized children anywhere is still limited [6,26].

Overall, around one third (31.5%) of the children tested were infected with intestinal parasites. Infection with intestinal protozoa was more common than helminthic infections and *G. duodenalis*, was by far the predominant parasite (23.9%), followed by *S. stercoralis* (4.1%), and *Cryptosporidium* sp. (3.4%).

Table 3. Frequency of identified pathogenic intestinal parasites (n = 831): relative frequencies (%); absolute frequencies (n).

Intestinal parasites	**n** (%)
*Giardia duodenalis**	199 (23.9)
Giardia duodenalis	109 (13.1)
Strongyloides stercoralis	34 (4.1)
Cryptosporidium sp.	28 (3.4)
Ascaris lumbricoides	13 (1.6)
Ancylostomatidae	10 (1.2)
Trichuris trichiura	5 (0.6)
Entamoeba histolytica/dispar	3 (0.4)
Hymenolepis diminuta	2 (0.2)
Cystoisospora belli	1 (0.1)
Hymenolepis nana (syn. *Rodentolepis nana*)	1 (0.1)

* Results obtained through RDT.

Table 2. Type of infection and type of pathogenic intestinal parasites diagnosed (N = 831): relative frequencies (%); absolute frequencies (n).

	n (%)
Infected children	263 (31.6)
Simple infection	230 (27.7)
Helminths	39 (4.7)
Protozoans*	192 (23.1)
Multiple infection	32 (3.9)
Helminths	4 (0.5)
Protozoans*	11 (1.3)
Helminths and Protozoans*	17 (2.0)
Non infected children	568 (68.4)

* All positive results for *G. duodenalis* obtained through RDT were included.

The prevalence in this study is higher than reported in other studies conducted in Africa (17.3% in Ethiopia, 15.1% in Tanzania, 17.7% in Saudi Arabia and 25.6% in Kenya) [6,27] and this outcome might be explained by the use of RDT for *Giardia* diagnosis. The use of RDT is a great advantage as it allows the detection of *G. duodenalis* antigen rather than in fresh stool samples and in those not properly preserved [28], which was the case for most of the analyzed samples. In Mozambique a study performed at the Central Hospital in Maputo reported a 16.1% prevalence of intestinal parasitic infections [21] and another study performed in a rural setting identified a prevalence of 16.6% [20]. In both studies, *G. duodenalis* was the predominant parasite and this finding is also supported by other studies [6,27,29-32]. In fact, this parasite may persist in water, even after conventional water treatment procedures, contributing to higher prevalence's, namely in children [33-35]. In the present study, no significant association was found between water treatment and protozoan infection, which could be mainly due to *G. duodenalis* resistance to treatment, as previously explained.

Several socioeconomic risk factors have been related to intestinal infections. In the current study, we were able to identify age as a risk factor for global intestinal parasitic infection. It is widely known that older children are more exposed to greater transmission risk due to the inadequate personal hygiene habits and greater contact with the soil than younger children [26,36,37,40]. It is believed that children harbour higher levels of intestinal pathogens and eggs of helminths, thereby exposure to their feces poses a greater risk to health [38,39]. Precarious child feces disposal is not only common in households without improved sanitation facilities but also in those with improved sanitation [38,40].

Table 4. Multiple logistic regression analysis for sociodemographic factors and intestinal parasitic infection.

Global intestinal parasitic infection (n = 263)	**Positive cases (%)**	***p*-value**	**aOR**	**95% CI**
Age (months)	n.a.	**0.005**	1.025	1.008 – 1.042
Gender				
Male	32.4	ref		
Female	30.7	0.806	1.042	0.750 – 1.447
Breastfeeding				
Exclusive	6.9	ref		
No breastfeeding or not exclusive	32.7	0.764	1066	0.704 – 1.615
Mother´s education				
No education	33.9	0.766	0.933	0.591 – 1.473
Primary	31.0	0.656	0.917	0.628 – 1.340
Secondary or higher	31.1	ref		
Density of people in housing				
< 3	30.4	ref		
≥ 3	39.7	0.206	1.345	0.850 – 2.128
Water treatment				
Yes	31.6	ref		
No	33.9	0.458	1.136	0.811 – 1.591
Open defecation				
Yes	40.7	ref		
No	30.8	0.432	1.230	0.734 – 2.063
Waste disposal				
Container	30.4	ref		
Open air	32.8	0.503	1.123	0.799 – 1.578
Protozoan infection (n = 203)	**Positive cases (%)**	***p*-value**	**aOR**	**95% CI**
Age (months)		0.147	1.014	0.995 – 1.032
Gender				
Male	24.6	ref		
Female	24.3	0.519	1.121	0.792 – 1.588
Breastfeeding				
Exclusive	6.9	ref		
No breastfeeding or not exclusive	25.2	0.541	0.871	0.559 – 1.357
Mother´s education				
No education	20.8	0.300	0.774	0.477 – 1.257
Primary	23.2	0.225	0.781	0.524 – 1.164
Secondary or higher	27.2	ref		
Density of people in housing				
< 3	23.5	ref		
≥ 3	30.6	0.202	1.368	0.846 – 2.214
Water treatment				
Yes	25.8	ref		
No	24.2	0.916	1.019	0.713 – 1.458
Open defecation				
Yes	25.6	ref		
No	24.5	0.849	1.056	0.603 – 1.849
Waste disposal				
Container	23.7	ref		
Open air	25.1	0.858	1.033	0.721-1.482
Helminthic infection (n=43)	**Positive cases (%)**	***p*-value**	**aOR**	**95% CI**
Age (months)		0.069	1.029	0.998 – 1.062
Gender				
Male	5.2	ref		
Female	5.1	0.992	1.004	0.492 – 2.046
Breastfeeding				
Exclusive	0.0	ref		
No breastfeeding or not exclusive	5.4	0.052	2.694	0.994 – 7.304

Table 4 (continued). Multiple logistic regression analysis for sociodemographic factors and intestinal parasitic infection.

Mother´s education				
No education	9.5	0.195	1,878	0.725 – 4.866
Primary	4.6	0.705	1.188	0.487 – 2.895
Secondary or higher	3.2	ref		
Density of people in housing				
< 3	5.2	ref		
≥ 3	5.0	0.752	0.851	0.312 – 2.317
Water treatment				
Yes	3.2	0.065	2.046	0.956 – 4.379
No	7.8	ref		
Open defecation				
Yes	14.0	0.157	1.925	0.777 – 4.767
No	4.1	ref		
Waste disposal				
Container	4.3	ref		
Open air	5.9	0.481	1.304	0.623 – 2.728

Every time child feces management is inadequate environmental contamination occurs affecting individuals living in the same household and neighborhood [40].

Other risk factors such as breastfeeding, mother's education, open defecation and waste disposal have also been cited in several works [38-40]. However, the present study did not show an association between those variables and children with intestinal parasitic infection, which may be due to several factors such as the studied population (children, adults, community or hospital-based studies, immunological status…) and the sensitivity of the diagnostic techniques applied.

The presence of the opportunistic *Cryptosporidium* sp. as the third most common parasite found in the studied children may be due to the high prevalence of HIV infected children as well as of undernourished children, which are more vulnerable to this type of infections.

One of the limitations of this study is that only a single stool sample was collected per children and therefore the frequency of infection may be underestimated. It is well known that, ideally, three samples per children should be collected, which would probably increase the sensitivity of the microscopic diagnosis, namely concerning the detection of the intestinal protozoan *G. duodenalis* from 60% to over 90% [41]. This is highly recommended because this intestinal protozoan presents an intermittent and low shedding [41-44]. Other limitation was related with the concentration technique, which should be complemented with the Kato-Katz technique, not only regarding an increase in sensitivity, but also to determine the helminthic infection burden.

Despite these limitations and to our knowledge, this is the first published work regarding intestinal parasitic infections in Northern Mozambique. Our findings revealed a high prevalence of intestinal parasites in children under five attending a Central Hospital and highlight the importance of *G. duodenalis* infection. Since microscopy is still the standard diagnostic procedure for protozoa identification there is a need of trained skilled technicians in order to perform an adequate diagnosis [45]. On the other hand, if the stools specimens are diarrheic microscopy may fail to detect *Giardia* trophozoites whenever the samples are not promptly observed. As this form disintegrates rapidly in the external environment the sample should be observed while still fresh, which was not possible for the great majority of the samples [28].

Conclusion

Although the findings of the present study were limited to a hospital, they may represent the population of Nampula, since the great majority of the participants were from all over this province and district (98.6%, 819/831 and 90.9%, 755/831, respectively, data not shown). Therefore, in order to address this issue and improve morbidity and mortality associated with it, there is an urgent need to improve not only its diagnosis, but also environment health, water and sanitation conditions, along with integrating behavior change and public policies focused in hygiene.

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