Annals of Tropical Medicine & Parasitology, Vol. 105, No. 6, 413–424 (2011)

Prevalence and epidemiology of intestinal parasitism, as revealed by three distinct techniques in an endemic area in the Brazilian Amazon

J. G. VALVERDE[*,†], A. GOMES-SILVA[*,†], C. J. DE CARVALHO MOREIRA[*,‡], D. LELES DE SOUZA[§,¶], L. H. JAEGER[*,†], P. P. MARTINS[*,#], V. F. MENESES[*,#], M. N. BÓIA[*,$] and F. A. CARVALHO-COSTA[*,&]

[*]*Programa de Pós-graduação em Medicina Tropical, Instituto Oswaldo Cruz — Fiocruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil*
[†]*Laboratório Interdisciplinar de Pesquisas Médicas, Instituto Oswaldo Cruz — Fiocruz, Rio de Janeiro, RJ, Brasil*
[‡]*Laboratório de Doenças Parasitárias, Instituto Oswaldo Cruz — Fiocruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil*
[§]*Departamento de Microbiologia e Parasitologia, Instituto Biomédico, Universidade Federal Fluminense, Macaé e Santos, RJ, Brasil*
[¶]*Laboratório de Paleoparasitologia — Escola Nacional de Saúde Pública, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil*
[#]*Laboratório de Hepatites Virais, Instituto Oswaldo Cruz — Fiocruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil*
[$]*Laboratório de Biologia e Parasitologia de Mamíferos Silvestres Reservatórios, Instituto Oswaldo Cruz — Fiocruz, Rio de Janeiro, RJ, Brasil*
[&]*Laboratório de Sistemática Bioquímica, Instituto Oswaldo Cruz — Fiocruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil*

Received 22 November 2010, Revised 21 July 2011, Accepted 27 July 2011

This survey aims to estimate the prevalence of intestinal parasitic infections in Santa Isabel do Rio Negro, Amazonian Brazil, through three distinct techniques, correlating the prevalence rates with family income and age groups as well as assessing the household clustering of infections. Prevalence rates were assessed through Graham (n=113), Baermann-Moraes (n=232) and Ritchie (n=463) methods. The Graham method was adopted only for children under 5 years old, 15% of whom were positive for *Enterobius vermicularis*. By the Baermann-Moraes technique, 5.6% of the samples were positive for *Strongyloides stercoralis* larvae. The Ritchie technique disclosed the following results: *Ascaris lumbricoides* (26%), *Trichuris trichiura* (22.5%), hookworms (9.5%), *Entamoeba histolytica*/*Entamoeba dispar* (25.3%), *Giardia lamblia* (12.5%) and *E. vermicularis* (0.6%). Children aged 5–14 years presented the highest prevalence for pathogenic parasites. Giardiasis and hookworm infection rates were inversely related to family income. The presence of positive contacts in the same household substantially increased the risk of infection by enteric parasites: odds ratio (OR)=2.70, 95% confidence interval (CI)=1.69–4.29 for ascariasis; OR=2.17, 95% CI=1.34–3.51 for trichuriasis; OR=2.13, 95% CI=1.08–4.17 for hookworm disease; OR=3.42, 95% CI=1.86–6.30 for giardiasis; and OR=2.16, 95% CI=1.35–3.47 for amoebiasis, supporting infection clustering in the home. Intestinal parasitoses are extremely frequent in the studied area, and routine methods for diagnosis may underestimate the prevalence of enterobiasis and strongyloidiasis.

Reprint requests to: F. A. Carvalho-Costa, Av. Brasil 4365, Pav. Leônidas Deane, sala 308, CEP 21045-900 Rio de Janeiro, Brazil.
E-mail: guaratiba@ioc.fiocruz.br.

© W. S. Maney & Son Ltd 2011
DOI: 10.1179/1364859411Y.0000000034

INTRODUCTION

Enteric parasitoses still represent major public health concerns and are associated with a lack of proper sanitation and education (Stephenson *et al.*, 2000; Hotez *et al.*, 2008). In this context, intestinal parasitism is among the many health problems observed in economically disadvantaged populations of developing countries, especially where population growth has not been accompanied by adequate improvements in sanitation and safe water supply (Bóia *et al.*, 2006; Miné and Rosa, 2008). It is estimated that approximately 3.5 billion people are affected by intestinal protozoa and/or helminthes, approximately 450 million of whom manifest symptoms of these infections (Schuster and Chiodini, 2001; Machado *et al.*, 2008). Furthermore, heavily infected individuals represent major sources of infection for the rest of the community (Montresor *et al.*, 2002), as well as also suffering most of the clinical consequences of the parasitism including nutritional and cognitive deficits, malabsorption syndromes and anemia (Stephenson *et al.*, 2000). In addition, acute severe complications like intestinal obstruction/perforations, cholangitis and rectal prolapse (de Silva *et al.*, 1997; Stephenson *et al.*, 2000) may plague individuals with high parasite burdens.

Previous analyses in the Amazon region revealed high prevalence of infection with intestinal parasites, Miranda and colleagues (Miranda *et al.*, 1998) reporting rates of 42.8% for ascariasis, 33.3% for hookworm, 65.0% for amoebiasis and 46.8% for giardiasis among Parakanã Indians. Enteric parasitoses were frequent among Indian peoples living in Iauareté, in the Negro River Basin (Bóia *et al.*, 2009). Agudelo-Lopez and colleagues (Agudelo-Lopez *et al.*, 2008) cited an infection prevalence of 56.0%for *Ascaris lumbricoides*, 53.0% for *Trichuris trichiura* and 54.0% for *Entamoeba histolytica*/*Entamoeba dispar* in rural Colombia. In addition, *Capillaria* spp. eggs have been recovered from stool samples obtained from Amazonian riverine communities (Carvalho-Costa *et al.*, 2009).

The urban heart of Santa Isabel do Rio Negro is the result of a demographic concentration in which hundreds of Amerindian descended families were relocated from riverine communities and Indian villages to the urban center where a sedentary lifestyle resulted. This process was driven by the missionaries throughout the twentieth century and was not accompanied by the implementation of sanitary infrastructure, allowing the emergence and spread of many infectious diseases, such as tuberculosis, malaria and enteric infections. This eco-epidemiological scenario justifies parasitological surveys to assess the epidemiology of intestinal parasitism in the region.

Soil-transmitted helminthiases, amoebiasis and giardiasis have been regarded as neglected and poverty-related conditions. In the present study, the Graham and Baermann-Moraes coproparasitological techniques were applied in order to better characterize the infection rates by *Enterobius vermicularis* and *Strongyloides stercoralis*, respectively. Coproparasitological surveys for strongyloidiasis and enterobiasis probably underestimate the prevalence of these infections, since pinworm eggs remain adhered to perineal skin and *S. stercoralis* larvae are rarely detected through conventional coproparasitological methods (Burkhart and Burkhart, 2005; Olsen *et al.*, 2009). On the other hand, the Ritchie method was carried out due to its ability to detect helminths ova and protozoa cysts.

Therefore, the objectives of this survey were to estimate the prevalence of intestinal parasitic infections in Santa Isabel do Rio Negro, Brazil, through three distinct techniques, correlating these prevalence rates with family income and age groups. Additionally, we aimed to assess the household clustering of infections.

PATIENTS AND METHODS

Setting and Population Studied

Santa Isabel do Rio Negro, situated 781 km from Manaus, the capital of the state of Amazonas (0°28′S and 65° 32′W), is in the midst of the Amazonian Rain Forest in a low-latitude tropical moist climate zone. The urban population of Santa Isabel do Rio Negro descends from Tukano Oriental- and Aruak-speaking pre-Columbian societies that were demographically concentrated, assimilated and educated by missionaries starting around a century ago. The whole municipality of Santa Isabel do Rio Negro encompasses 10 561 inhabitants, 4220 of whom reside in the six-district urban area and 6341 in riverine communities, including the Yanomami Indian Reservation. Sanitary structure is formidably deficient (Bóia *et al.*, 2006).

Study Design

This cross-sectional survey was conducted in July 2008. Urban domiciles were selected at random and visits were accompanied by communitarian health agents. The sample size, calculated upon an estimation of 50.0% for ascariasis, trichuriasis, hookworm, enterobiasis, giardiasis, amoebiasis and strongyloidiasis with a confidence level of 95% for an acceptable error of 10%, was based on 352 subjects for Ritchie and the Baermann-Moraes techniques. By means of systematic random sampling, 200 of the 700 households in the urban area were selected. Identified plastic vials without preservatives were distributed to 1281 people (expecting a ~30% adherence to the study), who were encouraged to return the fecal samples to the local laboratory. We received 463 fecal samples, of which 232 had enough material to allow examination by the Baermann-Moraes method (i.e. for the assessment of strongyloidiasis prevalence, we worked with an acceptable error of 12%). The plastic bottles with the fecal samples were returned to the laboratory within 24 hours after they were given to the subjects during the home visits.

A questionnaire was utilized to gather socioeconomic data in order to classify families into three different categories: no income, monthly income between one and two minimum Brazilian salaries (US$198–396) and monthly income of more than two minimum salaries (greater than US$396). During the interviews and home visits, the Graham method was also performed on children under 5 years of age.

Data were processed with EpiInfo 2000 v. 3.5.1. Rates of positivity for each intestinal parasite were compared within distinct income and age groups with the chi-square test for linear trend. The chi-square test was utilized to compare positivity rates according to the exposure status to an infected homemate, statistical significance defined by $P \leqslant 0.05$.

Laboratory Procedures

Samples were processed through the Ritchie (Ritchie, 1948) and Baermann-Moraes (Moraes, 1948) methods. In order to perform the Ritchie, 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. The Baermann-Moraes technique was performed in glass funnels containing 20 ml of water heated to 45°C, which were connected to 15 ml polypropylene tubes. Approximately 10 g of feces wrapped in gauze was placed in the heated water within the funnels. After 60 minutes, the water was collected, centrifuged (500*g*, 1 minute) and examined through light microscopy. Ritchie smears were stained with Lugol's iodine before reading. Fecal samples not containing enough material to perform both methods were processed only by the Ritchie method.

Additionally, the Graham method was performed on children under 5 years of age. This technique involves a clear adhesive cellulose tape application to the anal area in the early morning prior to defecation and bathing. The adhesive tape is attached to a

TABLE 1. *Prevalence of intestinal parasitoses assessed through the Ritchie method by family income, sex and age groups in Santa Isabel do Rio Negro, Amazonas, Brazil*

		Any geohelminth		Any pathogenic protozoa		*Ascaris lumbricoides*		*Trichuris trichiura*		Hookworms		*Giardia duodenalis*		*Entamoeba dispar/ Entamoeba histolytica*	
Variable	N^c	No.	% (95% CI)	No.	% (95% CI)	No.	% (95% CI)	No.	% (95% CI)	No.	% (95% CI)	No.	% (95% CI)	No.	% (95% CI)
INCOME CATEGORY															
No wage	19	8	42.1 (20.3–66.5)	11	57.9 (33.5–79.7)	6	31.6 (12.6–56.6)	2	10.5 3(1.3–3.1)	3	15.8 (3.4–39.6)	6	31.6 (12.6–56.6)	5	26.3 (9.1–51.2)
US$198–396	331	153	46.2 (40.8–51.8)	186	56.2 (50.7–61.6)	84	25.4 (20.9–30.5)	80	24.2 (19.7–29.2)	36	10.9 (7.8–14.9)	41	12.4 (9.1–16.5)	91	27.5 (22.8–32.7)
>US$396	102	36	35.3 (26.1–45.4)	45	44.1 (34.3–54.3)	29	28.4 (19.9–38.2)	15	14.7 (8.5–23.1)	5	4.9 (1.6–11.1)	8	7.8 (3.4–14.9)	17	16.7 (10–25.3)
P value*			0.109		0.042		0.790		0.250		0.005		0.016		0.049
SEX															
Male	209	93	44.5 (37.6–51.5)	102	48.8 (41.8–55.8)	53	25.4 (19.6–31.8)	48	23 (17.4–29.3)	27	12.9 (8.7–8.2)	29	13.9 (9.5–19.3)	49	23.4 (17.9–29.8)
Female	254	111	43.7 (37.5–50)	148	58.3 (51.9–64.4)	67	26.4 (21.1–32.3)	56	22 (17.1–27.7)	17	6.7 (3.9–10.5)	28	11 (7.5–15.5)	68	26.8 (21.4–32.7)
P value†			0.863		0.066		0.803		0.813		0.023		0.352		0.412
AGE CATEGORY (years)															
<5	68	21	16.9 (10.9–24.5)	28	41.2 (28.4–53.8)	13	19.1 (10.6–30.5)	12	17.6 (9.5–28.8)		0 (0–5.3)	13	19.1 (10.6–30.5)	10	14.7 (7.3–25.4)
5–9	99	50	46.3 (36.7–56.2)	58	58.6 (48.2–68.4)	35	35.4 (26–45.6)	25	25.3 (17.1–35)	4	4 (1.1–10)	19	19.2 (12–28.3)	23	23.2 (15.3–32.8)
10–14	61	34	54 (40.9–66.6)	30	49.2 (36.1–62.3)	20	32.8 (21.3–46)	15	24.6 (14.5–37.3)	7	11.5 (4.7–22.2)	4	6.6 (1.8–15.9)	17	27.9 (17.1–40.8)
15–24	44	25	53.2 (38.1–67.9)	26	59.1 (43.2–73.7)	17	38.6 (24.4–54.5)	19	43.2 (28.3–59)	6	13.6 (5.2–27.4)	3	6.8 (1.4–18.7)	14	31.8 (18.6–47.6)
25–39	85	30	34,1 (24.3–45)	44	51.8 (40.7–62.7)	14	16.5 (9.3–26.1)	12	14.1 (7.5–23.4)	14	16.5 (9.3–26.1)	6	7.1 (2.6–14.7)	20	23.5 (15–34)

TABLE 1. *Continued*

Variable	N^c	Any geohelminth No.	Any geohelminth % (95% CI)	Any pathogenic protozoa No.	Any pathogenic protozoa % (95% CI)	*Ascaris lumbricoides* No.	*Ascaris lumbricoides* % (95% CI)	*Trichuris trichiura* No.	*Trichuris trichiura* % (95% CI)	Hookworms No.	Hookworms % (95% CI)	*Giardia duodenalis* No.	*Giardia duodenalis* % (95% CI)	*Entamoeba dispar*/ *Entamoeba histolytica* No.	*Entamoeba dispar*/ *Entamoeba histolytica* % (95% CI)
40–59	61	28	40 (28.5–52.4)	39	63.9 (50.6–75.8)	13	21.3 (11.9–33.7)	13	21.3 (11.9–33.7)	10	16.7 (8.2–28.1)	9	14.8 (7–26.2)	18	29.5 (18.5–42.6)
>59	45	16	34 (20.9–49.3)	25	55.6 (40–70)	8	17.8 (8–32.1)	8	17.8 (8–32.1)	3	6.7 (1.4–18.3)	3	6.7 (1.4–18.3)	15	33.3 (20–49)
P value*			0.643		0.173		0.068		0.501		0.001		0.017		0.035
TOTAL	463	205	37.1 (33.1–41.3)	250	54 (49.3–58.6)	120	25.9 (22–30.2)	50	23 (17.6–29.2)	44	9.5 (7.1–12.6)	57	12.3 (9.5–15.7)	117	25.3 (21.4–29.5)

N^c = Number of tested samples in each category.
*Chi-square test for linear trend.
†Chi-square test.

microscope slide and read by light microscopy (Graham, 1941; Burkhart and Burkhart, 2005). This adhesive tape test was also performed on children's underwear.

Treatment

Research team physicians prescribed a 3-day course of mebendazole (100 mg twice a day) for intestinal helminthiases, except strongyloidiasis, which was treated with thiabendazole (50 mg/kg/day) twice daily for 3 consecutive days. A 5- or 7-day course of methronidazole (25 mg/kg/day) was adopted for giardiasis and amoebiasis, respectively. Pregnant women were excluded from the survey.

Ethical Approval

Written informed consent was given by parents or relatives. This study was approved by the Evandro Chagas Clinical Research Institute Committee for Ethics on Research (license no. 0011.0.009.000-3).

RESULTS

For the Graham method, performed on children under 5 years of age, out of 113 examinations, 17 (15.0%) were positive for *E. vermicularis* eggs. Regarding anal-tape-positive cases, seven (41.2%) were also positive by the underwear tape test. By the Ritchie method, three positive cases of enterobiasis were detected in subjects more than 5 years old (not shown). Seven children deemed positive for *E. vermicularis* eggs according to Graham smears were also examined by the Ritchie technique, which did not detect these ova.

Four hundred and sixty-three samples, corresponding to 11.3% of the urban population, were processed through Ritchie and 232 (5.6% of the urban population) through the Baermann-Moraes methods. From 463 fecal samples submitted to the Ritchie method, 204 (44.7%) were helminth-positive and 250 (54%) were pathogenic protozoa-positive. Income category, age class and sex-specific prevalence rates for intestinal parasitoses assessed through the Ritchie technique are presented in Table 1. The overall helminth detection rates were: *A. lumbricoides*, 26.0% (n=120), *T. trichiura*, 22.5% (n=104) and hookworm, 9.5% (n=44). For pathogenic protozoa, the detection rates were: *E. histolytica/E. dispar*, 25.3% (n=117) and *G. lamblia*, 12.3% (n=57). Non-pathogenic commensal protozoa detection rates were as follows: *Entamoeba coli*, 41.3% (n=191), *Blastocystis hominis*, 10.2% (n=47), *Iodamoeba butschlii*, 1.5% (n=7), *Endolimax nana*, 1.3% (n=6) and *Chilomastix mesnili*, 0.2% (n=1) (not shown). Through the Ritchie method, 123 persons were poly-parasitized with at least two pathogenic organisms, 88 (29%) were harboring two pathogenic species, 32 (6.9%) had three species and 3 (0.6%) had with four species. Table 2 displays the specific poly-parasitism rates found.

With respect to age distribution, 70.3% of the children 5–9 years old and 70.5% 10–14 years old, were infected with at least one pathogenic organism, while the highest frequency of *A. lumbricoides* and *T. trichiura* was detected in the 15- to 24-year-old age bracket. Giardiasis prevalence exhibited a linear trend to decrease with age (P=0.017), while amoebiasis and hookworm infections tented to increase with age (P=0.035 and P=0.001, respectively) (Table 1).

A statistically significant, inverse relationship between the rate of intestinal parasites and family income was found for individuals harboring either *G. lamblia* (P=0.016), hookworms (P=0.005) or at least one pathogenic parasite (P=0.002) (chi-square test for linear trend).

From 232 stool samples analysed by the Baermann-Moraes method, 5.6% (n= 13) were positive for *S. stercoralis* larvae. Strongyloidiasis was diagnosed in all age strata (median age: 16 years; interquartile range: 6–35 years; range: 3–75 years). Intriguingly, prevalence of strongyloidiasis was significantly higher among male subjects (10.6% versus 1.6%; P=0.004, chi-square test), as among the 13 positive persons, 11 were males. Moreover, strongyloidiasis was not associated with income.

The presence of an infected individual increased significantly the chance that other members of the same household would be infected. This association was observed for all parasite species studied: odds ratio (OR)=2.70, 95% confidence interval (CI)=1.69–4.29 for ascariasis; OR=2.17, 95% CI=1.34–3.51 for trichuriasis; OR=2.13, 95% CI=1.08–4.17 for hookworm infection; OR=3.42, 95% CI=1.86–6.30 for giardiasis; and OR=2.16, 95% CI=1.35–3.47 for amoebiasis.

DISCUSSION

This study demonstrated high prevalence rates for intestinal parasitoses among children living in the urban heart of Santa Isabel do Rio Negro. Among the helminthes, *A. lumbricoides* was detected most frequently, followed by *T. trichiura*. This finding is consistent with previous surveys of the same region (Bóia *et al.*, 2006; Carvalho-Costa *et al.*, 2007; Bóia *et al.*, 2009) and of other Amazonian regions (Crompton, 1988). Herein, potential biases may arise from distinct return rates of the samples with respect to neighborhoods and social strata. However, we believe that these biases were minimized, since the return rate was similar in all districts. Concerning the techniques employed, this survey reinforces the view that the Ritchie method is inefficient for detecting *E. vermicularis* ova and *S. stercoralis* larvae, suggesting that the Graham and the Baermann-Moraes techniques are preferable for estimating the prevalence rates of enterobiasis and strongyloidiasis,

TABLE 2. *Rates of infection with multiple parasites in 463 subjects living in Santa Isabel do Rio Negro, 2008*

	n	%
Number of parasites and species		
TWO PARASITES	88	19
A. lumbricoides + *T. trichiura*	26	5.7
A. lumbricoides + hookworm	3	0.6
A. lumbricoides + *G. lamblia*	3	0.6
A. lumbricoides + *E. histolytica*/*E. dispar*	16	3.2
T. trichiura + hookworm	4	0.8
T. trichiura + *G. lamblia*	5	1.0
T. trichiura + *E. histolytica*/*E. dispar*	12	2.4
Hookworm + *G. lamblia*	…	
Hookworm + *E. histolytica*/*E. dispar*	6	1.2
G. Lamblia + *E. histolytica*/*E. dispar*	13	2.6
THREE PARASITES	32	6.9
A. lumbricoides + *T. trichiura* + hookworm	10	2.0
A. lumbricoides + *T. trichiura* + *E. histolytica*/*E. dispar*	9	1.8
A. lumbricoides + hookworm + *E. histolytica*/*E. dispar*	2	0.4
A. lumbricoides + hookworm + *G. lamblia*	2	0.4
A. lumbricoides + *G. lamblia* + *E. histolytica*/*E. dispar*	4	0.8
A. lumbricoides + *G. lamblia* + *T. trichiura*	1	0.2
G. lamblia + *E. histolytica*/*E. dispar* + hookworm	1	0.2
G. lamblia + *E. histolytica*/*E. dispar* + *T. trichiura*	2	0.4
T. trichiura + *E. histolytica*/*E. dispar* + hookworm	1	0.2
FOUR PARASITES	3	0.6
A. lumbricoides + hookworm + *T. trichiura* + *E. histolytica*/*E. dispar*	2	0.4
A. lumbricoides + *G. lamblia* + *T. trichiura* + *E. histolytica*/*E. dispar*	1	0.2

respectively. Unfortunately, we could not perform Kato–Katz smears in this survey, in order to assess parasite burden in distinct age and income groups. Nevertheless, distinct prevalence rates of intestinal parasitosis might have a relationship with parasite burden in different groups (Guyatt and Bundy, 1991). A massive anti-helminthic administration was carried out in 2003 in Santa Isabel do Rio Negro and was successful in reducing the prevalence of intestinal parasitism found in 2004 compared to that in 2002 (Bóia *et al.*, 2006). Even though, in the present survey, prevalence of many helminthiases considerably increased compared to 2004.

We observed that, while ascariasis and trichuriasis were more frequent among children and young adults, hookworm disease increased significantly with age. Actually, subjects bearing hookworms were significantly older than those infected with *A. lumbricoides* and *T. trichiura* (not shown), which characterizes the distribution of this disease predominantly in adulthood. In addition, hookworm disease prevalence was lower than that of ascariasis and trichuriasis, and the detection rate of hookworm eggs was lower than observed in other settlements in the Rio Negro basin (Bóia *et al.* 2009), suggesting that hookworm transmission may be restricted to a specific eco-epidemiological niche in the studied area. The marked association between hookworm infection and family income corroborates this hypothesis. This should be linked to exposure to the infective stages in soil (larvae that penetrate the skin) during subsistence agriculture activities on farms where open defecation is largely practiced, instead of the fecal–oral route through which ascariasis and trichuriasis are transmitted, subjecting children to greater risk.

The Graham method is considered to be highly sensitive for detecting enterobiasis, and we found a prevalence of 15% among children under 5 years old with this method. Nonetheless, in this study, we could not assess the actual prevalence rate of enterobiasis in adults. Enterobiasis is a common childhood infection, affecting children of less than 10 years old, but, as has been reported, the actual rate of infection in adults is unknown and probably underestimated (Sato *et al.*, 2008). A previous study of older patients in a rural population of the Dominican Republic revealed a 12.5% prevalence of *E. vermicularis* infection using the Graham method (Collins and Edwards, 1981).

The prevalence of *S. stercoralis* in this work (5.6%) was similar to that in the Peruvian Amazon region (Yori *et al.*, 2006), whose population displays similar characteristics to ours. Interestingly, as observed for hookworm disease, strongyloidiasis was significantly more frequent in males. The cycle of *S. stercoralis* can be completed in the soil, leading to long-term environmental persistence of infective stages in endemic areas (Yori *et al.*, 2006). As well as hookworms, *S. stercoralis* larvae penetrate the skin, transmission most likely occurring in a similar epidemiological pattern.

Among protozoa, *E. histolytica*/*E. dispar* was the most frequently detected, mainly in adults. However, the parasitological techniques performed did not permit differentiation between pathogenic and non-pathogenic *Entamoeba* species belonging to this complex. The differentiation of these species can only be accomplished by immunological or molecular techniques. In this context, it has been argued that stool microscopy can over-diagnose amoebiasis, frequently leading to unnecessary treatment with anti-protozoal drugs (Parija *et al.* 2010). It has been demonstrated that many patients actually are infected with *E. dispar*, a commensal species that causes no tissue damage. Recently, however, it has been proposed that some *E. dispar* genotypes might be associated with amoebic liver abscess and intestinal amoebiasis in Mexico and Brazil, respectively (Ximénez *et al.*, 2010).

Giardiasis was diagnosed in 12.3% of the subjects. Among the enteric parasitoses, giardiasis seems to be the most harmful to the nutritional status of infected subjects. Cross-sectional studies aiming to assess the relationship between intestinal parasitism and anthropometric parameters often find a significant association of giardiasis with low height for age (stunting) and low weight for height (wasting), suggesting that *G. lamblia* is an important nutrient depleter (Carvalho-Costa *et al.*, 2007; Matos *et al.*, 2008; Nematian *et al.*, 2008; Botero-Garcés *et al.*, 2009). In addition, we demonstrated that persons harboring *G. lamblia* are significantly younger than those infected with *E. histolytica*/*E. dispar*. This reinforces the medical and epidemiological importance of giardiasis among children in the studied area. We argue that periodical massive deworming in conjunction with vaccination campaigns, performed without awareness of enteric protozooses prevalence rates in distinct areas, can convert giardiasis into the most prevalent enteric parasitosis, control programs of which pose a real challenge to public health policy makers. In consideration, although there have been relatively few new drugs available to treat giardiasis, the second generation of nitroimidazole derivatives, such as secnidazole and tinidazole, has proven to be highly effective in a single dose (Rossignol, 2010). Nevertheless, these drugs are not yet available for free distribution in Brazil, where giardiasis is currently treated with a 5-day course of metronidazole.

As expected, the highest rate of detection of pathogenic parasites occurred in children over 4 years of age and young adults, possibly in the case of children over 4 years old because they are more independent and spend time outside the home, where they are exposed to contaminated soil and water. In addition, they are less aware of the importance of personal hygiene (Montresor *et al.*, 2002). The importance of school-aged children as reservoirs of intestinal parasites has been demonstrated (Sowemimo and Asaolu, 2010). In this context, prevalence and intensity of infections increase with age until adolescence, then decreasing progressively. Our data indicate that prevalence of ascariasis and trichuriasis increases until the 15- to 24-year age group. It is important to note that adolescents and young adults usually are not targeted by deworming campaigns, mainly when the campaigns include vaccination. We argue that these age groups should receive more attention from programs to control intestinal parasites. As discussed, hookworm infection prevalence did not decrease in subjects over 25 years old.

Many factors are involved in the epidemiology of intestinal parasite infections, including environmental determinants and economic issues (WHO, 1981). Our study suggests that a disadvantaged economic situation increases the risk of giardiasis and hookworm disease, since the prevalence of these infections increased significantly among subjects with lower wages. We therefore infer that, in the studied area, at least some factors conducive to intestinal parasite transmission are economic, such as the ability to afford good sanitary conditions. As additional evidence, giardiasis was virtually absent in a district whose inhabitants had more privileged economic conditions (data not shown). The heterogeneous distribution of enteric diseases in distinct income groups has proven that these infections are closely related to socioeconomic circunstances (Quihui *et al.*, 2006; Sowemimo and Asaolu, 2010). Therefore, improving living conditions is probably the main factor in order to achieve sustainable control of intestinal parasitism (Fonseca *et al.*, 2010).

Mass treatment may still be considered as a practical short-term, low-cost measure. However, long-term improvements in sanitation and health education are required for sustained results. Furthermore, it has been proposed that anti-helminthic massive administration should be adopted on a strict regular basis, as a strategic public health intervention (Albonico *et al.*, 2008).

In this study, we demonstrated that enteric parasitic infections are, to some extent, distributed within family clusters, suggesting the importance of domestic and peridomestic environmental contamination with infective stages of the parasites. In this scenario, the absence of adequate latrines and septic tanks in the domiciles leads to the intra-familiar spread of enteric parasites. The subjects of this study are of heavily indigenous ancestry and are still undergoing an urbanization process. The agglomeration of populations without adequate planning for the provision of basic healthcare services generates poverty and the degradation of living conditions, as evidenced by the high rates of parasitic infections, similar results being obtained in other regions (Ugbomoiko *et al.*, 2009).

In conclusion, this survey exhibits and substantiates that intestinal parasitic infections constitute a major public health concern in the Brazilian Amazon. The Graham and Baermann-Moraes techniques, not usually applied in routine coprological surveys and clinical practice, can provide a more complete picture of intestinal parasite epidemiology in field investigations. Some pathogens like *G. lamblia* and *S. stercoralis*, not targeted by mass deworming campaigns, may be considered special challenges. Although the scope of this study was limited geographically, the eco-epidemiological picture described here could be extrapolated to many urban Amazonian communities where a lack of adequate sanitation and safe water supply coexist.

AUTHORS' CONTRIBUTIONS

FACC and MNB designed the study protocol, carried out the clinical assessment and prescribed treatment. JGV and FACC drafted the manuscript. All authors contributed to and read and approved the final manuscript. A demographic and socioeconomic questionnaire was applied by DLS as well as by students of the Tropical Medicine Post-graduation Program at Instituto Oswaldo Cruz/Fiocruz (JGV, AGS, CJCM, LHJ, VFM and PPM) as part of the field activities of the discipline Applied Epidemiology. These students also performed the laboratory procedures, as well as analysis and interpretation of data. JGV and FACC are guarantors of the paper.

FUNDING

The study on which this report is based was supported with federal funds from the Brazilian Federal Agency for Support and Evaluation of Graduate Education (CAPES) and the National Council for Scientific and Technological Development (CNPq). DLS, JGV, AGS, LHJ, VFM and PPM are recipients of scholarships from these institutions.

CONFLICTS OF INTEREST

None declared.

ACKNOWLEDGEMENTS. We would like to thank the people of Santa Isabel do Rio Negro, the communitarian health officials, the municipality's administrative staff for lodging the research team and Dr. Lindsay Carpp for critical review of the manuscript.

REFERENCES

*Agudelo-Lopez, S., Gómez-Rodríguez, L., Coronado, X., Orozco, A., Valencia-Gutierrez, C. A., Restrepo-Betancur, L. F., Galvis-Gómez, L. A. & Botero-Palacio, L. E. (2008). Prevalence of intestinal parasitism and associated factors in a village on the Colombian Atlantic Coast. *Revista de Salud Publica*, **10**, 633–642.

Albonico, M., Allen, H., Chitsulo, L., Engels, D., Gabrielli, A. F. & Savioli, L. (2008). Controlling soil-transmitted helminthiasis in pre-school-age children through preventive chemotherapy. *PLoS Neglected Tropical Diseases*, **2**, e126.

Bóia, M. N., Carvalho-Costa, F. A., Sodré, F. C., Eyer-Silva, W. A., Lamas, C. C., Lyra, M. R., Pinto-Junior, V. L., Cantalice Filho, J. P., Oliveira, A. L., Carvalho, L. M., Gross, J. B., Sousa, A. L., Moraes,

T. I., Bermudez-Aza, E. H., Martins, E. B. & Coura, J. R. (2006). Mass treatment for intestinal helminthiasis control in an Amazonian endemic area in Brazil. *Revista do Instituto de Medicina Tropical de São Paulo*, **48**, 189–195.

Bóia, M. N., Carvalho-Costa, F. A., Sodré, F. C., Porras-Pedroza, B. E., Faria, E. C., Magalhães, G. A. & da Silva, I. M. (2009). Tuberculosis and intestinal parasitism among indigenous people in the Brazilian Amazon region. *Revista de Saúde Pública*, **43**, 176–178.

Botero-Garcés, J. H., García-Montoya, G. M., Grisales-Patiño, D., Aguirre-Acevedo, D. C. & Alvarez-Uribe, M. C. (2009). Giardia intestinalis and nutritional status in children participating in the complementary nutrition program, Antioquia, Colombia, May to October 2006. *Revista do Instituto de Medicina Tropical de São Paulo*, **51**, 155–162.

Burkhart, C. N. & Burkhart, C. G. (2005). Assessment of frequency, transmission, and genitourinary complications of enterobiasis (pinworms). *International Journal of Dermatology*, **44**, 837–840.

Carvalho-Costa, F. A., Gonçalves, A. Q., Lassance, S. L., Silva Neto, L. M., Salmazo, C. A. & Bóia, M. N. (2007). *Giardia lamblia* and other intestinal parasitic infections and their relationships with nutritional status in children in Brazilian Amazon. *Revista do Instituto de Medicina Tropical de São Paulo*, **49**, 147–153.

Carvalho-Costa, F. A., Silva, A. G., de Souza, A. H., Moreira, C. J., de Souza, D. L., Valverde, J. G., Jaeger, L. H., Martins, P. P., de Meneses, V. F., Araújo, A. & Bóia, M. N. (2009). Pseudoparasitism by *Calodium hepaticum* (syn. Capillaria hepatica; Hepaticola hepatica) in the Negro River, Brazilian Amazon. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **103**, 1071–1073.

Collins, R. F. & Edwards, L. D. (1981). Prevalence of intestinal helminths and protozoans in a rural population segment of the Dominican Republic. *Transactions of the Royal Society of Tropical Medicine*, **75**, 549–551.

Crompton, D. W. (1988). The prevalence of Ascariasis. *Parasitology Today*, **4**, 162–169.

de Silva, N. R., Guyatt, H. L. & Bundy, D. A. (1997). Morbidity and mortality due to Ascaris-induced intestinal obstruction. *Transactions of the Royal Society of Tropical Medicine*, **91**, 31–36.

Fonseca, E. O., Teixeira, M. G., Barreto, M. L., Carmo, E. H. & Costa Mda, C. (2010). [Prevalence and factors associated with geohelminth infections in children living in municipalities with low HDI in North and Northeast Brazil]. *Cadernos de Saúde Pública*, **26**, 143–152.

Graham, C. F. (1941). A device for the diagnosis of *Enterobious* infection. *American Journal of Tropical Medicine and Hygiene*, **21**, 159–161.

Guyatt, H. L & Bundy, D. A. (1991). Estimating prevalence of community morbidity due to intestinal helminths: prevalence of infection as an indicator of the prevalence of disease. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **85**, 778–782.

Hotez, P. J., Bethony, J. M., King, C. H., Pearce, E. J., & Jacobson, J., (2008). Helminth infections: the great neglected tropical diseases. *Journal of Clinical Investigation*, **118**, 1311–1321.

Machado, E. R., Santos, D. S. & Costa-Cruz, J. M. (2008). Enteroparasites and commensals among children in four peripheral districts of Uberlândia, State of Minas Gerais. *Revista da Sociedade Brasileira de Medicina Tropical*, **41**, 581–585.

Matos, S. M., Assis, A. M., da Silva Prado, M., Strina, A., dos Santos, L. A., de Jesus, S. R. & Barreto, M. L. (2008). *Giardia duodenalis* infection and anthropometric status in preschoolers in Salvador, Bahia State, Brazil. *Cadernos de Saúde Pública*, **24**, 1527–1535.

Miné, J. C. & Rosa, J. A. (2008). Frequency of *Blastocystis hominis* and other intestinal parasites in stool samples examined at the Parasitology Laboratory of the School of Pharmaceutical Sciences at the São Paulo State University, Araraquara. *Revista da Sociedade Brasileira de Medicina Tropical*, **41**, 565–569.

Miranda, R. A., Xavier, F. B. & Menezes, R. C. (1998). Intestinal parasitism in a Parakanã indigenous community in southwestern Pará State, Brazil. *Cadernos de Saúde Pública*, **14**, 507–511.

Montresor, A., Crompton, D. W. T., Gyorkos, T. W. & Savioli, L. (2002). *Helminth control in school-age children*. Available at: http://www.who.int/wormcontrol/documents/helminth_control/en/ [accessed 29 January 2009].

Moraes, R. G. (1948). Contribuição para o estudo do *Strongyloides stercoralis* e da estrongiloidíase no Brasil. *Revista do Serviço de Saúde Pública*, **1**, 507–624.

*Nematian, J., Gholamrezanezhad, A. & Nematian, E. (2008). Giardiasis and other intestinal parasitic infections in relation to anthropometric indicators of malnutrition: a large, population-based survey of schoolchildren in Tehran. *Annals of Tropical Medicine and Parasitology*, **3**, 209–214.

Olsen, A., van Lieshout, L., Marti, H., Polderman, T., Polman, K., Steinmann, P., Stothard, R., Thybo, S., Verweij, J. J. & Magnussen, P. (2009). Strongyloidiasis — the most neglected of the neglected tropical diseases? *Transactions of the Royal Society of Tropical Medicine*, **103**, 967–972.

Parija, S. C., Garg, A., Pushpa, K., Khairnar, K. & Priya, T. (2010). Polymerase chain reaction confirmation of diagnosis of intestinal amebiasis in Puducherry. *Indian Journal of Gastroenterology*, **29**, 140–142.

Quihui, L., Valencia, M. E., Crompton, D. W., Phillips, S., Hagan, P., Morales, G. & Díaz-Camacho, S. P. (2006). Role of the employment status and education of mothers in the prevalence of intestinal parasitic infections in Mexican rural schoolchildren. *BMC Public Health*, **6**, 225.

Ritchie, L. S. (1948). An ether sedimentation technique for routine stool examinations. *Bulletin of the United States Army Medical Department*, **8**, 326.

Rossignol, J. F. (2010). *Cryptosporidium* and *Giardia*: treatment options and prospects for new drugs. *Experimental Parasitology*, **124**, 45–53.

Sato, M., Sanguankiat, S., Pubampen, S. & Kusolsuk, T. (2008). Enterobiasis: a neglected infection in adults. *Southeast Asian Journal of Tropical Medicine and Public Health*, **39**, 213–216.

Schuster, H. & Chiodini, P. L. (2001). Parasitic infections of the intestine. *Current Opinion in Infectious Diseases*, **14**, 587–591.

Sowemimo, O. A. & Asaolu, S. O. (2010). Current status of soil-transmitted helminthiases among pre-school and school-aged children from Ile-Ife, Osun State, Nigeria. *Journal of Helminthology*, **2**, 1–5.

Stephenson, L. S., Latham, M. C. & Ottesen, E. A. (2000). Malnutrition and parasitic helminth infections. *Parasitology*, **121**, 223–238.

Ugbomoiko, U. S., Dalumo, V., Ofoezie, I. E. & Obiezue, R. N. (2009). Socio-environmental factors and ascariasis infection among school-aged children in Ilobu, Osun State, Nigeria. *Transactions of the Royal Society of Tropical Medicine*, **103**, 223–208.

WHO. (1981). *Intestinal Protozoan and Helminthic Infections*. pp. 1–154. Geneva: WHO.

Ximénez, C., Cerritos, R., Rojas, L., Dolabella, S., Morán, P., Shibayama, M., González, E., Valadez, A., Hernández, E., Valenzuela, O., Limón, A., Partida, O. & Silva, E. F. (2010). Human amebiasis: breaking the paradigm? *International Journal of Environmental Research in Public Health*, 7, 1105–1120.

Yori, P. P., Kosek, M., Gilman, R. H., Cordova, J., Bern, C., Chavez, C. B., Olortegui, M. P., Montalvan, C., Sanchez, G. M., Worthen, B., Worthen, J., Leung, F. & Oré, C. V. (2006). Seroepidemiology of strongyloidiasis in the Peruvian Amazon. *American Journal of Tropical Medicine and Hygiene*, **74**, 97–102.