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Intestinal parasitosis, undernutrition and socio-environmental factors in schoolchildren from Clorinda Formosa, Argentina

Parasitosis intestinal, desnutrición y factores socio-ambientales en niños escolares de Clorinda Formosa, Argentina

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ABSTRACT

Objective To evaluate intestinal parasitosis, undernutrition and socio-environmental factors in schoolchildren from Clorinda (Formosa, Argentina).
Materials and Methods Serial fecal samples and anal swabs of 114 schoolchildren, canine feces and soil samples were analyzed. Body weight and height of 215 schoolchildren were measured and undernutrition was estimated according to the World Health Organization criteria. Socio-environmental variables were assessed by means of a semi-structured questionnaire.
Results 78.1% of children were infected by at least one of the 12 species identified and 70.8% had multiple parasitic infections. *Blastocystis* sp., *Giardia lamblia* and *Enterobius vermicularis* were the most prevalent. Additionally, 17.5% of children were infected by at least one geohelminth (e.g. *Ascaris lumbricoides*, *Trichuris trichiura*, hookworms). 64.3% of canine of canine feces were positive and six parasitic species were found; the most frequent were *Ancylostoma caninum*, *Uncinaria stenocephala* and *G. lamblia*. Furthermore, 37.5% of soil samples showed zoonotic parasites (i.e. *Ascaris* sp., *Toxocara* sp.). Finally, 10.0% of the children were undernourished and 85.7% of them had parasites. The risk for parasitosis was higher in children that lived in houses with inadequate solid waste disposal and whose parents were unemployed or had temporary jobs.
Conclusions The lack of environmental sanitation, unstable employment of parents and the presence of zoonotic species were the most relevant factors observed. Consequently, these conditions result in an increase of parasitic infections and negatively influence the growth of children.

Key Words: Parasites; malnutrition; environment; children *(source: MeSH, NLM).*

RESUMEN

Objetivo Evaluar la parasitosis intestinal, la desnutrición y los factores socio-ambientales en escolares de Clorinda (Formosa, Argentina).
Materiales y Métodos Se analizaron muestras fecales y escobillados anales seriados de 114 escolares, heces caninas y muestras de suelo. Se midió el peso corporal y la talla de 215 escolares y se evaluó la desnutrición según los criterios de la Organización Mundial de la Salud. Las variables socio-ambientales se evaluaron mediante una encuesta semiestructurada.
Resultados El 78,1% de los niños estuvieron parasitados por al menos 1 de las 12 especies identificadas y el 70,8% presentó parasitosis múltiples. *Blastocystis* sp., *Giardia lamblia* y *Enterobius vermicularis* fueron las más prevalentes. Además, el 17,5% de los niños estaban infectados con al menos un geohelminto (e.g. *Ascaris lumbricoides*, *Trichuris trichiura*, ancylostomideos). El 64,3% de las heces caninas resultaron positivas y se encontraron seis especies parasitarias; las más frecuentes fueron *Ancylostoma caninum*, *Uncinaria stenocephala* y *G. lamblia*. Además, el 37,5% de las muestras de suelo mostraron parásitos zoonóticos (i.e. *Ascaris* sp., *Toxocara* sp.). Finalmente, el

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10% de los niños estaban desnutridos y el 85,7% de ellos resultaron parasitados. El riesgo de parasitosis fue mayor en los niños que vivían en casas con eliminación inadecuada de desechos sólidos y cuyos padres estaban desempleados o tenían empleos temporales.
Conclusiones La falta de saneamiento ambiental, el empleo inestable de los padres y la presencia de especies zoonóticas fueron los factores observados más relevantes. En consecuencia, estas condiciones resultan en un aumento de las infecciones parasitarias e influyen negativamente en el crecimiento de los niños.

Palabras Clave: Parásitos; malnutrición; ambiente; niños *(fuente: DeCS, BIREME)*.

Intestinal parasitosis is a neglected infectious disease that is found worldwide. This disease is highly prevalent in tropical and subtropical areas of low-income developing countries. (1) Recent estimates are that more than one billion people of developing regions of Africa, Asia and America are infected by parasitic helminth species, of which more than 1 450 million are infected by at least a species of intestinal nematode; this number could be higher if pathogenic intestinal protozoa were considered. (2,3) Even if these parasitic infections do not lead to immediate death, they may have negative effects on the physical and cognitive development of children, such as malabsorption syndrome, anemia, anorexia, chronic inflammation, undernutrition and diarrhea. (4,5).

The constant growth of the population and urbanization, the increase of migrations, the lack of sanitary facilities, inadequate hygiene and limited access to health services favor the transmission of parasites, and the consequent infections (6). In this sense, parasitosis can be caused by the ingestion of infecting cysts or eggs present in the soil, water and raw food, or by the penetration of larvae from the soil through the skin. (3) Biological contamination of the environment with pet feces is also a serious problem for public health, since their parasitic forms can infect humans as well. One example is visceral larva migrans and ocular larva migrans caused by *Toxocara* sp., and cutaneous larva migrans caused by *Ancylostoma caninum*, *A. brasiliense*, and *Uncinaria stenocephala* (7).

In Argentina, a country with a wide geographical and climatic diversity, the prevalence of intestinal parasitosis shows a declining trend from north to south and from east to west that accounts for the complex mosaic of social, economic, and environmental variability of its territory. (8) In this context, the north of the country presents socio-economic and environmental conditions that favor the development of these infections. Since information about these infections in north-eastern Argentina is scarce, the aim of this study was to evaluate intestinal parasitosis, undernutrition and socio-environmental factors in schoolchildren from Clorinda (Formosa, Argentina).

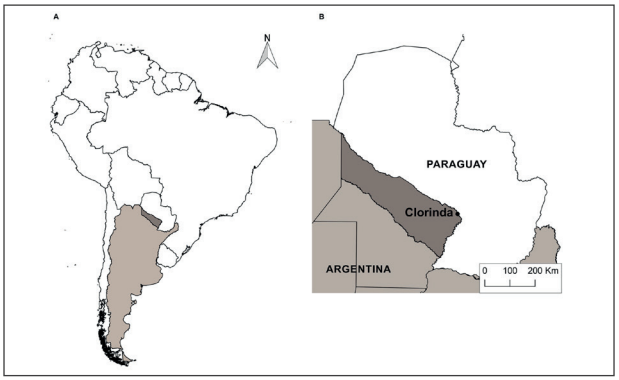
MATERIALS AND METHODS

Population and study area

The study was carried out in children of both sexes from school No. 372 in Clorinda. The school has 350 primary school students coming from peripheral neighborhoods.

Clorinda (in the department of Pilcomayo, province of Formosa) (25°17'S, 57°43'W) is a city located in the north-east of Argentina bordering Paraguay (Figure 1). The climate of the area is subtropical with an average annual temperature of 24°C, a marked seasonal variation and annual rainfall that can exceed 1 200 mm. Soils are clay-slime with poor to imperfect drainage.

Figure 1. (A) Geographic location of Argentina in South America and the province of Formosa in Argentina; (B) Location of Clorinda in Formosa.



A cross-sectional study was carried out in September 2014. The selection of the sample was non-probabilistic and largely determined by voluntary participation.

This study included children who had not received antiparasitic treatment at the time of the research and whose parents or legal guardians signed an informed consent. Children with no parental or guardian consent and with chronic diseases or pathological conditions were excluded.

Parasitological study

Meetings with adults and children were initially held to inform them about the biology and strategies to prevent intestinal parasites and aspects related to the nutritional status of children. Free parasitology tests for children and dogs were

offered. Every consenting family was provided with one vial per dog and two vials per child of formalin 10% for stool samples, and anal swabs to diagnose intestinal parasites.

The study included 114 children (51.8% boys and 48.2% girls) aged 1-14 and divided into 3 age groups (1.0-5.9, 6.0-9.9 and 10.0-14.9). Fecal and anal swab samples of children were collected by their parents or legal guardians during 5-7 days. Anal swabs were obtained from the perianal zone during the morning using sterile gauze. Feces from dogs (n=16) were collected during 3-5 days by their owners or by the research group immediately after deposition.

Coproparasitological samples were processed using concentration by sedimentation (Ritchie) and flotation (Willis) techniques. Anal swab vials were agitated vigorously and centrifuged for 10 minutes at 400g (gravity) to obtain a pellet with the highest possible concentration of eggs of *Enterobius vermicularis* (9,10).

Soil samples (n=16) were collected from the peridomiciliary area of the participating families using a grid of 20x20cm and 5cm depth. They were processed applying the decantation and centrifugation technique of Shurtleff & Averre (11).

Every sample was examined by experts using an optical microscope at 100X and 400X magnification, and eggs, cysts and larvae were identified based on their measures and morphological characteristics (9,12).

Anthropometric study

The study included 215 children (55.8% boys and 44.2% girls) aged 6-12 divided into 2 age groups (6.0-9.9 and 10.0-12.9). A single technician performed anthropometric measurements according to standard protocols. (13) Age, body weight and height were recorded: age was obtained from identification cards or school records, body weight was measured in kilograms (kg) using a digital scale (Tanita UM-061, 100g accuracy) with children lightly dressed (to correct for clothing, the weight of clothes was subtracted), and height was measured in centimeters (cm) using a portable vertical anthropometer (SECA, 1 mm accuracy).

The exact age of each child was calculated as a function of their birth date. Similarly, body mass index [BMI $=(W/H^2)$ (kg/m^2)] was estimated according to weight and height data. Underweight (low weight-for-age, LW/A), stunting (low height-for-age, LH/A) and low BMI-for-age (LBMI/A) were determined using the World Health Organization (WHO) reference charts and -2 Z scores as cut-off points (14).

Socio-environmental study

A semi-structured questionnaire was completed by the parents or legal guardians (n=113) to evaluate the socio-environmental characteristics with information regarding structural and physical facilities. These characteristics provided information about indoor and outdoor housing conditions. Moreover, parents' educational level, employment, governmental food and monetary support, hygiene and cultural practices were considered, among others (15-17).

Statistical analysis

Data were analyzed by software R (18). Parasitosis and parasitic species were analyzed in relation to sex and age group, socio-environmental characteristics, hygiene practices and nutritional status. The independence of the variables was evaluated using the Chi square test (x^2) or Fisher´s exact test at a significance level of $p<0.05$. Socio-environmental variables were examined by means of logistic regression models with a stepwise forward selection to determine risk factors for parasitic infection in children. The model with the lowest residual deviance was chosen and, in case of several competitive models, the most parsimonious was selected comparing Akaike information criterion and p-value. Association force was estimated by odds ratio (OR) values. Level of significance $p<0.05$ and confidence intervals (CI) were used. To perform a regression analysis, the educational level of parents was grouped into unschooled/primary (basic education) and secondary/tertiary/university (higher education); employment status was divided into unemployed/temporary and permanent/freelance.

Ethics statement

The study was carried out without affecting the physical, psychic and moral inte *Blastocystis* grity of the participants and protecting their privacy. The principles proclaimed in the Universal Declaration of Human Rights (1948), the ethical standards established by the Nüremberg Code (1947), the Declaration of Helsinki (1964) and its successive amendments were taken into consideration. Special attention was also paid to Article 5 of the Regulation Decree of National Law 25.326.

All families involved were given the results of the parasitology diagnosis, and positive cases were referred to the nearest healthcare center and to the school to receive the corresponding antiparasitic treatment.

RESULTS

Parasitological analysis in children

Of the total number of analyzed children, 78.1% (89/114) were infected with at least one intestinal parasite species. Infection was slightly more frequent in boys than in girls (51.7% vs. 48.3%; $p>0.05$), as well as in 6.0-9.9 year-old

children (58.4%) with respect to younger and older children (18.0% and 23.6%, respectively; p>0.05).

Twelve parasitic species were identified. Infection by pathogenic species was higher than infection by non-pathogenic species (76.3% vs. 1.8%), and the most prevalent were *Blastocystis* sp. (57.9%), *Giardia lamblia* (36.8%) and *E. vermicularis* (19.3%). *Entamoeba coli* were the most prevalent (21.1%) of the non-pathogenic protozoa. In addition, 17.5% (20/114) were positive for some species of geohelminth, and the most frequent were *Ascaris lumbricoides* and *Trichuris trichiura* (7.9% and 7.0, respectively) (Table 1).

Table 1. Prevalence[a] (%) of parasitic species in children from Clorinda, Formosa

Parasitic species	Positive cases	Prevalence (%)
Infected	89	78.1
Pathogenic protozoa		
Blastocystis sp.	66	57.9
Giardia lamblia	43	37.7
Non-pathogenic protozoa		
Entamoeba coli	24	21.1
Endolimax nana	16	14.0
Iodamoeba bütschlii	1	0.9
Chilomastix mesnili	1	0.9
Helminths		
Enterobius vermicularis	22	19.3
Hymenolepis nana	14	12.3
Geohelminths		
Ascaris lumbricoides	9	7.9
Trichuris trichiura	8	7.0
Hookworms	3	2.6
Strongyloides stercoralis	1	0.9

[a] Prevalence estimated in relation to total number of analyzed children (n=114)

Multiple parasite infections (children infected by two or more species) were more frequent than monoparasitosis (children infected by a single species) (70.8% vs. 29.2%; p>0.05), observing a maximum of 5 species in some cases. Multiple infections were more prevalent in girls than in boys (37.1% vs. 33.7%; p>0.05) and in children between 6.0-9.9 years of age (40.4%) with respect to younger and older children (12.3% and 17.9%, respectively; p>0.05).

Statistically significant associations were observed between *Blastocystis* sp./*G. lamblia* (x^2=4.2, p<0.05); *Blastocystis* sp./*E. coli* (x^2=4.6, p<0.05); *G. lamblia*/*E. coli* (x^2=4.9, p<0.05); and *E. vermicularis*/*A. lumbricoides* (Fisher= 0.01).

Parasitological analysis in dogs and soil

Of the total number of analyzed dogs, 68.7% (11/16) were infected with at least one species. Six species were identified and all of them were pathogenic. The most prevalent nematodes were *A. caninum* (62.5%) and *U. stenocephala* (37.5%), followed by *Dioctophyma renale* and *Aelurostrongylus abstrusus* (6.2%). Among the protozoa found, 25% of the samples were positive for *G. lamblia*. As for cestodes, eggs of *Spirometra* spp. were identified (12.5%).

Regarding the soil samples collected, 81.2% were positive (13/16) for at least some parasitic form: 37.5% showed parasites of zoonotic importance (i.e. *Ascaris* sp., *Toxocara* sp.), and 18.7% revealed eggs of veterinary importance (i.e. *Bunostomum* sp., *Cooperia* sp., *Ostertagia* sp.).

Anthropometric analysis

Of all children measured anthropometrically, 60.9% (131/215) were well-nourished and 10.2% (22/215) were undernourished.

The analysis of undernutrition revealed that 3.5% were underweight, 0.9% had low BMI-for-age, and 9.3% presented stunted growth. Age group and sex comparisons showed no significant differences (p>0.05) (Table 2).

Table 2. Prevalence[a] (%) of undernutrition by age group and sex in children from Clorinda, Formosa

Nutritional indicator	Age group					Sex			
	6.0-9.9	10.0-12.9	Total	X^2	p	Boys	Girls	X^2	p
Undernutrition	9.7	10.8	10.2	0.064	0.49	10.0	10.5	0.016	0.54
Underweight	3.5	0.0	3.5	0.037	0.96	1.6	5.9	1.536	0.23
Low BMI	0.9	1.0	0.9	0.005	0.72	0.8	1.1	0.028	0.69
Stunting	8.8	7.8	9.3	0.058	0.49	9.2	9.5	0.006	0.56

[a] Prevalence estimated in relation to total number of analyzed children (n=215)

Seventy-four children were anthropometrically and parasitologically evaluated and 9.5% (7/74) of them showed some type of undernutrition. Undernourished children had more parasitic infections than well-nourished children (85.7% vs. 51.3%) and they were also more infected by *Blastocystis* sp. (85.7% vs. 51.0%) and *A. lumbricoides* (14.3% vs. 8.2%). The percentages of infection by *G. lamblia* in well-nourished and undernourished children were similar (30.6% and 28.6%, respectively).

Socio-environmental analysis

Most families owned the houses they lived in, which were mainly made of brick masonry, and had concrete floors and access to piped water. However, most of them had septic tanks and latrines for wastewater disposal. Domestic solid waste was usually burnt for there was no public collection. Most roads were made of dirt and houses flooded frequently. The majority of the participants lived in overcrowded conditions and shared single beds (Table 3).

Table 3. Socio-environmental variables of the analyzed population from Clorinda, Formosa

Variables	Frequency[a]	
	n	%
Structural qualities and facilities		
Lodging or house tenure status		
House owner	61	54.0
Lease holder	6	5.3
Free lodging	20	17.7
Non-answered	26	23.0
Building materials		
Fired-brick masonry or prefabricated	79	69.9
Makeshift material	8	7.1
Non-answered	26	23.0
Flooring		
Concrete or other	73	64.9
Dirt	14	12.4
Non-answered	26	23.0
Wastewater disposal		
Septic tank	86	76.1
Latrine	21	18.6
Open-air defecation	5	4.4
Sewage system	0	0.0
Non-answered	1	0.9
Drinking water (main source)		
Piped water system	94	83.2
Protected well	1	0.9
Public tap	17	15.0
Solid waste disposal		
Open-air pits	6	5.3
Incineration or non-sanitary burial	87	77.0
Public waste collection	16	14.2
Non-answered	4	3.5
Flooding		
Never	42	37.2
Occasionally	48	42.5
Always	21	18.6
Non-answered	2	1.8
Roads condition		
Paved	5	4.4
Dirt	107	94.7
Non-answered	1	0.9
Overcrowding[b]	61	54
Bed-sharing	65	57.5
Socio-economic characteristics		
Mother's education		
Unschooled	4	3.5
Primary	68	60.2
Secondary	13	11.5
Tertiary/University	3	2.7
Non-answered	25	22.1
Father's education		
Unschooled	1	0.9
Primary	42	37.5
Secondary	10	8.8
Tertiary/University	5	4.4
Non-answered	55	48.7
Mother's employment		
Unemployed/housewife	50	44.2
Temporary	8	7.1
Employed or freelance	26	23.0
Non-answered	29	25.7
Father's employment		
Unemployed	1	0.9
Temporary	43	38.1
Employed or freelance	13	11.5
Non-answered	56	49.6
Monetary support	56	49.6
Food support	12	10.6
Farming practice		
Animal husbandry	22	19.5
Orchard	3	2.7
Health insurance	7	6.2
Pet ownership		
Dog	89	78.8
Cat	36	31.8

[a] Frequency estimated in relation to the total number of children with socio-environmental data (n=113).
[b] More than three people per room

With respect to the educational attainment of parents, the majority of them had only completed primary education. As for their employment status, 38.1% of fathers had temporary jobs and mothers were mostly unemployed or were housewives (44.2%) (Table 3).

The families received monetary and food support from the government (49.6% and 10.6%, respectively) and most of them had access to the public health system. Besides, most families owned pets and only a few practiced animal husbandry and orchard agriculture for personal consumption (Table 3).

As for hygiene practices, most adults stated that their children always washed their hands before eating (76.1%) and after going to the toilet (73.5%) and playing with their pets (58.4%). Additionally, they claimed that they washed fruits and raw vegetables before consumption (76.1%).

About cultural practices, 71.7% of children usually walked barefoot, 46.9% had onychophagia, and 79.6% played in the soil.

The risk of parasitic infection was higher in children that lived in houses where the disposal of domestic residues was inadequate (OR=4.4, 95% CI=1.4-13.7). The analysis of each parasitic species showed that the risk of infection by *Blastocystis* sp. and *G. lamblia* was greater in children whose parents were unemployed or had temporary jobs (OR=3.8, 95% CI=1.04-13.9 and OR=15.7, 95% CI=1.6-154.4, respectively). Furthermore, the risk of infection by *G. lamblia* was high in families that received no monetary support from the government (OR=4.7, 95% CI=1.2-18.3).

DISCUSSION

Of the total number of analyzed children, 78.1% were infected by at least one of the 12 parasite species identified, result that reveals disparity when compared with other populations. In this regard, studies in Venezuela and Paraguay reported a lower prevalence (63% and 35%, respectively) (19,20), but similar or slightly higher prevalence in studies performed in other Latin American countries. (21,22) In Argentina, prevalence was not uniform either since the distribution of parasitic infections changed according to region: values were over 80% in the north (16,23), between 60% and 70% in the center (24,25), and lower than 40% in the south (8).

In this study, boys turned out to be slightly more infected than girls, as well as children aged 6-9 years with respect to younger and older children. Moreover, and in agreement with other studies, no statistically significant association among the variables sex and age was found. (19,26) Despite this, the results suggested that school-age children were the most affected by intestinal parasitosis probably because they had not yet consolidated certain hygiene habits and/or had greater contact with the sources of infection (27).

Furthermore, 76.3% of the children included in this study were infected by pathogenic species, being *Blastocystis* sp. and *G. lamblia* the most prevalent protozoa, which are species also considered the most frequent by other researchers (19,28). Among the non-pathogenic species, *E. coli* showed the highest value; this type of protozoa associated significantly as they share the same way of transmission in water, soil and food when contaminated with feces (3,28). In this context, it should be noted that most of the population studied used septic tanks and latrines or practiced open defecation, and that most houses flooded frequently, favoring the dispersion of these parasites.

Among nematodes, *E. vermicularis* was the most frequently found. *Enterobius vermicularis* infection is favored by gaming habits and personal hygiene practices of children, as well as by bed-sharing and overcrowding. (29) As for the population studied, 50% lived in overcrowded conditions and shared single beds. Moreover, 47% of children had onychophagia.

As for geohelminth infection, 17.5% of children were positive for at least one geohelminth species with values that fluctuated between 0.9-7.9%. In this regard, the presence of these species is favored by warm and humid climate and abundant rainfall, as well as by deficient sanitary conditions such as inadequate excreta disposal, lack of drinking water and floods, all of which characterize and are common in Clorinda. In addition, the lack of access to information and a deficient nutritional status might increase the risk of infection by geohelminths (30,31) The presence of this helminths may also be related to the behavior of vulnerable populations whose hygienic and cultural practices, such as walking barefoot or not washing their hands, are relevant (32). Consequently, infection by geohelminth species in this study was not surprising after finding that more than 75% of children went around barefoot and played in the soil.

This research showed that 70.8% of the analyzed schoolchildren had multiple parasitic infections and the most frequent associations were among protozoa that are transmitted in a similar way (*Blastocystis* sp., *G. lamblia* and *E. coli*). It has been reported that multiple intestinal infections in children is a widespread phenomenon caused by the interaction of common environmental factors, means of infection, host exposure, susceptibility, as well as behavioral and socio-economic factors that facilitate the co-occurrence of diverse parasites (33).

About the analyzed dogs, 68.7% were infected, being *A. caninum*, *U. stenocephala* and *Giardia* sp. the most pre-

valent species. In addition, a small percentage of *D. renale* and *Spirometra* sp., as well as larvae of *A. abstrusus*, was identified. The finding of *D. renale* in feces was unusual; however, their presence could have been caused by cross-contamination with urine. *Spirometra* is a genus of pseudophyllid cestodes that infects canines and felines, but causes sparganosis in humans by either drinking water contaminated with infected copepods (intermediate host) or by consuming raw meat of infected second intermediate hosts or paratenic hosts (including fish, reptiles and amphibians) (34). *Aelurostrongylus abstrusus* is a strongylid nematode that affects the respiratory system of domestic cats and other wild felids, which acquire the infection by ingesting infective larvae encysted with paratenic hosts (usually snails or birds) (35). In this context, *Spirometra* sp. and *A. abstrusus* have been reported for the first time in Clorinda. Most of the species found in dogs were potentially zoonotic and, therefore, able to affect human health. This study also showed the presence of eggs of *Ascaris* sp. and *Toxocara* sp. in the soil. These results confirm that the presence of zoonotic species is favored by inadequate disposition canine feces, the lack of environmental sanitation, a close contact with pets, and, eventually, the presence of intermediate hosts essential to complete the life cycle of some parasitic species (i.e. *D. renale*, *Spirometra* sp. and *A. abstrusus*) (17,34,36).

The prevalence of undernutrition (10.2%) was high compared to other provinces of Argentina. (37,38) Moreover, prevalence of underweight (3.5%) and stunting (9.3%) showed that children experience serious nutritional deficiencies as a consequence of the limited socio-economic conditions of their families, inadequate hygiene practices and sanitation. This scenario was reflected in undernourished children who were the most infected, especially by *Blastocystis* sp. and *A. lumbricoides*. Other studies also confirmed an association between malnutrition and parasitism, and stated that it could be due to the negative effect that enteroparasites have on the physical and cognitive development of children, or by the effect of the nutritional deficit on the immune response, which in turn increases the chances of acquiring parasitic infections (5,39).

Lastly, the regression analysis showed that the risk of being infected was higher in children who lived in houses where the disposal of domestic residues was inadequate and whose parents were unemployed or had temporary jobs. Other researches demonstrated that access to housing with basic sanitary services (e.g. drinking water, adequate excreta and solid waste disposal) and having educated and employed parents have a direct impact on the health of children (40).

The results show that the lack of environmental sanitation, unstable employment of parents and the presence of zoonotic species were the most relevant factors in the analyzed population. Consequently, these conditions result in an increase of parasitic infections and influence the growth of children negatively. This study aims at increasing the parasitological knowledge of the region to improve the nutritional status of children and the sanitation of the environment through a comprehensive approach to prevent and control intestinal parasitosis.

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