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ORIGINAL ARTICLE

PREVALENCE OF INTESTINAL PARASITES IN A CLINICAL ANALYSIS LABORATORY IN SOUTHERN BRAZIL: A RETROSPECTIVE STUDY

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ABSTRACT

The population's living conditions, basic sanitation, hygiene, and poor socioeconomic status, are determining factors for diseases´ transmission, such as intestinal parasitic infections which constitute one of the main public health problems in Brazil. These diseases are considered endemic in several areas of the country, presenting a wide geographic distribution, varying according to environmental conditions and parasites´ species. Therefore, the present study aimed to evaluate the prevalence of intestinal parasites in individuals attending a clinical analysis laboratory in a municipality located in Southern of Brazil. A retrospective study was carried out through the analysis of 2,247 reports of parasitological stool examination from individuals who attended a clinical analysis laboratory located in Veranópolis, Rio Grande do Sul, from September 1st, 2018, to December 31st, 2020. Parasitic structures were found in stool samples from 181 (8.1%) individuals. The protozoans *Endolimax nana* and *Entamoeba coli* were the most prevalent parasites, being present in 58.0% and 29.8% of individuals, respectively. A higher prevalence of intestinal parasites was observed in women (52.5%), aged 21 to 60 years old (62.4%). Biparasitism or polyparasitism was present in 7.2% (13/181) of individuals and only 31.7% (713/2247) of patients collected three fecal samples for examination. The low prevalence of intestinal parasites found in the present study may be an indicator of improvements in sanitary, environmental, and health education conditions.

KEY WORDS: Parasitic diseases; helminths; parasites; protozoans.

INTRODUCTION

The increasing prevalence of parasitic diseases represents one of the main concerns in public health, especially in developing countries, reaching approximately 24% of the world population (WHO, 2020). Intestinal parasitic diseases are considered endemic in several areas of Brazil, and present a wide

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geographic distribution, varying according to parasites´ environment and species. Moreover, they are related to socioeconomic status and the lack of basic sanitation infrastructure (Zardeto-Sabec et al., 2018; Cordeiro et al., 2019). Several parasitic infections, such as soil-transmitted helminths and schistosomiasis, are still classified as neglected tropical diseases due to the low funding and support in control, research, and medicine production, and the most affected countries do not present efficient health education programs (Maia & Hassum, 2016; Lima et al., 2020).

Intestinal parasitic infections are mainly caused by protozoans and helminths belonging to the Protozoa, Platyhelminthes, Nematoda, and Acanthocephala phyla. They colonize the human gastrointestinal tract and induce chronic diarrhea, abdominal pain, anemia, and malnutrition, as well as other more severe complications, e.g. intestinal obstruction, rectal prolapse, and formation of extra-intestinal abscesses. The living conditions including basic sanitation are determining factors for the dissemination of these parasites (Andrade et al., 2010; Ludwig et al., 2016; Silva et al., 2018; Munareto et al., 2021). Regarding the prevention of intestinal parasitic infections, some aspects should be considered, such as basic sanitation, improvements in infrastructure, health education, proper treatment of solid waste, water and sewage, also careful management of soil related to irrigation and fertilization. Besides personal hygiene habits including handwashing, avoid consuming raw or rare meat and contaminated water might reduce parasitic diseases (Azevedo et al., 2013; Santana et al., 2014).

The helminths *Ascaris lumbricoides, Trichuris trichiura,* hookworms, and the protozoans *Entamoeba histolytica* and *Giardia intestinalis* are the main agents of intestinal parasitic infections (WHO, 2020). The transmission is through water or food contaminated by parasites ingestion and/or by the active penetration of larvae through the skin or gut (Soares et al., 2018). Thus, epidemiological aspects are important factors that contribute to parasitic infections, and they are related to the host and parasite conditions and environment. In this context, poor sanitation and water contamination play an essential ecological role in several parasites and vectors life cycle (Antunes et al., 2020).

The prevalence of intestinal parasites varies in the Brazilian regions from 5.3% to 68% according to previous studies, based on health, education, local social and economic conditions, climate, populational index, and individuals´ hygiene habits (Alves et al., 2014; Ludwig et al., 2016; Sampaio & Barros, 2017; Sousa et al., 2019; Ferlito & Dalzochio, 2020). Nonetheless, only a few studies have assessed the occurrence of parasites in the Southern region in Brazil. Thus, the present study aimed to evaluate the prevalence of intestinal parasites in individuals attending a clinical analysis laboratory located in the city of Veranópolis, RS.

MATERIAL AND METHODS

A descriptive and retrospective study was carried out through the analysis of 2,247 parasitological stool examination (PSE) reports from individuals attending a clinical analysis laboratory located in the municipality of Veranópolis in Rio Grande do Sul (RS). The reports issued from September 1st, 2018 to December 31st, 2020 were included in the analysis. This laboratory was chosen because it has five units in different cities and they attend a microregion that comprises approximately 90 thousand inhabitants. The population attended by the laboratory consists mainly of rural and industry workers, including food handlers from collective feeding units. In addition, a laboratory branch is located in a community hospital and it has *Organização Nacional de Acreditação* (ONA) level 3 certification, which provides higher reliability in the quality of the services. The present study was approved by the Ethics Committee from *Centro Universitário CNEC, Bento Gonçalves*, protocol n. 4.854.813.

The laboratory processes stool samples routinely by the centrifugal-sedimentation method. Initially, a small amount of stool is collected by the individuals and stored in a proper flask provided by the laboratory. For the exam, the samples are diluted in 10% formalin and then homogenized. After 30 minutes, 10 mL are transferred to a conic tube and centrifuged for 5 minutes at 672 g. For microscopical analysis, one drop of the sediment is deposited on a slide with a Pasteur pipette, stained with Lugol's iodine, and covered with a coverslip. The slides are analyzed by at least two experts and an atlas of parasitology was used for comparisons.

The following variables were collected from the reports: individuals´ gender and age, number of requested/analyzed samples, and the analysis result. Data were expressed as absolute and relative (%) numbers. Statistical analysis was performed using the Prism GraphPad software, version 8.0.1. The chi-squared test (χ^2) was used to verify the association between the variables, and $p<0.05$ was considered significant.

RESULTS

Considering the 2,247 reports of PSE, one or more parasitic structures were found in 181 individuals´ samples, corresponding to a prevalence of 8.1%. Among the helminths, *Ascaris lumbricoides* was the most prevalent parasite, whereas *Endolimax nana* was the most prevalent protozoan (Table 1). Other parasites less frequently found include the helminths *Enterobius vermicularis*, *Taenia* sp. and *Hymenolepis nana*, and the protozoans *Blastocystis hominis, Cystoisospora (Isospora) belli*, and structures similar to *Enteromonas hominis.*

Table 1. Distribution of helminths and protozoans in individuals attending a clinical analysis laboratory located in Southern Brazil, between September 1st, 2018 and December 31st, 2020.

Species	n	%*
Helminths		
Ascaris lumbricoides	7	3.9
Trichuris trichiuria	4	2.2
Strongyloides stercoralis	2	1.1
Other	3	1.7
Protozoans		
Endolimax nana	105	58.0
Entamoeba coli	54	29.8
Iodamoeba butschlii	9	5.0
Entamoeba histolytica/dispar	6	3.3
Giardia intestinalis	5	2.8
Other	5	2.8

* Percentage calculated according to the total number of positive samples.

Among the individuals with positive samples, 7.2% (13/181) presented biparasitism or polyparasitism, and the most prevalent association was *Endolimax nana* + *Entamoeba coli*, found in 38.4% of individuals (Table 2).

Table 2. Prevalence of intestinal parasites in individuals with bi or polyparasitism attending a clinical analysis laboratory located in Southern Brazil, between September 1st, 2018 and December 31st, 2020.

Species	n	%
Endolimax nana + *Entamoeba coli*	5	38.4
Endolimax nana + *Entamoeba coli* + *Iodamoeba butschlii*	4	30.8
Hymenolepis nana + *Entamoeba coli*	1	7.7
Ascaris lumbricoides + *Entamoeba coli*	1	7.7
Endolimax nana + *Entamoeba histolytica/dispar*	1	7.7
Endolimax nana + *Iodamoeba butschlii*	1	7.7
Total	13	100

A higher prevalence of parasites was found in female individuals, corresponding to 52.5% (95/181), whereas a prevalence of 47.5% (86/181) was observed in male individuals (Table 3). However, there was no significant association between these variables (occurrence of parasites and gender) (p=0.13).

Table 3. Comparison of the prevalence of intestinal parasites between individuals gender attending a clinical analysis laboratory located in Southern Brazil, between September 1st, 2018 and December 31st, 2020.

	Positive		Negative		Total		p value
	n	%	n	%	n	%	
Male	86	47.5	861	41.7	947	42.1	0.13
Female	95	52.5	1205	58.3	1300	57.9	
Total	181	100	2066	100	2247	100	

The average age of individuals with positive samples was 32.9±19.7 years old and the median corresponded to 33 years old. To assess the most affected age group in the study and to analyze a potential association between age and bi/polyparasitism and monoparasitism, ages were divided into groups (Table 4). Adults (21 to 60 years old) were the most affected by intestinal parasites, presenting a prevalence of 62.4% (113/181), whereas the older adults (>60 years old) were less affected. However, there was no significant association between the age group and the presence of one or more parasitic structures in the fecal samples (p=0.23).

Table 4. Association between monoparasitism and bi/polyparasitism according to the individuals age group attending a clinical analysis laboratory located in Southern Brazil and the positive result of the parasitological stool examination (PSE) for intestinal parasites.

	Bi/polyparasitism		Monoparasitism		Total		p value
Age group	n	%	n	%	n	%	
<10 years old	1	7.7	34	20.2	35	19.3	0.23
11 to 20 years old	3	23.1	16	9.5	19	10.5	
21 to 60 years old	9	69.2	104	61.9	113	62.4	
>61 years old	0	0	14	8.3	14	7.7	
Total	13	100	168	100	181	100	

Regarding the number of samples collected to perform the exam, only 31.7% (713/2,247) of individuals collected three samples, and from these, 51 had positive results for the presence of parasitic structures (Table 5). However, there was no significant association between these variables (p=0.07).

Table 5. Association between the number of fecal samples collected/analyzed per patient and the result of the parasitological stool examination (PSE).

	PSE positive		PSE negative		Total		p value
Number of samples collected/analyzed	n	%	n	%	n	%	
One sample	130	71.8	1,362	65.9	1,492	66.4	
Two samples	0	0	42	2.0	42	1.9	0.07
Three samples	51	28.2	662	32.1	713	31.7	
Total	181	100	2,066	100	2,247	100	

DISCUSSION

Despite the high levels of morbidity and mortality related to parasitic diseases, they are still classified as neglected tropical diseases. Therefore, studies on the prevalence of intestinal parasites are necessary to provide local epidemiological information on the subject, and also to guide strategies related to health education.

The prevalence of intestinal parasites found in this study, corresponding to 8.1%, was equal to another study conducted in the city of Sananduva, RS, where 8.1% (44/546) of individuals presented positive results for parasites, and the most frequent structures were *E. nana* and *E. coli* cysts, found in 44.4% and 24.4% of fecal samples respectively (Bellin & Grazziotin, 2011). Similarly, another study evidenced the same prevalence of 8.1% (10/124) in individuals from Ipê municipality, also located in RS (Zanotto et al., 2018). A lower prevalence was found in the city of Bento Gonçalves, RS, corresponding to 5.3% (96/1808), where the most prevalent parasites were *E. nana, Giardia intestinalis* and *E. coli* (Ferlito & Dalzochio, 2020). In contrast, higher prevalences were reported in studies conducted in Brazil Northeast region. Filho et al. (2017) found a prevalence of 12.73% (840/6,596) of parasites in individuals from the metropolitan region of Fortaleza, Ceará (CE). Accordingly, another study observed that 23.3% (295/1,266) of samples analyzed from individuals using the *Sistema Único de Saúde* (SUS), in the city of Limoeiro do Norte, CE, were positive for parasites, where *E. nana* and *E. coli* were observed in 44.2% and 27.4% of samples respectively (Maia et al.,

2015). Other studies conducted in the Southeast and North regions of Brazil also reported higher prevalence of parasites, corresponding to 17.5% and 32.4% respectively (Faria et al., 2017; Lima et al., 2020). These data demonstrate that the occurrence of parasites in the population might be associated with local sanitary conditions, public water supply, domestic waste collection and access to health and education services (Andrade et al., 2010).

Regarding the parasites, the commensal protozoan *E. nana* was the most prevalent, corroborating studies conducted in Brazilian Southern (Casavechia et al., 2016; Ferlito & Dalzochio, 2020), North (Lima et al., 2020), and Northeast (Lima et al., 2016; Silva et al., 2018). It is relevant to highlight that non-pathogenic amoebas demontrated the same transmission routes as other potential pathogenic parasites. Despite the lack of clinical importance, their identification has epidemiological significance because they are related to socioeconomic, environmental, and poor sanitary conditions to which humans are exposed (Pereira et al., 2011). Among the pathogenic protozoan species, *G. intestinalis* and *Entamoeba histolytica/dispar* cysts were found, being the later (*E. dispar*) commensal, but morphologic undistinguished from *E. histolytica* which might cause intestinal and extra-intestinal amoebiasis (Dulgheroff et al., 2015).

Considering the helminths, a higher prevalence of *A. lumbricoides* was also observed, which corroborates studies conducted in other Brazilian States, such as Bahia, Maranhão, and Amazonas (Gonçalves et al., 2016; Sousa et al., 2019; Moreira et al., 2020). Usually, this parasite is more frequent in children due to the constant contact with soil and water for recreational activities (Cavagnolli et al., 2015). *A. lumbricoides* is a geo-helminth transmitted by the ingestion of water and/or food contaminated with viable and infective eggs (Silva et al., 2019). This parasite can cause diarrhea, nausea, Löeffler syndrome (a condition similar to pneumonia), intestinal obstruction, and severe infection in children, besides nutritional and cognitive deficits (Andrade et al., 2010; Soares et al., 2018; Teixeira et al., 2019).

The prevalence of biparasitism/polyparasitism observed in this study was 7.2%, thus lower in comparison to other studies, in which biparasitism was found in 44.6% (58/130) and polyparasitism in 32.5% (64/197) (Martins et al., 2014; Melo et al., 2015). Among the cases of biparasitism, the association between the commensal protozoans *E. coli* and *E. nana* was the most frequent. This same association was reported in a study conducted in the city of São Francisco, Minas Gerais, but with higher frequency (in 48.9% of individuals with two parasites) (Souto et al., 2012). Only one case of polyparasitism was found in the present study consisting of the association of *E. nana, E. coli,* and *Iodamoeba butschlii.* Nonetheless, another study reported a prevalence of polyparasitism in 21.4% of individuals from the city of Santa Cruz in the Northeastern region (Lima et al., 2020). Thus, the variation in the prevalence of parasitic diseases among the Brazilian regions is evident. Such variation

might be due to local sanitary conditions, access to health services, as well as to methodological differences related to the number of stool samples analyzed per individuals and the technique used for the detection of parasites. In addition, data extracted from previous studies in Brazil should be analyzed with some caution, once they were limited, isolated, and usually they reflect the results from small towns and/or of restricted groups (day-care centers, schools, indigenous tribes, small hospitals, and so forth) (Faria et al., 2017).

In the present study, adults from 21 to 60 years old and female individuals were mostly affected by parasites. Despite the higher prevalence in these groups, there was no significant association between the occurrence of parasites and the variables of gender and age. Previous studies conducted by Lima et al. (2020), Cordeiro et al. (2019), and Silva et al. (2018) corroborate this data, which prevalence in these groups corresponded to 43.9%, 47%, and 36% respectively. It is likely that the transmission of intestinal parasites in this age group is associated with poor hygiene conditions (personal and/or environmental), and occurs through the ingestion of eggs or cysts present in water or raw food (Cunha & Amichi, 2014; Silva et al., 2018). It is important to highlight that although children are more susceptible to parasitic infections, individuals attending the laboratory of clinical analysis located in Veranópolis, RS, were mainly adults. Thus, it was expected to observe a lower prevalence of parasites in children.

Although there was no significant association between the PSE results and the number of samples analyzed, it is well known that the analysis of one single sample per individuals results in a lower diagnosis efficacy, since parasites (when present) may be intermittently eliminated by the host leading to false-negative results and consequently to underestimated data. Thus, some authors assert that the analysis of multiple samples collected every other day improves laboratory diagnosis (Escobar-Pardo et al., 2010; Menezes et al., 2013).

Some of the limitations of this study include the use of one single method for the detection of parasitic structures in fecal samples. According to the technical manager responsible for the laboratory, a second method is not employed due to financial costs, which occurs at the majority of laboratories in Brazil, where it is necessary to reduce some exams cost. Another limitation is the lack of structure to perform differential staining and/or measure microscopic structures.

In conclusion, a low prevalence of intestinal parasites was found in comparison to previous studies conducted in Brazil. The occurrence of protozoan was higher than helminths and, adult females were the most affected group. However, data from this study might be underestimated due to the analysis of one single fecal sample in most individuals and the use of only one method for parasitological analysis. Other factors that might explain the low prevalence of intestinal parasites include the living conditions from

the population studied. In Brazil, as in other countries, parasitic infections are still a public health concern, although their frequency has decreased as a consequence of basic sanitation improvement in some areas. Thus, the implementation of educational practices in local communities, as well as the treatment of asymptomatic individuals may contribute to parasitic diseases prevention.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest to disclose.

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