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THE EFFECT OF HOOKWORM INFECTION AND URINARY SCHISTOSOMIASIS ON BLOOD HEMOGLOBIN CONCENTRATION OF SCHOOLCHILDREN LIVING IN NORTHERN MOZAMBIQUE

Verónica CASMO(1), Gerito AUGUSTO(1), Rassul NALA(1), Acácio SABONETE(1) & Filipe Anibal CARVALHO-COSTA(2)

SUMMARY

This study aims to assess the association between schistosomiasis and hookworm infection with hemoglobin levels of schoolchildren in northern Mozambique. Through a cross-sectional survey, 1,015 children from five to 12 years old in the provinces of Nampula, Cabo Delgado and Niassa were studied. Hookworm infection and urinary schistosomiasis were diagnosed, through Ritchie and filtration methods, with a prevalence of 31.3% and 59.1%, respectively. Hemoglobin levels were obtained with a portable photometer (Hemocue®). The average hemoglobin concentration was 10.8 ± 1.42 g/dL, and 62.1% of the children presented levels below 11.5 g/dL, of which 11.8% of the total number of children had hemoglobin levels below 9 g/dL. A multiple linear regression analysis demonstrated negative interactions between hemoglobin levels and ancylostomiasis, this being restricted to the province of Cabo Delgado ($\beta = -0.55$; $p < 0.001$) where an independent interaction between hemoglobin levels and urinary schistosomiasis was also observed ($\beta = -0.35$; $p = 0.016$). The logistical regression model indicated that hookworm infection represents a predictor of mild (OR = 1.87; 95% CI = 1.17-3.00) and moderate/severe anemia (OR = 2.71; 95% CI = 1.50 - 4.89). We concluded that, in the province of Cabo Delgado, hookworm and *Schistosoma haematobium* infections negatively influence hemoglobin levels in schoolchildren. Periodical deworming should be considered in the region. Health education and improvements in sanitary infrastructure could achieve long-term and sustainable reductions in soil-transmitted helminthiasis and schistosomiasis prevalence rates.

KEYWORDS: Hookworms; *Schistosoma haematobium*; Anemia; Hemoglobin; Mozambique.

INTRODUCTION

Urinary schistosomiasis is a major health concern in sub-Saharan Africa²², while hookworm disease afflicts about 740 million people in the developing world²⁹. Control initiatives for schistosomiasis and soil-transmitted helminth infections have been implemented in many sub-Saharan African countries with technical and financial support, in part, from the Schistosomiasis Control Initiative, a charitable institution⁹.

Helminthic infections, as well as poly-parasitism, are associated with anemia⁷, protein-energy malnutrition¹² and cognitive deficits²⁴. In this context, it has been demonstrated that hookworm infection, due to its pathogenic mechanisms, is the main intestinal parasitosis related to iron deficiency anemia⁴.

Hookworm species infecting humans in the African continent include *Ancylostoma duodenale* and *Necator americanus*, whose eggs are indistinguishable through light microscopy. Adult worms attach themselves to the intestinal mucosa which results in blood loss from the host, which may be significant in high parasitic burdens, and aggravated by iron-deficient diets based on starch intake, other infectious diseases

like malaria and sickle-cell anemia which have a high prevalence in Africa.

Schistosomiasis morbidity is mainly associated with egg obstruction and fibrosis in various organs, depending upon the species of schistosomes. In addition, urinary schistosomiasis, caused by *Schistosoma haematobium* (a species that most often inhabits the venous plexus of the bladder) is a leading cause of hematuria. Blood loss through the urinary tract can be formidable and associated with anemia. Again, high intensity of infection has been an important factor that has contributed to morbidity in children¹⁴. The control of helminthic infections in children can supposedly be achieved by strategically reducing the potential for worm burden and transmission via periodical mass administrations of a single-dose of orally-active anti-helminthic drugs targeting 75-100% of school-aged children²⁸.

In a previous report, we demonstrated that urinary schistosomiasis, hookworm disease and other intestinal parasitic infections are highly prevalent in Mozambican schoolchildren in most provinces of the country¹. Our survey aims to assess hookworm infection and urinary schistosomiasis associated anemia in schoolchildren living in three provinces of northern Mozambique.

(1) Laboratório de Parasitologia Intestinal e Vesical do Instituto Nacional de Saúde de Moçambique, Av. Eduardo Mondlane 1008, Cidade de Maputo, Moçambique.

(2) Laboratório de Epidemiologia e Sistemática Molecular/Laboratório de Virologia Comparada e Ambiental, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Av. Brasil 4365, Pav. Leônidas Deane, Sala 308; 21045-900 Rio de Janeiro, RJ, Brazil.

Correspondence to: Filipe Anibal Carvalho-Costa. E-mail: guaratiba@ioc.fiocruz.br

METHODS

Setting. The study was carried out in the provinces of Nampula, Cabo Delgado and Niassa, situated in northern Mozambique. Nampula has 3,985,285 habitants distributed among 18 districts and five municipalities, Cabo Delgado has 1,650,270 inhabitants distributed among 16 districts and three municipalities and Niassa has a population of 1,027,037 spread among 15 districts and three municipalities. More information about the characteristics of these provinces has been presented in a previous report¹.

Strategy for children recruitment and sampling. This study was carried out in a subgroup of the population analyzed by AUGUSTO *et al.* 2009 from August 2005 to June 2007 by the Schistosomiasis and Soil-Transmitted Helminthiasis Laboratory of the National Institute of Health of the Ministry of Health, Mozambique. In the studied areas, each district or municipality is divided into small areas called Pedagogical Influence Zones (PIZ), each containing an average of eight schools. One school from each PIZ was randomly selected for this study from which one class from each grade was included. The sampling was intended to cover the whole province as well as urban and rural zones including children belonging to families with distinct incomes. The research team remained in each of the districts and municipalities for two days, carrying out hemoglobin measurements, gathering demographic data and collecting feces and urine samples. In Nampula, children living in the districts/municipalities of Ilha de Moçambique, Malema, Meconta, Mecubúri, Mogovolas, Mongicual, Murrupula, Nacala Porto, Nacala-a-Velha and Nacaroa were recruited. In Cabo Delgado, children in this cross-sectional survey attended schools in the districts/municipalities of Macomia, Mecufi, Meluco, Mueda, Muindumbe, Namuno, Nangade, Palma, Chiure, Quissanga, Pemba, Mocimboa da Praia and Montepuez. In the province of Niassa the children lived in the districts/municipalities of Cidade de Lichinga, Cuamba, Distrito de Lichinga, Lago, Mandimba, Marrupa, Maúa, Mecanhelas, Mecula, Meterica, Muembe, Ngauma, Nipepe and Sanga.

Collection and processing of stool and urine samples. The children's parents were encouraged to return the fecal and urine samples in previously provided wide-topped polythene identified bottles to members of the research team. Urine samples were collected between 10:00AM and 2:00PM. Stool and urine examinations were conducted in each district by trained members of the research team on samples from 1,015 schoolchildren aged five to 12 years-old. A single stool specimen was taken from each child, the Ritchie technique was applied in order to detect hookworm eggs. Briefly, gauze-filtered stool suspensions were centrifuged and sediments re-suspended in 5 mL of water, shaken and centrifuged again. Sediments were then re-suspended in 10% formalin and 3 mL of ether was added to the suspensions. Tubes were shaken and centrifuged again and the sediment was examined through light microscopy. Furthermore, one urine sample was obtained from each subject to diagnose *S. haematobium* infection using the filtration method and light microscopy (10 mL of urine was passed through a nylon filter [4 µm pore size] and the eggs counted under light microscopy). *S. haematobium* infection intensity was measured according to the number of eggs per 10 mL of urine. *S. haematobium* worm burdens were classified into light (1-49 eggs/10 mL of urine) or heavy (≥ 50 eggs/10 mL of urine)¹⁸.

Hemoglobin measurements. Blood hemoglobin concentration levels were determined from finger prick blood samples with a Hemocue

Photometer (HemoCue AB, Ängelholm, Sweden). Anemia was defined by values below 11.5 mg/dL³¹. Severe anemia was designated for hemoglobin levels < 9 mg/dL.

Statistical analyses. Multiple linear regression analysis was employed to investigate the association between hemoglobin levels and the presence of hookworm infection or urinary schistosomiasis maintaining age and gender in the model. In addition, the interaction between anemia/severe anemia and the parasitoses was assessed through logistic regression analysis. We present odds ratios (ORs) and 95% confidence intervals (CIs). All analyses were done with the statistical software SPSS version 15.0 (SPSS, Inc., Chicago, IL).

RESULTS

Hookworm disease and urinary schistosomiasis presented prevalence rates of 31.3% and 59.1%, respectively, and the rate of co-infection with both parasites was 12.7%. Prevalence rates according to gender, age group and province are in Table 1. Hookworms infection was significantly lower in Niassa, and urinary schistosomiasis was less frequent in Cabo Delgado. Detection rates of enteric protozoa were as follows: 3.3% *Giardia duodenalis* (n = 34), 2% *Entamoeba histolytica* (n = 20), 1.1% *Iodamoeba bütschlii* (n = 11), 1.3% *Endolimax nana* (n = 13) and 34.9% *Entamoeba coli* (n = 354) (not shown).

Hemoglobin concentration averages were lower in Cabo Delgado and among children from five to six years old. Also, the anemia prevalence rate was significantly higher in Cabo Delgado (Table 1).

As presented in Table 2, a multivariate analysis through multiple linear regression demonstrated independent negative associations between hemoglobin levels and both hookworm ($\beta = -0.55$; $p < 0.001$) and *S. haematobium* ($\beta = -0.35$; $p = 0.016$), after considering age and gender. These findings were only in the province of Cabo Delgado. Additionally, as presented in Tables 3 and 4, the logistic regression model suggests that, in Cabo Delgado, hookworm disease represents a risk for the development of mild (OR = 1.87; 95% CI = 1.17-3.00) and moderate/severe anemia based on hemoglobin level < 9mg/dL (OR = 2.71; 95% CI = 1.50 - 4.89).

DISCUSSION

Results from this study suggest that hookworm and *S. haematobium* infections are independently associated with lower hemoglobin levels among schoolchildren living in the Cabo Delgado province, northern Mozambique. Furthermore, hookworm disease proved to be a predictor of both mild and severe anemia in this province but not in Nampula and Niassa provinces. The main limitation of this study, however, is the fact that almost 25% of the children in the studied provinces do not attend schools. Therefore, prevalence rates and the interaction of hookworm disease and schistosomiasis with hemoglobin levels in this subgroup could not be assessed. As observed in many cross-sectional surveys aiming to evaluate the relationships between helminthic infections with anemia and other nutritional parameters, cumulative effects of previously resolved infections could not be evaluated.

The main finding of this survey is the distinct influence that the studied helminth infections exerted over the hemoglobin levels in

Table 1

Prevalence of hookworm infection, urinary schistosomiasis, anemia and severe anemia and mean blood hemoglobin levels by gender, age group and province in Northern Mozambique

Characteristic	Hookworm infection, n (%)	Urinary schistosomiasis, n (%)	Hookworm + <i>Schistosoma haematobium</i> co-infection	Anemia, n (%)	Moderate/Severe anemia, n (%)	Mean hemoglobin level ± standard deviation
Gender						
Male	145 (29.4)	287 (58.2)	63 (12.8)	236 (47.9)	62 (12.6)	10.88 ± 1.52
Female	173 (33.1)	313 (60)	66 (12.6)	245 (46.9)	58 (11.1)	10.91 ± 1.53
Age groups, years						
5-6	1 (5.9)	10 (58.8)	1 (5.9)	12 (70.6)	5 (29.4)	10.11 ± 1.26
7-8	90 (32.5)	163 (58.8)	52 (18.8)	150 (54.2)	39 (14.1)	10.72 ± 1.54
9-10	140 (33.3)	242 (57.6)	61 (14.5)	182 (43.3)	52 (12.4)	10.93 ± 1.52
11-12	87 (28.9)	185 (61.5)	15 (5)	137 (45.5)	24 (8)	11.05 ± 1.49
Province						
Nampula	110 (33.6)	201 (61.5)	73 (22.3)	147 (45)	41 (12.5)	10.98 ± 1.63
Cabo Delgado	139 (35)	207 (52.1)	56 (14.1)	214 (53.9)	54 (13.6)	10.69 ± 1.47
Niassa	69 (23.7)	192 (66)	0 (0)	120 (41.2)	25 (8.6)	11.07 ± 1.42

Table 2

Multiple linear regression analysis of blood hemoglobin concentration by *Schistosoma haematobium* and hookworm infections, age and sex of schoolchildren in three provinces in northern Mozambique

	β	Standard-error	p-value
Total population			
Urinary schistosomiasis	-0.07	0.097	0.469
Hookworm infection	-0.334	0.102	0.001
Age	0.107	0.03	< 0.001
Sex	0.034	0.095	0.722
Nampula			
Urinary schistosomiasis	0.192	0.185	0.299
Hookworm infection	-0.163	0.191	0.392
Age	0.164	0.057	0.004
Sex	-0.008	0.18	0.963
Cabo Delgado			
Urinary schistosomiasis	-0.351	0.145	0.016
Hookworm infection	-0.554	0.152	< 0.001
Age	0.056	0.045	0.210
Sex	0.063	0.147	0.670
Niassa			
Urinary schistosomiasis	-0.136	0.18	0.451
Hookworm infection	-0.086	0.201	0.667
Age	0.094	0.055	0.088
Sex	0.047	0.169	0.778

Table 3

Logistic regression analysis assessing the relationships between urinary schistosomiasis and hookworm infection and anemia defined by blood hemoglobin concentration below 11.5 g/dL, controlling for age and sex

	Odds ratio	95% Confidence Interval	p-value
Total population			
Urinary schistosomiasis	1.0576	0.8155 - 1.3714	0.673
Hookworm infection	1.3793	1.0419 - 1.8258	0.024*
Age	0.9072	0.8374 - 0.9827	0.017*
Sex	1.1362	0.8802 - 1.4668	0.326
Nampula			
Urinary schistosomiasis	0.8959	0.5633 - 1.4249	0.642
Hookworm infection	1.0694	0.6621 - 1.7273	0.783
Age	0.8777	0.7607 - 1.0128	0.074
Sex	1.6507	1.0518 - 2.5907	0.029*
Cabo Delgado			
Urinary schistosomiasis	1.4522	0.9451 - 2.2313	0.088
Hookworm infection	1.8731	1.1689 - 3.0015	0.009*
Age	0.9491	0.8314 - 1.0835	0.439
Sex	1.0777	0.6987 - 1.6624	0.734
Niassa			
Urinary schistosomiasis	1.1021	0.6697 - 1.8136	0.702
Hookworm infection	1.0754	0.616 - 1.8772	0.798
Age	0.9203	0.7895 - 1.0727	0.287
Sex	0.8367	0.5243 - 1.3351	0.454

*Statistically significant values.

Table 4

Logistic regression analysis assessing the relationships between urinary schistosomiasis and hookworm infection and moderate/severe anemia defined by blood hemoglobin concentration below 9 g / dL, controlling for age and sex

	Odds ratio	95% Confidence Interval	p-value
Total population			
Urinary schistosomiasis	1.2079	0.8105 - 1.8003	0.353
Hookworm infection	1.7376	1.1729 - 2.574	0.005*
Age	0.8295	0.7352 - 0.9359	0.002*
Sex	0.8604	0.5853 - 1.2648	0.444
Nampula			
Urinary schistosomiasis	0.9697	0.4929 - 1.9078	0.928
Hookworm infection	1.2952	0.6559 - 2.5579	0.456
Age	0.8761	0.7074 - 1.085	0.225
Sex	0.9041	0.4671 - 1.7502	0.764
Cabo Delgado			
Urinary schistosomiasis	1.3679	0.7539 - 2.482	0.302
Hookworm infection	2.7448	1.522 - 4.9501	<0.001*
Age	0.8471	0.7068 - 1.0152	0.072*
Sex	0.7003	0.3838 - 1.2777	0.245
Niassa			
Urinary schistosomiasis	1.9013	0.7169 - 5.0422	0.196
Hookworm infection	0.7629	0.2689 - 2.1642	0.610
Age	0.7886	0.6027 - 1.0318	0.083
Sex	1.2445	0.5319 - 2.8912	0.618

*Statistically significant values.

different provinces. Unfortunately, we could not perform Kato-Katz smears in order to better characterize worm burdens, which would have been extremely elucidating. In this context, we believe that the average intensity of hookworm infection in Cabo Delgado must be higher than in Nampula and Niassa. In addition, we observed that the prevalence rate of hookworm infection was higher in Cabo Delgado, and significantly lower in Niassa.

According to the Multiple Indicator Cluster Survey (MICS) carried out in Mozambique in 2008, Cabo Delgado had a worse sanitary background than either Nampula or Niassa¹¹. The proportion of families with access to improved sanitary conditions facilitating solid organic waste disposal (*i.e.* improved latrines) was 5.6% in Cabo Delgado, 15.2% in Nampula and 15.4% in Niassa. In Cabo Delgado, 4.3% of the families defecate on the beach, 25.3% in bushes and 64.7% have rudimentary latrines unable to contain solid excrement. The prevalence of hookworm infection was significantly lower in the Niassa province. According to the MICS, Niassa is the province where there is the lowest proportion of open sky defecation (*i.e.* defecation made directly into the soil, leaving the feces exposed to the environment)¹¹. Additionally, Niassa is a province where the population is more dispersed, probably making hookworm transmission more difficult. Furthermore, in Cabo Delgado only 29.9% of the families have access to improved sources of drinking

water, this proportion reaching 43.1% in Nampula and 44.1% in Niassa. Cabo Delgado also presents the highest child mortality rate in northern Mozambique (180 / 1,000 live births), this rate is 140 / 1,000 in Nampula and 123/1,000 in Niassa. Cabo Delgado is also plagued with the highest proportion of chronic malnutrition (stunting) among children under five years old, 56%, defined by height-for-age z-scores below - 2 vs. 51% in Nampula and 45% in Niassa¹¹. Taking into account all this data serves to highlight atrocious sanitary scenario in Cabo Delgado. In this context, a higher level of environmental contamination with the infective stages of hookworms (*i.e.* larvae that penetrate the skin) and, consequently, higher parasite burdens should be expected in the studied districts of the Cabo Delgado province, parasite burden being intrinsically linked to continuous re-infections.

The interactions between hookworm infections and anemia were demonstrated in primary schoolchildren living in India²⁰, Zimbabwe¹⁷ and Tanzania²⁶. Nevertheless, some surveys that attempted to characterize the relationship between hookworm disease and hemoglobin levels failed to find a conclusive association between these variables. In this context, studies performed in distinct countries included preschool and schoolchildren in Zanzibar^{13,15}, preschoolchildren in the Brazilian Amazon⁵ and South Africa⁸ and pregnant women in Kenya²⁸. Factors leading to such incongruent results are probably distinct intensities (often not assessed through quantitative stool examinations, *e.g.* Kato-Katz smears) of helminthic infections in different studies and potential biases such as distinct socio-economic, dietary, demographic, epidemiologic and sanitary circumstances. Also, studies assessing the impact of anti-helminthic administration on anemia prevalence and hemoglobin levels reveal conflicting results. It has been demonstrated that anti-helminthic drugs significantly decrease the burden of helminth infections and reduce anemia prevalence among schoolchildren in the Ivory Coast²². However, this effect was not observed in pregnant women in Uganda, probably due to low hookworm burdens¹⁹.

Taking everything into consideration, it has been proposed that the influence of soil-transmitted helminthiases on nutritional deficits, such as iron imbalance, should be taken into account in the context of interrelated determinants. The main factors are poor dietary intake and infectious diseases, which are affected by family access to good water, sanitation and adequate health services²⁷.

In the provinces of northern Mozambique prevalence rates of anemia were also very high (above 60%) among non-parasitized children who, also, presented an average hemoglobin level below 11.5 mg/dL. This demonstrates that other factors besides helminthic infections strongly contribute to anemia in the studied area. Malaria and inadequate iron intake due to a predominantly starch-based diet are probably other determinants of anemia in Mozambican children living in the province of Cabo Delgado. In Niassa and Nampula, these two considerations should be viewed as the main causes of anemia, since no relationship between the studied parasitoses and hemoglobin levels was established. The affliction of malaria and its possible effects on hemoglobin levels of children in northern Mozambique can be estimated indirectly. During a demographic survey in 2008¹¹, 26.7% of the children in Nampula, 20.1% in Cabo Delgado and 13.9% in Niassa presented a fever during the 14 days before the survey, of which 57.6% of the children in Nampula, 47.4% in Cabo Delgado and 26.5% in Niassa were treated with anti-malarial drugs. This demonstrated that malaria reaches hyperendemic

levels in the studied area and should be viewed as a determinant of the high prevalence of anemia as has been extensively demonstrated in other regions in Africa^{3,16}. In addition, the effect of malaria and enteric helminth infections on anemia prevalence among schoolchildren was exhibited in Ethiopia⁶.

The interaction between urinary schistosomiasis and iron deficiency was reported in Kenya²⁴ and Niger²¹. However, an extensive study performed in Cameroon did not demonstrate an association between *S. haematobium* and anemia in primary schoolchildren, probably due to low parasitic loads².

Soil-transmitted helminth infections and schistosomiasis associated morbidity mitigation focusing on worm burden control must be considered as a cost effective short-term initiative that can be linked to vaccination campaigns. In many developing countries, periodical mass treatments are extensively applied¹⁰. In rural Mozambique, vaccine coverage reaches 83% for tuberculosis (BCG), 71% for the DPT3 vaccine (diphtheria-pertussis-tetanus) and 70% for the oral poliomyelitis vaccine¹¹. These rates could allow for an inference on the coverage of deworming campaigns. Massive deworming must, however, be accompanied by projects focusing on health education and improvements in sanitary infrastructure in order to achieve long term and sustainable reductions in soil-transmitted helminthiasis as well as schistosomiasis prevalence rates. Very distinct proportions of families with access to safe solid waste disposal are evident between urban (47%) and rural (6%) zones in Mozambique¹¹.

We conclude that hookworm infection and urinary schistosomiasis are major public health concerns in the studied area. Periodical deworming should be performed on a strictly regular basis in conjunction with the vaccination campaigns.

RESUMO

Infecções por ancilostomídeos e *Schistosoma haematobium* e sua correlação com a concentração sanguínea de hemoglobina em crianças moçambicanas

Este estudo tem como objetivo avaliar a relação entre a ancilostomíase e a esquistossomíase urinária com as concentrações sanguíneas de hemoglobina em crianças escolares no norte de Moçambique. Em estudo transversal, 1.015 crianças com idade entre cinco e 12 anos foram incluídas, nas Províncias de Nampula, Cabo Delgado e Niassa. A ancilostomíase e a esquistossomíase urinária foram diagnosticadas através das técnicas de Ritchie e de filtração da urina, respectivamente; prevalências de 31,3% e 59,1% foram observadas. As concentrações sanguíneas de hemoglobina foram obtidas com um fotômetro portátil (Hemocue). A concentração média de hemoglobina foi $10,8 \pm 1,42$ g/dL, 62,1% das crianças apresentaram concentração abaixo de 11,5 g/dL e 11,8% apresentaram nível abaixo de 9 g/dL. A regressão linear múltipla demonstrou interações negativas entre os níveis de hemoglobina e i) a infecção por ancilostomídeos ($\beta = -0,55$; $p < 0,001$) e ii) a esquistossomíase urinária ($\beta = -0,35$; $p = 0,016$), ambas associações restritas à Província de Cabo Delgado. Também em Cabo Delgado, o modelo de regressão logística demonstrou que a infecção por ancilostomídeos representa um preditor de anemia leve (OR = 1,87; 95% CI = 1,17-3,00) e anemia moderada/grave (OR = 2,71; 95% CI = 1,50 - 4,89). O estudo conclui

que em Cabo Delgado, Moçambique, as infecções por ancilostomídeos e *Schistosoma haematobium* estão significativamente associadas a uma menor concentração sanguínea de hemoglobina em crianças em idade escolar. A administração periódica de anti-helmínticos deve ser feita regularmente. Melhorias na infraestrutura sanitária das regiões estudadas são as medidas mais eficazes para controle destas parasitoses.

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CONFLICTS OF INTEREST

None declared.

ETHICAL APPROVAL

This study was previously approved by the National Bio-ethics Committee for Health of Mozambique. Informed consent was obtained from parents or legal guardians of all children.

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