

HOOKWORM AND THREADWORM INFECTIONS AND THEIR ASSOCIATION WITH HEMOGLOBIN AND EOSINOPHIL CONCENTRATIONS IN RESIDENTS OF SALVADOR-BAHIA, BRAZIL

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SUMMARY

Parasitic infections are responsible for substantial mortality and morbidity worldwide. In most healthy individuals, little overt pathology is observed during infection with *S. stercoralis*. However, the symptoms in advanced cases may include gastrointestinal bleeding. Anemia is most commonly associated with hookworm infection, especially when several hundred worms are present. Our study evaluates the relationship between the hookworm or *S. stercoralis* infection status and the hemoglobin concentration of individuals examined by a private network of laboratories in Salvador, Bahia, Brazil. We examined 374,120 samples from middle-class individuals living in Salvador City from January 2004 to April 2008. The stool samples were analyzed by the Lutz and Baermann-Moraes methods, and the blood samples were analyzed for hemoglobin concentration and eosinophil counting. The prevalence of hookworm and *S. stercoralis* were 0.27% (1,027) and 0.34% (1,286), respectively. The prevalence of hookworm and *S. stercoralis* infection was significantly higher in males than in females and increased with age. Eosinophilia was a common laboratorial finding in individuals infected with hookworm and *S. stercoralis*. The hemoglobin concentration was lower in the hookworm-infected individuals than in non-infected ones, but none of the examined patients were anemic. Lack of anemia could be a consequence of the socioeconomic status of these patients.

KEYWORDS: Hookworms; *S. stercoralis*; Eosinophilia; Anemia.

INTRODUCTION

Parasitic infections are responsible for substantial mortality and morbidity worldwide and represent one of the most prevalent pathological phenomena in humans. However, the worldwide distribution of the effect is not uniform, as residents of developing countries are the most impacted²⁹. Many parasites are spread through the fecal contamination of food and water. This type of infection occurs most frequently in areas with inadequate sanitation and education. Certain parasites, such as hookworms (*Necator americanus* and *Ancylostoma duodenale*) and threadworms (*Strongyloides stercoralis*), are common species of soil-transmitted helminthes, and infection can cause significant nutritional deficiencies, delayed physical and cognitive development during childhood and reduced productivity in adults^{15,25,37,47,53}.

S. stercoralis has a cosmopolitan distribution in tropical and subtropical regions. It is estimated that 100-200 million people are infected worldwide. However, it is likely that this infection rate is underestimated. The diagnosis of strongyloidiasis is difficult because the majority of cases involve the chronic form, low-level infection and very small numbers of larvae intermittently eliminated^{27,31,43}.

In a fairly narrow range of conditions, notably in allergic states and infection with helminthes, eosinophil production increases drastically^{18,38,46}. According to RANQUE *et al.* (1998)⁴⁰ and ANANE (2006)³, helminth infection should be suspected as the etiology of eosinophilia. In most immunocompetent individuals, *S. stercoralis* causes little overt pathology, and eosinophilia may be the only symptom⁵. This parasite can persist in the host for decades, leading to the development of gastrointestinal, pulmonary or cutaneous symptoms. Disseminated infection causes severe clinical manifestations and is frequently observed in immunocompromised or malnourished individuals^{5,28,48,52}.

The association between *S. stercoralis* infection and anemia is rare, except in cases of hyperinfection^{28,34,39,43,52}. In contrast, hookworm infection induces iron deficiency, which constitutes one of the world's major nutritional problems^{2,21,42,49}. Hookworm infection affects approximately one billion people worldwide, mainly in developing countries. Its prevalence increases with age in children, typically reaching a plateau in late adolescence, whereas the intensity of infection may continue to increase throughout adulthood¹¹. The tropical climate of Brazil provides excellent conditions for the development of soil-transmitted helminthes. Therefore, the risk of infection is substantial even for inhabitants who have education and access to health care,

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although there is a lack of epidemiological studies on this population. The relationship between helminth infection and anemia has been demonstrated in epidemiological studies^{21,33,49,51}, mainly in patients with a high worm burden⁵⁰.

We conducted an analytical, cross-sectional study of helminth infections (hookworms and *S. stercoralis*) and the relationship between hemoglobin concentration and eosinophilia among individuals analyzed by a private network of laboratories in Salvador, Bahia, Brazil.

MATERIALS AND METHODS

Study population: This study was conducted between January 2004 and April 2008. Samples were collected from individuals attending a routine laboratory service in Salvador City, Brazil. Stool samples were analyzed from 374,120 patients with a mean age of 34.04 years, all living in Salvador City and the surrounding metropolitan area. The laboratory network is composed of 11 posts throughout Salvador and its metropolitan region and serves the socioeconomically higher segment of the population, which has access to basic sanitation, a good nutritional status and income averaging five times the minimum wage. Salvador is the third most populous city in Brazil and the ninth in Latin America, with a population of 2,675,656²⁶. It has a warm and humid tropical rainforest climate with no discernible dry season and temperatures that are relatively constant throughout the year, which is conducive to the growth of soil-transmitted helminthes.

Samples processing: A single fresh stool specimen from each patient was analyzed by both the BAERMANN-MORAES^{6,36} and LUTZ³² methods. In this study, monoinfected patients were defined as individuals of either sex with stool samples that were either positive for hookworms as diagnosed by spontaneous sedimentation, or positive for *S. stercoralis* as diagnosed by the Baermann-Moraes technique. Poliparasited individuals were excluded from the study. Healthy patients were defined as individuals with negative results for eggs and larvae of helminthes, and cysts or trophozoites of protozoan. Knowledge of the age and sex of the patients and an adequate quantity of stool for diagnosis by both methods were the minimum inclusion criteria.

The hemoglobin concentration was measured using an automated method (Sysmex XT-2000i, Roche). According to the hemoglobin concentration, patients were divided into five groups as follows: infant (< 2 years; reference range: hemoglobin > 10.5 g/dL); preschool (2-6 years;

reference range: hemoglobin > 11.5 g/dL); schoolchild (7-12 years; reference range: > 11.5 g/dL); teenager (13-18 years; reference range for males: > 13.0 g/dL; reference range for females: > 12 g/dL); and adult (> 19 years; reference range for males: > 13.5 g/dL; reference range for females: > 12.0 g/dL)¹⁷. To examine the red blood cell morphology, the smear was stained with panoptic dye and the white blood cells were counted. An eosinophil count less than 5% or 500/mm³ was considered to be normal¹⁷.

Statistical analysis: The data were managed and analyzed using the Statistical Package for the Social Sciences (SPSS) 15.0 Chicago software. The variables were compared using the Student's t-test and the Mann-Whitney test. A two-tailed *p*-value less than 0.05 was considered to be significant.

RESULTS

Stool samples of 374,120 patients, examined by the spontaneous sedimentation and Baermann-Moraes techniques, revealed infection rates of 0.27% (1,027) for hookworm and 0.34% (1,286) for *S. stercoralis*. The prevalence of hookworm and *S. stercoralis* infection was significantly higher in males than in females (75.8% vs. 24.2%, *p* < 0.001; 78.6% vs. 21.4%, *p* < 0.001, respectively), and the prevalence of hookworm and *S. stercoralis* infection increased with age (Table 1). These prevalences ranged from 0.44% to 0.20% and from 0.42% to 0.27% for hookworms and *S. stercoralis*, respectively, over the four-year course of this study (Fig. 1). The stool samples were categorized by calendar month to evaluate seasonal variation over the years. The mean infection rates for each of the 52 months comprising the study period were computed separately. To assess the statistical significance of the differences in infection rates between 12 calendar-year months, we combined the total number of specimens listed and the total number of positive specimens for each month across each of the 4.4 study years. The overall rates for hookworm infection ranged from a low of 0.18% (September) to a high of 0.37% (June). Similar results were observed for *S. stercoralis* infection, in which the positivity rates ranged from 0.25% (September) to 0.43% (July) (Fig. 2). Statistical analyses indicated that the monthly infection rates did not vary considerably over time.

The hemoglobin concentration (g/dL) was normal for all of the groups of hookworm- and *S. stercoralis*-infected individuals. Neither hookworm nor *S. stercoralis* infections were associated with any risk of decreased hemoglobin levels (OR=1.11 95% CI=0.86-1.43) and 1.12 (95% CI 0.87-1.45), respectively.

Table 1
Prevalence of hookworm and *Strongyloides stercoralis* infections in 374,120 individuals from Salvador, Bahia, Brazil, according to age and sex

Age (years)	Hookworms			<i>S. stercoralis</i>		
	Males	Females	Total	Males	Females	Total
< 2	3 (0.3)	0	3 (0.3)	0	0	0
2-6	14 (1.4)	4 (0.3)	18 (1.7)	10 (0.8)	5 (0.4)	15 (1.2)
7-12	27 (2.6)	14 (1.4)	41 (4.0)	15 (1.2)	8 (0.6)	23 (1.8)
13-18	27 (2.6)	21 (2.0)	48 (4.6)	11 (0.9)	2 (0.1)	13 (1.0)
> 19 years	707 (68.9)	210 (20.5)	917 (89.4)	975 (75.7)	260 (20.3)	1,235 (96.0)
Totals	778 (75.8) ^a	249 (24.2) ^a	1,027 (100)	1,011 (78.6) ^a	275 (21.4) ^a	1,286 (100)

^a*p* < 0.05. Statistic difference between males and females groups (Student's t-test).

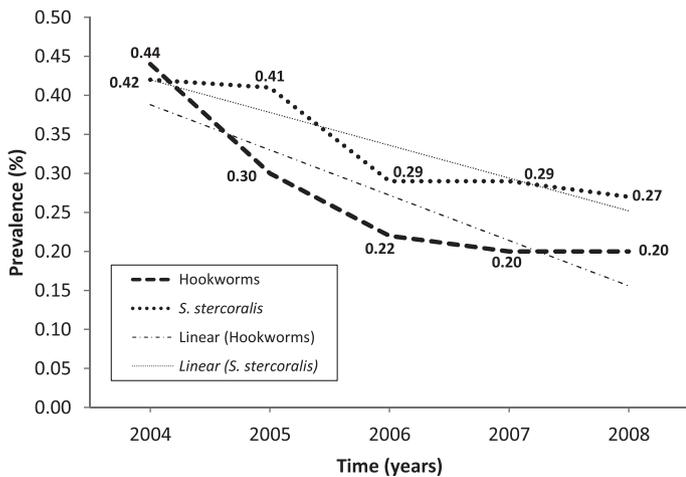


Fig. 1 - Prevalence of hookworms and *S. stercoralis* in individuals attending a laboratory network from Salvador, Bahia, Brazil (n = 374,120).

The increase in the blood eosinophil levels in hookworm- (OD = 6.46 95% CI = 5.46-7.64) and *S. stercoralis*-infected individuals (OD = 10.45 95% CI = 8.87-12.30) ($p < 0.01$) is shown in Table 3.

DISCUSSION

Helminthic infections are widely distributed throughout tropical and

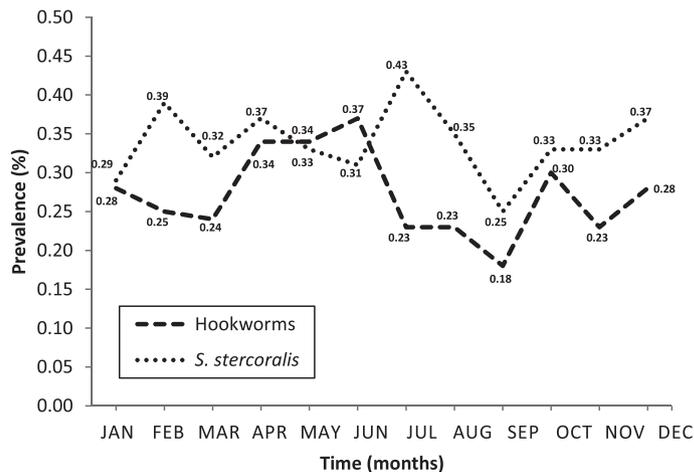


Fig. 2 - Mensal prevalence of hookworm and *S. stercoralis* infection in 374,120 individuals attending a laboratory network from Salvador, Bahia, Brazil, based on the mean obtained for each month in the 52 months of the study (January 2004 - April 2008).

subtropical areas, particularly in developing countries where sanitary education actions are ineffective. This study evaluated the prevalence of hookworm and *S. stercoralis* in 374,120 patients. Of these, 1,027 (0.27%) patients were positive for hookworm and 1,286 (0.34%) patients were positive for *S. stercoralis*. These data are similar to those obtained by an investigation conducted in São Paulo City studying a population

Table 2
Hemoglobin levels (g/dL) in hookworm- and *S. stercoralis*-infected patients compared with a healthy control group

Variables	Hookworms		<i>S. stercoralis</i>		Uninfected group	
	n	Hb	n	Hb	n	Hb
Infant Male	3	12.6	0	-	642	12.2
Infant Female	0	-	0	-	613	12.1
Preschool Male	7	12.2 *	5	12.6	9,385	12.4
Preschool Female	3	12.9	4	11.9	8,559	12.4
Schoolchild- Male	22	12.7	13	13.1	10,465	13.0
Schoolchild- Female	13	12.0 ^a	5	12.1	10,318	13.0
Teenage Male	21	13.8	9	14.2	6,364	13.9
Teenage Female	12	13.6	2	12.6	6,328	13.1
Adult Male	605	14.8 ^a	838	14.7 ^a	65,050	15.0
Adult Female	164	13.2	196	13.3	92,024	13.2

Infant (< 2 years); Preschool (2-6 years); Schoolchild (7-12 years); Teenager (13-18 years); Adult (> 19 years). ^a $p < 0.05$. Statistic difference between infected and non-infected groups (Mann-Whitney and Student's t-test); Hb = Hemoglobin level.

Table 3
Eosinophil levels in hookworm-infected patients compared to a healthy group

Variables	Hookworms		<i>S. stercoralis</i>		Uninfected group		p value
	n	EOS	n	EOS	n	EOS	
Male	632	10.12	843	11.34	125,244	5.11	0.000*
Female	183	8.35	210	10.50	148,816	3.78	0.000*

*Student's t-test. EOS = Eosinophil count.

with access to health care³⁵. Other projects in several regions of Brazil showed 1.7% in patients in Aracaju (Sergipe State)⁵³; 2.3% in patients living in Salvador (Bahia) proceeding from the Public Health System⁴⁴; 2.9% in patients in Minas Gerais State¹⁴; 6.6% in patients in Ipirá City (Bahia State)⁴⁵; 9.0% in patients in the Amazon region⁴; and 15.3% in children living in municipalities with low human development indices in North and Northeast Brazil¹⁹. On the other hand, other authors found as high a prevalence as 88.4% of inhabitants of four villages in São Lourenço da Mata (Pernambuco State)²³ and 91.9% of inhabitants of Ilha da Marambaia (Rio de Janeiro State)¹⁶. With regard to *S. stercoralis* prevalence, our results are comparable to those of MONTEIRO *et al.* (1988)³⁵ obtained in São Paulo City (0.3%), ROCHA *et al.* (2000)⁴¹ in the town of Bambuí, Minas Gerais State (0.2%), and ARAÚJO & FERNÁNDEZ (2005)⁴ in the Amazon region (1.0%). The observed differences in hookworm prevalence could be explained by the fact that the studies were conducted in distinct areas and on different populations with varied exposure to risk factors and socioeconomic status. In fact, a study has shown that the income level of a population influences the distribution of intestinal helminths¹. SANTOS *et al.* (2007)⁴⁴ observed that the prevalence of *S. stercoralis* in patients from the Public Health System (4.6%) was 7.7 times higher than that of those attended to by the Private Health System (0.6%).

Infections by hookworms and *S. stercoralis* were associated with age and sex. The 30- to 50-year age group was the most commonly infected by hookworms, and the 40- to 60-year age group was the most commonly infected by *S. stercoralis*. Children can be infected with hookworms and *S. stercoralis* at an age as young as six months, and this infection prevalence increases with age, plateauing in adulthood¹¹. In this study, the youngest infected patient was eight months old. The highest prevalence was observed in adulthood. However, different results have been observed by others worldwide. In China and Southeast Asia, the highest prevalence was observed in the middle-aged or even in individuals over 60 years old^{8,10,20,30}. Such age profiles of hookworms contrast to the rapid increase in prevalence of *Ascaris lumbricoides* and *Trichuris trichiura*, for which the prevalence reaches a maximum in 5- to 10-year-olds and remains relatively stable throughout adulthood¹².

In accordance with other infectious diseases, it has been suggested that males are more commonly infected with hookworm and *S. stercoralis* than females^{13,22}. It is likely that this discrepancy arises because males perform more activities that lead to greater risk of exposure⁸. However, other factors may contribute to susceptibility to infections, such as immunological response and nutritional status¹². Our results also demonstrated that infections with hookworm and *S. stercoralis* are more common in males than in females, possibly due to the higher risk of exposure of males.

In the last year of the study, we observed an apparent decrease in the prevalence of both helminths. From 2004 to 2008, the hookworm prevalence decreased by 0.24% (from 0.44 to 0.20%), and the *S. stercoralis* prevalence decreased by 0.15% (from 0.42 to 0.27%). This decrease could be explained by the positive impact of a project called Bahia Azul⁷. This program was implemented in 1996 to improve sanitation infrastructure and environmental preservation. The reduction in prevalence between time periods showed that changes in environmental health significantly decreased the occurrence of intestinal parasites in residents from Salvador City and its metropolitan area.

The most serious cause of iron loss from the body is chronic hemorrhage; estimates show that a blood loss of 15-20 mL per day into the lumen of the small intestine results in a state of negative iron balance. *Ancylostoma duodenale* and *Necator americanus* have been estimated to cause a daily loss into the small intestine of 0.14-0.26 mL and 0.02-0.07 mL per worm, respectively⁵⁴. When several hundred hookworms are present in the small intestine, the daily blood loss is sufficient to cause anemia, even in well-nourished children or adults⁵⁴. We observed that the hemoglobin level of certain infected groups was less than that observed in the corresponding uninfected group ($p < 0.05$). Nevertheless, no anemia case was verified (hemoglobin < 10 g/dL). We believe that the values of hemoglobin found are consistent with patients from the upper and middle classes, who have access to education, iron-rich foods and health programs. Controversially, the *S. stercoralis*-infected groups were not significantly different from the control group, except the adult male group. The development of anemia in *S. stercoralis*-infected individuals is correlated with alcoholism, malnutrition, the breakdown of local immune responses, and/or alterations in intestinal barriers^{24,39,52}. Our population is characterized by a high socioeconomic status with access to basic sanitation and a good nutritional status.

An increase in the peripheral blood eosinophils has long been recognized as a characteristic feature of helminth infection, consumption of medications, allergic disorders, and autoimmune and malignant diseases^{18,34,38,46}. However, parasitic infection is the major cause of eosinophilia in developing countries. In healthy individuals, eosinophils comprise approximately 1-5% of white blood cells. In this study, the majority of infected patients exhibited higher percentages than uninfected patients; 10.12% (male) and 8.35% (female) to hookworm, and 11.34% (male) and 10.50% (female) to *S. stercoralis*-infections.

It is widely accepted that helminths and their antigens induce the T helper cell type 2 (Th 2) response, resulting in the production of interleukin (IL)-4, IL-5 and consequently the development of strong immunoglobulin E (IgE), eosinophil and mast cell responses. The eosinophils release large amounts of potent mediators, such as major basic protein, eosinophil cationic protein, eosinophil peroxidase, and eosinophil neurotoxin, all of which may directly damage tissues in addition to damaging the infectious worms^{9,18,38}. Perhaps the increase in peripheral blood eosinophils in infected patients reflects the control of the infection but is not in itself sufficient to eliminate the parasite.

We conclude the following: (1) The prevalences of hookworm and *S. stercoralis* were 0.27% and 0.34%, respectively, and infection was more frequent in adults (21-60 years) and in males; (2) The hemoglobin concentration was lower in the hookworm-infected group than in the uninfected group, whereas a decrease in the hemoglobin levels of *S. stercoralis*-infected patients was shown only in adult males; (3) Eosinophilia is a common secondary diagnosis in hookworm- and *S. stercoralis*-infected patients; and (4) Anemia was not observed in any hookworm-infected patient, most likely as a result of their high socioeconomic status.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

RESUMO

Associação entre infecções por ancilostomídeos e *Strongyloides stercoralis* e concentração de hemoglobina e eosinófilos em residentes de Salvador-Bahia, Brasil

As infecções parasitárias são responsáveis por importantes quadros de morbimortalidade em todo o mundo. A maioria dos indivíduos saudáveis infectados pelo *S. stercoralis* não apresenta sintomas clínicos evidentes. Entretanto, em casos mais avançados sangramentos gastrintestinais podem estar presentes. A anemia é mais comumente associada à infecção causada pelos ancilostomídeos, especialmente quando centenas de vermes estão presentes. Neste trabalho, a associação entre infecções causadas por ancilostomídeos ou *S. stercoralis* e as concentrações de hemoglobina e de eosinófilos foi investigada em indivíduos atendidos em uma rede de laboratórios de Salvador, Bahia, Brasil. Foram examinadas 374.120 amostras de indivíduos de classe média residentes na cidade de Salvador, de janeiro de 2004 a abril de 2008. As amostras de fezes foram analisadas pelos métodos de Lutz e Baermann-Moraes e as de sangue foram avaliadas para determinação da concentração de hemoglobina e do número de eosinófilos. A prevalência de ancilostomídeos e de *S. stercoralis* foi de 0,27% (1.027) e 0,34% (1.286) respectivamente. A prevalência de infecções, tanto por ancilostomídeos quanto por *S. stercoralis*, foi significativamente maior em homens que em mulheres e aumentava com o aumento da idade. A eosinofilia foi um achado comum tanto em indivíduos infectados por ancilostomídeos como por *Strongyloides*. A concentração de hemoglobina foi menor nos indivíduos infectados por ancilostomídeos quando comparado aos indivíduos não infectados, embora nenhum paciente apresentasse anemia. A ausência de anemia pode ser devida às boas condições socioeconômicas dos pacientes analisados.

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