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# Prevalence of Intestinal Parasites Among Children 13-And-Under Residents in the Indigenous Land of Xakriabá, Brazil

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## Abstract

**Introduction:** This study reports the prevalence of intestinal parasite infections in Xakriabá indigenous children living in the southeastern region of Brazil.

**Methodology:** Stool samples of 2973 children 13-and-under were analyzed. Associations between environment factors and parasites were evaluated by multivariate Poisson regression model.

**Results:** The prevalence of protozoa was high (*Entamoeba histolytica/dispar* 16.9%; *Giardia duodenalis* 18.4%), whereas that of helminthes was low (*Schistosoma mansoni* 1.2%; *Hymenolepis nana* 2.2%; hookworm 3.7%; *Strongiloydes stercoralis* 0.4%; *Ascaris lumbricoides* 0.4%; *Enterobius vermicularis* 1%). The risk of protozoan infection increased if the house was located in clay and rocky terrain; it decreased with increasing education of the mother and the presence of toilet in the house. Sex, clay-and-rocky terrain, dry-sump and toilet in the house were associated with prevalence of helminthes. Conclusion: The high prevalence of protozoa infection suggests contamination of water sources with feces, probably due to deficient sanitation and poor hygiene.

**Keywords:** Indigenous population; Parasite diseases; Prevalence

## Introduction

Indigenous population count in Brazil increased from 294131 in 1991 to 817963 people in 2010 due to the criteria of self-declaration of ethnicity adopted by the Brazilian Institute of Geography and Statistics - IBGE for the demographic census [1]. of the 48720 self-declared indigenous in Minas Gerais state in 2000, 8880 live in one of the seven Demarcated Indigenous Lands (DIL). The Xakriabá comprise 85% of total indigenous population in the state. They live in the semiarid region by the river São Francisco, in the municipality of São João das Missões, north of the state [2]. Demographic distribution in the DIL is determined by natural water availability, which can be scarce and often subject to exhaustion of the reservoirs [3], forcing the use of rainwater usually stored for very long periods.

The recent history of the indigenous populations in Brazil has been marked by rapid socioeconomic and environmental changes, with consequences to public health. When compared to the general population, health indicators of indigenous populations tend to be worse, despite improvements observed in the 10 years following the foundation of the health subsystem dedicated to indigenous populations [4]. Childhood health indicators reveal high prevalence of height-for-age deficit, anemia, high frequency of hospitalization during the first year and diarrhea during the first week of life [5].

It has been suggested that the epidemiology of intestinal parasites among indigenous populations depends on factors such as climate, socio-economical, educational and sanitation [6]. Those characteristics determine a range of health conditions, including chronic diarrhea and malnutrition, especially in younger ages [5]. A major obstacle for the development of appropriate health policies and responsive healthcare delivery is the lack of population specific data. Despite the size of the indigenous population in Minas Gerais and its importance in the national nosology scenario [1,3], little is known about the epidemiology of intestinal parasites in this population. The results reported here might help to reduce parasitic infections in children living in the studied area. The main objective of this study is to determine the prevalence of intestinal parasites and the risk factors related to protozoan and helminth infection in children 13-and-under living in the Demarcated Indigenous Land of Xakriabá (DILX).

## Materials and Methods

### Sample and Study Design

A general overview of the Indigenous Population of Xakriabá (ILX) living conditions is presented. Sanitation, housing, water sources, education, income, economic activity and parasite infections are discussed. The study took place in the DILX in São João das Missões-MG, Brazil, in the micro-region of Peruaçu valley, part of the São Francisco river watershed (Figure 1). The study population was 6854 people, living in 1250 houses, distributed in 52 villages, served by five Basic Healthcare Units (BHU): Brejo Mata Fome, Sumaré, Itapicuru, Pindaíbas and Rancharia.

A cross-sectional study was performed in 2009. All children 13-and-under were invited to participate (N=2973). TF-Test kits were used for stool sampling. The adults responsible for the participating children received instructions for triple sample collection, one sample a day on alternate days, using one new tube for each sample. TFT offers a reliable estimate of the prevalence of protozoan infection, and exhibits higher or comparable positivity rates compared to Kato-Katz (KK), Hoffman-Pons-Janer (HPJ), and Willis techniques for helminth detection [7].

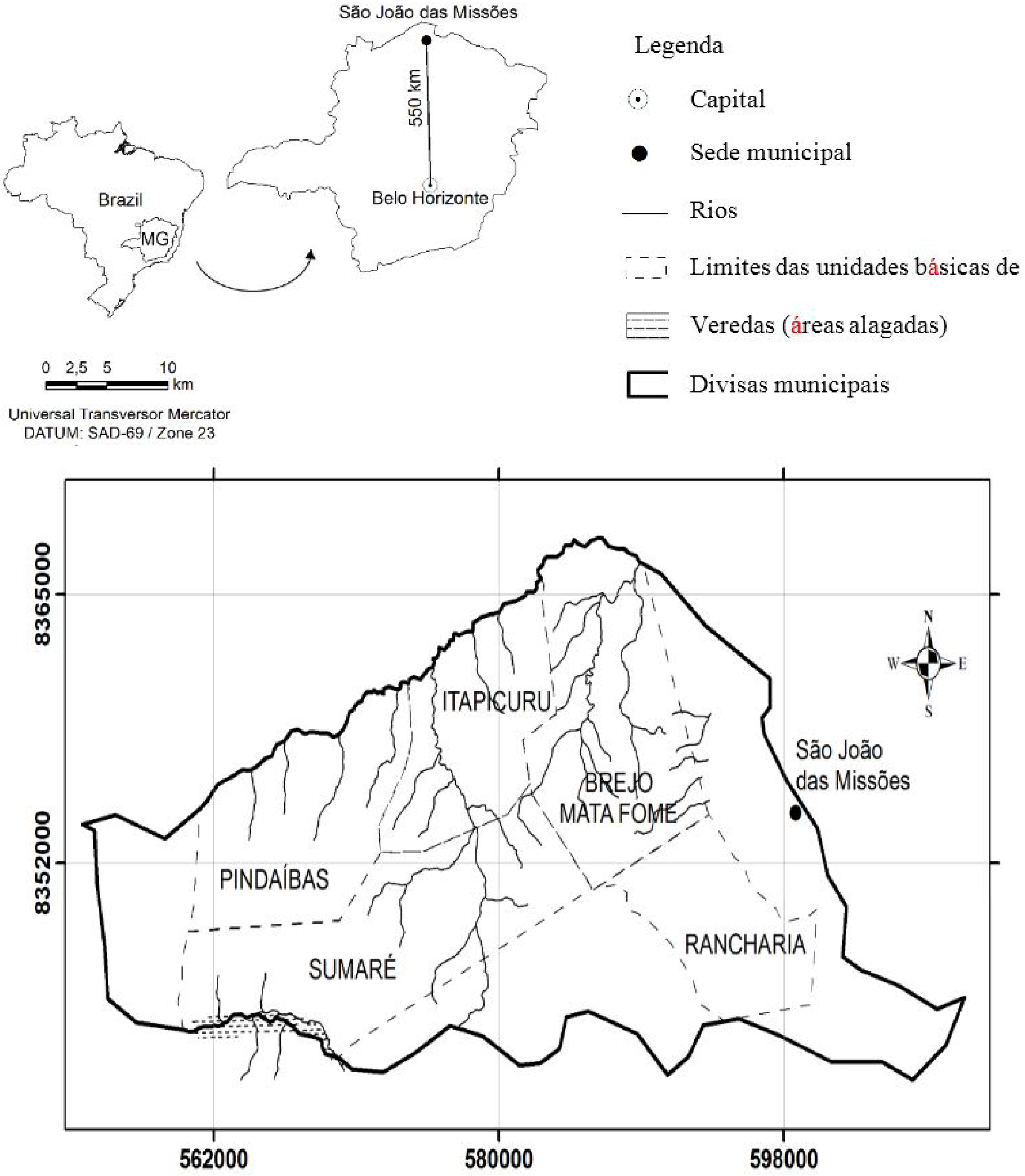

**Figure 1:** Geographic location and the basic healthcare units' coverage in the Xakriabá indigenous land in the north of Minas Gerais state, Brazil.

## Parasitological Techniques

All samples were processed following TFT kit manufacturer instructions. A TFT kit includes three collector tubes containing one small scoop and neutral buffered formalin (patient kit), and a conical flask with a double-filtering system composed of one 400μm and one 200μm meshes for parasite concentration by centrifugation (laboratory kit). The scoop is calibrated to collect approximately 1g of feces. The TFT kit was developed to process simultaneously three fecal samples collected on alternate days. Approximately 1g of feces was collected and added to each of the three tubes. The tube was vigorously agitated for homogenization. Each tube received a drop of neutral detergent plus 3ml ethyl acetate and was attached to the lid of the conical flask in order to transfer the content of the tubes by inversion. The flask was centrifuged for 1min at 1500rpm. The supernatants were discarded and the sediments were homogenized with normal saline solution. For each sample, one drop of the homogenized material was transferred to a microscope slide containing one drop of Lugol's solution. Slides were subsequently examined in an optical microscope.

## Data

Interviewers were trained to collect socio-demographic, housing and environment information, using a pre-tested and standardized form of questionnaire. Variables recorded were sex, weight, height, age, level of education of the mother, and residence. Housing characteristics such as agglomeration, electric power, type if roof (straw or canvas), type of wall (adobe or mud hand), type of floor, availability of kitchen, toilet, water source, drinking water filtered or boiled were also recorded. The variables related to the environment around the villages and BHU were main type of terrain and forestation. Severe or moderate chronic and acute protein-energy malnutrition in children 9-and-under was defined by the height-to-age index and weight-to-age index, respectively, using the Z-score criteria, with a cut-off of-2 [8], according to the Multicentre Growth Reference Study (MGRS) of the World Health Organization [9]. Anthro Plus software was used to generate anthropometric indexes.

## Statistical Analysis

Prevalence of intestinal parasites was estimated by different demographic and environmental factors. Chi-square tests explored associations between the prevalence of intestinal parasites (pathogenic protozoa and helminth) with sex, age and Basic Health Unit. A two-stage analysis statistical modelling was used to further explore the association of intestinal parasites with potential socio-demographic, housing and environmental risk factors. The first stage was used to filter out irrelevant variables. The model built in the second stage allowed a formal description of a functional relation between the variables screened for relevance in the first stage and the outcome. At the screening stage, associations of parasites with seventeen potential risk factors were examined with the use of univariate random intercept Poisson regression model with robust variance [10] set to account for the degree of clustering of individuals within twenty-five villages and four BHU [11]. Association of potential risk factors with parasite infection showing a p-value lower than 0.20 in the univariate analysis (screening stage) were selected for inclusion in a multivariate random intercept Poisson regression model with robust variance (model building stage) [12]. All statistical analyses were performed with STATA 12 (Stata Corporation, College Station, Texas USA).

## Ethics Approval

The present work was approved by the local indigenous leadership, the local council of the Main Health Unit of São João das Missões, the Ethics on Research Board of the Universidade Federal de Ouro Preto (Statement nº 2005/58), the National Ethics on Research Board (Statement nº 902/2006, Register 12827), and the National Indigene Foundation (Statement nº 323/CGEP/06). Written informed consent was collected from the adult responsible for each child.

# Results

Distribution according to age was as follows: 216 (7.1%) under 1; 1168 (38.5%) 1 to 5 y-o; 999 (33.0%) 6 to 9 y-o and 647 (21.3%) 10-12 y-o. There were 1546 (51%) boys and 1484 (49%) girls.

Stools samples were provided by 2106 children and 1066 (50.6%) were positive. Geographical distribution of parasite positivity is shown in (Figure 2). Itapicuru had the highest prevalence (57.1%), and Pindaíbas, the lowest (44.5%) (Table 1). Prevalence of infection by pathogenic protozoa (*Entamoeba histolytica/ dispar* and *Giardia duodenalis*) (32.5%) was significantly higher compared to helminthes (7.7%). Prevalence of multiple parasite infection was very low, 0.5% for helminthes and 3.0% for co-infection with *E.histolytica/E. dispar* and *G. duodenalis*. Brejo Mata Fome had the highest prevalence of multiple infection for pathogenic protozoa (4.4%); however, infection with one single protozoa was more frequent in Itapicuru (34.2%).

| Group of parasites | Result | Basic health unit | | | | | Total N=2106 (%) | P value |
|---|---|---|---|---|---|---|---|---|
| | | Brejo Mata Fome N=773 (%) | Itapicuru N=424 (%) | Sumaré N=559 (%) | Pindaíba N=137 (%) | Rancharia N=213 (%) | | |
| **General** | Positive | 354 (45.8) | 242 (57.1) | 309 (55.3) | 61 (44.5) | 100(46.9) | 1066(50.6) | < 0.001 |
| **Pathogenic protozoa** | Negative | 536 (69.3) | 262 (61.8) | 369 (66.0) | 94 (68.6) | 159 (74.6) | 1420 (67.4) | 0.001 |
| | Mono parasitism | 203 (26.3) | 145 (34.2) | 180 (32.2) | 41 (29.9) | 53 (24.9) | 622 (29.5) | |
| | Poliparasitism | 34 (4.4) | 17 (4.0) | 10 (1.8) | 2 (1.5) | 1 (0.5) | 64 (3.0) | |
| **Helminths** | Negative | 731 (94.6) | 400 (94.3) | 487 (87.1) | 131 (95.6) | 196 (92.0) | 1945 (92.4) | < 0.001 |
| | Mono parasitism | 41 (5.3) | 24 (5.7) | 63 (11.3) | 6 (4.4) | 17 (8.0) | 151 (7.2) | |
| | Poliparasitism | 1 (0.1) | 0 (0.0) | 9 (1.6) | 0 (0.0) | 0 (0.0) | 10 (0.5) | |

**Table 1:** Prevalence of pathogenic protozoa and helminths in children under 13, according to basic health unit in the Xakriabá indigenous land, São João das Missões, Minas Gerais state, Brazil.

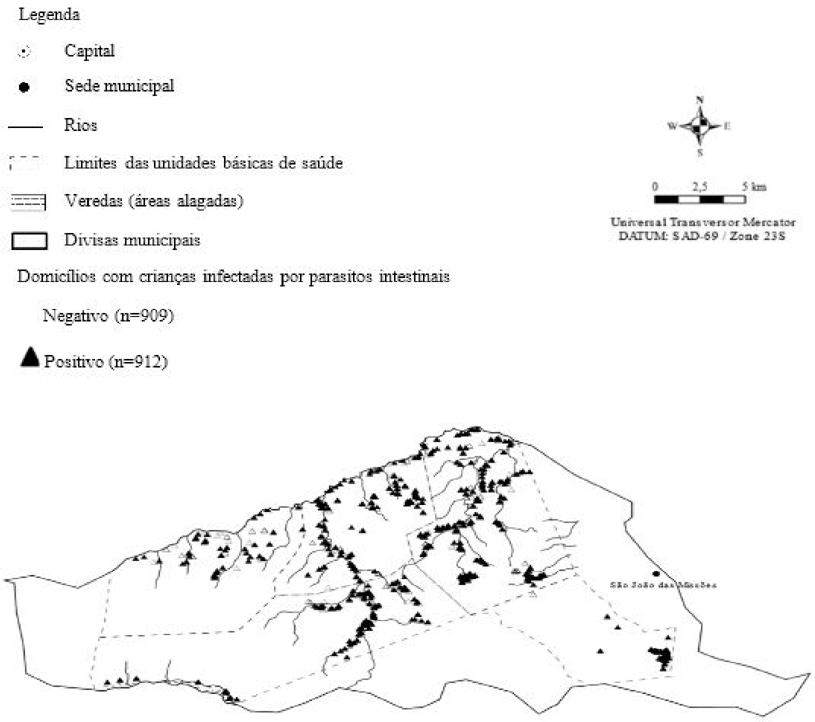

**Figure 2:** Distribution of parasites in the basic healthcare units' coverage in the Xakriabá indigenous land in the north of Minas Gerais state, Brazil.

The highest prevalence ($p<0.001$) by age was in the 6-9 y-o group (Table 2). The prevalence of both pathogenic protozoa and helminthes increased with age, except for *G. duodenalis* that predominated in the 1-5 y-o group. *Entamoeba coli*, a nonpathogenic protozoon, was the most prevalent with 30% overall positivity.

| Parasite | Age group (years) | | | | |
|---|---|---|---|---|---|
| | <1[a] | 1-5[a] | 6-9[b] | 10-12[b] | Total |
| | N=142 | N=821 | N=727 | N=416 | N=2106 |
| General parasites | 12 (8.5) | 392 (47.7) | 410 (56.4) | 252 (60.6) | 1066 (50.6) |
| *Entamoeba histolytica / E. dispar* | 1 (0.7) | 98 (11.9) | 163 (22.4) | 91 (21.9) | 353 (16.8) |
| *Giardia duodenalis* | 7 (4.9) | 181 (22.0) | 136 (18.7) | 73 (17.5) | 397 (18.9) |
| *Entamoeba coli* | 4 (2.8) | 198 (24.1) | 275 (37.8) | 155 (37.3) | 632 (30.0) |
| *Iodamoeba butschlii* | 0 (0.0) | 9 (1.1) | 14 (1.9) | 12 (2.9) | 35 (1.7) |
| *Endolimax nana* | 1 (0.7) | 24 (2.9) | 30 (4.1) | 25 (6.0) | 80 (3.8) |
| *Taenia* sp | 0 (0.0) | 0 (0.0) | 2 (0.3) | 1 (0.2) | 3 (0.1) |
| *Hymenolepis nana* | 0 (0.0) | 17 (2.1) | 18 (2.5) | 10 (2.4) | 45 (2.1) |
| *Schistosoma mansoni* | 0 (0.0) | 3 (0.4) | 4 (0.6) | 5 (1.2) | 12 (0.6) |
| *Ascaris lumbricoides* | 0 (0.0) | 2 (0.2) | 6 (0.8) | 1 (0.2) | 9 (0.4) |
| *Trichuris trichuria* | 0 (0.0) | 0 (0.0) | 1 (0.1) | 0 (0.0) | 1 (0.05) |
| Hookworm | 0 (0.0) | 18 (2.2) | 31 (4.3) | 26 (6.3) | 75 (3.6) |
| *Strongiloydes stercoralis* | 0 (0.0) | 3 (0.4) | 5 (0.7) | 0 (0.0) | 8 (0.4) |
| *Enterobius vermicularis* | 0 (0.0) | 3 (0.4) | 13 (1.8) | 6 (1.4) | 22 (1.0) |
| a,b: the letters indicate the age groups merged for analysis | | | | | |

**Table 2:** Prevalence (%) of intestinal parasites by age group, in the Xakriabá indigenous land, São João das Missões, Minas Gerais state, Brazil.

There was evidence that parasite infection increased with age (data not show). A statistically significant negative association of intestinal parasites with the number of years that mothers attended school was also observed. Positivity for helminthes was twice as frequent in malnourished children defined by weight-for-age index compared to eutrophic children. Pathogenic protozoa were more frequent in more populated houses. Children and adolescents living in villages with a sandy terrain had higher positivity for helminthes compared to those living in villages with clay or rocky terrain. Protozoa infection occurred more frequently in houses with straw or canvas roof, dirty floor, dry sump or outdoor toilet. No association was detected between helminthes infection and the housing variables (all p-values>0.05) or between sex and intestinal parasites (data not show).

Multivariate analysis found negative association of protozoan infection with the number of years the mother had attended school. It found positive association with living in villages with clay and rocky terrain, and with the presence of sanitary toilet in the house. Prevalence of helminthes was approximately 52% higher in boys compared to girls ($p=0.016$), 0.41 times lower in villages with clay terrain ($p=0.002$) and was associated with houses without sanitary toilet or dry sump. Similar results were observed when Health Polo Base was considered as level 2 unit in the analysis (Table 3).

| | **Helminth** | | | | **Protozoan** | | | |
|---|---|---|---|---|---|---|---|---|
| | **PRR*** | **95% LCI** | **95% UCI** | **p-value** | **PRR*** | **95% LCI** | **95% UCI** | **p-value** |
| **Sex** | 0.47 | 0.49 | 1.55 | <0.001 | 1.02 | 0.89 | 1.17 | 0.767 |
| **Age group (6-12 years)** | 0.87 | 0.15 | 1.08 | 0.636 | 1.01 | 0.82 | 1.24 | 0.913 |
| **Mother schooling** | 0.40 | 0.56 | 2.07 | 0.072 | 0.74 | 0.64 | 0.86 | <0.001 |
| **Agglomeration** | | | | | 1.03 | 0.77 | 1.39 | 0.831 |
| **Argillous soil** | 0.54 | 0.42 | 1.17 | <0.001 | 0.93 | 0.83 | 1.05 | 0.233 |
| **Argillous and rocky soil** | | | | | 1.25 | 1.14 | 1.36 | <0.001 |
| **Sanitary toilet in the peridomiciliary** | | | | | 0.87 | 0.79 | 0.95 | 0.002 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Dirt floor** | | | | | 1.04 | 0.94 | 1.14 | 0.458 |
| **Dry sump in the household** | 0.70 | 0.96 | 2.15 | 0.169 | 1.07 | 0.73 | 1.57 | 0.716 |
| **Sanitary toilet in the peridomiciliary** | 1.43 | 0.97 | 1.00 | 0.08 | 1.00 | 0.82 | 1.22 | 0.994 |
| **% Households without sanitary toilet** | 0.98 | 0.97 | 1.00 | 0.015 | 0.98 | 0.95 | 1.03 | 0.255 |

*Prevalence Risk Ratios (PRR) and 95% confidence intervals are estimated with the use of a multivariate random intercept Poisson regression model with robust variance.

**Table 3:** Effect of potential risk factors on intestinal parasites among indigenous children under 13 years old in São João das Missões, Minas Gerais state, Brazil.

## Discussion

The Xakriabá indigenous children have high prevalence of intestinal protozoa infection and low prevalence of helminthes. Dirty floor, dry sump and houses supplied with untreated natural surface water were significantly associated with higher prevalence of protozoan. Clay and rocky terrain and male sex were associated with high prevalence of helminthes.

Factors related to the environment and housing conditions, the individual, the parasites, and local health policies could explain the high prevalence of protozoa. Poor hygienic habits of children, living in houses with dirty floor and dry sump that allow the proliferation of germs [13]; the long survival of cysts in the environment [14]; the use of pluvial water stored for long periods for home supply; and the non-adherence to clinical protocols for anti-protozoa treatment due to the high toxicity related to the drugs commonly available in the health services could be listed. The high prevalence of protozoa is associated with the fecal-oral mode of transmission. The host eliminates infective cystic forms in the feces, allowing interpersonal contamination even in good environmental sanitary conditions [15].

Infection with *E. histolytica/E. dispar* is distributed worldwide, closely related to poor sanitation and unsafe water supply. The agent usually lives in the human intestine, where it causes dysentery, colitis and enterocolitis. Haematogenous dissemination may occur and affect organs such as the liver and brain, causing inflammation and abscesses [16].

Infection by *G. duodenalis* must not be neglected. It may cause diarrhea, malabsorption of nutrients, vitamins, folate, iron and zinc, and hinder children development [16]. Cysts of *G. duodenalis* remain viable in wet environment for three months, and are resistant to treatment with chloride [14]. Giardiasis is common in children under 10, both in developed and developing countries. The prevalence in Xakriabá children was 18.9%. The high infectivity rates can be worrisome, since one single infected subject can disseminate the parasite in the domestic environment, allowing the agent to spread outside the domicile [14]. The prevalence of *E. coli* was high in the DILX and can be explained by interpersonal contamination with infecting cysts. It has been considered a human intestinal commensal like *Endolimax nana* and *Iodamoeba butschlii*, but it is currently accepted as a parasite of variable pathogenicity. Depending on the intensity of infection, it can be associated with diarrhea in children [15]. Nonetheless, despite the low pathogenicity the three agents are considered indicators of poor sanitary conditions, alerting the risk of fecal-oral transmission of diseases [16].

Contrary to general expectation, the Xakriabá children had low prevalence of helminthiasis. One possible explanation could be the routine administration of wide spectrum anti-helminthic drugs that, in opposition to anti-protozoa drugs, have very low toxicity [17]. The unfavorable environment for the maintenance of eggs could add to the explanation [14]. DILX is located in the semi-arid region of the São Francisco river with average precipitation as low as 75.25mm per year registered in a center near the Xakriabá land. In a similar ecosystem in the northeast region of Brazil, Alves et al. [18] observed that the dissemination of helminthic infections is correlated with soil humidity and the long duration of the dry season limits the proliferation of parasites. On the other hand, geohelminthes show characteristics that potentially increase the chances of reinfection, such as high fertility, resistance of the embryonated eggs under adverse conditions and wide capacity of dispersion, both out and indoors [17].

Regarding *Schistosoma mansoni* infection, all positive samples proceeded from the Dizimeiro and Peruaçu villages, served by the Sumaré BHU. These two villages completely lack basic sanitation. They are located in wetlands by the Peruaçu river, infested with snails of the *Biomphalaria* genus (data not shown). The area is considered endemic for *S. mansoni* because of continued local transmission. Chronic forms of disease may present liver and spleen involvement and in some cases upper digestive bleeding secondary to the rupture of esophageal varices. Therefore, actions for the control of schistosomiasis endemic are strongly desirable in this area. Hookworm eggs were detected in 75 children, most of them (53.3%) living in Sumaré. The area presents favorable environment for the maintenance of infective larval forms, such as humidity, luminosity and sandy soil [19]. Low prevalence of *Ascaris lumbricoides* and *Trichuris trichiura* was observed. As opposed to our study, high prevalence of both parasites has been described in other indigenous populations [6,20]. The synergism between the two species could be explained by the similarities of their life cycles, the high production of eggs and their capacity to survive adverse environmental conditions. Low prevalence of helminthes has also been observed by other authors [21] and could be explained by frequent mass treatment with anti-parasitic drugs in all the DILX. The low prevalence for *Strongiloydes stercoralis* and *Enterobius vermicularis* in Xakriabá children could be explained by limitations of the coproscopic method used in this study. Stool samples are immersed in conservative solution in the TF-test method, which may destroy the larvae of *S. stercoralis*. On the other hand, anal swab examination would be the most appropriate method to detect *E. vermicularis*, according to the biological behavior of the parasite [7].

Positivity in the parasitological survey did not show significant difference between boys and girls in the DILX. Similar results were reported in children under 13 in ethnic groups from the Amazonia region [22]. As opposed to the children, prevalence is usually higher in teenager boys, mainly for schistosomiasis. It is usually explained by the differences in behavior. Teenager boys are usually in contact with the environment more often than girls and younger children, who tend to stay near the house [23].

Infection with multiple parasites has negative consequences to the general health, including malnutrition and anemia (5,21). Multiple parasite infection in the DILX was restricted to protozoa species, and may be attributed to poor sanitation. Nonetheless, considerable improvement had been implemented years before this survey. The National Health Foundation (FUNASA) is in charge of the health care of indigenous populations since the year 2000. Actions were taken to improve sanitation, including the building of sanitary modules, tubular wells, the distribution of filters for drinking water, to list a few, that benefitted at least 1000 people. In 2002, the government started the program for the surveillance of quality of water, SISAGUA (Information System on Water for Human Supply). The program improved the quality of water in the DILX. Currently, nearly all houses receive treated running water, except for the villages in the critical area near Peruaçu river. The improvements mentioned above could also be responsible for the low prevalence of helminthes in the DILX. But the high prevalence of protozoa infections indicates that building interventions alone are not sufficient to control protozoa infections.

In order to examine associations between parasitic diseases and potential demographic, housing and environmental factors, a random intercept Poisson model with robust variance was employed to take into account the dependence of individuals between villages. One of the major assumptions of the tests of significance used in the multilevel models is normality of the error distributions involved. Another assumption is the sufficient number of sample size for the higher-level variables. A potential limitation for using the multi-level model approach is that the localities studied are not a random sample of a number of villages. However, random intercept and random coefficient models are a flexible and powerful tool to tackle the effect of clustering in multi-level data [24] and it has been suggested that a size of 30 in each level (in our case village-level), provide accurate standard error estimates for the regression coefficients of the fixed part of the multilevel model [25].

In addition to the loss of 30% of indigenous participation, the main limitation of the present study is the restriction to estimate the prevalence of *S. stercoralis* and *E. vermicularis*, because of the parasitological method employed. TF-Test is not recommended for detection of those parasites. The use of combined methods such as Baermann & Moraes or Rugai for *S. stercoralis*, and anal swab for *E. vermicularis* would have allowed to estimate the prevalence of these parasites [7]. In addition, the use of a quantitative method such as Kato-Katz [6] for positive samples would allow to estimate the parasite burden, important to evaluate the sanitation programs implemented by FUNASA. Yet, the use of the TF-test method was important because it is more sensitive to detect cystic forms of protozoa, commonly underestimated by quantitative or sedimentation methods.

The variations observed in prevalence between the BHU could be explained by differences in adherence to study protocol. On average, adherence was 71%. No significant difference in the proportion of participants was observed between the BHU.

## Conclusion

The present data point to the continuing poor sanitation conditions in the DILX despite recent improvements, resulting not only in high levels of parasitism, but also in the frequent occurrence of multiple parasite infections. It demonstrates the urgent need to complete the building of the sanitation system and to implement consistent actions of basic education for health. Isolated measures tend to prove unsuccessful as demonstrated in the DILX, where high prevalence of water borne protozoa infections are still observed.

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