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Research Article

Prevalence of Opportunistic and Non-Opportunistic Intestinal Parasites in HIV/AIDS Patients in Cochabamba, Bolivia

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Abstract

Background: People living with HIV frequently have opportunistic infections; intestinal parasites are the most common infections that impair their health status. This study examined prospectively the prevalence of intestinal parasites in patients with the Human Immunodeficiency Virus (HIV) who were treated at the Reference Assistance Service (CDVIR) of Cochabamba-Bolivia, and analyzed its correlation with clinical, laboratory, and socio-epidemiological parameters.

Methods: A total of 503 patients were enrolled. Stool sample for each has been analyzed by several methods, including direct observation methods, and specifics staining; Kinyoun's or the Diddier modified method.

Results: The prevalence of intestinal parasites was 30.0% including the following species, *Giardia lamblia*(4.0%), *Chilomastix mesnili*(1.6%), *Strongyloides stercoralis*(1.2%), *Isospora belli*(0.6%), *Ascaris lumbricoides*(0.6%), *Hymenolepis nana* (0.4%), *Trichuris trichiura*(0.2%) as pathogens. *Blastocystis Homminis*(10.1%), *Entamoeba histolytica/dispar*(6.6%), *Entamoeba coli*(0.2%), *Trychomona homminis*(0.4%), *Endolimax nana*(0.2%) as non-pathogens.

Multivariate analysis, showed statistical significance in relation to a parasitic infection with the fact of being on antiretroviral therapy (OR = 3.82% CI, 1.01 to 14.46, P = 0.05). The current CD4 cell count (OR = 1.11% CI, 1.00 to 1.23, P = 0.05). The liquid appearance of the faeces at the time of direct examination (OR = 0.44% CI, 0.24 to 0.83, P = 0.01).

Conclusion: In conclusion, health assistance and the extensive use of antiretroviral therapy allows improving immunological states. The prevalence of parasitic infections found in this study generates substantial evidence of a need to implement additional laboratory tests in the monitoring and treatment of patients in order to reduce the risk of infections in this population.

Keywords: Opportunistic ; Infections; Parasites; HIV; Prevalence; Reference laboratory

Abbreviations: ART-Anti-Retroviral Therapy; CDVIR-Reference center for HIV control and prevention in Cochabamba; CI-Confidence intervals; HAART-Highly active antiretroviral therapy; HIV/AIDS-Human Immunodeficiency Virus**;** IIBISMED-Institute of Biomedical Research; LABIMED-Reference laboratory

in the city of Cochabamba; PLWHIV-People living with HIV/AIDS; PNC/VS-National HIV/AIDS Control Program; UMSS-Universidad Mayor de San Simón**;** UNAIDS-Joint United Nations Program on HIV/AIDS; SPSS -Statistical Package for Social Science

Introduction

The global HIV/AIDS control strategies promoted by UNAIDS consider that ending the AIDS epidemic is more than a historic obligation to the 39 million people who have died from the disease. It also represents a momentous opportunity to lay the foundation for a healthier, more just and equitable world for future generations. Ending the AIDS epidemic will inspire broader global health and development efforts, demonstrating what can be achieved through global solidarity, evidence-based action and multi-sectorial partnerships[(UNAIDS)., 2017 #1;(UNAIDS)., 2017 #1]. Although many strategies will be needed to close the book on the AIDS epidemic, one thing is certain: it will be impossible to end the epidemic without bringing HIV treatment to all who need it.

According to this strategy by 2020, 90% of all people living with HIV will know their HIV status. 90% of all people with diagnosed HIV infection will receive sustained antiretroviral therapy and 90% of all people receiving antiretroviral therapy will have viral suppression [1].

The Latin American reports on the HIV epidemic in 2017 show 1.8 million [1.4 million-2.1 million] people living with HIV (PLWHIV) in this region. In 2016, approximately 97,000 [79,000-120,000] new HIV infections occurred in the region. The number of new HIV infections did not vary from 2010 to 2016. Over this period, the number of AIDS-related deaths in the region experienced a decrease of 12% [2].

The human immunodeficiency virus (HIV) and the consequent acquired immunodeficiency syndrome (AIDS) in the world, has modified the epidemiological behavior of intestinal parasite infections. Severe episodes of diarrhea and malnutrition are now a prognostic marker, since it is an evolutionary predictor of AIDS [3].

Parasitic infections are an important cause of morbidity and mortality, especially with the emergence of immunosuppressive diseases [4]. The magnitude of intestinal parasitic infection in acquired immunodeficiency syndrome patients requires careful consideration in developing countries where poor nutrition is associated with poor hygiene and several tropical diseases. In Bolivia, the climatic conditions favor the spread of parasites.

The introduction of highly active anti-retroviral therapy (HAART) has improved the immune status and viral load. Additionally, secondary prophylaxis has reduced opportunistic infections and mortality rates associated with HIV.

Despite the availability of ART, Opportunistic Infections (OIs) continue to cause considerable morbidity and mortality in the world. Because many HIV-infected persons are unaware of their HIV infection and present with an OI as the initial indicator of their disease. Some individuals are aware of their HIV infection, but do not take ART due to psychosocial or economic factors; and some patients are enrolled in HIV care and prescribed ART, but do not attain an adequate virologic and immunologic response due to inconsistent retention in care, poor adherence, unfavorable pharmacokinetics, or unexplained biologic factors [5,6].

HIV-infected people have a high prevalence of *Cryptosporidium sp., Microsporidium sp*, and *Isospora* infection in low-income countries and patients with diarrhea, reinforcing the importance of routine surveillance for opportunistic intestinal protozoa in HIV-infected people [7].

Several protozoan species belonging to *Cryptosporidium, Microsporidia* and *Isospora*, are common causative pathogens responsible of significant morbidity and mortality in HIV patients [7,8].

With a worldwide distribution of *Cryptosporidium, C. parvum and C. hominis* are the most common species detected in humans, though other species [7]. Despite the use of ART in many countries, the infection rates of *Cryptosporidium* in HIV patients are still high, accounting for up to a third of diarrhea cases in HIV patients [9].

Cryptosporidium is the most common parasite encountered in the immunocompromised host, followed by *Cyclospora* and *Isospora*. In recent years, *Microsporidia* and *Blastocystis* have also emerged as important players, due in part to improved molecular diagnostic assays [10].

Bolivia is a low income country, HIV / AIDS is currently a public health problem in Bolivia due to the constant and continuous spread of the disease. The available information for the year 2016 allows us to determine that approximately 1 in 4 PLWHA would be receiving antiretroviral treatment, even if official new notifications and new initiations mentions not exceeding 37% [2].

Official data indicate that the estimated HIV prevalence is 0,15% with an annual incidence of approximately 0.01% (1000 new cases per year), meaning that approximately 10 out of every 10,000 people in the country would have been notified as HIV cases, of which about 2,400 were on antiretroviral treatment in the public health system [11]. In this context, 16.640 confirmed cases of HIV were reported since June 2016 in the health system and 1.015 deaths for causes related to AIDS.

According to the 2017 global AIDS update [2], the Knowledge of status among all people living with HIV has reached 79%, 82%

of people living with HIV know their status and are on treatment, and 77% on treatment are virally suppressed. In Cochabamba, the HIV / AIDS program of the Departmental Health Service (SEDES), registered a total of 408 new infections in 2015 [11].

Investment in health is low and does not reach 6% of the general budget of the state [11] this results in an evident lack of infrastructure and equipment, for this reason despite having technical experts, advanced diagnostic methods for the detection of diseases are routinely not available. This is evident in HIV care, investment in this aspect comes from almost 95% of the global fund and international cooperation grant.

The reference laboratories are limited to monitoring indicators such as CD4 leukocyte count, viral load and routine laboratory tests, including a direct corproparasitological examination to investigate intestinal parasitic infections with great limitations that make it difficult using this technique for detection of HIV marker diseases such as Cryptosporidium and Microsporidium (Coccidias). Physicians must resort to specific tests that are not subsidized and the patient assumes cost of diagnosis and treatment.

Although molecular biology methods can identify parasitic infections when they are standardized and there are adequate conditions, their clinical application in limited resource settings is restricted by feasibility, technical availability and cost. The reference laboratory for HIV program in Cochabamba (LABIMED) does not currently perform the detection of parasitic infections by molecular biology methods and these diagnostic methods are not available in the country.

Publications referring to the presentation of parasitic diseases in PLWHA at the national level are not available in Bolivia, but there is research that shows a high prevalence of helminths in populations living in high altitudes [12].

To date there have been no systemic study on the prevalence of intestinal parasites in PLWHA in Bolivia. Our aim was to assess the prevalence and evaluate the epidemiologic factors related to HIV-intestinal parasites co-infection in Cochabamba.

Methods

Settings

Cochabamba is a city in central Region of Bolivia. It is the third most populated department with 1.758.143 population in 2012, according to official data from the National Institute of Statistics of Bolivia. The regional prevalence of HIV of 0.16% in 2014, making Cochabamba the second city of HIV prevalence in Bolivia [11].

The increasing use of antiretroviral therapy (ART) means that HIV is becoming a chronic disease, but many health services in Bolivia do not currently count with personnel or equipment for the management of complex health conditions.

The health system in Bolivia centralizes the care of HIV patients in regional referral centers (CDVIR), where we recruited all the patients and the study was implemented in Cochabamba.

The study participants were adult patients (aged over 18 years old) of both genders, with HIV confirmed infection, who had not used anthelmintic in the previous three months and were followed up at the center for reference of HIV/AIDS and Sexual transmitted diseases (CDVIR) where the study was conducted in the city of Cochabamba, Bolivia.

From December 2017 through March 2018 all consecutive patients with confirmed HIV infection consulting at the CDVIR were enrolled in the study if they provided written informed consent.

At the time of carrying out the clinical controls in the reference center (CDVIR), patients were referred to the laboratory (LABIMED) for collecting the stool sample and to maintain optimal conditions for their analysis. Microbiological tests were performed by two observers (Parasitologists) separately as a standard procedure in this reference laboratory.

After their consent, a questionnaire was administered to each participant by the team who had been specifically trained for this task and who also clarified all the study objectives and the patients doubts. A stool sample was collected from each of the participants and used for four different intestinal parasites testing. All participants were offered professional counseling before parasitological testing. All diagnostic results were kept strictly confidential. Deworming treatments (Albendazole, Nitazoxanida) were proposed to all participants found to be infected.

Patients were instructed on how to collect their stool samples and received vials identified with codification; such system was used in the health system for the SPECTRUM reports in Bolivia. Fecal samples were stored, further processed and analyzed in the Parasitology Laboratory of the school of medicine at "Universidad Mayor de San Simon" (Cochabamba, Bolivia).

Laboratory Procedures

Techniques for parasitological diagnosis of fecal samples were performed for each fresh stool specimen and processed using the following methods: a) Direct observation of fresh feces, b) Concentration method (modified Ritchie) c) Immunochromatography d) Specific stains (Kinyou/Ziehl Modified Nielseen, modified Diddier and Giemsa) e) Molecular biology methods; for technical feasibility reasons, these last techniques could not be performed.

Stool samples were analyzed by direct microscopy with physiological saline and iodine. After concentration methods (i.e., formalin ether or formalin-ethyl acetate), the spontaneous sedimentation allowed the detection of helminth eggs and larvae,

as well as protozoan cysts and also *S. stercoralis* and *hookworm* larvae. The Ritchie technique was used on concentrate samples to prepare fecal smears, coupled with 3 different stains. Modified Ziehl Neelsen technique (Kinyoun), modified Diddier technique and Giemsa were used to identify *Cryptosporidium sp, I. belli*, and *C. cayetanensis.* (*Coccidias and microsporidium sp.*). Three slides of each sample were prepared and examined by two analysts. When no parasites and/ or commensals were found in the sample, two additional slides were prepared to confirm the result.

Data were registered in CsPro 7.0 and analyzed with Statistical Package for Social for the Science (SPSS) version 24.0 statistical software. Data were analyzed by the t-test (student), chi-square, one-way ANOVA and the Fisher's exact test when appropriate. Multivariate logistic analysis was used to evaluate the association between parasitic infection and clinical and socio-epidemiological characteristics. The magnitude of association between variables was estimated by the odds ratio (OR) with a 95% confidence interval (95% CI). The level of significance was set at $p < 0.05$.

Results

Complete data were collected from all participants who had provided stool samples as well as answered the questionnaires. The summary of these data are presented in Table 1.

Features		All recruited patients (N=503)
Sex-no. (%)		
	Male	318(63.2%)
	Men/Women Ratio	1.72
Age-yrs		
	Median (Range)	34(17;88)
	Average ±SD	36±13
Scholarship No. yrs		
	Median (Range)	11(0;22)
	Average ±SD	10±4
Therapy time (ART)-yrs		
	Median (Range)	2.6(0;14.4)
	Average ±SD	2.9±2.3
Time since the last control-days		
	Median (Range)	0131(0;1675)
	Average ±SD	142±133
Number of controls-no		
	Median (Range)	4(0;9)
	Average ±SD	4±2
Actual CD4 count-no.(cells/mm^3)		
	<350	254(50.5)
	350-500	110(21.9)
	>500	139(27.6)
	Average ±SD	356(267)
ART yes-no.(%)		493(98.0)
Undetectable Viral Load yes-no.(%)		291(57.9)
Actual Scheme of ART		
	AZT/3TC/EFV	17(2.2)
	TDF/3TC/EFV	476(95.8)
Income / month (USD)		
	Median (IQ Range)	288(144;360)
	Average (SD)	263±145
BMI		
	Median (IQ Range)	23.7(4.1)
	Average (SD)	23.8±5.2

Table1: Characteristics of patients with HIV/AIDS recruited in the study.

Fecal samples were collected from each one of the 503 patients, 318 (63.2%) participants were male, with a ratio male/female of 1.72, their age ranged from 17 to 88 years old, with mean 36.1 ± SD 13.0.

Of the total of patients recruited 57.9% (291) have undetectable viral loads, 98% (493) currently receive ART, 96.3% of this patients receiving the Tenofovir/Lamivudin/Efavirenz (TDF/3TC/EFV) scheme. The median number of controls attended in the CDVIR during the last year was 4. Respect to the CD4 cell count 50.5% does not reach 350 cells/mm^3, 21.9% are between 350-500 cells/mm^3 and 27.6% have counts> 500 cells/mm^3, the BMI average was 23.7 SD +/- 13.6, the average monthly income was 263(USD) with a median of 288 and SD +/- 144 Table1.

HIV-infected patients of both genders were positive for intestinal parasites: 97/318(30.5%) infected men and 69/185(37.3%) infected women, showing a lower risk of infection in male gender but without statistical significance OR=0.74 IC 95% (0.50 to1.08 P=0.12). Age was not a determinant of infection by intestinal parasites P=0.34. Likewise, the current CD4 cell count (P=0.17), antiretroviral therapy (P = 0.07), body mass index

(P=0.46) and income level (P = 0. 36) did not show a significant association with intestinal parasite infection in this study. On the other hand, a significant association was found respect to parasitic infection and the absence of clinical symptoms or diarrhea in the recruited patients OR = 0.49 IC 95% (0.27-0.89 P=0.017) Table2.

Features		**Parasites**			
		Yes	**OR**	**IC (95%)**	**P value**
Sex					0.12
	Female	69/185(37.3)	1		
	Male	97/318(30.5)	0.74	(0.50-1.08)	
Age					0.34
	≤29 Yr	67/187(35.8)	1		
	30-39 yr	52/152(34.2)	0.93	(0.59-1.45)	
	≥40 yr	47/117(28.7)	0.72	(0.46-1.13)	
Consistence Liquid (Feces)					0.02
	Yes	24/50(48.0)	1		
	No	142/453(31.3)	0.49	(0.27-0.89)	
CD4 T cells count					0.17
	< 350 per mm3	93/254(36.6)	1		
	350-500 per mm3	35/110(31.8)	0.80	(0.50-1.30)	
	>500 per mm3	38/139(27.3)	0.65	(0.41-1.02)	
ART					0.07
	No	6/10(60.0)	1		
	Yes	160/493(32.5)	0.32	(0.89-1.15)	
BMI- Kg/M^2					0.46
	Under weight (<20)	10/22(44.5)	0.58	(0.24-1.39)	
	Normal (20-25)	97/298(32.6)	1		
	Over weight (>25)	59/181(32.6)	1.00	(0.68-1.49)	
Income					0. 36
	< Minimum wage	75/2242(31.0)	1		
	> Minimum wage	53/186(28.5)	0.84	(0.58-1.22)	

Table 2: Characteristics of patients with HIV/AIDS and their association with prevalence of intestinal parasites.

*Fisher exact test

Multi-parasitism was rare in our population. In the HIV infected group, most participants were infected by only one parasite (33.0% of the patients), while 7.8% were infected with two or more species.

The study shows a general prevalence of intestinal parasites in the PLWHIV population of 33.0%. A total of 12 species were identified during the study period, using the method of direct observation of stool. For parasites considered as pathogenic species, we detected *Giardia lamblia* (4.0%), *Chilomastix mesnili* (1.6%), *Strongyloides stercoralis* (1.2%), *Isospora belli* (0.6%), *Ascaris lumbricoides*

(0.6%), *Hymenolepis nana* (0.4%), *Trichuris trichiura* (0.2%). *Blastocystis Homminis* (10.1%), *Entamoeba histolytica/dispar* (6.6%), *Trychomona Homminis* (0.4%), *Entamoeba coli* (0.2%), *Endolimax nana* (0.2%) as non-pathogens. The parasitic species identified in the study are shown in Table 3.

		VIH (+) (n=503)
Outcomes		
Parasite Infection -no(%)		
	Any	166(33.0)
	Polyparasitism	39(7.8)
Parasites species-no(%)		
Direct Observation		
	Blastocystis homminis	51(10.1)
	Entamoeba histolytica/dispar	33(6.6)
	Giardia lamblia	10(4.0)
	Chilomastix mesnili	8(1.6)
	Strongyloides stercolaris	6(1.2)
	Isospora belli	3(0.6)
	Ascaris lumbricoides	3(0.6)
	Hymenolepis nana	2(0.4)
	Entamoeba coli	1(0,2)
	Trychomona homminis	2(0.4)
	Trichuris trichura	1(0.2)
	Endolimax nana	1(0.2)
Immunochromatography		
	Giardia lamblia	26(5.8)
	Ameba SP.	2(0.4)
	Cryptosporidium sp.	2(0.4)
Stains		
	(1) Kinyou (Modified Ziehl Neelsen)*	
	Microsporidium sp.	45(8.9)
	Isospora belli	18(3.6)
	Strongyloides stercolaris	7(1.4)
	CRIPTOSPOR	4(0.8)
	(2) Diddier / Modified***	
	Microsporidium sp.	45(8.9)
	Isospora belli	17(3.4)
	Strongyloides stercolaris	7(1.4)
	Cryptosporidium sp.	4(0.8)
	(3) Giemsa**	
	Isospora belli	13(2.6)
	Microsporidium sp.	8(1.6)

Table 3: Prevalence of Intestinal parasitic infections by species in adult patients with HIV, Cochabamba-Bolivia.

*Kappa for Stain 1 y 2 =0.99 P<0.001

**Kappa for Stain 1 y 3 =0.40 P<0.001

***Kappa for Stain 2 y 3 =0.41 P<0.001

For reasons of technical availability, the Immunochromatography method was established only for the detection of *Giardia lamblia, Entamoeba histolytica/dispar* and *Cryptosporidium*. Prevalence of *Giardia lamblia* was 5.8% highly concordant with the direct observation method (Kappa = 0.77 P <0.001). *Entamoeba histolytica/dispar* was 0.4% and had a low concordance with direct observation (Kappa = 0.11 P <0.001). *Cryptosporidium sp*. was 0.4% and had low concordance with direct observation Kappa = 0.005, P = 0.92. Table 3.

In addition, staining methods have been implemented in this study specifically for the detection of species that cannot be identified by direct methods such as *Coccidias, Microporidium* and *nematodes*. The results for Kinyou staining (Modified Ziehl Neelsen) showed a higher prevalence of *Microsporidium. sp.* (8.9%), *I. belli* (3.6%), *S. stercoralis* (1.4%) and *Cryptosporidium* sp. (0.8%). Modified Diddier staining showed a prevalence of *Microsporidium sp.* Of 8.9%, *I. belli* 3.4%, *S. stercoralis* 1.4% and *Cryptosporidium sp.* 0.8%, these two methods showed a high concordance (Kappa = 0.99, P <0.001). Giemsa stain showed *I. belli* (2.6%) and Mic*rosporidium sp.* (1.6%), but showing a low concordance with the other two stains (Kappa= 0.41, P <0.001), Table 3.

Associations between the incidence of different intestinal parasitic infections and socio-epidemiological factors in patients with HIV/ AIDS are presented in Table 4.

		Parasite Infection		
	β±SE	**OR**	**(95% IC)**	**P value**
Men (yes=1)	-0.38±0.22	1.50	0.96 to 2.23	0.08
Age (per 10 yrs)	-0.15±0.09	1.16	0.98 to 1.37	0.09
Scholarship No. yrs	-0.47±0.25	1.60	0.97 to 2.60	0.06
ART(yes=1)	-1.34±0.68	3.82	1.01 to14.46	0.05
Therapy time (ART) yrs (per 10 yrs)	0.02±0.05	0.99	0.90 to1.08	0.76
Time since the last control yrs (per yr)	0.55±0.30	0.58	0.32 to 1.04	0.07
Number of Controls- last year	0.38±0.22	1.04	0.90 to 1.19	0.51
>5		1		0.66
1-4	0,05±0.53	1.05	0.37 to 3.00	0.92
0	0,21±0.24	1.23	0.78 to 1.96	0.38
Initial Viral load (per million)	-0.11±0.11	1.11	0.90 to 1.38	0.33
Actual Viral load (per million)	0.10±0.16	0.90	0.66 to 1.23	0.52
INITIAL CD4 counts (per hundred)	0.04±0.06	0.96	0.86 to 1.07	0.47
ACTUAL CD4 counts (per hundred)	-0.11±0.05	1.11	1.00 to 1.23	0.05
Income ((per thousand USD)	0.09±0.07	0.92	0.80 to 1.06	0.24
BMI (per ten)	-0.12±0.20	1.12	0.76 to 1.65	0.55
Liquid faeces (yes =1)	0.81±0.32	0.44	0.24 to 0.83	0.01
unintentional weight loss- last yr.	-0.11±0.05	1.12	1.01 to 1.24	0.03

Table 4: Associations between the incidence of intestinal parasitic infections and socio-epidemiological factors.

Intestinal parasitic infections were frequent but not statistically significant in men (OR = 1.5% CI, 0.96 to 2.23, P = 0.08). Also the age expressed higher risk in populations under 29 years.

Whereas, in multivariate logistic regression analysis, the factors that showed statistical significance in relation to a parasitic infection were the fact of being on antiretroviral therapy (OR = 3.82% CI, 1.01 to 14.46, P = 0.05). The current CD4 cell count (OR = 1.11% CI, 1.00 to 1.23, P = 0.05). The liquid appearance of the faeces at the time of direct examination (OR = 0.44% CI, 0.24 to 0.83, P = 0.01).

Discussion

As the under 40 years of age group corresponds to active individuals with respect to their capacity to work, procreate, and have sexual intercourses, the prevalence of HIV-infected people in this age group can result in significant impairment to the population and the economic development of the country [13].

Opportunistic infections are common in patients with HIV/AIDS and are related to a high morbidity in this population. Before ART was freely distributed to HIV patients, intestinal parasites, opportunistic or not, were common in HIV patients. Even after free ART availability, intestinal parasites are still a big concern [14]. The HIV virus induces an immunodeficient state that favors infection by enteroparasites, which, in turn, contribute to worsen the patient clinical condition by causing malnutrition, weight loss, and chronic diarrhea [15].

Malnutrition is common in the later stages of HIV infection [16]. Hypercatabolic states generally associated with aggregate infectious processes should be considered among the main factors responsible for the malnutrition linked to HIV. Absorption deficit are caused by parasites such as *G. lamblia*. Villous atrophy and metabolic alterations in addition to a deficient intake. These factors contribute to generate the so-called wasting syndrome.

In this study, *G. lamblia* reports one of the highest prevalence (4%), in relation to the pathogens identified by direct methods and Immunochromatography.

Intestinal parasites are a diverse group of microorganisms that include single-cell protozoans and multi-cellular intestinal worms, capable of disrupting the absorption of nutrients. The waterborne diseases, especially those caused by intestinal protozoa, are among the most relevant pathogens described in PLWHIV [10].

The poor management of water and sewage services, associated with the lack of regulation and public policies represent a serious problem in Bolivia. The quality of drinking water has been a concern in the past and it is still one today. Although remarkable progress has been made in the last decade by governments in providing good quality water to population, these remain insufficient.

Good hygiene habits are the best way to avoid contamination and reinfection by intestinal parasites. Health security is necessary for the prevention of diseases transmitted by water and food. The high positivity rate for commensals observed in the present study may be considered as an indicator of poor hygiene and sanitation, as well as of consumption of contaminated water and food by these patients.

The present study examined the occurrence of intestinal parasites in patients with HIV/ AIDS who were treated at the CDVIR. The socio-epidemiological profile of the studied patients, corroborates the national data reported by the Ministry of health.

Our study aimed at finding the epidemiology of HIV-intestinal parasites co-infection in Cochabamba and to evaluate the factors associated to this co-infection. The overall prevalence of intestinal parasites was 33.0%. Our data showed that the major predisposing factor for intestinal parasitic infections was the number of years of schooling. ART revealed an association between the probability of having parasitic infection and antiretroviral therapy, in the same way the presence of diarrhea. Monoparasitism predominated among the study participants (25.2%). No significant correlations were found between parasite presence and CD4+ T cell counts or HIV RNA levels.

Detection of *S. stercoralis, Cryptosporidium sp.* and *I. belli* in fecal samples of patients with HIV/ AIDS was lower than the prevalence reported by Soúza Junior and Garcia-Zapata in a similar study conducted in Goiânia, Goiás-Brazil [8]. It is important to note that there are few studies on the prevalence of intestinal parasites, including S. stercoralis and intestinal coccidia, in patients with HIV/ AIDS in this geographical context.

The Coccidia life cycles are suited to waterborne and foodborne transmission and their oocysts are resistant to conventional water treatment protocols [17].

Antiretroviral therapy has gradually reduced the incidence of opportunistic infections in patients with HIV/ AIDS. However, poor adherence to treatment negatively affects the effectiveness of antiretroviral therapy and the prognosis of patients. In this study, the use of antiretroviral therapy provided protection against parasites (OR=3.83, p=0.05). Patients' high adherence to antiretroviral therapy markedly improves the immune response, slows down the progression of disease and reduces the susceptibility to opportunistic infections [8]. It must be taken into account that currently the ART is administered once the HIV infection is diagnosed in such a way that immuno-suppression levels are currently not as low.

The presence of diarrhea shown significant correlation with infection by intestinal parasites, corroborating the findings of Pavie et al In contrast Bachur et al. and Wanyiri et al. [19] who reported a positive relationship between diarrhea and intestinal parasites positive samples, Cardoso et al., [18].

Cryptosporidiosis can cause severe chronic diarrhea leading to electrolyte imbalance, mal-absorption of nutrients, and profound weight loss. The physiopathology of the infection is not well-understood. Non-specific mechanisms such as the atrophy of villi and inflammatory reaction could be contributing factors to this mal-absorption process, therefore, orally taken ARV treatment will not be an exception and thus will also suffer poor absorption leading to lower ART efficacy.

A low prevalence of *Isospora belli* reported was observed in our study, in line with the study done in Yaoundé [19] where only 1.9% prevalence was reported and in Congo by Wumba et al. in 2010 where a prevalence of 1.7% was reported [20]. In contrast this prevalence was as high as 4.5% in Thailand [21] and 7% in Brazil [22,23]. In two studies done in China, I belli was not reported [24,25].

Although molecular biology methods can identify parasitic infections when they are standardized and when there are adequate conditions, their clinical application is restricted by feasibility, technical availability and cost in limited resource settings. This implies a great importance for the clinical practice regarding the microbiological studies in this context.

In Bolivia, the subsidy of laboratory studies for PLWHA only allows direct microscopic studies in the reference laboratory (LABIMED), molecular biology techniques are not available in this context, performing the controls to PLWHA only through direct microscopy methods.

Conclusions

To conclude, the prevalence of intestinal parasitic infections found in this study generates substantial evidence on the need to implement additional laboratory tests in the monitoring and treatment of patients with HIV/AIDS, the additional tests must have greater performance.

On the other hand, this study shows which factors were related to infection by enteric parasites according to the epidemiological scenario in Cochabamba, highlighting the need to implement preventive measures to reduce infection rates and prevent the worsening of patients' clinical conditions.

From these results, we suggest the inclusion of methods of concentration, staining, Immunochromatography and immunofluorescence, in addition to standardizing and achieving the availability of molecular biology methods, especially for the identification of microsporidium species, for its clinical implications regarding its therapeutic. These tests should be prescribed appropriately to reduce the prevalence of these infections and their consequences, such as wasting syndrome and alterations of the intestinal microbiome.

Declarations

Ethical Approval and Consent to Participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study protocol was approved by the "Comité de Bioética de la investigación de la Universidad Mayor de San Simón" (CBI) under the registration N° CBI-2016-015, and the Commission d'éthique biomédicale hospitalo-facultaire de l' UCLouvain. (CEHF) Cliniques universitaries Saint-Luc Bruxelles.

Informed consent: "Informed consent was obtained from all individual participants included in the study."

Availability of Data and Materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing Interests

The authors declare that they have no competing interests.

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Authors' Contributions

Conceptualization: [Jorge Avilés]; Methodology, statistical analysis and were a major contributors in writing the manuscript: [Annie Robert, Jorge Avilés]; Formal analysis and investigation: [Annie Robert, Jean Cyr Yombi, Héctor Rodriguez, Jorge Avilés]; Writing - original draft preparation: [Jorge Avilés, Carlos Erostegui, Marcelo Rojas]; Writing - review and editing: [Daniel Mercado; Daniel Illanes]; Funding acquisition: [Annie Robert, Jorge Avilés]; Resources: [Académie De Recherche Et D'Enseignement Supérieur]; Supervision and performed the microbiological and molecular examination of the stool samples laboratory test: [Maricruz Torrico; Jorge Avilés]. All authors read and approved the final manuscript.

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References

1. UNAIDS, JUNPoHA (2014) 90-90-90 An ambitious treatment target to help end the AIDS epidemic.
2. UNAIDS, JUNPoHA (2017) UNAIDS 2017 | REFERENCE - UNAIDS DATA 2017. UNAIDS/JC2910E 248.
3. Wilcox CM (2000) Etiology and evaluation of diarrhea in AIDS: a global perspective at the millennium. World J Gastroenterol 6: 177–186.
4. WHO (2013) Sustaining the drive to overcome the global impact of neglected tropical diseases, Second WHO report on neglected tropical diseases. WHO reference number: WHO/HTM/NTD/2013.1.
5. John C. Hall BJH, Cockerell CJ (2011) HIV/AIDS in the Post-HAART Era: Manifestations, Treatment, and Epidemiology.
6. Marcos LA, Gotuzzo E (2013) Intestinal protozoan infections in the immunocompromised host. Current opinion in infectious diseases 26: 295-301.
7. Wang ZD, Liu Q, Liu HH, Li S, Zhang L, et al. (2018) Prevalence of Cryptosporidium, microsporidia and Isospora infection in HIV-infected people: a global systematic review and meta-analysis. Parasites & vectors 11: 28.
8. Barcelos NB, Silva LFE, Dias RFG, Menezes Filho HR, Rodrigues RM (2018) Opportunistic and non-opportunistic intestinal parasites in HIV/AIDS patients in relation to their clinical and epidemiological status in a specialized medical service in Goias, Brazil. Revista do Instituto de Medicina Tropical de Sao Paulo 60: e13.

9. Buchacz K, Baker RK, Palella FJ, Jr., Chmiel JS, Lichtenstein KA, et al. (2010) AIDS-defining opportunistic illnesses in US patients, 1994-2007: a cohort study. AIDS (London, England) 24: 1549-59.

10. Zorbozan O, Quliyeva G, Tunali V, Ozbilgin A, Turgay N, et al. (2018) Intestinal Protozoa in HIV-Infected Patients: A Retrospective Analysis. Turkiye parazitolojii dergisi 42: 187-190.

11. Bolivia MdS (2014) Informe Nacional De Progresos En La Respuesta Al VIH/SIDA, 2014 Seguimiento a la Declaración Política sobre el VIH/sida 2011. 73.

12. Asai T, Cordova Vidal C, Strauss W, Ikoma T, Endoh K, et al. (2016) Effect of Mass Stool Examination and Mass Treatment For Decreasing Intestinal Helminth and Protozoan Infection Rates in Bolivian Children: A Cross-Sectional Study. PLoS neglected tropical diseases 10: e0005147.

13. Gentilini M, Chieze F (1990) Socioeconomic aspects of human immunodeficiency virus (HIV) infection in developing countries. Bulletin de l'Academie nationale de medecine 174:1209-1219; discussion 19-21.

14. Bachur TP, Vale JM, Coelho IC, Queiroz TR, Chaves Cde S (2008) Enteric parasitic infections in HIV/AIDS patients before and after the highly active antiretroviral therapy. The Brazilian journal of infectious diseases : an official publication of the Brazilian Society of Infectious Diseases 12: 115-122.

15. Willemot P, Klein MB (2004) Prevention of HIV-associated opportunistic infections and diseases in the age of highly active antiretroviral therapy. Expert review of anti-infective therapy 2: 521-532.

16. Ford N, Shubber Z, Meintjes G, Grinsztejn B, Eholie S, et al. (2015) Causes of hospital admission among people living with HIV worldwide: a systematic review and meta-analysis. The lancet HIV 2: e438-444.

17. Cama VA, Mathison BA (2015) Infections by Intestinal Coccidia and Giardia duodenalis. Clinics in laboratory medicine 35: 423-444.

18. Cardoso LV, Galisteu KJ, Schiesari Junior A, Chahla LA, Canille RM, et al. (2011) Enteric parasites in HIV-1/AIDS-infected patients from a Northwestern Sao Paulo reference unit in the highly active antiretroviral therapy era. Revista da Sociedade Brasileira de Medicina Tropical 44: 665-669.

19. Vouking MZ, Enoka P, Tamo CV, Tadenfok CN (2014) Prevalence of intestinal parasites among HIV patients at the Yaounde Central Hospital, Cameroon. The Pan African medical journal 18: 136.

20. Wumba R, Longo-Mbenza B, Mandina M, Odio WT, Biligui S, et al. (2010) Intestinal parasites infections in hospitalized AIDS patients in Kinshasa, Democratic Republic of Congo. Parasite (Paris, France) 17: 321-328.

21. Punpoowong B, Viriyavejakul P, Riganti M, Pongponaratn E, Chaisri U, Maneerat Y (1998) Opportunistic protozoa in stool samples from HIV-infected patients. The Southeast Asian journal of tropical medicine and public health 29: 31-34.

22. Pacheco FT, Silva RK, Martins AS, Oliveira RR, Alcantara-Neves NM, et al. (2013) Differences in the detection of Cryptosporidium and Isospora (Cystoisospora) oocysts according to the fecal concentration or staining method used in a clinical laboratory. The Journal of parasitology 99: 1002-1008.

23. Silva CV, Ferreira MS, Borges AS, Costa-Cruz JM (2005) Intestinal parasitic infections in HIV/AIDS patients: experience at a teaching hospital in central Brazil. Scandinavian journal of infectious diseases 37: 211-215.

24. Tian LG, Chen JX, Wang TP, Cheng GJ, Steinmann P, et al. (2012) Co-infection of HIV and intestinal parasites in rural area of China. Parasites & vectors 5: 36.

25. Tian LG, Cheng GJ, Chen JX, Cai YC, Guo J, Tong XM, et al. (2012) [Survey on co-infection with HIV and intestinal parasites in high prevalence areas of HIV/AIDS, China]. Zhongguo xue xi chong bing fang zhi za zhi = Chinese journal of schistosomiasis control 24: 168-72.