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Research Article

Prevalence of *Schistosoma haematobium* and Soil-Transmitted Helminths Infections among School-Aged Children in Quelimane and Gurue Districts, Central Mozambique

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ABSTRACT

Schistosomiasis and soil-transmitted helminths are endemic in Mozambique, affecting mostly children and young people from suburban and rural areas where socioeconomic and sanitary conditions are deficient. The study aimed to determine the prevalence of *Schistosoma haematobium* and soil-transmitted helminths infections among school-aged children, through parasitological and molecular methods. Urine and stool samples were collected from 350 and 234 children respectively. The investigation of *Schistosoma haematobium* eggs was performed by the urine filtration technique, and PCR was used to detect the parasite DNA in the samples. The detection of soil-transmitted helminths in stools was performed using the Kato-Katz method. The data were analyzed using the Statistical Package for Social Sciences (SPSS), version 24. The global prevalence of *Schistosoma haematobium* by filtration method was 38.4% and 73.4% (68.1-78.1 95% CI) by PCR technique. For soil-transmitted helminths the overall prevalence was 32.1% for *Ascaris lumbricoides*, 35.5% for *Trichuris trichiura* and 5.1% for Hookworms. The study indicates that *Schistosoma haematobium* and soil-transmitted helminths continue to have a significant prevalence in these districts, requiring additional control measures, particularly the strategic use of anthelmintics, improvement of sanitary conditions and health education.

Keywords: *Schistosoma haematobium*; Hygiene; Public health; Sanitation; Parasites

INTRODUCTION

Schistosomiasis is a neglected tropical disease, caused by worms of the genus *Schistosoma*, with major public health impacts in tropical regions of Africa, Asia and Latin America [1]. Globally, five species of importance in human health are known, namely *Schistosoma haematobium*, *Schistosoma mansoni*, *Schistosoma intercalatum*, *Schistosoma mekongy* and *Schistosoma japonicum* [2]. The disease is estimated to affect 260 million people worldwide, of which about 90% of cases occur in sub-Saharan Africa [3]

Through the implementation of effective control programs based on large-scale chemotherapy, sanitary measures and mollusc control, the prevalence of schistosomiasis has been considerably reduced in endemic areas of Latin America and Asia. However, this has not been the situation in sub-Saharan Africa where prevalence and morbidity remain high [4].

On the other hand, soil-transmitted helminths are among the leading causes of global health problems especially among the poorest communities where implementation of control measures is weak [5]. Globally, over one billion people are infected by at least one of the commonest species namely: *Ascaris lumbricoides* (the roundworm), *Trichuris trichiura* (the whipworm) *Strongyloides stercoralis* (threadworm) and the hookworms; *Ancylostoma duodenale*

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and *Necator americanus* [6]. Environmental survival of STH eggs and larvae including hatching and embryonation are determined by warm temperatures and adequate moisture. Human infection is influenced by poverty, poor personal hygiene, inadequate sanitation and overcrowding [7].

Mozambique is an endemic country for schistosomiasis and intestinal parasites, and they mainly affect children in suburban and rural areas, where numerous challenges related to environmental sanitation and drinking water supply persist. Data from epidemiological survey carried out in 2009 revealed that these parasites were more prevalent in central and northern provinces of the country, specifically in Nampula, Niassa, Zambezia and Cabo Delgado, with a prevalence that reached 77.7%, compared to the provinces of the South, with a record of maximum prevalence of 34.2% [8].

Thus, actions were recommended to be taken, with greater focus on annual mass treatment campaigns with Praziquantel and Albendazole for all school-aged children and education campaigns, the objective of changing the risk behaviors. This study aimed to evaluate the prevalence of *Schistosoma haematobium* and soil-transmitted helminths infections after the interventions started in 2009 in Quelimane and Gurue, central Mozambique.

MATERIALS AND METHODS

Study area and population

This cross-sectional study was carried out between February and September 2016, in districts of Quelimane and Gurue, Zambezia province in Mozambique. The selection criteria of these districts were based on the high prevalence of infection by soil-transmitted helminth and *Schistosoma haematobium* from the findings of the epidemiological survey carried out in 2009. In each district, two schools were randomly selected; Manhaua Expansao and Icidua primary schools in Quelimane and in Gurue were selected Projecto and Moneia schools. A total of 357 school children aged between 5 and 15 years, were involved in the study. The determination of this sample size was based on the prevalence reported in the study developed by Augusto (2009) (Figure 1).

Collection of urine and stool samples

After sensitizing the children and parents, appropriate containers were provided for the collection of urine and stool samples, and the urine was collected between 10 am and 2 pm, which corresponds to the period of maximum elimination. The same children were informed about the collection procedures, highlighting the performance of an exercise, with the aim of facilitating the elimination of *Schistosoma haematobium* eggs. The stool sample was collected on the following day and, similarly, they were placed in an isothermal box with ice accumulators and then immediately sent to the Parasitology laboratory of General Hospital of Quelimane and the Rural Hospital of Gurue for further analysis.

Sample processing

The urine and stool samples were processed and examined using the filtration and Kato-Katz technique, for detection of *Schistosoma haematobium* eggs and Soil-transmitted helminths, respectively [9]. After applying the filtration technique, the urinary sediment was collected into eppendorfs containing 70% ethanol and the same was kept in boxes containing dry ice, and later the samples were sent to the Molecular Biology laboratory of Hygiene and Tropical Medicine Institute at Nova University of Lisbon, in Portugal, where the extraction and detection of *Schistosoma haematobium* DNA by molecular methods were carried out.

Extraction of DNA in urine samples

DNA extraction in urine was done based on protocol proposed by Stothard with some modifications [10]. Briefly 150 µl of urinary sediment were centrifuged for 5 minutes at 4°C, and the pellet was dissolved by the addition of 600 µl of CTAB and 8 µl of proteinase K and incubated for 90 minutes at 55°C. Then 750 µl of isoamyl chloroform was added, followed by centrifugation steps, removal of aqueous layer to new tubes, and addition of 1000 µl and 500 µl of pure ethanol and 70% respectively to wash the pellet. After evaporation of the ethanol 50 µl TE was added to dissolve the pellet. The extracted DNA was analyzed by electrophoresis in 1% agarose gel and later visualized in the transilluminator (Aphalmager®HP, Alpha Innotech). Quantification and purity evaluation of the extracted DNA was done in Nanodrop and the samples stored at -20°C until use.

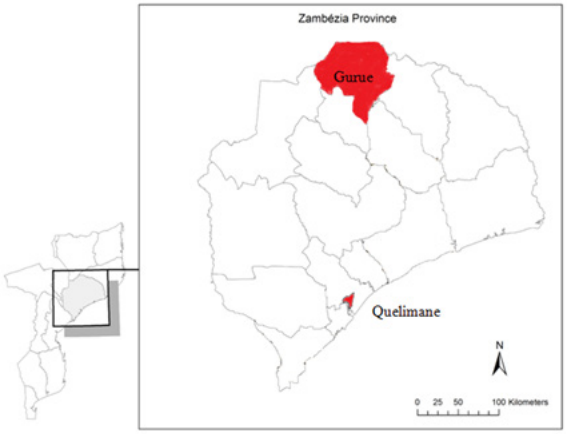

Figure 1: Map of the study area, showing Quelimane and Gurue districts, in Zambezia Province.

PCR reaction

The PCR reaction was performed using specific primers (forward: 5' GATCTCACCTATCAGACGAAAC-3' and reverse: 5' TCACAACGATACGACCAAC–3'), developed by Hamburger, intended for specific amplification of 121 bp of *S. haematobium* [11]. Samples were prepared to a volume of 25 µl each, consisting of 0.5 µl of Taq polymerase, 10 µl of buffer, 4.5 µl of MgCl2, 3 µl of dNTP, 0.5 of each of the primers, 1 µl of DNA and made up to volume with 5 µl of double-distilled water. A total of 30 urine samples, representative of light (1-10 eggs/10 ml urine), moderate (11-49 eggs/10 ml urine) and heavy (≥ 50 eggs/10 ml urine) parasite load, were used as a positive control, with double distilled water used as a negative control. The amplification of the fragments was performed in a thermocycler (AVISO®GmbH), according to the following protocol: denaturation at 95°C for 10 minutes, followed by 35 cycles of 95°C for 30 seconds, annealing (pairing) at a temperature of 53°C for 90 seconds, followed by the step of stretching at 72°C for 60 seconds and the final extension at 60°C in a period of 5 minutes. Finally, the amplification products were analysed on a 1.5% agarose gel, stained with ethidium bromide and visualized with ultraviolet light on a transilluminator.

Data analysis

The data were analysed using the Statistical Package for Social Sciences (SPSS), version 24 for windows. The chi-square test ($\chi 2$) was applied to assess the frequency and differences of parasitism between groups of children. Univariate logistic regression analysis was employed to assess social and behavioural factors associated with *S. haematobium* infection and Soil-transmitted helminths.

Ethical approval

The study was approved by the National Bioethics Committee for Health (Nº 101/CNBS/2015 and Ref: 05/CNBS/2016). It also received authorization from the Office of the Minister of Health of Mozambique (note 321/GMS/002/2016). Participants were previously informed about the importance of schistosomiasis and intestinal parasites and informed consent was obtained from the children's parents and guardians. Individuals who tested positive were sought from the nearest health centres and treated with Praziquantel or Albendazole [12,13].

RESULTS

Population and their housing conditions

From the universe of 357 children involved in the study, 187 (52.4%) were from Quelimane district and the remaining 170 (47.6%) were from Gurue district. Most of them, 216 (60.5%) were male and 141 (39.5%) were female (P=0.010), ranging in age from five to 15 years. The group older than 10 years was significantly larger (P≈0.000) than those under age. Most of the children attended high school and were living in a peri-urban setting. As for sanitation conditions, the absence of piped water in the dwelling were mentioned by 246 children, while 102 had no toilets in the residence. In all parameters there were statistically significant differences (P<0.01).

Prevalence of *Schistosoma haematobium* by urine filtration technique

From the universe of 357 children involved in the study, 350 children provided urine samples and, after laboratory analysis, *S. haematobium* eggs were found in 134, giving an overall prevalence of infection equal to 38.4%, with the confidence interval (95% CI) ranging from 33.3%-43.5%. Statistically significant differences (χ^2, P=0.002) were observed at the district level, with the highest prevalence of infection seen in Gurue with 47.1% (80/170), compared to Quelimane which had a prevalence of 30% (54/180), as shown in Table 1.

Regarding gender, although the prevalence was higher in males (41.6%, 89/214) compared to females (33.1%, 45/136), no statistically significant differences were observed (P>0.05). With regard to age group, children aged (>10 years) were the most affected with an equivalent prevalence of 39.3% (100/254) compared to younger children (≤ 10 years) where a prevalence of 35.4% (34/96) was observed with no significant differences (P=0.550).

Prevalence of S. haematobium by PCR technique

Of the 216 negative samples by the urine filtration technique, 159 were analysed by the PCR technique, and it was not possible to analyse the remaining 57 that had spillage during transport. Of these, 81 (50.9%) samples were positive for *S. haematobium*, and the other 78 (49.1%) were confirmed to be negative. The positive samples showed bands with a molecular weight of 121 bp, which correspond to *S. haematobium*, and which are coincident with the 30 urine samples used as positive control and representative of light parasite load (1-10 eggs/10 ml of urine), moderate (11-49 eggs/10 ml of urine) and heavy (≥ 50 eggs/10 ml of urine), confirming the results obtained by filtration. Thus, it was possible to detect parasite DNA in samples that were considered negative by the filtration method and to discriminate them from those that were truly negative.

Thus, with the application of the two techniques, urine filtration and PCR, it was found that 215 children were parasitized, giving an estimated prevalence of *S. haematobium* in the analysed population of 73.4%, [68.1-78.1 CI 95%;], a value that is considerably higher than that obtained by filtration alone (38.4%), as shown in Table 2.

Prevalence of soil-transmitted helminths

Of the 234 samples analysed for soil-transmitted helminths, it was found that 118 (50.4%) were positive for geohelminth eggs with the following global prevalence of infection: 32.1% (75/234) for *Ascaris lumbricoides* [95% CI 26.4-38.3], 35.5% (83/234) for *Trichuris trichiura* [95% CI 29.6-41.8] and 5.1% (12/234) for hookworms [95% CI 2.9-8.8], no *S. mansoni* eggs were found. Among the districts, Quelimane had the highest prevalence of *A. lumbricoides* 54% (67/124), as well as *T. trichiura* 66.1% (82/124) compared to Gurue with values of 7.3% and 0.9% respectively, revealing statistically significant differences ($\chi 2$, P≈0.000). The presence of cases of hookworm parasitism was only found in Gurue with 10.9% (12/110) (Table 3). With gender, as observed for *S. haematobium*, the infection was more frequent in the male population, with a prevalence of 34.9% and 40.4% observed in *A. lumbricoides* and *T. trichiura*, respectively, when compared to that obtained in females, 27.3% for both parasitic species.

Table 1: Prevalence of *Schistosoma haematobium* in school-aged children in the district of Quelimane and Gurue, using the urine filtration technique.

Variable		No. of tested	Positives	Percentage (95% CI)	P-value
Age	>10	254	100	39.3%	0,55
	≤10	96	34	35.4%	
Gender	Male	214	89	41.6%	0,55
	Female	136	45	33.1%	
District	Quelimane	180	54	30%	0,002
	Gurue	170	80	47.1%	
School	Manhaua	89	29	32.6%	0,002
	Icidua	91	25	27.5%	
	Projeto	95	49	51.6%	
	Moneia	75	31	41.3%	

Table 2: Comparison of the prevalence of *Schistosoma haematobium* infection by the urine filtration technique and PCR.

Method	Samples	Positives	Prevalence	95% CI
Filtration	350	134	38.4	33.3-43.5
Filtration+PCR	293	215	73.4	68.1-78.1

Table 3: Prevalence of soil-transmitted helminths infection in school-aged children in the district of Quelimane and Gurue, Zambezia.

Variable		No. of tested	*A. lumbricoides*			*T. trichiura*			Hookworms		
			Pos	(%)	P*	Pos	(%)	P*	Pos	(%)	P*
District	Quelimane	124	67	54	0.000a	82	66.1	0.000a	0	0	NA
	Gurue	110	8	7.3		1	0.9		12	10.9	
Gender	Male	146	51	34.9	0.224b	59	40.4	0.042a	6	4.1	0.363b
	Female	88	24	27.3		24	27.3		6	6.8	
Age	≤ 10 Years	75	36	48	0.000a	48	64	0.000a	3	25	0.591b
	> 10 Years	159	39	24.5		35	22		9	75	

Note: a: With significant differences; b: No significant differences; NA: Not Applicable; P*: P value, Pos: Number of positive samples.

Regarding the age group, significant differences were observed in terms of the prevalence of infection, with the group aged 10 years or younger being the most parasitized, either by *A. lumbricoides* (48%) or by *T. trichiura* (64%) than those of older age (P≈0.000). As for hookworms, no statistically significant differences were observed in the level of infection between both gender (P=0.363) or between the age groups (P=0.591).

Risk factors associated with infection by *S. haematobium* and soil-transmitted helminths

After applying univariate logistic regression analysis, it was noted that frequent contact with freshwater courses (rivers and lakes) was a potential risk factor for acquiring *S. haematobium*. In fact, the probability of infection associated with swimming or personal hygiene in watercourses made individuals 3 times more prone to infection than those not exposed (OR=3.33; 95% CI 1.76-6.28 and OR=3.67; 95% CI 1.51-8.87) respectively. For soil-transmitted helminths, it was seen that the lack of piped water and basic sanitation represented potential risk factors for infection in the school-aged children.

DISCUSSION

The study examined 350 children from 4 schools in two districts, Quelimane and Gurue, in Zambezia province. The overall prevalence of *S. haematobium* infection of 38.4% (134/350) was observed by the parasitology exam of urine filtration. The district of Gurue had the highest prevalence of infection, 47.1% (80/170), and significantly higher ($\chi 2$, P=0.002) than that detected in Quelimane, 30% (54/180). This difference in the prevalence may be associated with the greater exposure of the child population of Gurue to freshwater courses for various hygiene, domestic and leisure activities (bathing, washing clothes or household items, swimming and fishing), which is less common in Quelimane city. Frequent contact with water sources containing infected snails has been recognized as a key factor in the transmission of schistosomiasis, including *Schistosoma haematobium* [14,15].

The analysis of gender revealed that *S. haematobium* infection was higher in males 41.6% (89/214) compared to females 33.1% (45/136), but without significant differences (P> 0.05). These results are in agreement with the findings of Phillips, in which they

also obtained greater infection by the parasite in male children (68.1%) than in female children (60.1%), in Cabo Delgado, northern Mozambique. Similar observations were reported by other authors in Mozambique and as in other endemic regions, suggesting that this difference may be related to the existence of cultural and behavioural factors, such as swimming, fishing and playing in stagnant waters or with little current, which contributes to longer exposure of boys to outbreaks of transmission [16-19].

In relation to the age group, it was found that children over 10 years of age were the most infected 39.3% (100/254) compared to the youngest (≤ 10 years) with 35.4% (34/96), and the parasite load mostly corresponding to mild infection, but without statistical significance (P=0.550). Concordant results were obtained in a study carried out in Ethiopia, in which the authors found that the prevalence of *S. haematobium* among children increased with age [20]. Augusto also observed similar results, with the prevalence of infection reached its peak in schoolchildren aged between 10 and 14 years old ($\chi 2$=0.08, P=0.96). This could be explained by the greater involvement of older children in domestic and leisure activities which contribute to greater exposure of this group.

The study reported no eggs of *S. mansoni* in the analysed stool samples, both from the district of Quelimane and from Gurue, confirming the low endemicity of *S. mansoni* in Mozambique and in Zambezia province. After applying the PCR technique for the detection of *S. heamatobium* DNA in urine samples that were considered negative by filtration, this was effective, allowing the confirmation of positivity in 81 (50.9%) samples that previously had been considered negative.

Therefore, considering the two methods simultaneously (urine filtration and PCR) it was found that 215 children were parasitized, giving an estimated prevalence of *S. haematobium* in the analysed population of 73.4%, [68.1-78.1 95% CI]. This may reveal that to detect the real prevalence of schistosomiasis infection, it is essential to associate several diagnostic methods. Similar results were found in a study carried out in Angola, in which 142 samples analysed revealed no eggs of *S. haematobium* by the parasitological methods. However, when using the PCR technique, it was possible to detect parasitic DNA in 105 samples, thus revealing the low sensitivity of the filtration method [21, 22].

In addition to detecting the DNA bands corresponding to *S. haematobium* (121 bp), there was also amplification of another larger fragment (200 bp) using the same primers as those used in the work developed by Ibironke [23].

In relation to soil-transmitted helminths, the overall prevalence of infection in this study was 32.1% for *A. lumbricoides*, 35.5% for *T. trichiura*, and 5.1% for hookworms. The district of Quelimane had the highest prevalence of infection with 54% (67/124) for *A. lumbricoides* and 66.1% (82/124) for *T. trichiura*. These differences are significant ($\chi 2$, P≈0.000) in relation to Gurue, in which the prevalence was 7.3% (8/110), 0.9% (1/110) and 10.9% (12/110) for *A. lumbricoides*, *T. trichiura* and hookworms, respectively. The observations results are in agreement with the prevalence of intestinal parasites reported in Zambezia in previous studies. It can be assumed that the high prevalence of these intestinal parasites in Quelimane is directly associated with poor basic sanitation conditions. This hypothesis is supported by the fact that in the neighborhoods of Manhaua and Icídua, where the two schools studied are located in Quelimane city, 54.5% (102/187) of the children surveyed revealed that they did not have a latrine in the house and when asked about the excreta evacuation site, they responded that they used mangrove areas (in Icidua) or rice fields (in Manhaua) for this purpose.

Comparing the prevalence of infection by *S. haematobium* and geohelminths between 2009 and the current study in Zambezia province, using only parasitological methods, it appears that there was an apparent decrease from 60.1% to 38.4% in the case of *S. haematobium*, however, associating the PCR technique, the prevalence obtained was 73.4%, higher than that found in the survey carried out in 2009. Regarding geohelminthiasis, the prevalence remained practically unchanged, with 50.3% in 2009 and 50.4% in the current study. These results indicate that despite the efforts that have been made to reduce these diseases, through annual deworming campaigns with PZQ and ALB in children aged between five and 15 years, their effectiveness is insufficient.

CONCLUSION

In conclusion, the study found that despite the cyclical deworming campaigns with Praziquantel and Albendazole in school children, urogenital schistosomiasis and soil-transmitted helminths continue to have expressive prevalence in the districts of Quelimane and Gurue. It is urgent to strengthen the sanitary surveillance system and improve sanitary conditions. It was also clear that the PCR technique had more sensitive in the detection of *S. haematobium* compared to parasitological methods of urine filtration, and this can confirms that the exclusive application of this method may underestimate the real prevalence of the parasitosis, being PCR useful in the diagnosis of infection in areas of low endemicity and after mass treatment, for the detection of mild infections that still prevail in the population after administration of praziquantel.

CONFLICT OF INTEREST

Authors state no conflict of interest.

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