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Research Article

Enteric parasitic infections among HIV-positive patients that are receiving care at the Single Specialized Assistance Health Service in Pelotas - RS, Brazil.

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Abstract: Enteric parasitic infections are important issues on public health, mainly when they are associated with the Human Immunodeficiency Virus (HIV). The objective of this study was to understand the prevalence and the risk factors associated with enteric parasites among HIV-positive patients who were attending at the single specialized assistance health service in HIV/AIDS in Pelotas, a city in southern Brazil. Out of 273 patients who were interviewed in this study, 19.8% had enteric parasites, and the most frequent pathogens were *Trichuris trichiura*, *Giardia lamblia* and *Ascaris lumbricoides*. The prevalence of opportunistic enteric parasites (*Cryptosporidium* spp., *Cystoisospora belli* and *Strongyloides stercoralis)* was low; and it was 1.9% and 2.4%, respectively, among who were and were not undergoing antiretroviral therapy (HAART). This difference was not statistically significant. People in socially vulnerable conditions, who had domestic animals and were not undergoing HAART, would have more chance to suffer from enteric parasitic infection when contrasted with the compared groups. The study has shown that it is necessary to adopt and carry out preventive and diagnosis actions among HIV-positive patients.
Keywords: HIV, enteric parasites, parasitology, cross-sectional study and humans, HAART, opportunistic parasites, immunodeficiency virus

INTRODUCTION

The acquired immunodeficiency syndrome (AIDS) is the clinical manifestation of the infection caused by human immunodeficiency virus (HIV), with progressive decrease in cellular immunity and subsequent opportunistic infections and malignancies [1, 2]. These opportunistic infections include intestinal parasites, such as those caused by *Cryptosporidium spp.*, *Cystoisospora belli* and *Strongyloides stercoralis*. The enteric infections implicate in immunological changes that favor the progression of the infection from seroconversion of AIDS, which demonstrates the mutual interaction between enteric parasites and AIDS [3, 4].

Enteric parasites represent an important public health problem, especially in developing countries where sanitation conditions are deficient [5, 6] and socioeconomic conditions are poor [8], highlighting fecal contamination of soil and water [7]. One of the clinical manifestations associated with enteric parasites is diarrhea, which is directly linked to malnutrition [6, 8, 9].

High prevalence of opportunistic enteric parasitic infections in AIDS patients were recorded in the 1980s and early 1990s, which presented important clinical manifestation of diarrhea that could evolve to death, and the most prevalent agents were *Giardia lamblia* (26.7%), *Cryptosporidium parvum* (24.4%), *Cystoisospora belli* (6.7%) and *Strongyloides stercoralis* (6.7%) [7,10]. Highly active antiretroviral therapy (HAART) had been gradually introduced from 1996, making it possible to control AIDS and subsequent reconstitution of the immunological system of these individuals, which led to a decrease in the prevalence of enteric parasites in this population [11]. Even with HAART, the prevalence of enteric parasites in HIV positive patients is high, ranging between 24% and 47.5% [3, 10, 12, 13].

After the introduction of HAART, few studies identifying the profile of HIV-infected patients with enteric parasites were found in the literature [14, 15, 16]. Therefore, knowing the profile of HIV-infected patients with enteric parasites, attending a Specialized Assistance Service (SAE), will develop and direct strategies for primary and secondary prevention in public health in developing countries such as Brazil.

MATERIALS AND METHODS

It was conducted a cross-sectional study in HIV-positive patients treated at Specialized Assistance Service (SAE) - Federal University of Pelotas (UFPel)/Municipal Health Department of Pelotas (SMS), Brazil, during the period of April 2013 to March 2015. This service (SAE) is accredited by the Unified Health System (SUS) and it is a reference for patient care in the city and region.

The HIV seropositive patients treated at SAE, which received the request to perform CD4 T lymphocyte count and viral load, were invited to participate in the study, provided recipients for the collection of clinical material (three stool samples) and instructions for the collection and wrapping (refrigerated between 4 and 8°C). The stool samples were performed on alternate days, taking into account the intermittent disposal of cysts and oocysts from protozoa. Subsequently, patients gave the vials with samples at the Municipal Laboratory of Pelotas, accredited by SUS. In this place, structured questionnaires were applied to patients by trained interviewers.

On the day of arrival of the samples, a larval migration method was performed (Baermann-Moraes Technique) to search for larvae of *Strongyloides stercoralis* and method of flotation solution of zinc sulfate - 1.18 density (Faust Technique) [17] to search for helminths eggs and protozoan cysts. Besides this, the Laboratory of Parasitology, Department of Microbiology and Parasitology - Biology Institute - Federal University of Pelotas (UFPel) was used as a technical support for diagnostic confirmation.

Thereafter, fecal samples were preserved in formalin solution (three parts 10% formalin and one part of faeces) for performing the method of centrifugal sedimentation with formalin-ethyl acetate (modified Ritchie Technique) as recommendations of the Center for Disease Control and Prevention, Atlanta, USA (CDC, 2009) [18]. Part of the sediment was used to search for eggs, larvae helminth and protozoan cysts. In case of doubt on the identification of the cysts, a Trichrome coloration technique was performed [18]. Part of the sediment was used to oocysts of protozoans' research (coccidia).

The research on oocysts was performed at the Laboratory of Parasitology, Biomedical Sciences Interdisciplinary Area (AICB) – Medical School (FAMED) – Federal University of Rio Grande, by Kinyoun acid fast staining and morphometry. It was also performed by safranin staining and the sporulation technique with a solution of potassium dichromate 2.5% to accomplish differential diagnosis of *Cryptosporidium spp.* and *Cyclospora cayetanensis* [18, 19].

The sample size calculation was based on the total of 708 patients followed at the SAE, prevalence of enteric parasites in 40% of HIV-positive patients, with an acceptable error of 5 percentage points and a significance level of 95%, increased by 10% for losses, requiring a total of 266 patients. To evaluate associations of independent variables with the outcome, we used "skin color", with a ratio exposed / unexposed 1:3 and prevalence of enteric parasites 9% in unexposed (white), statistical power of 80% to detect prevalence ratios of at least 3, with a confidence level of 95%. 10% were added for losses and 15% for confounding factors, requiring a sample of 253 patients.

The data entry and cleaning were performed using Epi Info 6.04d (CDC, Atlanta, 2001), while the analysis was performed by Stata 8 (Stata Corp, College Station, TX, 2001). A descriptive analysis was performed, examining frequencies and measures of central tendency and dispersion of variables. The gross analysis characterized the associations between risk factors studied and the prevalence of pathogenic enteric parasites. The adjusted analysis was performed using backward unconditional logistic regression, following the conceptual model proposed, in which the more proximal level were the demographic variables (age and sex); the second level, the socioeconomic variables (economic level according to the Brazil Economic Classification Criterion, Brazilian Association of Research Companies – ABEP [20] and skin color) and the most distal level were the variables "type of household sewage," "habit of washing vegetables", "having domestic animals", "type of sexual relationship", "being or not being treated with antiretroviral ", "value of CD4 T lymphocyte "and" viral load - CV". During the statistical modeling, the significance of each predictor was calculated with adjustment for variables in the same level and upper levels, respecting the conceptual model, and those which presented tests with a value between 5 and 20% were retained in the analysis to control confounders.

The project was approved by the Research Ethics Committee of the Medical School - UFPel (Letter 05407) and the SAE, asking the respondent informed consent (signed by the participant) to the questionnaire and collecting information from his medical record. The confidentiality of information and the right of refusal were granted to the respondent.

RESULTS

The study interviewed 273 patient and collected 682 fecal samples, with an average of 2.5 samples per individual. Overall, 53.1% were male. As regards age, 7% were under 13 years of age, 8.8% were between 13 and 25 years of age, 63.4% were between 26 and 49 years of age and 20.9% over 50 years of age. To classify them as socioeconomic status, 79.7% belonged to the lower economic levels (D and E classes, according to ABEP), 68.5% were classified as white skin and 31.5% non-white. Among the sample, 84.2% lived in the city of Pelotas. About housing aspects, 84.9% lived in brick houses, 49.3% had public sewage network and 76.8% had some domestic animal. With regard to sexual behavior, 9% (25/272) reported ever having had sexual intercourse, 8% (21/272) reported homosexual, 4% (11/272) reported bisexual relationship and 79% reported having heterosexual intercourse. Overall, 62.2% of the respondents were making use of antiretroviral, 33.7% had CD4 T lymphocyte count above 500 cells / mm3 and 80.7% had a viral load smaller than 10.000 copies RNA/ml. Regarding the outcome, 19.8% (54/273) of HIV-positive patients treated at SAE, Pelotas, Brazil had some pathogenic enteric parasite and 27.8% (76/273) had some type of nonpathogenic enteric parasite.

Among nematodes, the higher prevalence rates were *Trichuris trichiura* (9.2%) and *Ascaris lumbricoides* (4.4%), while the most frequent protozoan was *Giardia lamblia* (7.7%). The prevalence rates of *Cryptosporidium spp.*, *Cystoisospora belli* and *Strongyloides stercoralis*, considered opportunistic parasites, ranged from 0.4 to 1.1% (Figure 1).

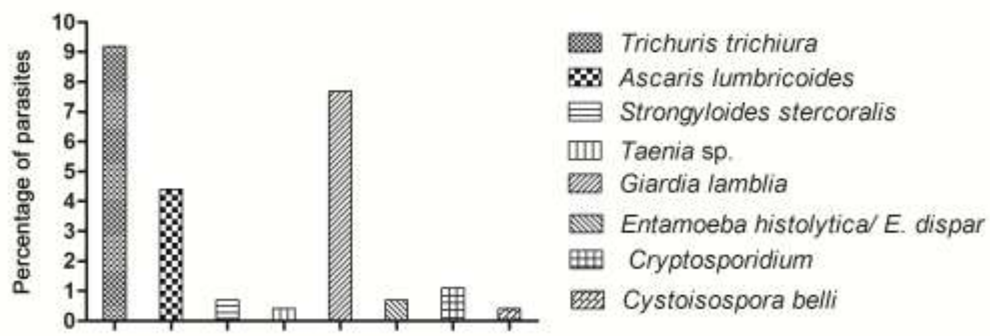

Fig. 1: Prevalence of pathogenic enteric parasites in HIV + patients treated at Specialized Assistance Service (SAE), Pelotas, RS, Brazil. (N = 273)

Among the 273 HIV-infected patients, 15.8% (43) had single infections and 4% (11) had multiple intestinal parasites, confirmed that both the simple infections as in multiple intestinal parasites there was a predominance of *T. trichiura, A. lumbricoides* and *G. lamblia* occurred (Figure 2). The proportion of single and associated infections of each gender or species of pathogenic enteric parasites identified in parasitological stool examinations can be observed in Table 1.

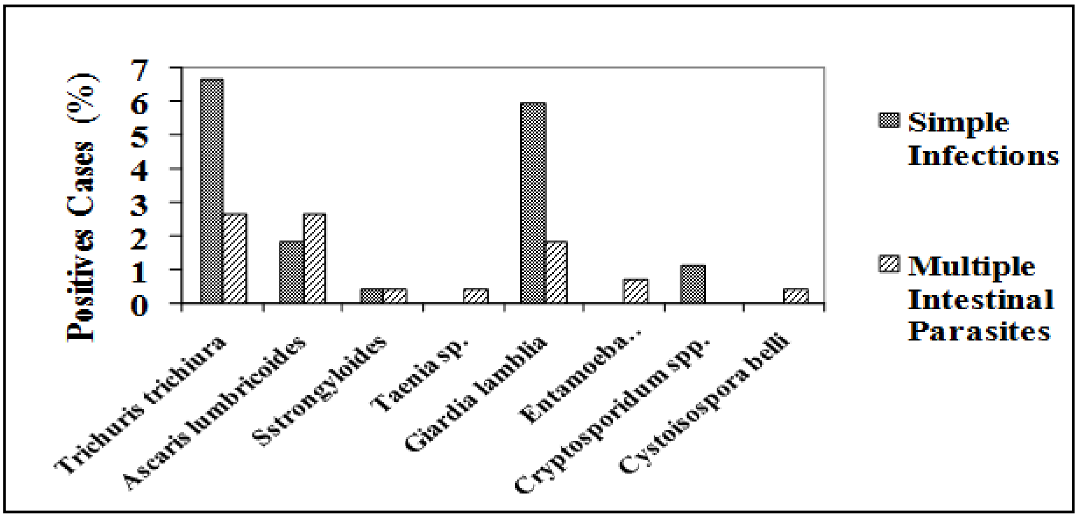

Fig. 2: Prevalence of simple infections and multiple intestinal parasites by gender or species of pathogenic enteric parasites in HIV + patients treated at Specialized Assistance Service (SAE), Pelotas, RS, Brazil. (N = 273).

Table 1: Percentage of simple and associated infections of each gender or species of pathogenic enteric parasites identified in parasitological stool examinations of HIV + patients treated at Specialized Assistance Service (SAE), Pelotas, RS, and Brazil. (N = 54).

Enteric parasites	+	%
Ascaris lumbricóides	5	9.3
Trichuris trichiura	18	33,3
Strongyloides stercoralis	1	1.9
Giardia lamblia	16	29.6
Cryptosporidium spp	3	5.6
Giardia lamblia / Entamoeba histolytica/E. dispar	2	3.7
Giardia lamblia / Cystoisospora belli	1	1.9
Giardia lamblia / Trichuris trichiura	1	1.9
Giardia lamblia / Ascaris lumbricóides	1	1.9
Trichuris trichiura / Ascaris lumbricoides	4	7.4
Trichuris trichiura / Ascaris lumbricoides / Strongyloides stercoralis	1	1.9
Trichuris trichiura / Ascaris lumbricoides l Taenia sp.	1	1.9
Total	54	100

The prevalence of non-pathogenic enteric parasites was 27.8% (76/273), mainly *Enteromonas hominis* (15.8%) and *Entamoeba coli* (8.4%). Also cysts were identified *Iodamoeba buetschlii* and *Endolimax nana* (Table 2). These protozoa were identified in stool samples of 29.6% (16/54) of patients who were positive for pathogenic enteric parasites. The prevalence of opportunistic enteric parasites was 1.9 and 2.4%, respectively, between those who were and were not treated with HAART. This association was not statistically significant.

Table 2: Prevalence of simple and associated infections of each gender or species of nonpathogenic enteric parasites identified in parasitological stool examinations of HIV + patients treated at Specialized Assistance Service (SAE), Pelotas, RS, Brazil. (N = 273).

	N=273	
Nonpathogenic enteric parasites	+	%
Entamoeba coli	23	8.4
Enteromonas hominis	43	15.8
Endolimax nana	4	1.5
Iodamoeba buetschlii	1	0.4
Entamoeba coli / Enteromonas hominis	2	0.7
Entamoeba coli / Endolimax nana	1	0.4
Entamoeba coli / Iodamoeba buetschlii	1	0.4
Enteromonas hominis / Endolimax nana	1	0.4
Total	76	100

The analysis of the associations between the independent variables and the outcome (the presence of pathogenic enteric parasites) showed that people with lower socioeconomic status (class E) were four times more likely (RO:4,1, IC95%:1,3-12,3) of having the outcome when compared with people of higher socioeconomic status (B and C classes). Moreover, people who reported having domestic animals had 3.6 times (95% CI: 1.3 - 9.8) more likely to have the outcome than those who had no domestic animals. It was also demonstrated that patients who were not taking antiretroviral drugs were 2.6 times more likely (95% CI: 1.4 - 5.2) to develop pathogenic enteric parasites than people who were on antiretroviral therapy (Figure 3). Age, sex, skin color, type of household sewage and viral load (average of 20,940) were not associated with outcome in the current study. The variables "habit of washing vegetables", "type of sex" and "value of CD4 T lymphocyte" (average of 507,9) were not taken to the multivariate analysis model for presenting p-value> 0.2 during bivariate analysis.

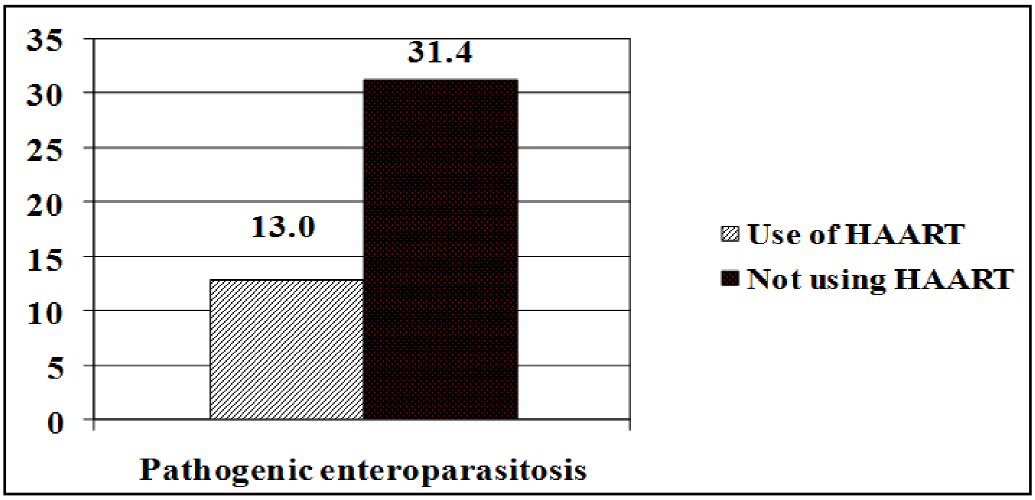

Fig. 3: Prevalence of pathogenic enteric parasites according to the use of antiretroviral therapy (HAART) in HIV + patients treated at Specialized Assistance Service (SAE), Pelotas, RS, Brazil (N = 273).

DISCUSSION

The study shows that the pathogenic enteric parasites were present in one-fifth of the interviewees. Factors that were significantly associated with a higher prevalence of the outcome were: lower economic level, having domestic animals and not being treated with antiretroviral drugs. In this study, the sample was age and sex distribution similar to that found in another study in the same service (SAE Pelotas) [21].

The prevalence found in this study was similar to that found for Zali *et al* [22] and lower than that reported by Sarfati *et al* [13]. This variation may be due to the population studied in this research regularly consult the SAE, where they can receive treatment for enteric parasites and other diseases related to HIV [4]. Furthermore, the study was conducted in the south of the country where the prevalence of enteric parasites is lower than the northeast, probably due to differences in socio-economy, health and climate of these regions [3, 23, 24, 25].

These results agree with some studies [4, 7, 26, 27], which reported that among nematodes, the most prevalent are *Trichuris trichiura* and *Ascaris lumbricoides*, and, between the intestinal protozoan, the most prevalent is *Giardia lamblia*. However, Bachur *et al* [3] found a higher prevalence of *Strongyloides stercoralis* among nematodes in a study conducted in northeast Brazil, where the tropical climate provides that people walk barefoot longer, favoring skin penetration of infective larvae [17, 25]. The opportunistic parasites most frequently found were similar to those found by Sarfati *et al* [13], except for microsporidia, which were not investigated in this study.

There was no association between HAART and opportunistic infection by parasites, possibly due to lack of statistical power of the study, being demonstrated this association in the work of Sarfati *et al* [13].

The prevalence of multiple parasitic infections was similar to that observed in another study conducted in Brazil, where the enteric parasites most frequently associated with multiple parasitic infections were *Giardia lamblia, Ascaris lumbricoides* and *Entamoeba coli* [10].

The frequency of non-pathogenic enteric parasites of almost 30% in the population studied is relevant, because although these parasites are not pathogenic to humans, they suggest fecal contamination, in addition to potentiating the transmission of other parasites [27].

Regarding the associations between the independent variables and pathogenic enteric parasites, this study is consistent with the literature. The low socioeconomic status reflects worst level of education, health, nutritional health, which may provide greater exposure to enteric parasites [22, 28, 29]. The association between having domestic animal and the presence of enteric parasites in immunocompromised patients in this study has been reported, possibly due to contamination by the fecal oral route [30, 31, 32].

Many authors have demonstrated the association between HAART and lower prevalence of outcome [3, 13, 31, 33]. According to Bachur *et al* [3], the prevalence of opportunistic enteric parasites declined after the advent of HAART, as a result of improved immune system responses and decrease in HIV replication. Another study conducted at the University Hospital of Rio Grande, Brazil, measured the prevalence of *Cryptosporidium spp.* in the pre-HAART (1994 to 1997) and post-HAART (2000 to 2004), and recorded a reduction from 22% to 5%, respectively [34].

The association between low CD4 T lymphocytes and the high prevalence of pathogenic enteric parasites were found by some authors [24, 28, 35]. However, as in the current study, Cimerman *et al* [10] did not find this association. Hosseinipour *et al* [18] also found no association between the level of viral load (CV) and the outcome. These results can be explained due to the study population be composed of individuals presenting no extreme immunodeficiency (i.e., extremely low values of CD4 T lymphocyte and very high values of CV). Furthermore, it is possible that the isolated measurement values of CD4 T lymphocyte and CV are not the best parameter to evaluate the immune response of the individual as compared with the monitoring of the evolution of these values. In a country like Brazil, where the National Program on Sexually Transmitted Diseases (STD / AIDS) is recognized worldwide, with specialized services to treat HIV-positive patients, provided free of charge, it is necessary to pay more attention to prevention and treatment of enteroparasitosis to increasingly reduce their prevalence, especially in immunocompromised populations, where enteric parasites can pose greater risk.

CONCLUSION

The study conducted in HIV-positive patients treated at SAE, Pelotas, shows that a fifth of the population is infected with pathogenic enteric parasites, particularly *Ascaris lumbricoides* and the *Trichuris trichiura*; being the intestinal protozoan *Giardia lamblia* the most frequent. Factors that were significantly associated with higher prevalence of pathogenic enteric parasites were lower economic level, having domestic animals and not being treated with antiretroviral drugs.

Non-pathogenic intestinal parasites such as *Entamoeba coli* and *Enteromonas hominis* infect 27.8% of these patients, indicating the risk of fecal contamination to which they are exposed. The profile of opportunistic infections by enteric parasites in HIV positive patients revealed low prevalence of infection by *Cryptosporidium spp.*, *Cystoisospora belli* and *Strongyloides stercoralis*.

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