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Intestinal parasitic infections among school children in northern Madagascar

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A – Research concept and design, B – Collection and/or assembly of data, C – Data analysis and interpretation,

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Abstract

Introduction and Objective. According to the World Health Organization (WHO) more than 95% of all parasitic infections reported globally result from poverty, poor personal hygiene practices, open defecation practices, consumption of contaminated drinking water, and improper handling of food. The rates of parasitic infections are particularly high in the paediatric population. One of the low-income African countries, where 75% of residents live below the poverty level, is the island of Madagascar. The aim of the study was to assess the prevalence of intestinal parasitic infections among school children living in northern Madagascar.

Materials and Method. The screening was conducted in October 2023 and involved a group of 241 school children aged 5–15 years, inhabiting the Mampikony district of Madagascar. Single stool samples were collected from study participants, the samples were fixed in SAF solution, transported from Africa to Europe, and analyzed by light microscopy using 3 different diagnostic methods (direct smear, decantation with distilled water, Fülleborn method) at the Department of Epidemiology and Tropical Medicine, Military Institute of Medicine – National Research Institute in Poland.

Results. The overall prevalence of intestinal parasites in the study group was found to be 71.0%. Pathogenic intestinal parasites were detected in 65 patients (27.0%), with Giardia intestinalis being the most prevalent (21.2%). Most infections were caused by potentially pathogenic stramenopila Blastocystis spp. (48.6%).

Conclusions. The prevalence of intestinal parasitic infections is high in the community of school children in northern Madagascar. There is an urgent need to implement long-term prevention measures which would effectively limit the spread of new cases, and reduce the prevalence rates of intestinal infections in the local community.

Key words

intestinal parasites, school children, Madagascar

INTRODUCTION

Intestinal parasitic infections (IPIs), such as soil-transmitted helminths (STHs), intestinal protozoa and stramenopila, have been described as the major worldwide causes of illnesses and diseases in tropical and sub-tropical regions. According to the World Health Organization (WHO) more than 95% of all parasitic infections reported globally result from poverty, poor personal hygiene practices, open defecation practices, consumption of contaminated drinking water, improper handling of food, and cultural practices [1]. Global epidemiological data demonstrate that the rates of IPIs are particularly high in the paediatric populations, and that IPIs often result in malnutrition, anaemia and growth retardation, and may also lead to a variety of other health problems [2-4]. Compared to adults, children are more susceptible to an infection with intestinal parasites because they have a higher exposure to pathogens (due to the lack of hygiene during meals, and also when urinating and defecating) [5]. Countries in Sub-Saharan Africa have one of the highest

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rates of intestinal parasitic infections [6, 7]. However, the distribution of IPIs varies across countries depending on environmental and socio-economic conditions, as well as geographic location.

Madagascar is a Sub-Saharan African island located in the Indian Ocean, off the south-eastern coast of Africa, and classified by the United Nations (UN) as a low-income country [8]. The leading causes of death in Madagascar include perinatal disorders, malnutrition and infectious illnesses, including parasitic infections [9]. It is estimated that 75% of the country's population live in extreme poverty (i.e. below the poverty level); 35% do not have access to toilets, and 52% have limited access to safe drinking water. The country's economy is dominated by traditional agriculture, which uses faeces or manure as fertilizers; the use of such fertilizers is the major source of IPIs in Madagascar [10]. There are few publications available in the world's literature on the prevalence of IPIs in Malagasy children, and no publications on the prevalence of such infections in Mampikony, one of 114 districts in Madagascar.

Annals of Agricultural and Environmental Medicine 2024, Vol 31, No 4

Wanesa Richert, Daniel Kasprowicz, Daria Kołodziej, Danuta Zarudzka, Krzysztof Korzeniewski. Intestinal parasitic infections among school children in northern Madagascar

OBJECTIVES

The aim of the study is to assess the prevalence of intestinal parasitic infections among school children living in the Mampikony district in northern Madagascar, and to compare it with the prevalence of intestinal infections reported in other parts of the country.

MATERIAL AND METHODS

Study design and settings. The screening for the prevalence of intestinal parasites was carried out during three weeks in October 2023 in two different schools in Mampikony (urban municipality with the population 29,000 inhabitants, located 84 km from Ambondromamy on the Route Nationale 6, at the elevation of 55 meters above sea level; coordinates: 16°5'30"S 47°38'50"E) in Sofia Region in northern Madagascar (Fig. 1).



Figure 1. Map of Madagascar with location of Mampikony

Study participants. The study involved a group of 241 children aged between 5–15 years old attending private (n=130) and public schools (n=111). Each school child could participate in the screening, regardless of health condition. Information for the patient and informed consent form were translated into the Malgasy language. The mean age of the study subjects was 8.7 years. Females accounted for 51.5% of the study group (n=124), while males represented 48.5% of the sample (n=117).

Data collection. The biological material for coprological diagnostics consisted of single stool samples provided by the children. The samples were delivered to the healthcare

facility (Clinique Medicale Beyzym in Manerinerina, Ambatoboeny district) on the day of collection. Next, the material was fixed in SAF fixative (sodium acetate-acetic acid-formalin), and two weeks later transported to the Department of Epidemiology and Tropical Medicine of the Military Institute of Medicine – National Research Institute in Poland, where laboratory examination by light microscopy was performed using three different diagnostic methods (direct smear in Lugol's solution, decantation with distilled water, the Fülleborn method) [11]. In total, 723 parasitological tests were performed. Microscopic examination allowed for determining the number of parasitic infections in the study group and their percentage distribution.

Study variable The correlation between intestinal parasite infections, gender and age was examined. As STHs are one of the important causes of anaemia in children [12], it was therefore assessed whether the occurrence of IPIs was associated with a low haemoglobin (Hb) level in the study group. Hb concentration was measured in the field from a finger-prick blood sample with a portable DiaSpect TM analyzer (EKF Diagnostics, Cardiff, UK), following standard procedures.

Statistical analysis. All statistical calculations were performed using StatSoft Inc. (2014) STATISTICA version 12.0 (www.statsoft.com) and an Excel spreadsheet. Quantitative variables were characterized by the arithmetic mean. The variables of the qualitative type were presented in terms of counts and percentages. Chi-squared tests for independence were employed to analyze qualitative variables. In all calculations, the level of statistical significance was set at p=0.05.

Ethical approval. The protocol of the study was approved by the Malgasy Ministry of Public Health in Antananarivo (Ref. No. 256–23/MSANP, dated 3 July 2023). Parental consent was obtained for each child to participate in the study. The collection of samples was supervised by the medical personnel employed at the Clinique Medicale Beyzym from Manerinerina (Ambatoboeny district, Boeny Region, northern Madagascar). The study was conducted in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki).

RESULTS

Microscopic examination of the samples obtained from the 241 schoolchildren revealed that 171 (71.0% of the study group) were infected with intestinal parasites. The total number of infections was 300. Potentially pathogenic stramenopila *Blastocystis* spp. were responsible for most infections (48.6%). Coprological examination of the stool specimens revealed 10 different species of intestinal parasites, with non-pathogenic protozoa being predominant (49.0%). A significant number of infections was caused by *Giardia intestinalis* protozoa (21.2%). There was a relatively low number of helminthic infections (5.8%) among study participants; invasion with *Ascaris lumbricoides* (2.9%) was responsible for the majority of helminthiases (Tab. 1; Fig. 2).

A total of 90 children involved in the study were found to be infected with more than one parasite (polyparasitism); the Wanesa Richert, Daniel Kasprowicz, Daria Kołodziej, Danuta Zarudzka, Krzysztof Korzeniewski. Intestinal parasitic infections among school children in northern Madagascar

Intestinal parasites	Number (percentage) of infections	Percentage of infected school children (n=171)	Percentage of tested school children (n=241)
Nematodes	13 (4.3)	7.6	5.4
Ascaris lumbricoides	7 (2.3)	4.1	2.9
Hookworm	3 (1.0)	1.7	1.2
Trichuris trichiura	2 (0.7)	1.2	0.8
Enterobius vermicularis	1 (0.3)	0.6	0.4
Cestodes	1 (0.3)	0.6	0.4
Hymenolepis nana	1 (0.3)	0.58	0.4
Trematodes	0 (0.0)	0.0	0.0
Pathogenic protozoa	51 (17.0)	29.8	21.2
Giardia intestinalis	51 (17.0)	29.8	21.2
Non-pathogenic protozoa	118 (39.3)	69.0	49.0
Entamoeba coli, Endolimax nana	118 (39.3)	69.0	49.0
Potentially pathogenic stramenopila	117 (39.0)	68.4	48.6
Blastocystis spp.	117 (39.0)	68.4	48.6
Total		300 (100.0)	

Table 1. Distribution of intestinal parasites detected in the school children (n=241) in northern Madagascar, 2023

co-infection of *Blastocystis* spp. with protozoa was found to be the most common. The distribution of intestinal parasites detected in school-age children was compared depending on the schools they attended (private vs. public). Statistical analysis demonstrated that infections with potentially pathogenic *Blastocystis* spp. were most often found in public school students (private 43.1% vs. public 54.9%), but the distribution of pathogenic intestinal parasites (helminths + protozoa) was comparable in both schools (private 27.7% vs. public 26.1%) (Tab. 2). The female vs. male ratio was 1.06. The distribution of intestinal parasites detected in school-age children was compared depending on gender (female 75% vs. male 66.7%). Statistical analysis demonstrated that infections with pathogenic intestinal parasites (helminths + protozoa)

Table 2. Monoparasitism and polyparasitism detected in school children (n=241) in northern Madagascar, 2023

Intestinal parasites	Total	Private school	Public school	P-value	Female	Male	P-value
No. of tested school children	241	130	111		124	117	
				0.1357			0.1544
Negative (-)	70 (29.0%)	43 (33.1%)	27 (24.3%)		31 (25.0%)	39 (33.3%)	
Positive (+)	171 (70.9%)	87 (66.9%)	84 (75.7%)		93 (75.0%)	78 (66.7%)	
Helminths	14 (5.8%)	7 (5.4%)	7 (6.3%)	0.7604	5 (4.0%)	9 (7.7%)	0.2248
Ascaris lumbricoides	5	1	4		2	3	
Ascaris lumbricoides + Blastocystis spp. + Entamoeba coli	1	0	1		0	1	
Ascaris lumbricoides + Blastocystis spp. + Endolimax nana	1	1	0		0	1	
Ancylostoma duodenale/Necator americanus	1	1	0		1	0	
Ancylostoma duodenale/Necator americanus + Enterobius vermicularis + Entamoeba coli + Blastocystis spp.	1	1	0		0	1	
Ancylostoma duodenale/Necator americanus + Blastocystis spp.	1	0	1		0	1	
Trichiuris trichiura	2	2	0		1	1	
- Hymenolepis nana + Blastocystis spp.	1	0	1		1	0	
Pathogenic protozoa	51 (21.2%)	29 (22.3%)	22 (19.8%)	0.6374	23 (18.5%)	28 (23.9%)	0.3065
Giardia intestinalis	20	12	8		10	10	
Giardia intestinalis + Blastocystis spp.	8	2	6		5	3	
Giardia intestinalis + Blastocystis spp. + other non-pathogenic protozoa	23	15	8		8	15	
Potentially pathogenic parasites (stramenopila)	117 (48.5%)	56 (43.1%)	61 (54.9%)	0.0659	61 (49.2%)	56 (47.9%)	0.8364
Blastocystis spp.	66	28	38		32	34	
Blastocystis spp. + other (non-pathogenic) protozoa	51	28	23		29	22	
Non-pathogenic protozoa (Endolimax nana, Entamoeba coli, Retortamonas intestinalis)	118 (49.0%)	68 (52.3%)	50 (45.0%)	0.0775	67 (54.0%)	51 (43.6%)	0.1051

Wanesa Richert, Daniel Kasprowicz, Daria Kołodziej, Danuta Zarudzka, Krzysztof Korzeniewski. Intestinal parasitic infections among school children in northern Madagascar

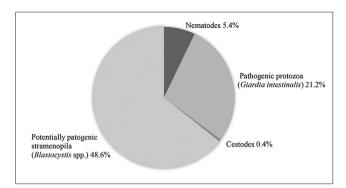


Figure 2. Distribution of intestinal parasites detected in the school children (n=241) in northern Madagascar, 2023

were most often found in the males (31.6% vs. 22.6%), while the distribution of potentially pathogenic *Blastocystis* spp. was comparable in both genders. Helminths were most often found in 10 – 11-year-old children (8%), but not found in any older than 13-years of age. Giardia intestinalis infections were the most common in children aged 10 years, with the prevalence decreasing in older children. There was a positive correlation between age and *Blastocystis* spp. infections. In children over 13 years of age, the incidence of Blastocystis spp. was more than 2.5 times higher than in children under the age of 6 years. There was no correlation between IPIs and low haemoglobin levels. 92.1% of the examined children had a haemoglobin concentration >12 g/dl, and only 2 children were infected with Giardia intestinalis and one infected with Blastocystis spp. who had a hemoglobin concentration of <11 g/dl.

The use of the basic direct smear method resulted in the detection of intestinal parasites in 158 of the children. The application of the other 2 testing methods increased the detection rates by 5% (171 infected children). The number of infections increased from 264 when only the direct smear method was used, to 300 when all 3 diagnostic methods were applied (Tab. 3).

DISCUSSION

High rates of intestinal parasitic infections are primarily associated with the low socio-economic status of a given population, poor social and living conditions, limited access to healthcare, and a lack of health awareness [13]. 1.5 billion people (24% of the world's population) are affected by IPIs, and over 613 million school-age children worldwide are at risk of STH infections. The prevalence of IPIs is also strongly correlated to the geographic location, and therefore differs between countries and regions [14]. Infections are widespread in tropical and subtropical areas, with the highest numbers occurring in sub-Saharan Africa, the Americas, and Asia [15]. According to studies, the prevalence of IPIs in Africa ranges from 6.6% – 86% [4, 16]. The present study, which was conducted in northern Madagascar, found that 71.0% of the participants were infected with intestinal parasites, and that 27% of those infections were caused by pathogenic parasites (5.8% helminthic and 21.2% protozoan infections), and 48.5% by the potentially pathogenic stramenopila. Comparable findings, but among adults, were reported by Greigert et al. [17] who studied a group of patients hospitalized at the department of gastroenterology in Mahajanga (north-west Madagascar); 77% of their study subjects were infected with either pathogenic or non-pathogenic IPIs and 7.9% of the patients were infected with helminths. For comparison, the frequency of IPIs in children living in other continents and countries with low sanitary and epidemiological conditions was 31% in Bolivia [18], 70.9% in Argentina [19], 60.7% in Colombia [20], 38% in Iran [21], 31.7–37.2% in Turkey [22], 37.3% in Thailand [23], 33.9% in India [24] and 82% in Pakistan [25]. In children living in European countries, this rate IPIs was 5.9% [26]. Blastocystis spp. infections were the most common in both the present study and the study by Greigert et al. Only Giardia intestinalis infections were demonstrated to be more common in children from Mampikony than in patients from Mahajanga, with prevalence rates estimated at 21.2% and 7.9%, respectively. Low rates of helminthic infections in children living in northern Madagascar may be attributable to the geographic location of the island and its isolation from the African continent. Similar trends have been observed for HIV prevalence. In fact, the geographic

Table 3. Comparison of 3 coprological methods used for detection of intestinal parasites in school children (n=241) in northern Madagascar, 2023

	Diagnostic methods (number of infections %)					
-	Direct smear in Lugol's solution	Decantation in distilled water	Fulleborne's flotation	Total (3 methods)		
Nematodes	4 (1.7)	5 (2.1)	13 (5.4)	13 (5.4)		
Ascaris lumbricoides	2 (0.8)	3 (1.2)	7 (2.9)	7 (2.9)		
Hookworms	0 (0.0)	0 (0.0)	3 (1.2)	3 (1.2)		
Trichuris trichiura	2 (0.8)	1 (0.4)	2 (0.8)	2 (0.8)		
Enterobius vermicularis	0 (0.0)	0 (0.0)	1 (0.4)	1 (0.4)		
Cestodes	1 (0.4)	1 (0.4)	1 (0.4)	1 (0.4)		
Hymenolepis nana	1 (0.4)	1 (0.4)	1 (0.4)	1 (0.4)		
Trematodes	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)		
Pathogenic protozoa	46 (19.1)	51 (21.2)	0 (0.0)	51 (21.2)		
Giaridia intestinalis	46 (19.1)	51 (21.2)	0 (0.0)	51 (21.2)		
Potentially pathogenic parasites	104 (43.2)	117 (48.6)	0 (0.0)	117 (48.6)		
Blastocystis spp.	104 (43.2)	117 (48.6)	0 (0.0)	117 (48.6)		
Non-pathogenic protozoa	106 (44.0)	118 (49.0)	5 (2.1)	118 (49.0)		

isolation of Madagascar is considered to be the major factor responsible for the low prevalence of HIV and AIDS infections on the island (estimated at only 0.2%) [9].

The results of the present study are surprising as they demonstrate that there is a relatively low prevalence of helminthic infections in Madagascar (5.8%) in comparison to other African countries, where the rates of such infections are much higher, e.g. 85% in the Central African Republic [13], 24.3% in Ethiopia [27], 23% in Rwanda [28] and 22.2% in Gabon [29].

A study conducted in western Madagascar between 1996 -1997 showed that the prevalence of infections with cestodes (mostly Hymenolepis nana, Taenia spp.) and trematodes (Schistosoma mansoni) in the study subjects was 23.4% [30]. A decrease in the number of IPIs in Madagascar could also result from a mass de-worming campaign initiated in 2002, and involved the mass administration of mebendazole and albendazole. Sartorius et al. [31] reported a significant reduction in the prevalence of geohelminths (soil-transmitted helminths, STH) in children aged 5 – 14 in Sub-Saharan Africa, from 44% in 2000 to <2% in 2018. The study incorporated data from 3,882 facilities located across Africa which are responsible for implementing programmes for IPIs control and prevention. The data suggest that the prevalence of STH infections in Madagascar remains high (>2%). The findings are supported by the results of the author's own studies, as well as the data generated by Razafiarimanga et al. [32] and Habib et al. [33].

In school children from Mampikony district in Madagascar, *Giardia intestinalis* was the most common intestinal pathogen, which is similar to children under 10 years old living on the west coast of Madagascar (21.2% and 19.2 %, respectively) [20]. Both studies demonstrated a high prevalence of non-pathogenic protozoan infections, such as *Entamoeba coli* infection (49.0% and 32.7%, respectively). Although the parasite is not pathogenic, it reflects the standards of sanitation and personal hygiene in the affected communities. In the schoolchildren from Mampikony disrict, the predominant infection was from the *Blastocystis* spp. Other studies also support a high prevalence of infections with this parasite across different parts of Madagascar.

The pathogenicity of *Blastocystis* spp. is controversial. The parasite often occurs in co-infections with other species of intestinal parasites, which makes it difficult to prove beyond doubt that it is pathogenic. However, because the presence of *Blastocystis* spp. infections in a given area reflects poor standards of sanitation and personal hygiene, it is a useful tool for the assessment of the epidemiological situation in a specific region or a community. *Giardia intestinalis, Entamoeba coli*, and *Blastocystis* spp. are good markers of faecal contamination of soil and water, and their presence indicates poor sanitation practices among local people, and the regular consumption of contaminated food and water [17, 32, 33].

The present study found that polyparasitism was predominant (52.6%) among the study participants, which is in line with other parasitological studies conducted among the Malagasy population [17, 30, 32, 33]. They concluded that the major risk factors responsible for the high prevalence of polyparasitism include the use or consumption of contaminated water, the presence of family members infected with IPIs, not washing fruit and vegetables before consumption, not having a toilet at home, walking barefoot outdoors, and not washing one's hands before eating.

The present study demonstrated an increased prevalence of polyparasitism in children from both private and public schools, which suggests poor standards of sanitation in the Mampikony District. The differences in the prevalence rates and the types of intestinal parasitic species in Madagascar between various studies can be explained by the choice of testing methodology. A study conducted in Mahajanga, for example, found infections with Dientamoeba fragilis, Chilomastox mesnili, and Enteromonas hominis protozoa, whereas no such infections were detected in the present study. It should, however, be pointed out that Greigert et al. [17] used cultivation techniques and molecular biology methods (PCR tests) for the identification of intestinal parasites. In contrast, in the present study, light microscopy methods were used, which are the gold standard for parasitological diagnosis as they allow the diagnostician to identify the developmental stage of a detected intestinal parasite (cyst, oocyst, trophozoite, larva, egg) [34]. The precision of the diagnosis will rely on the proficiency and experience of the technician performing the microscopic test, as well as the scope of the diagnostic methods used. The use of multiple testing methods increases the chances of detecting a parasite, which was supported by a study by Korzeniewski et al. [13]. The present study demonstrated that the application of 3 different diagnostic methods instead of only the direct smear method, increased the parasite detection rate by 5%. The use of the Fülleborn method (flotation), used for the identification of helminths, made the detection of hookworms and pinworms possible; neither the direct smear test nor the decantation test revealed the presence of these worms.

Limitations of the study. One limitation was that only one stool sample from each schoolchild was analyzed (the sensitivity of microscopy technique is increased by analyzing multiple stool samples for parasite detection). Another limitation was the 2-week interval between collecting biological material from the children and the examination of each stool by experienced staff in the parasitological laboratory.

CONCLUSIONS

The prevalence of intestinal parasitic infections is high in the community of school children in northern Madagascar. Limited access to uncontaminated drinking water and safe food, low economic status and poor sanitation contribute to the rapid spread of parasitic diseases in the Malagasy people. Therefore, it is recommended to implement effective control measures, introduce regular chemoprophylaxis (mass-scale deworming of the entire population), and promote health awareness and the importance of personal hygiene practices in the Malagasy community. Such measures are an effective tool to reduce the prevalence rates of intestinal infections and limit the spread of new cases among local residents.

Conflict of interest. The authors declare that they no financial or personal connections with other persons or organizations that might negatively affect the contents of this publication, and/or claim to author's rights to this publication.

Annals of Agricultural and Environmental Medicine 2024, Vol 31, No 4

Wanesa Richert, Daniel Kasprowicz, Daria Kołodziej, Danuta Zarudzka, Krzysztof Korzeniewski. Intestinal parasitic infections among school children in northern Madagascar

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