

Enteric Pathogens in Patients with Acquired Immunodeficiency Syndrome from Porto Velho City, Rondonia State, Western Amazon, Brazil

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Abstract

Background: Patients with human immunodeficiency virus (HIV), mainly those who live under poor sanitary and socioeconomic conditions, are often diagnosed with Gastrointestinal (GI) tract diseases. The lowest CD4+ T-cell counts are not found in the plasma, but in the GI tract, the biggest HIV source, thus allowing opportunistic infections. Therefore, the aim of the present study was to identify the epidemiological factors of GI infections and the prevalent pathogens in HIV patients from Porto Velho City, Rondônia State.

Methods: Observational, cross-sectional study conducted with 207 patients treated in inpatient and outpatient services, in referral centers for HIV/ acquired immunodeficiency syndrome (AIDS) patients, between May 2014 and May 2018. Sociodemographic information was collected at admission time. Fresh stool samples were subjected to direct smear microscopy. Modified Ziehl-Neelsen, Hoffman's spontaneous sedimentation and formalin-ether techniques were also performed to identify intestinal parasites. Routine microbiological and biochemical tests were applied to identify bacterial infestation. Colonies suggestive of *E. coli* were subjected to polymerase chain reaction testing in order to identify virulence factors.

Results: Data indicated that 38.1% (79/207) of patients had elementary education, 82,1% (170/207) received up to two minimum wages, and 81,6% (169/207) did not have access to household sewage treatment. *Endolimax nana, Blastocystis hominis, Giardia intestinalis*, and *Entamoeba histolytica* were identified in 22.2% (46/207), 13.6% (28/207), 11.1% (23/207), and 6.3% (13/207) of patients, respectively. *Cryptosporidium* spp. and *Cystoisospora belli* were the most prevalent among coccidia, present in 10.1% (21/207) and 3.9% (8/207) of patients respectively. Diarrheagenic *E. coli* (DEC) was found in 27.9% (34/122) of bacterial isolates, of these 41.2% (14/34) were Enteroaggregative *E. coli*, 35.3% (12/34) Enteropathogenic *E. coli*, 17.6% (6/34) Diffusely-adherent *E. coli*, and 5.9% (2/34) were Enteroinvasive *E. coli. Entamoeba histolytica* was associated with diarrheal diseases (p = 0.0072) and with stool occult blood (p < 0.0001).

Conclusions: The study may contribute to a understanding of the role that enteropathogens play in HIV patients and may aid in strategic planning for controlling the disease in such regions.

Keywords: HIV/Aids; Enteric Pathogens; Western Amazon

List of abbreviations: AIDS: Acquired Immunodeficiency Syndrome; ART: Antiretroviral therapy; CEMETRON: Tropical Medicine Center; DEC: *Diarrheagenic E. coli*; EAEC: Enteroaggregative *E. coli*; EIEC: Enteroinvasive *E. coli*; EPEC: enteropathogenic *E. coli*; GI: Gastrointestinal; HAART: Highly active antiretrovial therapy HIV: Human Immunodeficiency Virus; INI: Inibidores da integrasse; NNRTI: Non-nucleoside Reverse Transcriptase Inhibitors; NRTIs: Nucleoside Analog Reverse-transcriptase Inhibitors; PI: Protease inhibitors; SAE: Specialized Assistance Service; SS: Salmonella-Shigella; UNIR: Rondonia Federal University; WHO: World Health Organization; XLD: Xylose Lysine Deoxycholate

Introduction

According to World Health Organization (WHO), human immunodeficiency virus (HIV) continues to be a major global public health issue [1]. However, with increasing access to effective prevention, diagnosis, treatment, and care, has become a manageable chronic health condition, enabling people living with HIV to lead long and healthy lives. There are an estimated 38.0 million people living with virus, as of the end of 2019, and the disease has claimed almost 33 million lives so far [1]. The HIV and AIDS epidemic in Brazil is classified as stable at the national level; however, prevalence varies geographically. In 2019, 920,000 people were living with HIV compared to 640,000 people in 2010. In 2019, there were 48,000 new infections and 14,000 people died due to AIDS-related illnesses [2]. Many etiologic agents behave as opportunists in HIV patients. Accordingly, pathogens causing infections in the gastrointestinal (GI) tract, mainly those affecting populations living under poor socioeconomic and sanitary conditions, should be thoroughly assessed [3,4].

The GI tract plays a key role in HIV infection, since it is the largest human lymphoid organ [5]. The acute HIV phase is marked by a decrease in GI defense cells, which affects enterocytes, allows microbial translocation, and weakens the intestinal mucosa [6,7]. This condition can be aggravated in the chronic phase of untreated HIV infection.

Antiretroviral therapy (ART) has globally increased the survival rates of HIV patients [8]. However, GI diseases still affect their quality of life [9]. Studies suggest that 50% to 80% of HIV-positive patients suffer from diarrhea, which is one of the main morbidity risk factors in HIV patients, and indicate that approximately 90% of patients from developing countries suffer from diarrhea during the HIV infection [7-10]. This condition still affects one-third of the world's population infected with HIV and it becomes chronic in most of them [11].

Chronic diarrhea lasts for more than one month and can persist for several months, thus leading to progressive weight loss and overall health impairments [12]. Under such conditions, CD4 T-cell counts can be lowered to a range of 200 to 300 cells/mm³ [9].

GI disease etiologies in HIV-positive patients can be either infectious or non-infectious. Non-infectious diarrhea implies GI injury associated with antiretroviral therapy [13,14].

GI infections in HIV patients are mostly caused by the etiologic agents *Giardia intestinalis, Entamoeba histolytica*, coccidia, such as *Cryptosporidium* spp., *Cystoisospora* spp., *Salmonella* spp., *Shigella* spp., *Yersinia* spp., *Clostridium difficile, Campylobacter* spp., and *Escherichia coli* [15,16]. Virulence of enteric pathogens contributes to the most common GI infection symptoms, namely weakness, fever, vomiting, abdominal pain, anorexia, weight loss, and diarrhea [17]. These infections mainly affect populations from socioeconomically disadvantaged regions or those living under poor sanitary conditions, given their low immunity to diseases [3].

Enteric pathogens can further progress from HIV to AIDS, due to lack of basic sanitation in Northern Brazil, mainly in the Amazon region. In this context, data on HIV-related GI disorders in the Brazilian Amazon are scarce. Therefore, the present study assessed the epidemiological profile of HIV patients infected with enteric pathogens and the health issues resulting from it.

Materials and Methods

Study location and patients

Samples from 207 patients were analyzed. Sample size was defined based on studies that showed DEC prevalence in 16% of HIV carriers, on average; it was done by taking into consideration the population of 2,528 HIV carriers recorded in Porto Velho City at the beginning of the study. The study adopted sampling error margin of 5%, at 95% confidence level, based on simple random sampling. Sample size calculations have shown that the sample should comprise at least 191 patients.

Study participants comprised patients older than 18 years, who were regularly followed-up at the Specialized Assistance Service (SAE) and at the Tropical Medicine Center (CEMETRON) - which are excellence infectious and tropical disease treatment centers in Porto Velho City, Rondônia State - from May 2014 to May 2018.

All patients were invited to meet the research and answered a structured sociodemographic questionnaire at the time of admission. The questionnaire required the following data: age, sex, education, current and history of diarrhea, clinical signs and symptoms, and antiretroviral therapy adherence. Fecal samples were collected from 207 HIV-1 patients with acute gastroenteritis. Samples were registered and stored, and further processed in the Microbiology Laboratory of Oswaldo Cruz Foundation in Rondônia State. The study was approved by the Ethics Committee of Rondônia Tropical Medicine Research Center under protocol No. 30782514.9.0000.0011.

Intestinal protozoa detection

Intestinal protozoa were detected in fresh stool. Stool samples were subjected to direct smear microscopy and modified Ziehl-Neelsen staining technique to detect trophozoites and protozoan cysts. These techniques mainly stain enteric coccidia, such as *Cryptosporidium* spp. and *Cystoisospora belli* [18]. Hoffman's spontaneous sedimentation, centrifugal sedimentation, and formalin-ether techniques were also performed.

Bacteriological analysis

All specimens were processed using routine microbiological and biochemical tests obtained from bioMérieux, France (API 20E) to identify *E. coli, Salmonella* spp., and *Shigella* spp. strains collected from Salmonella-Shigella (SS), xylose lysine deoxycholate (XLD), and Brilliant Green agars (HiMedia Laboratories, Mumbai, India).

Detection of E. coli virulence factors by multiplex polymerase chain reaction (PCR)

Multiplex PCR tests were performed as previously described to assess diarrheagenic E. coli prevalence [19].

Statistical Analysis

Statistical analysis was performed using the R-3.4.3 and GraphPad Prism version 6.0 softwares. Data were analyzed by chi-square, and Fisher's exact test was performed to compare categorical variables in 2×2 contingency tables. The magnitude of association between variables was estimated by the odds ratio (OR) with a 95% confidence interval and 0.05 significance level.

Results

Fecal samples were collected from 207 HIV patients, of whom 30% (62/207) were admitted to SAE and 70% (145/207) to CEMETRON Hospital. The mean age of participants was 42.3 years. As for gender, 54.1% (112/207) were male and 45.9% (95/207) were female.

The time since HIV diagnosis was longer than 20 years for 1.9% (4/207) of patients and shorter than five years for 53.6% (111/207). A total of 93.2% (193/207) of patients had some education and 6.8% (14/207) of them were illiterate. Eighty-two percent of patients (170/207) received up to two minimum wages. In addition, 81.6% (169/207) of patients did not have access to sanitary sewage treatment (Table 1).

Adherence to regular ART was confirmed in 51.7% (107/207) of patients. Moreover, 28.0% (58/207) said they did not receive the ART and 18.4% (38/207) said they received it irregularly. As for ART, 53.6% (111/207) of patients used combined nucleoside analog reverse-transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTI) (Table 1).

Among the assessed symptoms, diarrhea was reported in 55.6% (115/207) of patients, followed by abdominal pain in 39.6% (82/207), headache in 24.6% (51/207), fever in 24.2% (50/207), and vomiting in 13% (27/207). Detected protozoa included *Endolimax nana* in 22.2% (46/207) of the patients, *Blastocystis hominis* in 13.53% (28/207), *Giardia intestinalis* in 11.11% (23/207), and *Entamoeba histolytica* in 6.3% (13/207). *Cryptosporidium* spp. was identified in 10.14% (21/207) and *Cystoisospora* spp in 3.9% (8/207) of the patients. *E. histolytica* was significantly associated with diarrheal disorders (p = 0.0072; OR 10.6 and CI 1.351 to 83.17%) and stool occult blood (p < 0.0001; OR 10500 OD and CI 200.3–550700).

The most common enteric bacterium was *E. coli* in 59% (122/207) of the patients. Diarrheagenic *E. coli* was found in 27.9% (34/122) of stool samples, which comprised the following pathotypes, enteroaggregative *E. coli* (EAEC) in 41.2% (14/34), enteropathogenic *E. coli* (EPEC) in 35.3% (12/34), diffusely-adherent *E. coli* (DAEC) in 17.6% (6/34), and enteroinvasive *E. coli* (EIEC) in 5.9% (2/34). *Salmonella* spp. was found in 4.35% (9/207) of the samples. The analysis revealed a significant association between the patients' high viral loads and the assessed enteric pathogens (Table 2).

Total	n = 207 (%)							
Admission of HIV/AIDS patients								
SAE	62 (30)							
CEMETRON	145 (70)							
Gender								
Female	112 (54.1)							
Male	95 (45.9)							
Mean age (SD)	42.3 (11.7)							
Education level								
Illiterate	14 (6.7)							
Elementary School	79 (38.1)							
High School	99 (47.8)							
Higher Education	15 (7.2)							
Per capita income								
No income	5 (2.4)							
1 to 2 minimum wages	170 (82)							
3 to 4 minimum wages	31 (14.9)							
Profession								
Retired	19 (9 2)							
Freelancer	27 (10 3)							
Unemployed	31(15)							
Homemaker	51 (24.6)							
Not informed	79 (38 2)							
Drinking water	/ / (00.2)							
Untreated	61 (29.4)							
Mineral	93 (44 9)							
Filtered	38 (18.4)							
Chlorinated	15 (7 2)							
Sewage treatment	10 (7.2)							
No sewage treatment	169 (81.6)							
Access to sewage treatment	38 (18 4)							
Time since HIV diagnosis	50 (10.1)							
Not informed	2(1)							
> 20 years	$\frac{2(1)}{4(1.9)}$							
	4(1.7)							
5 10 years	45 (21.7)							
	45 (21.7)							
< 5 years Highly Active Antiretroviral Therapy (HA	APT) adherence							
Not informed								
Vac	107 (51 7)							
No	59 (29 0)							
Into	38 (28.0)							
Antiantravial the anerest tame	38 (18.4)							
Not informed	2(1)							
No adharanca	2 (1) 59 (28 5)							
	9 (20.)							
	0 (3.9)							
NR11, INI	1 (0.5)							
NK11, PI	16 (7.7)							
NKII, NNKII	111 (53.6)							
NRTI, NNRTI, INI	1 (0.5)							

NRTI, NNRTI, PI	8 (3.9)
NRTI, NNRTI, PI, INI	1 (0.5)

INI: Integrase inhibitors; PI: Protease inhibitors; NRTI: Nucleoside analog reverse-transcriptase inhibitors; NNRTI: Non-Nucleoside Analogue Reverse Transcriptase Inhibitor

 Table 1: Sociodemographic and clinical characteristics of HIV/AIDS patients

Parameter	To Popul	tal lation	Diarreio E.col	ogenica i++	Salmo sp	onella pp	Gia Intest	rdia tinales	Crypt iun	osporid 1 spp	Cystoisospera spp		Entamoeba histolytica		Entamoeba coli	
Clinical Symptoms	(207)	(%)	N (34)	(%)	(9)	(%)	(23)	(%)	(21)	(%)	(8)	(%)	(13)	(%)	(12)	(%)
Fever	50	24.2	6	12.0	4	8.0	5	10	4	8	2	4	5	10	2	4
Diarrhea	115	55.6	10	8.7	4	3.4	9	7.8	10	8.6	7	6	12§	10.4	7	6
Vomit	27	13.0	1	3,7	4	14.8	2	7.4	3	11.1	1	3.7	2	7.4	2	7.4
Headache	51	24.6	11	21.5	5	9.8	9	17.6	11	21.5	3	5.8	4	7.8	5	9.8
Abdominal pain	82	39.6	8	9.7	6	7.3	7	8.5	10	12.2	3	3.6	7	8.5	6	7.3
TCD4+T (cells/mm ³)																
> 500	48	23.1	12	25	3	6.25	6	12.5	4	8.3	2	4.1	3	6.25	2	4.1
200-500	50	24.2	9	18	1	2	5	10	11	22	4	8	6	12	8	16
50-200	56	27.1	4	7.1	4	7.14	7	12.5	2	3.5	1	1.7	0	0	2	3.5
< 50	53	25.6	7	13.2	1	1.9	5	9.4	3	5.6	1	1.8	4	7.5	0	0
Viral Load (copies/m ³)																
Unknown	37	17.9	3	8.1	0	0	1	2.7	0	0	0	0	4	10.8	3	8.1
< 50	57	27.5	11	19.2	4	7	7	12.3	11	19.2	2	3.5	0	0	3	5.2
50-5.000	91	44.0	9	9.8	1	1	0	0	1	1	0	0	1	1	4	4.3
> 5.000*	22	10.6	9	40.9	4	18.1	15	68.1	9	40.9	6	27.2	6	27.2	2	9
Aspect of faeces																
Watery	65	31.4	14	21.5	4	6.1	10	15.3	4	6.15	4	6.1	11	17	5	7.6
Pasty	113	54.6	12	10.6	5	4.4	10	8.8	4	3.5	4	3.5	0	0	5	4.4
Formed	29	14	6	20.6	0	0	3	10.3	0	0	0	0	2	6.8	2	6.8
Blood stool	13	6.3	2	15.3	1	7.6	1	7.6	1	7.6	0	0	11\$	84.6	2	15.3

§E. histolytica was statistically associated with diarrheal syndrome (OR 10.6; 95% CI: 1.351 to 83.17; p = 0.0072) and with blood occult in stool (OD 10500; 95% CI 200.3 to 550700; p < 0.0001). ++DEC was statistically associated with CD4+T cells > 500 (OR 2.32; 95% CI 1,037 to 5,178; p 0.043). * All enteropathogens were associated with high viral load. Diarrheagenic *E. coli* (OR 4,41; IC 95% 1.715 to 11.44; p = 0.0033), *Salmonella* spp (OR 0.0086; IC 95% 1.970 to 32.49; p < 0.0001), *G. Intestinales* (OR 9.98; IC-95% 15.11 to 148.8; p < 0.0001) *Cryptosporidium* spp (OR 9.98; IC-95% 3.556 to 28.01; p < 0.0001), *C. belli* (OR 34.31 IC 95% 6.393 to 184.2, p < 0.0001) *E. histolytica* (OR 9.54; 95% CI 2.859 to 31.81; p = 0.0008), except *Entamoeba coli* - Confidence interval, *p* value - significant when p < 0.05.

Table 2: Frequency of enteric pathogens in relation to the HIV status of study subjects

Confirmed deaths were assessed through a medical record survey. In total, 16.4% (34/207) of patients died during the study period, of whom 58.8% (20/34) had CD4+ T-cell count < 50 cells/mm³ and 23.5% (8/34) from 50 to 199 cells/mm³, the highest death rates. With respect to viral load, 26,5% (9/34) of patients who died had loads of > 50 up to 100 copies (Table 3). Patients who did not use HAART, or who used it on an irregular basis, recorded higher mortality rate than those who adhered to the therapy (p <0.0001). However, given the clear and expected association between patient mortality and HAART, the influence of using antibiotics in association with other drugs on patient mortality rate remains unclear, since there was also higher mortality rate among patients who did not use HAART, or who did it on an irregular basis in association with antibiotics (p = 0.0005) and with other drug types (Table 4).

	Death			
Variables	No	Yes	p value	
	N = 173 (%)	N = 34 (%)		
Gender*			0.707	
Female	95 (54.9)	17 (50)		
Male	78 (45.1)	17 (50)		
Age**			0.753	
Mean age (SD)	42.2 (11.5)	42.9 (12.5)		
CD4 T-cell count****			< 0.001	
> 500 cells/mm ³	44 (25.9)	1 (2.9)		
From 200 to 500 cells/mm ³	45 (26.5)	5 (14.7)		
From 50 to 199 cells/mm ³	48 (28.2)	8 (23.5)		
< 50 cells/mm ³	33 (19.4)	20 (58.8)		
Viral load***			0.0134	
Not assessed	27 (15.6)	10 (29.4)		
< 50 copies/mL	55 (31.8)	2 (5.9)		
From 50 to 100.000 copies /mL	50 (28.9)	9 (26.5)		
From 100.001 to 500.000 copies/mL	25 (14.5)	7 (20.6)		
> 500.000 copies/mL	16 (9.2)	6 (17.6)		

* Fischer's exact test; ** t test; *** Chi-square test. **Table 3:** Risk factors associated with clinical variable

The sum out is a shown of	Death		Tatal			
inerapeutic scheme	Yes No		Total	p-value		
HAART therapy	5 (4.7)	102 (95.3) 107				
No HAART therapy	14 (24.1)	44 (75.9)	58	< 0.0001***		
Irregular HAART therapy	15 (39.5)	23 (60.5)	38			
HAART therapy + ATB*	4 (8)	46 (92)	50			
No HAART therapy + ATB*	7 (18.9)	30 (81.1)	37	0.0005***		
Irregular HAART therapy + ATB*	13 (44.8)	16 (55.2)	29			
HAART therapy + Dg**	0 (0)	15 (100)	15			
No HAART therapy + Dg**	6 (54.5)	5 (45.5)	11	-		
Irregular HAART therapy + Dg**	2 (28.6)	5 (71.4)	7			
Sample	34 (16.4)	173 (83.6)	207			

* Antibiotics; ** Drugs other than antibiotics; *** Chi-square test **Table 4:** Risk factors associated with adherence to therapy

Discussion

Opportunistic infections associated with environmental, socioeconomic, and epidemiological conditions are key factors in determining morbidity and mortality rates among HIV patients. The present study has investigated the prevalence of enteropathogens in 207 HIV patients, 112 women and 95 men. Results did not show significant difference between HIV patients based on sex; however, it is possible suggesting trend to feminization of HIV cases, as observed in several studies. Nowadays, the heterosexual transmission route is the HIV disease factor mostly observed in Brazil. The dynamics of the HIV epidemic has relevant expression in all Brazilian regions and has significantly contributed to the increased number of cases among women [20-22].

As for monthly income, 82% of patients received up to two minimum wages. This finding is in agreement with an HIV epidemiology study carried out in São Paulo, which reported mean individual income below or equal to two minimum wages and demonstrated that this income directly affects patients' quality of life [23].

Patient schooling was considered average, since 93.2% of them had some education level and 6.7% (14/207) were illiterate. Studies carried out in Piauí State, Northeast Brazil, reported low schooling, since only 38.4% of 146 patients went to high school [24]. Schooling is directly correlated to socioeconomic conditions and high morbidity and mortality rates [25].

Education in Brazil is under constant evolution: its illiteracy rate dropped from 6.8% in 2018 to 6.6% (approximately 200 thousand people) in 2019. However, there are still 11 million illiterates in the country. Accordingly, the influence of education on health conditions remains evident in HIV patients [26].

Socio-demographic assessment showed that 38.6% of the patients lived in the suburbs of Porto Velho City and 81.6% of them did not have access to household sewage treatment. These data are in agreement with those reported by Trata Brazil, which defines Porto Velho City as one of the capitals with the worst basic sanitation rates in Brazil. It has also been proven that Rondônia State is among the states with the worst Brazilian sewage treatment rates (only 6.33%) [27].

There is a global consensus that ART has improved the quality of life of HIV patients and has globally reduced HIV mortality rates [28]. However, there was an increase in the mortality rate per 100 thousand inhabitants in Northern Brazil from 2000 to 2014 [29,30]. These rates are substantiated by our findings, which indicated a 16.4% (34/207) mortality rate from 2014 to 2018. Mortality was statistically associated with low CD4+ T-cell count (p value < 0.001; viral load 0.0134, and HAART adherence < 0.0001). These data demonstrated that immunosuppression risk factors and non-adherence to ART are decisive to HIV patient death.

In the present study, HAART use was assessed; 28.0% of patients did not adhere to treatment and 18.4% irregularly adhered to HAART. These results are representative of irregular HAART adherence, which may reach up to 66% in Brazil. Consequently, Brazil remains far from eradicating HIV by 2030, which is a goal advocated by the Joint United Nations Programme on HIV/AIDS (UNAIDS) [29-31]. All these factors are key in understanding the findings of the enteric pathogenesis. It is well established that antibiotic therapy is an effective prophylactic against pneumonia and against other bacteria and has been extensively used in HIV-positive individuals around the world [32]. In this study, the use of HAART associated with antibiotic therapia showed as expected that they are protective factors of morbidity and mortality of the HIV patient.

Clinical manifestations caused by infectious diarrhea were evaluated, since the GI tract is one of the most affected sites in HIVpositive patients. The most common intestinal parasites were *Blastocystis hominis* (13.53%), *Cryptosporidium* spp. (10.14%), *Giardia intestinalis* (11.11%), *Entamoeba histolytica* (6.2%), and *Cystoisospora* spp. (3.86%). *Endolimax nana* was the most common nonpathogenic species found in the current study. Thus, the pathogenic potential of this species should be assessed in immunodeficient patients [33].

Studies on *Blastocystis* spp. prevalence in HIV patients are limited and outdated, due to false-negative laboratory results, since only a few laboratories can assess the morphological structure of this parasite through microscopy. A study conducted in Southeastern Brazil detected *Blastocystis* spp. in 17.8% of its sampled population [34]. Studies on *Blastocystis* spp. prevalence conducted in Rome with 156 HIV patients showed that 25% of them were HIV-positive and *Blastocystis* spp. was not correlated with viral or epidemiological risk factors [35].

A study carried out in children in Kenya showed that the overall prevalence of *G. intestinalis* in HIV-infected children tended to be higher (22.8% - 28/123) than in non-infected ones (18.0% - 20/111) [36]. A case report showed *G. intestinalis* in the anal pap test of a 41-year-old man without GI symptoms [37]. This pathogen is very common due to its resistance to external conditions and ease in causing fat-soluble vitamin malabsorption as a result of mucosal injury.

Cystoisospora spp. (coccidia subclass) was detected in a patient who suffered from weight loss and chronic diarrhea for two years. Negative routine laboratory results were found in the patient's medical records, which indicated that the patient's quality of life had been compromised due to lack of high-quality assessment methods [12]. *Cystoisospora* spp. infections can lead to chronic intermittent diarrhea and life-threatening diarrhea complications [38,39]. Standard HIV laboratory tests are essential for the following achievements: broad diagnosis, clinical assessment methods, such as sedimentation and staining techniques, are more effective as well as techniques involving other enteric pathogens, such as co-cultures, enteric pathogens, and stool occult blood [40]. HIV spread leads to opportunistic infections and increases the prevalence of potentially harmful opportunistic protozoa, such

as *Cryptosporidium* spp. and *Cystoisospora* spp. (coccidia subclass), whose persistence is directly related to poor basic sanitation and tropical climates.

Entamoeba histolytica can cause ulcerative colitis in acute HIV-infected patients. In the present study, *E. histolytica* was statistically associated with diarrhea and stool occult blood (p < 0.0001; 10500 OR at 200.3–550700 CI). Accordingly, *E. histolytica* can behave aggressively, as it invades the colon wall and destroys epithelial cells. This attack causes severe colon inflammation, which leads to bloody diarrhea [41].

As for diarrheagenic *E. coli* pathotypes, EAEC was found in 41.24%, EPEC in 35.3%, DAEC in 17.6%, and EIEC in 5.9% of isolates. The prevalence of DEC in the HIV population is also statistically underestimated due to limited routine laboratory methods [3]. Another study carried out with HIV patients in South Africa analyzed 58 stool samples, of which 30 (51.7%) had EAEC and only two (3.4%) had EPEC [31]. EAEC strains have been identified as etiologic agents of diarrhea and enteropathy in HIV-positive children and adults from developing countries [42]. Other identified enteric pathogens included *Salmonella* spp., *Shigella* spp., and *Klebsiella* spp. According to the literature, these bacteria can modulate mucosal immunity and overlap HIV pathogenic features, and induce inflammation in the GI microenvironment, which is responsible for cell proliferation and resistance [43].

Enteric pathogens were statistically associated with a high viral load. A systematic review reported a decrease in viral load when major co-infections were treated. An interesting study conducted in Ethiopia showed that the intestinal helminth infection treatment is effective in reducing plasma viral load in HIV co-infected patients, and that mean baseline HIV-RNA levels are associated with helminth infection severity [44]. Many studies agree that co-infection treatment or suppression can reduce HIV viral load, since prevalent co-infection treatments in developing countries can help reduce HIV-RNA levels, which are high enough to substantially affect public health [45].

Conclusion

In conclusion, sanitary and social conditions are decisive for HIV patients' quality of life. This is demonstrated in regions without basic sanitation, such as the conditions found in this study. Accordingly, routine standardization is key for proper and early laboratory diagnosis, since in-depth examination can truly reduce patients' morbidity and substantially improve public health.

Conflict of interest

None declared.

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