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EPIDEMIOLOGY OF Schistosoma mansoni INFECTION IN A LOW-ENDEMIC AREA IN BRAZIL: CLINICAL AND NUTRITIONAL CHARACTERISTICS

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SUMMARY

A cross-sectional case-control study designed to evaluate the role of malnutrition in the association between the intensity of **Schistosoma mansoni** infection and clinical schistosomiasis, was conducted in an area with both low frequency of infection and low morbidity of schistosomiasis in Brazil. Cases (256) were patients with a positive stool examination for **S. mansoni;** their geometrical mean number of eggs/gram of feces was 90. Controls (256) were a random sample of the negative participants paired to the cases by age, sex and length of residence in the area. The clinical signs and symptoms found to be associated with **S. mansoni** infection, comparing cases and controls, were blood in stools and presence of a palpable liver. A linear trend in the relative odds of these signs and symptoms with increasing levels of infection was detected. Adjusting by the level of egg excretion, the existence of an interaction between palpable liver and ethnic group (white) was suggested. No differences in the nutritional status of infected and non-infected participants were found.

KEY WORDS: Schistosomiasis mansoni; Infection intensity; Clinical signs and symptoms; Malnutrition; Epidemiological case-control studies.

INTRODUCTION

Schistosoma mansoni infection is endemic in Brazil. According to the Brazilian Ministry of Health the number of infected persons in the country is about 12 million. In most of the endemic areas the intensity of infection is considered to be low to moderate[38]. A large proportion of infected individuals remains clinically asymptomatic. There are evidences that the infection's intensity is the main determinant of clinical manifestations in schistosomiasis mansoni[38]. Several studies had shown an association between intensity of infection (estimated by the number of eggs/gram of feces) and more severe clinical forms of the disease [5, 7, 16, 17, 21, 23, 26, 33, 34, 45]. Other investigators, however, had not reported the same relationship [6, 11, 13, 14, 19, 28, 32, 37, 46]. Recently it was shown the intensity of S. **mansoni** infection to be associated only to the presence of blood in stools and to a palpable and hardened liver[41].

The importance of the human host nutritional status and its possible association with schistosomiasis clinical manifestations is controversial. Some studies have described such relationship, while in other investigations no association was found. However, the anthropometric measures and clinical signs of malnutrition used were not always similar, which prevented meaningful comparisons [7, 8, 14, 24, 48].

The objective of the present investigation, a cross-sectional case-control study was to evaluate,

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in the human host, the role of protein-caloric malnutrition (estimated by clinical, laboratory and anthropometric methods) in the relationship between the intensity of **S. mansoni** infection (estimated by the number of eggs/gram of feces as a surrogate of the worm burden) and clinical manifestations of schistosomiasis. The study was carried out in an area presenting a low to moderate endemicity level of this infection in Minas Gerais State, Brazil.

MATERIAL AND METHODS

This investigation was conducted in Ribeirão das Neves, a small city of about 14,000 people living in its urban area, belonging to the Metropolitan Region of Belo Horizonte, the capital of Minas Gerais State, Brazil. No cases of malaria or kalazar were ever reported from this area.

The minimum sample size needed to detect a relative risk of 2, estimated by the relative odds (RO), for clinical schistosomiasis, assuming 20 percent of the local population to be malnourished (below the 5 percent of Brazilian standards[2]), with an α =.05 and β = .10, was estimated to be 229 participants in each group[44].

The frequency of **S. mansoni** infection among schoolchildren in this area was 22.5%[18]. The distribution of the infection typical of most Brazilian endemic areas shows a sharp decline of infection rates with increasing age[25-27]. In order to obtain the necessary number of infected participants, a stool examination survey including approximately 4,000 inhabitants had to be carried out.

Cases were defined as subjects with a positive stool test for **S. mansoni.** Controls were selected from the pool of negatives and paired to the cases by age (within 3 years), by sex and by length of residence in the area (within one year). A detailed description of the procedures used in selecting participants, in conducting the stool examination survey, in selecting and matching controls can be seen in a previous publication[41].

Cases and controls were examined and interviewed following a pre-coded standard clinical and nutritional form, for signs and symptoms which occurred in the last 30 days, specially those related to the gastrointestinal system. One clinical examination was carried out. The interview included questions about alcohol intake and history of schistosomiasis mansoni treatment. A 10 ml blood sample was collected. The clinical classification adopted and the methods of liver and spleen palpation were already described[41].

The malnutrition signs recorded were those classsified as Group I [15]. The anthropometric measures taken were weight, height, arm circumference and triceps skinfolds using a Harpenden Caliper [20, 43, 47].The total area, muscular and fatty sections of the arm were also calculated[12].

In all participants, skin tests in the volar surface of the left arm were done: schistosomin (LE/BH strain, adult worm antigen), oligomycin, streptokinase and PPD-5TU; the readings were considered *positive* if the induration was equal or greater than 5 mm for oligomycin and streptokinase, equal or greater than 10 mm for PPD-5TU[30] and equal or greater than 1.0 cm^2 for schistosomin[36].

The laboratory tests carried out were: erythrocyte count, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), leukocyte count (global and differential), blood group and Rh factor typing. In a random subsample, immunoglobulin G, M and A (IgG, IgM, IgA) and complement component 3 (C_3) were also measured using the radial immunodiffusion technique.

During the clinical and nutritional examinations, neither the examiner nor the patients was informed about the results of any laboratory tests. The laboratory personnel was also not aware of the patients' status as cases or controls.

Frequency distributions and statistical tests were done using the SPSS Statistical Package for the Social Sciences[31] software. The RO and its confidence intervals were obtained using a Hewllet-Packard model 67 calculator[42]. Bivariate (Mantel-Haenszel) and multivariate comparisons (multivariate logistic analysis, conditional method), were accomplished using the EpiInfo[9] and the MULTLR Microcomputer Program for Multiple Logistic Regression by Unconditional and Conditional Maximum Likelihood Methods[4] software, respectively. In both analysis, two models were used: (1) **S. mansoni** infection as the outcome variable, blood in stools as the primary predictor variable (yes/no) and (2) **S.mansoni** infection as the outcome variable, palpable liver (yes/no) as the primary predictor variable. Secondary predictor variables included in the multivariate

models were: previous schistosomiasis mansoni treatment (yes/no), malnutrition signs (hair and face, yes/no), and ethnic groups (white/nonwhite). **S. mansoni** infection in the multivariate logistic models was stratified as controls (reference), less or equal 100 eggs/gram of feces and more than 100 eggs/gram of feces.

RESULTS

The characteristics of the study population are presented in Table 1; of the 4,141 persons identified in the study census, only 2,503 had stool samples examined for S. **mansoni** eggs (in study number order), as the proportion of infected subjects (14%) was higher than initially expected, yielding the necessary number of cases to be included in the study. From the 357 ascertained cases, 256 (72%) were included in the matched analysis and 282 (79%) in the stratified (unmatched) analysis; the remaining were excluded for various reasons.

Table 1
Epidemiology of **Schistosoma mansoni** infection. Identification of the study population. Ribeirão das Neves, MG.

Urban Area Population (1980 Census)			14,036
Identified (Study Census)			4,141
Stool examination survey			2,503
Positive for **S. mansoni**			357 (14%)
Excluded:	Volunteers	16	
	Migrants	9	
	No controls[1]	26	
	Refusals	50	
Cases included in the matched analysis			256 (72%)
Matched[2] Controls from pool of negatives			256
Cases included in the unmatched analysis			282 (79%)
Controls from pool of negatives			275

[1]excluded from the matched analysis
[2]age within 3 years; sex; length of residence within 1 year

The atributes of the cases, in relation to S. **mansoni** burden (estimated by the number of eggs/gram of feces) and the clinical classification of the disease, can be seen in Table 2. The geometrical mean number of eggs/gram of feces was 90; among those with low (12-99 epg), moderate (100-499epg) and heavy (≥ 500 epg) infection was 33, 221 and 1,212 respectively. The observed results (86% of cases shedding < 500 eggs/gram of feces and 89% classified as Type I clinical form) characterize Ribeirão da Neves as an area with both low frequency of infection and low morbidity for schistosomiasis mansoni[18]. Similar results were obtained for cases included in the unmatched analysis.

Table 2
Classification of schistosomiasis mansoni cases according to the number of eggs per gram of feces and the clinical form of the disease. Ribeirão das Neves, MG.

Characteristic	Cases of schistosomiasis mansoni			
	Matched Analysis		Unmatched Analysis	
	Number	%	Number	%
Eggs per gram of feces				
12 - 99	147	57.4	167	59.2
100 - 499	74	28.9	80	28.4
≥ 500	35	13.7	35	12.4
Total	256	100.0	282	100.0
Clinical form[*]				
Type I	226	89.0	251	89.7
Type II	23	9.0	23	8.2
Type III	5	2.0	6	2.1
Total	254	100.0	280	100.0

[*] in 2 cases the liver palpation was not done

The association of geometrical mean number of eggs/gram of feces and the clinical disease classification is shown in Table 3. The analysis of gradient in proportions for samples quantitatively ordered[10], when Type II and Type III patients where pooled together, showed a statistically significant trend. This finding suggests a linear increase in the proportion of patients presenting more severe forms of the disease with heavier infections (Figure 1). However, only 18% of the patients with heavier infection were classified as having Type II/III clinical disease.

Table 3
Clinical forms of schistosomiasis mansoni according to the intensity of infection (eggs/gram of feces). Ribeirão das Neves, MG.

Clinical Forms	Intensity of infection		
	Low (12-99 epg)	Moderate (100-499 epg)	Heavy (≥ 500 epg)
Type I	153	70	28
Type II/III	13	10	6
prop Type II/III	0.08	0.13	0.18
ln mean epg	3.5	5.4	7.1

trend analysis: b = 0.0282
X^2_{1gl} slope = 3.94 p<.05
X^2_{1gl} linearity = 0.03 NS

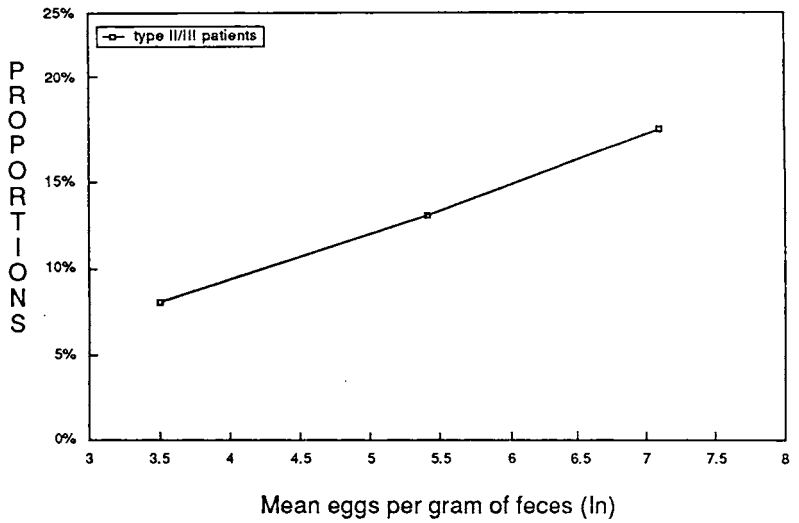

Figure 1
Proportions of type II/III patients according to **S. mansoni** egg excretion

Tables 4 to 8 present the results of the basic case-control comparisons (matched analysis). Among the demographic characteristics studied, cases showed a lower proportion of whites and a higher proportion of mulattoes. Pertaining to clinical signs and symptoms, a higher proportion of cases reported having blood stools and presented a palpable and hardened liver; hardened liver was only present among cases presenting a palpable liver. No differences in protein-caloric malnutrition clinical signs and anthropometric measures were found. Regarding laboratory tests, cases presented a higher mean value of IgG and a lower mean value of erythrocyte count; a higher proportion of cases also had more than 4% of eosinophil.

A similar proportion of cases and controls re-

Table 4
Comparison of demographic and anthropometric variables between schistosomiasis mansoni cases and controls (matched analysis). Ribeirão das Neves, MG.

Anthropometric Characteristics[1]	Cases (N)	Cases %	Controls (N)	Controls %
Ethnic Group: White	(97)	38	(134)	52
Mulatto	(137)	54	(103)	40
Black	(21)	8	(19)	7
Other	(1)	-	(0)	-
Weight kg (mean)	(252)	49.8	(252)	48.3
Height cm (mean)	(250)	155.8	(250)	152.4
Arm circumference cm (mean)	(253)	24.8	(253)	24.5
Triceps skinfold mm (mean)	(245)	11.3	(245)	11.6
Arm: Total area mm^2 (mean)	(244)	5086	(244)	5019
Muscular section mm^2 (mean)	(244)	3759	(244)	3712
Fatty section mm^2 (mean)	(244)	1389	(244)	1426

[1] refusals and not stated removed from Table
[*] significant at .05 level: means paired "t" test
proportions X^2_{1gl} Yates correction

Table 5
Comparison of the proportions of clinical signs and symptoms between schistosomiasis mansoni cases and controls (matched analysis). Ribeirão das Neves, MG.

Clinical Signs and Symptoms [1]	Cases (N)	Cases %	Controls (N)	Controls %
Headache	(26)	10	(16)	6
Abdominal pain	(53)	21	(42)	16
Diarrhea	(19)	7	(9)	4
Constipation	(3)	1	(6)	2
Blood in stools	(42)	16	(11)	4[*]
Enteralgia	(3)	1	(3)	1
Melena	(0)	-	(0)	-
Hematemesis	(0)	-	(0)	-
Dizziness	(14)	5	(14)	6
Weakness	(1)	-	(1)	-
Palpable liver	(28)	11	(9)	4[*]
Hardened liver	(7)	3	(0)	-[*]
Other	(17)	7	(16)	6
Asymptomatic	(125)	49	(163)	64[*]

[1] refusals and not stated removed from Table
[*] significant at .05 level: X^2_{1gl} with Yates correction

Table 6
Comparison of protein-caloric malnutrition clinical signs between schistosomiasis mansoni cases and controls according to anatomical site (matched analysis). Ribeirão das Neves, MG.

Anatomical Site[1]	Cases		Controls	
	(N)	%	(N)	%
Hair	(17)	7	(18)	7
Face	(46)	18	(38)	15
Eyes	(7)	3	(8)	3
Lips	(8)	3	(9)	4
Tongue	(0)	-	(2)	-
Teeth	(3)	1	(5)	2
Gums	(2)	-	(1)	-
Thyroid	(2)	-	(1)	-
Parathyroid	(0)	-	(0)	-
Skin	(1)	-	(0)	-
Nails	(0)	-	(0)	-
Subcutaneous edema	(2)	-	(0)	-
Muscular-skeletal system	(0)	-	(0)	-

[1] refusals and not stated removed from Table
[*]significant at .05 level: X^2_{1gl} with Yates correction

Table 7
Comparison of selected laboratory tests between schistosomiasis mansoni cases and controls (matched analysis). Ribeirão das Neves, MG.

Laboratory Tests [1]		Cases		Controls	
		(N)	%	(N)	%
Blood Groups	A	(75)	32	(78)	33
	B	(43)	18	(32)	14
	AB	(6)	3	(6)	3
	O	(114)	48	(117)	50
Rh Positive		(219)	92	(207)	90
Skin Tests: Oligomicym		(130)	62	(141)	65
Streptokinase		(50)	24	(54)	25
PPD-5TU		(35)	17	(40)	18
IgG mg% (mean)		(54)	1,623	(54)	1,380[*]
IgA mg% (mean)		(46)	220	(46)	216
IgM mg% (mean)		(46)	254	(46)	220
C_3 mg% (mean)		(40)	133	(40)	122

[1]refusals and not stated removed from Table
[*] significant at .05 level: means paired "t" test
proportions X^2_{1gl} Yates correction

Table 8
Comparison of selected laboratory tests between schistosomiasis mansoni cases and controls (matched analysis). Ribeirão das Neves, MG.

Laboratory Tests[1]	Cases		Controls	
	(N)	%	(N)	%
Erythrocytes mm^3 (mean)	(230)	$4.6x10^6$	230	$4.7x10^{6(*)}$
Hematocrit % (mean)	(218)	41.3	(218)	41.9
Hemoglobin g% (mean)	(230)	13.8	(230)	14.0
MCV mm^3 (mean)	(218)	91.7	(218)	88.1
MCHC % (mean)	(218)	33.4	(218)	33.6
Leukocytes mm^3 (mean)	(230)	$6.5x10^3$	(230)	$6.2x10^3$
Juvenile neutrophils > 4%	(65)	23	(52)	19
Segmented neutrophils > 57%	(54)	22	(44)	18
Lymphocytes > 35%	(239)	99	(244)	100
Monocytes >5%	(59)	25	(67)	27
Eosinophils > 4%	(196)	82	(171)	70 [(*)]
Basophils > 0.2%	(20)	8	(30)	12

[1]refusals and not stated removed from Table
[*]significant at .05 level: means paired "t" test
proportions X^2_{1gl} Yates correction

ported use of alcohol (19.2 and 21.5% respectively), without specification of quantity and frequency. In regard to receiving therapy against schistosomiasis mansoni, 14.5% of the cases and 14.8% of the controls related being previously treated, but were unable to recall specific details.

The assessment of the association between selected clinical signs and symptoms and **S. mansoni** infection can be seen in Table 9; only the RO for the presence of blood in stools and a palpable liver were statistically significant. Cases, classified by the infection's intensity (eggs/gram of feces) were

Table 9

Schistosoma mansoni infection and clinical signs and symptoms of schistosomiasis mansoni (matched analysis). Ribeirão das Neves, MG.

Clinical Signs and Symptoms	**S. mansoni** infection Cases + Controls -	Cases - Controls +	Relative Odds	95% CI
Headache	23	13	1.8	0.8 - 3.9
Abdominal pain	40	28	1.4	0.8 - 2.5
Diarrhea	18	8	2.2	0.9 - 6.3
Constipation	0	6	0.5	-
Blood in stools	40	9	4.4	2.0 - 10.8
Enteralgia	3	3	1.0	-
Dizziness	13	13	1.0	-
Weakness	1	1	1.0	-
Palpable liver	26	7	3.7	1.4 - 10.6

compared with the control group as reference. Significant trends (Figure 2) in the estimated RO were observed for blood in stools ($X^2_{1df} = 31.5$, $p < .001$) and palpable liver ($X^2_{1df} = 12.3$, $p < .01$), showing a linear increase in the proportion of cases presenting these signs and symptoms with heavier infections.

The comparison of serum mean values (in mg%) of IgG by egg excretion levels showed significant differences (controls, 1380; 12-99 epg, 1539; 100-499 epg, 1700 and ≥ 500 epg, 1822); the multiple comparison test (Scheffé) revealed the discriminating level to be ≤ 100 and > 100 eggs/gram of feces.

Stratified analysis (unmatched) did not detect confounding; some of the studied risk factors were associated to both exposure and disease under the two models studied, but the estimated adjusted and crude RO did not differ.

The estimates stratum specific RO for ethnic groups (white and non-white) and previous schistosomiasis treatment (yes/no) suggested the presence of effect modification (interaction), specially for the palpable liver model among whites; however the X^2 of heterogeneity was not significant. Results for the ethnic group analysis can be seen in Figure 3. This suggestion of effect modification was more evident stratifying by egg excretion levels (Figure 4), but again, no statistical significance was attained. It can be seen that the ROs were always higher among whites when compared to non-whites; for the blood in stools model, the

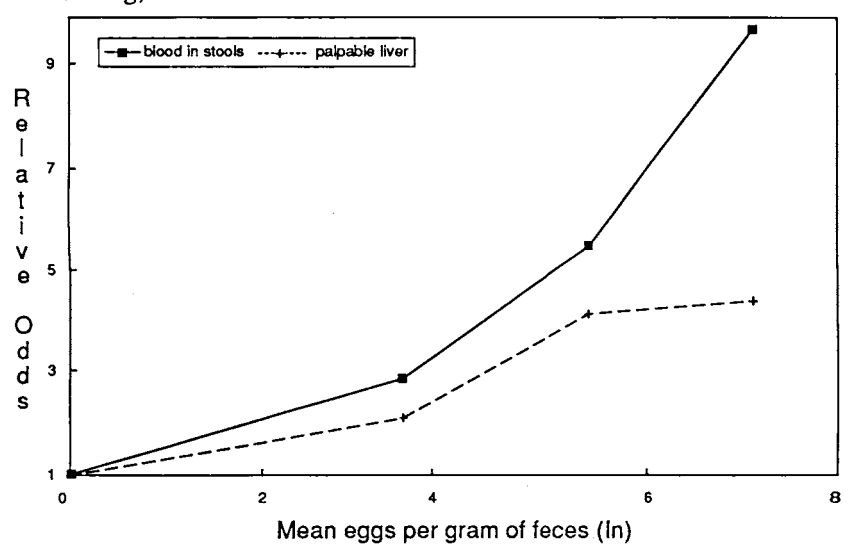

Figure 2 - Relative Odds of blood in stools and palpable liver by egg excretion

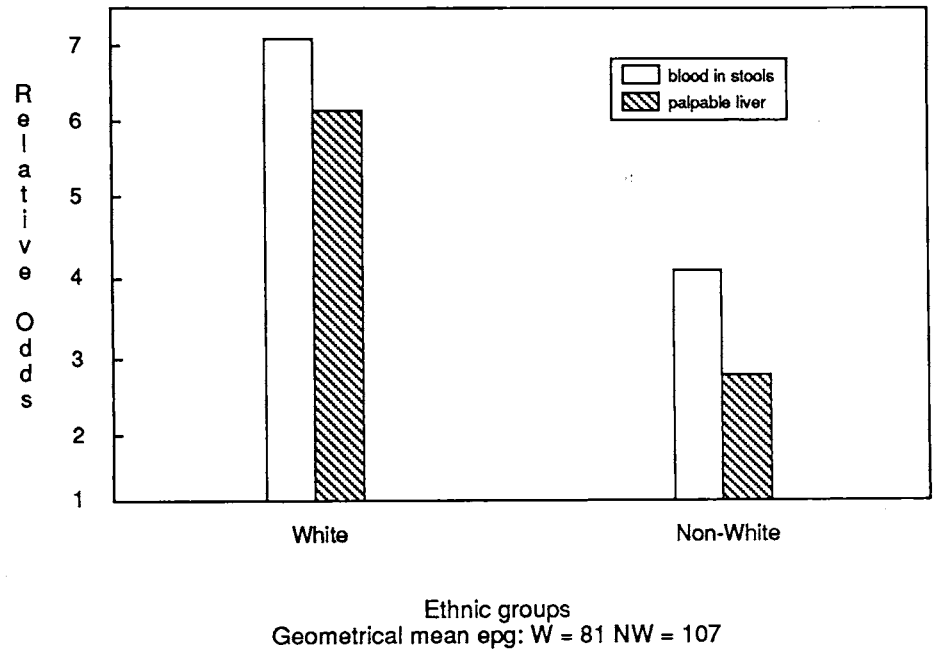

Figure 3 - Blood in stools and palpable liver by ethnic groups

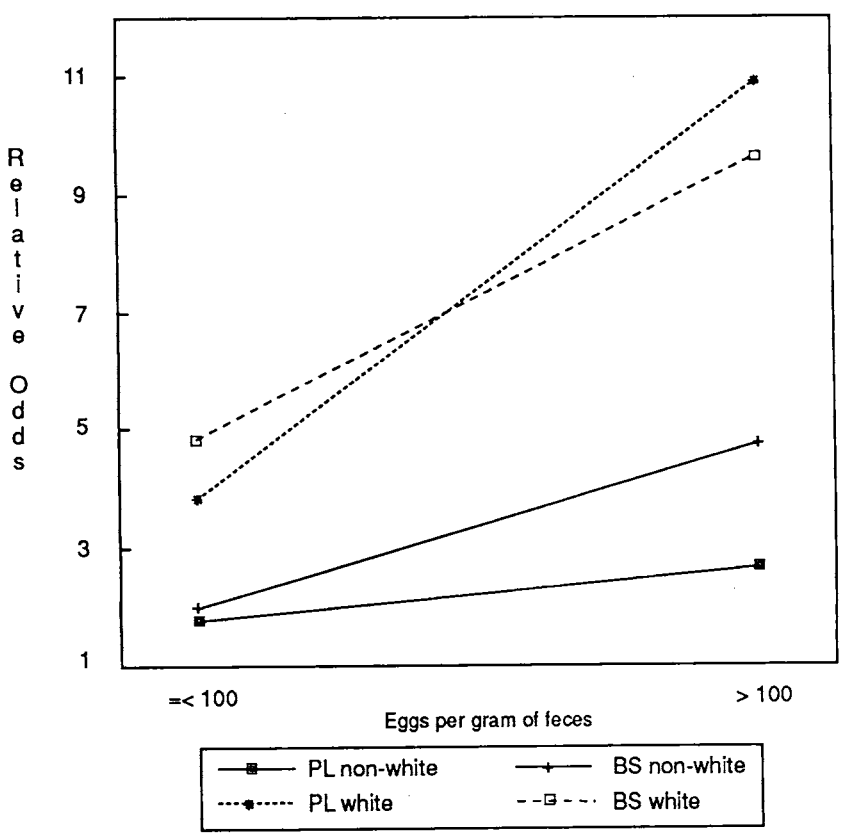

Figure 4 - Blood in stools and palpable liver by ethnic groups according to egg excretion.

increase in the ROs according to egg excretion was the same for both ethnic groups (paral lellines) whereas for the palpable liver model, the increase in the ROs was not equal, suggesting the existence of a positive interaction between ethnic group (whites) and heavier **S. mansoni** infection. Significant trends in the estimated RO were observed for blood in stools among whites ($X^2_{1df} = 15.5$, $p<.001$) and non-whites ($X^2_{1df} = 11.4$, $p<.001$) and for palpable liver among whites ($X^2_{1df} = 13.3$, $p<.001$); among non-whites, the trend in the estimated RO for palpable liver was not significant ($X^2_{1df} = 3.3$, NS). This finding also points out in the direction of an existing interaction between ethnic group (white) and heavier **S.mansoni** infections.

Similar results were obtained using conditional logistic and multivariate logistic methods (matched analysis); the interaction terms with ethnic groups and previous schistosomiasis treatment induced changes in the estimated RO of both blood in stools and palpable liver models, but were not statistically significant.

DISCUSSION

Cases and controls included in this study, because of the selection process used, are likely to represent Ribeirão das Neves' urban population. The reasons why the investigation was restricted to urban inhabitants were (1) the existence of street maps of the central area of Ribeirão das Neves, which allowed the identification of a sampling frame and (2) the presence of a more stable population (only 2.5% of losses due to migration).

As age, sex and length of residence in endemic areas have been shown to be associated to both S. **mansoni** infection and clinical manifestations of schistosomiasis [26, 27], matching on these factors assured control of any possible confounding.

The diagnostic test used to identify S. **mansoni** infected cases is routinely utilized as the technique of choice in Brazil. Although the ideal procedure would be to do stool examinations in two different occasions [19], the possible gain in validity would be diminished by increased costs and decreased compliance. The possible misclassification due to the inclusion of false-negative participants in the control group would have biased the risks estimates towards the null value. The calculated RO may be, therefore, a conservative estimate of the associations detected.

The clinical and physical examinations, as well as the interviews, were carried out blindly by one observer; the laboratory tests were done in a similar manner. Thus, the possibility of a differential misclassification can be ruled out.

In this investigation, nutritional condition seemed not to play an important role in the relationship S. **mansoni** infection-severity of clinical schistosomiasis. None of the methods used in measuring nutritional indicators, either anthropometrical, clinical or laboratorial, although adequate to field work[15], suggested the existence of a different nutritional status between cases and controls. Similar results were obtained ordering cases by egg excretion levels and by clinical disease. Different results, showing an association between nutritional anthropometric indicators with splenomegaly and socioeconomic variables as well as between heavy egg excretion and height were reported from another endemic area in Minas Gerais State[24]. Splenomegaly was practically nonexistent in the present investigation. Socioeconomic variables were not measured, but the restriction to an urban population together with the selection process used, probably guaranteed homogeneity in relation to these factors.

The previous suggestions of different host-parasite relationship according to ethnic groups[1,3,29,35,39,40]. were confirmed in this study (Figure 3). In addition, a differential increase in the risk of clinical schistosomiasis (estimated by palpable liver) was found, after adjusting by the level of egg excretion, among white subjects (Figure 4). The plausibility of this interaction is strengthened by (1) the control of possible confounders in the design and analysis, (2) a "dose-response" effect, (3) the magnitude of the estimated ROs and (4) similar results obtained using either the unmatched bivariate or the conditional (matched) multivariate methods of data analysis. The possible misclassification due to the subjective use of skin color as a surrogate for ethnic groups was shown to be small in Brazil[22]. Until a better understanding of the mechanisms involved in this association - either biological, environmental, sociocultural or behavioral - ethnic group should be regarded as a potential effect modifier and/or confounder in epidemiological and clinical investigations conducted in areas with similar racial composition.

In summary, the results obtained in this investigation have revealed that (1) S. **mansoni** infec-

tion is associated only to blood in stools and palpable liver as its clinical manifestations; (2) the proportions of patients with clinical schistosomiasis increase with the infection's intensity but only 18% of those with heavier infection are classified as having severe disease; (3) the proportion of participants with clinical manifestations and the increase in the risk of developing clinical schistosomiasis according to infection's intensity, differed according to their ethnic characteristics, suggesting the existence of an interaction between whites and heavier **S. mansoni** infection and (4) nutritional indicators, as assessed, did not differ between infected and non-infected subjects, even when adjusting by egg excretion levels and clinical signs and symptoms. These results, conceivably, can be only generalized to areas with similar characteristics (low to moderate endemicity level and morbidity), but nevertheless, these are the characteristics of most of the Brazilian endemic regions.

RESUMO

Epidemiologia da infecção pelo *Schistosoma mansoni* em área de baixa endemicidade no Brasil: Características clínicas e nutricionais

Um estudo epidemiológico seccional, tipo caso-controle, planejado para avaliar o papel desempenhado pelo estado nutricional do paciente na associação entre a intensidade da infecção pelo **Schistosoma mansoni** e as manifestações clínicas da esquistossomose, foi conduzido em uma área do Estado de Minas Gerais que apresentava uma baixa freqüência desta parasitose e uma baixa morbidade devido a esta infecção. Casos (256) foram definidos como participantes que apresentavam exame de fezes positivo para **S. mansoni**, com uma média geométrica de 90 ovos/grama de fezes. Controles (256) foram definidos como uma amostra aleatória dos pacientes com exames de fezes negativo, pareados aos casos por idade, sexo e tempo de residência na área. Os sinais e sintomas clínicos assinalados como associados à infecção pelo **S. mansoni**, comparando-se casos e controles, foram a presença de sangue nas fezes e de fígado palpável. Foi detectada uma tendência linear nas *odds* relativas destes sinais e sintomas e níveis crescentes de infecção. Ajustando pelo número de ovos de **S. mansoni** excretados, a existência de uma interação entre grupo étnico (brancos) e fígado palpável foi sugerida. Não foram encontradas diferenças significativas no estado nutricional dos casos e dos controles.

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