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Original Article

Giardia intestinalis infection associated with malnutrition in children living in northeastern Brazil

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Abstract
Introduction: The present study aimed to determine the prevalence and factors associated with *Giardia intestinalis* infection, verifying its impact on the nutritional status of children in northeastern Brazil.
Methodology: A cross-sectional study was conducted to obtain parasitological, sociodemographic, and anthropometric data in two municipalities in the states of Piauí and Ceará, northeastern Brazil.
Results: Prevalence of giardiasis was 55/511 (10.8%). *G. intestinalis* was more frequent in people living in poverty (30/209 [14.4%], p = 0.041), performing open evacuation (26/173 [15%], p = 0.034), and drinking rainwater stored in cisterns (9/56 [16.1%], p = 0.005). The proportion of stunting and being underweight in children infected with *G. intestinalis* was significantly higher than that in uninfected children (5/23 [21.7%] vs. 10/179 [5.6%], p = 0.017, OR = 4.69, 95% confidence interval [CI] = 1.44–15.25 and 5/23 [21.7%] vs. 13/179 [7.3%], p = 0.038, OR = 3.54, 95% CI = 1.13–11.09, respectively). Infection with *G. intestinalis* remained significantly associated with stunting and being underweight after adjustment for poverty, municipality, sex, and age in a logistic regression multivariate model.
Conclusions: In rural areas in northeastern Brazil, giardiasis has acquired great public health importance in the soil-transmitted helminths control era, impacting the nutritional status of children and requiring new approaches to diagnosis and treatment and translational research that could generate applicable solutions at the community level.

Key words: *Giardia intestinalis*; nutritional status; intestinal parasites; northeastern Brazil; Brazil; poverty

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Introduction
Intestinal parasitoses are neglected conditions linked to poverty and poor sanitation, being more prevalent in developing countries [1,2]. Among the protozoan parasites, *Giardia intestinalis* is present in distinct sociodemographic settings, being a potentially zoonotic pathogen [3-6]. This protozoan is transmitted by ingestion of cysts that are highly resistant and can survive several months in harsh environments. Trophozoytes emerging from ingested cysts inhabit the proximal small intestine, where they multiply by binary fission [7].

G. intestinalis presents high levels of genetic diversity, and its genotypes are clustered into distinct assemblages. Parasites isolated from humans have been characterized into two assemblages, A and B, both of which are distributed globally [8].

Although *G. intestinalis* causes diarrhea in travelers, a comprehensive study in developing countries did not observe any association between acute diarrheal disease and *G. intestinalis* in children, possibly because infections are often chronic and apparently asymptomatic [9]. *G. intestinalis* pathogenicity is poorly understood and appears to be mediated by complex mechanisms, including enterocyte apoptosis and epithelial cell damage, which lead to malabsorption [7].

Giardiasis has been associated with protein-energy malnutrition, micronutrient deficiency, iron deficiency anemia, and growth failure [10-12]; it is one of the most harmful intestinal parasitosis to the physical development of children [10,13-15].

Policies for controlling intestinal parasites focus on soil-transmitted helminths (STHs) and schistosomiasis, and do not take into account the burden of protozoan parasites, which require different drugs/dosages and treatment durations [16-18]. The aims of the present study were to assess the prevalence and factors associated with *G. intestinalis* infection and to determine its impact on the nutritional status of children in rural northeastern Brazil.

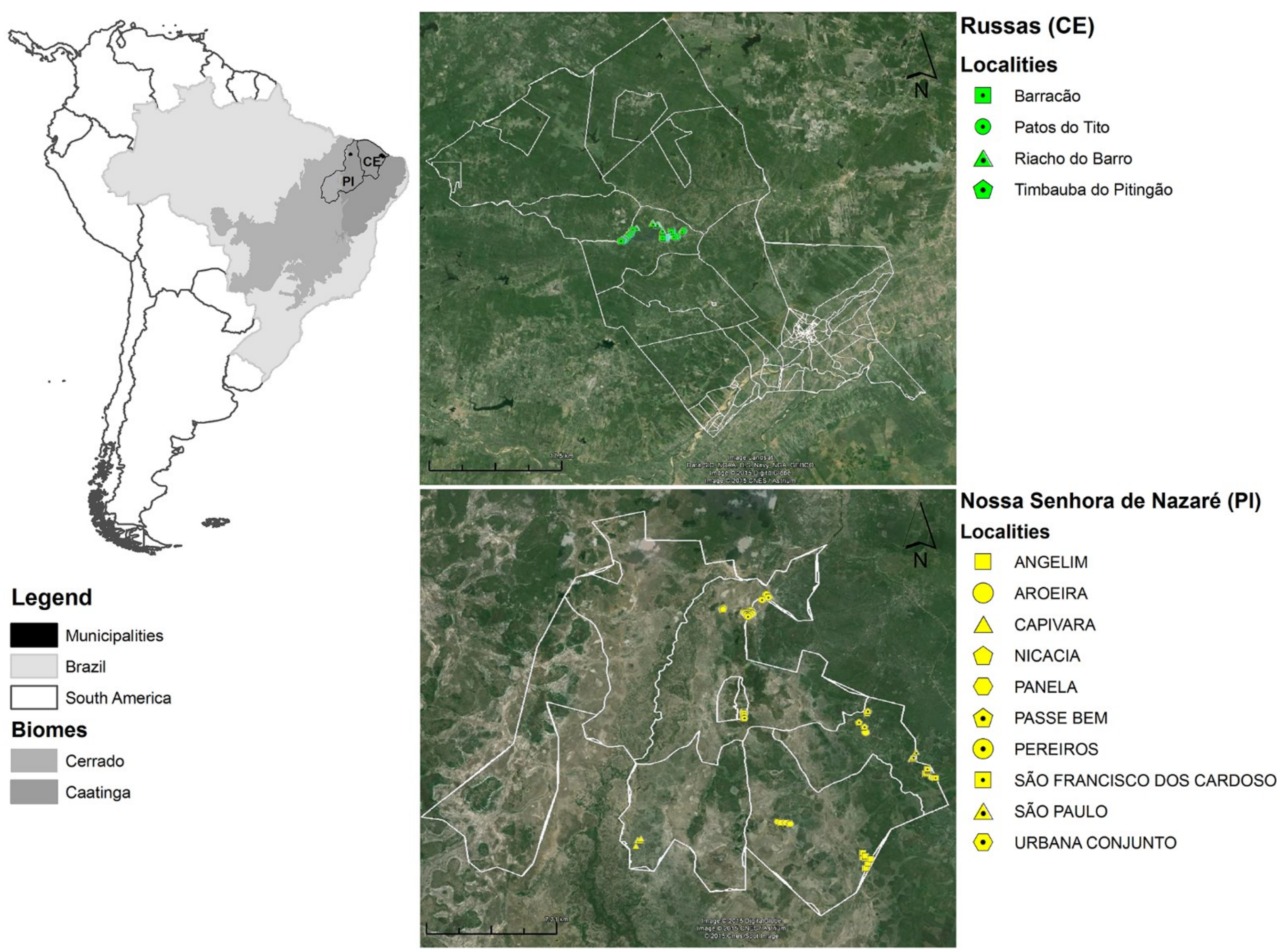
Methodology

Setting and study design

This cross-sectional survey was performed in Russas (RSS), in the state of Ceará, and Nossa Senhora de Nazaré (NSN), in the state of Piauí (Figure 1). RSS has 69,833 inhabitants and a human development index (HDI) of 0.674. RSS has a tropical semiarid climate and belongs to the semiarid region in the Caatinga biome, "Bsh", in the Köppen-Geiger system [20], and is subjected to prolonged periods of drought. NSN has 4,556 inhabitants, a HDI of 0.586, and a tropical hot climate, "As" [19], located in a transition region of Caatinga to Cerrado biome.

The study included four rural communities of RSS in 2013 (n = 213) and eight rural and one urban community of NSN in 2014 (n = 298). The sampling strategy was visiting all the houses that have children. All the residents were invited to participate, and written informed consent forms were signed.

Figure 1. Map showing the localizations of the studied municipalities in northeastern Brazil: Russas, in the state of Ceará, and Nossa Senhora de Nazaré, in the state of Piauí.



Sociodemographic and sanitation data were obtained by questionnaires. Per-capita family income stratified subjects into two groups: families receiving less than USD 38.5 per month (corresponding to the Brazilian poverty line) [20] and families with an income above USD 38.5.

Collection and processing of stool samples

During domicile visits, containers without preservatives were distributed for stool collection. Samples were processed through Ritchie's modified ethyl acetate sedimentation technique [21], the zinc sulfate centrifugal flotation technique [22], the Kato-Katz thick smear [23], and the Baermann-Moraes technique [24].

Anthropometric measurements

Weight, height, and arm circumference measurements were obtained from individuals between 12 months and 14 years of age. Weight was measured to the nearest 0.1 kilogram using a digital floor scale. Height and mid-upper arm circumference (MUAC) were measured to the nearest 0.1 cm. MUAC was measured by wrapping a flexible tape around the left arm. Standing height was measured with a steel millimetered tape coupled to a steel framing square.

Standard deviation scores (Z-scores) of height-for-age (HAZ), weight-for-height (WHZ), weight-for-age (WAZ), and MUAC-for-age (MUACZ) were calculated using the NutStat Module on EpiInfo 2000 (Centers for Disease Control and Prevention, Atlanta, USA) and the

Table 1. Distributions of *Giardia intestinalis* infection based on sociodemographic characteristics in Russas and Nossa Senhora de Nazaré, 2013 and 2014.

	Russas (Ceará)		**Nossa Senhora de Nazaré (Piauí)**		**Both cities**	
	N of *Giardia intestinalis*-positive subjects/examined subjects (% positive)	**P**	**N of *Giardia intestinalis*-positive subjects/examined subjects (% positive)**	**P**		**P**
Characteristic						
Age group (years)						
1–6	0/26 (0.0%)		7/70 (10%)		7/96 (7.3%)	
7–14	10/56 (17.9%)		9/80(11.2%)		19/136 (14%)	
15–21	1/16 (6.2%)	0.150	1/17 (5.9%)	0.127	2/33 (6.1%)	0.786
22–40	9/57 (15.8%)		6/76 (7.9%)		15/133 (11.3%)	
41–60	6/40 (15%)		2/39 (5.1%)		8/79 (10.1%)	
> 60	4/18 (22.2%)		0/16 (0%)		4/34 (11.8%)	
Sex						
Female	19/107 (17.8%)	0.167	9/160 (5.6%)	0.092	28/267 (10.5%)	0.887
Male	11/106 (10.4%)		16/138 (11.6%)		27/244 (11.1%)	
Income per capita per month (USD) *USD 1 = BRL 4						
Below the poverty line (≤ 38.5)	13/55 (23.6%)	0.024	17/154 (11%)	0.098	30/209 (14.4%)	0.041
Above the poverty line (> 38.5 and ≤ 330)	17/158 (10.8%)		8/144 (5.6%)		25/302 (8.3%)	
Site of defecation						
Latrines	20/166 (12%)	0.151	9/172 (5.2%)	0.033	29/338 (8.6%)	0.034
Open defecation	10/47 (21.3%)		16/126 (12.7%)		26/173 (15%)	
Main source of drinking water						
Desalinated brackish water	13/138 (9.4%)		-		13/138 (9.4%)	
Rainwater from cisterns	9/56 (16.1%)		-		9/56 (16.1%)	
Dam	1/4 (25%)	0.001	-	1.000	1/4 (25%)	0.005
Water trucks	7/15 (46.7%)		-		7/15 (46.7%)	
Individual water well	-		2/29 (6.9%)		2/29 (6.9%)	
Collective water well / cisterns	-		23/269 (8.6%)		23/269 (8.6%)	
Total	**30/213 (14.1%)**		**25/298 (8.4%)**	**0.044**		**55/511 (10.8%)**

World Health Organization's 1978 growth chart [25]. Stunting, wasting, and being underweight were defined by -2 standard deviations from mean HAZ, WHZ, and WAZ, respectively, of the reference population [26].

Statistical analysis

G. intestinalis positivity in different sociodemographic settings and frequencies of stunting and being underweight in *G. intestinalis*-positive and *G. intestinalis*-negative children were compared using Fisher's exact test. A logistic regression model included age, sex, municipality, and socioeconomic status as independent variables. Statistical analyses were performed using SPSS version 17.0 software (IBM, Armonk, USA). Statistical significance was established at $p < 0.05$.

Ethics

This study was approved by the Ethics Committee in Research with Humans Oswaldo Cruz Institute – Fiocruz (CAAE: 12125713.5.0000.5248).

Results

Frequency and distribution of G. intestinalis infection

The overall prevalence of giardiasis was 55/511 (10.8%); giardiasis was significantly more prevalent in RSS (30/213 [14.1%]) than in NSN (25/298 [8.4%]) (p = 0.044). As presented in Table 1, infection with *G. intestinalis* was more frequent in people living in poverty, performing open evacuation, and drinking rainwater stored in cisterns.

The frequency of infection with at least one enteric protozoa was 64/213 (30%) in RSS and 75/298 (25.2%) in NSN. In RSS and NSN, the intestinal protozoa frequencies were: *Endolimax nana*, 7.0% and 4.4%; *Entamoeba coli*, 12.2% and 14.1%; *E. histolytica/E. dispar*, 10.3% and 1.3%; and *Iodamoeba butschlii*, 4.2% and 2.7%, respectively.

Helminths were detected in 12/213 (5.6%) subjects in RSS and 52/298 (14.4%) subjects in NSN. Neither *Ascaris lumbricoides* nor *Trichuris trichiura* was found. Hookworm (3.7% in RSS and 14.1% in NSN), *Hymenolepis nana* (1.4% in RSS and 0.3% in NSN), *Strongyloides stercoralis* (0.5% in RSS and 0.3% in NSN), and *Enterobius vermicularis* (0.5% in RSS and 3% in NSN) infections were also detected. The rates of coinfection with *G. intestinalis* were as follows: hookworms, 1/30 (3.3%) and 2/25 (8%); and *Entamoeba histolytica/E. dispar*, 7/30 (23.3%) and 1/25 (4%) in RSS and NSN, respectively.

Association of giardiasis with nutritional status in children

Among the 511 study subjects, 45.4% were children under 14 years of age. The nutritional status could be determined for 53 of the children in RSS and 149 in NSN. The anthropometric parameters of these children are presented in Table 2, including the proportions of stunted, underweight, and wasted children.

The rates of stunting in RSS and NSN were 9.4% and 6.7%, respectively. As presented in Table 3, the proportion of stunting in children infected with *G. intestinalis* was significantly higher than that in uninfected children (5/23 [21.7%] vs. 10/179 [5.6%]; p = 0.017; OR = 4.69; 95% confidence interval [CI] = 1.44–15.25). In addition, the proportion of being underweight in children with giardiasis was significantly higher than that in children without giardiasis (5/23 [21.7%] vs. 13/179 [7.3%]; p = 0.038; OR = 3.54; 95% CI = 1.13–11.09). No association was observed between giardiasis and wasting. Infection with *G. intestinalis* remained significantly associated with stunting and being underweight after adjustment for poverty, municipality, sex, and age (Table 3). Although two children had coinfection of *G. intestinalis* and hookworm, this factor does not change the analysis,

Table 2. Nutritional status of children in Russas and Nossa Senhora de Nazaré, 2013 and 2014.

	Russas (Ceará)	Nossa Senhora de Nazaré (Piauí)	P
Height-for-age z-score			
Mean ± standard deviation	-0.60 ± 0.99	-0.38 ± 1.23	0.250*
Proportion of < - 2 (stunting)	5/53 (9.4%)	10/149 (6.7%)	0.546**
Weight-for-age z-score			
Mean ± standard deviation	-0.21 ± 1.39	- 0.48 ± 1.31	0.222*
Proportion of < - 2 (underweight)	5/53 (9.4%)	13/149 (8.7%)	1.000**
Weight-for-height z-score			
Mean ± standard deviation	0.28 ± 1.77	-0.35 ± 1.20	0.047*
Proportion of < - 2 (wasted)	1/37 (2.7%)	5/112 (4.5%)	1.000**
Mid-upper arm circumference z-score			
Mean ± standard deviation	-0.10 ± 1.21	-0.36 ± 1.01	0.213*

Table 3. Bivariate and multivariate logistic regression analysis of stunting and underweight according to Giardia intestinalis infection status, poverty, age group, sex, and municipality of children in Russas and Nossa Senhora de Nazaré.

	Stunting						Underweight					
	Crude			Adjusted			Crude			Adjusted		
	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P
Giardia intestinalis												
Uninfected	1			1			1			1		
Infected	4.69	1.44–15.25	0.017	5.66	1.57–20.33	0.008	3.54	1.13–11.09	0.038	3.616	1.09–11.936	0.035
Income												
Above the poverty line	1			1			1			1		
Below the poverty line	3.22	0.86–12.10	0.087	3.56	0.88–14.26	0.073	1.47	0.54–3.96	0.471	1.449	0.51–4.081	0.483
Sex												
Female	1			1			1			1		
Male	1.93	0.63–5.88	0.289	1.76	0.50–6.16	0.375	2.60	0.89–7.58	0.086	2.687	0.890–8.115	0.080
Age (years)												
1–6	1			1			1			1		
7–14	0.93	0.32–2.67	1.000	0.74	0.22–2.48	0.628	0.62	0.23–1.66	0.458	0.542	0.195–1.507	0.240
Municipality												
Nossa Senhora de Nazaré	1			1			1			1		
Russas	1.44	0.47–4.44	0.54	0.38	0.10–1.39	0.147	1.09	0.36–3.21	1.000	0.714	0.227–2.249	0.565

since both had normal Z-scores, as did children infected only with other intestinal parasites.

Discussion

This study shows that *G. intestinalis* was the most frequently identified intestinal parasite in rural locations in two states in northeastern Brazil. Interestingly, we did not identify common STHs (*e.g.*, *A. lumbricoides* or *T. trichiura*), but did detect hookworms. *G. intestinalis* infection was observed in all age groups, mainly in children 7–14 years of age. This downward trend in the prevalence of STHs associated with persistent protozoan infections has been observed in other countries and may represent a challenge for the control of parasitic intestinal infections [17].

Recent assessments of the prevalence of enteric parasitoses have shown that the intestinal protozoa, despite their significant impact on health, have not been effectively targeted by large-scale interventions against neglected tropical diseases [18]. Importantly, giardiasis has not been effectively targeted by the intestinal parasite control initiatives recently implemented by the Brazilian Ministry of Health [27], which have consisted of the distribution of a single 400 mg albendazole dose in schools and health centers. Although albendazole is effective and has been successfully used to control STHs, a five-day regimen is required to treat giardiasis [28]. Thus, the persistence of endemic giardiasis in rural areas may be due to limitations in the policies to control intestinal parasites.

The failure of these initiatives to successfully target *G. intestinalis* is likely due to the inherent difficulties in achieving this goal via massive preventive chemotherapy. Major operational hurdles must be overcome to target *G. intestinalis* and other protozoa. The 5-nitroimidazolic compound most widely used to treat giardiasis, metronidazole, cannot be administered in a single dose. Instead, a five-day course is needed [28], which poses logistical hurdles. Although three other 5-nitroimidazolic derivatives – secnidazole, ornidazole and tinidazole, all of which are marketed in many countries [28] – are effective in single doses against *G. intestinalis*, the cost of these drugs, their poor tolerability, and their adverse effects preclude their use in large-scale chemoprevention strategies.

A major strength of our study is that we detected *G. intestinalis* infection through traditional parasitological techniques, including centrifugation in ethyl acetate and centrifugal flotation in zinc sulfate. These techniques are time consuming and labor intensive; however, they provide valuable information because they can detect protozoan cysts and are thus the gold standard for intestinal protozoa diagnosis. Typically, parasitological surveys aiming to characterize the prevalence rates of STHs employ the Kato-Katz smear, which is operationally more convenient [29]. Therefore, not only are mass treatments more difficult for intestinal protozoa, but accurate diagnoses of these protozoa are also more complicated, meaning that it is extremely difficult to characterize the prevalence rates in different regions. In many regions, it is possible that the STH control strategies, which are performed without parasitological diagnoses, are contributing to the neglect of basic laboratory infrastructure and the diversion of personnel training away from parasitological diagnoses. When used in cross-sectional studies, molecular diagnostic techniques generally yield higher prevalence rates due to their improved sensibility, suggesting that some infections are not detected by light microscopy [30]. However, the higher cost of diagnostic PCR prohibits its use at the community level in many developing countries. Thus, the development of economical molecular diagnostic techniques is an important goal for achieving accurate detection of intestinal protozoa infections.

This study showed that children infected with *G. intestinalis* are more frequently stunted and underweight compared with non-infected children, even after controlling for potential confounders such as income. Several studies have demonstrated the impact of *G. intestinalis* infection on the nutritional status of children. For instance, the anthropometric parameter HAZ has been shown to be markedly negatively influenced by giardiasis in the Amazon region of Brazil [10]. *G. intestinalis* infection was also observed to influence nutritional status in northeastern Brazil [31-33]. In Rwanda, *G. intestinalis* infection was identified as a predictor of being underweight and severe malnutrition [30]. Similarly, giardiasis was also a strong predictor of low HAZ in Colombia and Iran, and has also been shown to be significantly associated with lower body weight, serum zinc levels, and serum iron levels in Egypt [11,13,34]. Thus, giardiasis is perhaps currently the most harmful intestinal parasitosis to the physical development of children in endemic areas with poor sanitation conditions. In the present study, the age group most frequently affected was school-aged children. This finding is important because children in this age group are at the highest risk for linear growth disruption by this parasite.

We observed that infection with *G. intestinalis* was more frequent among people living below the poverty line and people who practice open defecation, suggesting that improvements in income, sanitation, and hygiene could significantly reduce the prevalence of giardiasis. Rainfall is another factor that potentially influences the prevalence of giardiasis. For instance, we observed that giardiasis was significantly more prevalent in RSS, an area under substantial water stress, than in NSN. In RSS, giardiasis was more frequent among inhabitants who drink rainwater stored in cisterns. This water was collected during the rainy season and stored for nearly 10 months during drought, potentially favoring contamination with *G. intestinalis* cysts. Thus, we hypothesize that sanitary conditions contribute to an increased prevalence of *G. intestinalis* infection due to the required long periods of water storage during the dry season, although further studies are needed to test this hypothesis.

Our work highlights the public health importance of giardiasis in rural areas in northeastern Brazil, demonstrating that giardiasis impacts the nutritional status of children. In the STH control era, overcoming the burden of giardiasis necessitates the development of novel diagnostic tools and treatments. In addition, a focused effort on translational research would have great potential to generate effective solutions at the community level.

Conclusions

In rural areas in northeastern Brazil, giardiasis has acquired great public health importance in the STHs control era. *G. intestinalis* affects the nutritional status of children, since chronic and apparently asymptomatic infections are not diagnosed and consequently not treated. Infection is associated with poverty and water scarcity in the semi-arid Caatinga biome. This ecoepidemiological scenario points to the need for new approaches to diagnosis and treatment. Translational research could generate applicable solutions at the community level.

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