

Original Research Article

GASTROINTESTINAL TRACT OPPORTUNISTIC PARASITIC INFECTIONS AMONG HIV/AIDS PATIENTS UNDER ANTI-RETROVIRAL THERAPY IN THE DSCHANG HEALTH DISTRICT

ABSTRACT

Background: The analyses of the recurrence of opportunistic gastrointestinal parasitic infections is crucial for the effective management of HIV infection in sub-Saharan African countries where intestinal parasites are very common. More and recent information on the interested field is needed in order to better understand the magnitude of the problem. The present analytical case control study was designed in order to determine the prevalence of gastrointestinal opportunistic parasites and detect the associated risk factors among HIV positive patients in 4 sanitary centres in Dschang Health District (west Cameroon).

Methodology: Stool specimens were collected from 305 individuals, 97/305(31.8%) HIV positive patients and 208/305(68.2%) HIV negative participants. These specimens were examined for the research of gastrointestinal opportunistic parasites using the sedimentation concentration techniques followed by modified ZeihlNeelsen staining technique for the detection of different oocyst of the parasites. Statistical analysis performed included the Chi-square test and logistic regression. $P > 0.05$ was considered significant.

Results: Out of the 97 HIV positive patients, 16/97(16.49%) were infected with opportunistic intestinal parasites. Out of the 208 negative participants, 40/208(19.4%) were infected. There was no significant association between the occurrence of opportunistic intestinal parasitic infections and HIV sero-status ($OR=0.815$; $IC=0.410-1.620$; $p=0.559$). The parasite species of *Cryptosporidium parvum*, *Microsporidium*, *Cyclospora cayetanensis* and *Cycloisospora belli* were detected in 38(12.5%); 11(3.6%); 4 (1.3%) and 3(1%) participants respectively in the overall population. Drinking water obtained from springs ($OR=8.46$; $CI=1.56-14.34$; $p=0.00$), boreholes ($OR=3.129$; $CI=1.24-7.881$; $p=0.00$) and wells ($OR=4.01$; $CI=3.77-4.26$; $p=0.00$) were associated to opportunistic parasitic infections. Opportunistic parasitic infections in HIV positive patients were not statistically associated with CD4 count, viral load, type and duration of antiretroviral therapy with respective p values of 0.15; 0.58; 0.08 and 0.54.

Conclusion: The study revealed that HIV positive individuals continue to experience gastrointestinal infections although antiretroviral therapy reduces the risk of parasitic infections. Regular screening and treatment of intestinal opportunistic parasitic infections coupled with Adherence to highly active antiretroviral therapy is very vital in improving the overall quality of care of HIV/AIDS patients

KEYWORDS: HIV, Parasites, Opportunistic, seropositive, gastrointestinal tract.

UNDER PEER REVIEW

INTRODUCTION

Neglected tropical diseases, including intestinal parasitic infections are among the most common infections worldwide. They are a significant cause of morbidity and mortality in low and middle income countries of tropical and subtropical regions[1].

Acquired immune deficiency syndrome (AIDS) and its etiologic agent Human immune deficiency Virus (HIV) is one of the most important pandemic worldwide[2]. AIDS pandemic took a life every minute in 2021, with 650 000 AIDS-related deaths despite effective HAART and strategies used for preventing and managing opportunistic infections. Globally, there was an estimate of 38.4 million people living with HIV at the end of 2021[2].

AIDS is a condition in humans in which progressive failure of the immune system allows life-threatening infections to thrive[3]. Lifelong Antiretroviral HIV infection free is associated to increased vulnerability of patients to other infections, known as opportunistic infections [4]. The opportunity offered by the patient's weakened immune system for opportunistic infections is due to the fact that HIV infects vital cells of the immune system, including helper CD4 T cells[1], macrophages[5].

Cryptosporidium parvum, *Cycloisospora belli*, *Cyclospora cayetanensis*, *Microsporidium*, species such as *Enterocytozoon bieneusi* have been incriminated as causes of prolonged diarrhea, especially in AIDS patients, although they are thought to cause self-limiting diarrhea in immunocompetent individuals[6].

Few similar studies, have been carried out in Cameroun in Yaounde, Douala, Fako division, Bamenda, and South West regions, giving a prevalence of 9.7%[5]; 7.4%[7]; 82.6%[8], 34.5%[6]; 53.7%[9] respectively. In the Dschang district, West Region of Cameroon by Nkenfouet *al*, 2011 found a prevalence of 19.0% [10].

The objective of this study was to determine the prevalence of intestinal opportunistic parasitic infections among HIV positive patients and to investigate the clinical, laboratory, and epidemiologic characteristics associated with the different parasitic infections.

I. MATERIALS AND METHODS

I.1. Study area

This analytical case control study was conducted in Cameroon. More specifically in the 4 principal health centres of the Dschang health district. This is a district located in the Menoua division in the west region of Cameroon. The town of Dschang is located between latitude 5°20' to latitude 5°28' in the north and between longitude 10°3' and 10°6' in the East and at an altitude of 1382-1500m in the high land of west Cameroon. Its climate type is the soudano-guineen with two seasons: a dry season (November to March) and a raining season (March to November). The Dschang health district is one of the major health districts in the West region. It extends over approximately 1060 km². It is bounded to the east by the health district of Penka Michel, to the northeast by the health district of Batcham, to the west by the health district of Fontem, to the southeast by the health district of Bandja, and to the south by the health district of Santchou. It is made up of 22 health areas and 322 communities. Of the six districts of the Menoua department, it covers four (districts of Dschang, Fokoué, Nkong-ni and Fongo-tongo) and the Fondonera group.

I.2. Strategies for the recruitment of the studied population

An ethical clearance delivered by the RERCHH (Regional Ethics and Research Committee for Human Health) was obtained. After obtaining participants consents and assents, they filed prepared questionnaires.

I.2.1. Inclusion criteria

- Every person living with HIV/AIDS under ART of age 12 and above without gender distinction, pregnant or not coming for consultation or receiving ART at the DREAM (Drug Resource Enhancement against AIDS and Malnutrition) for our group of HIV positive patients

- Every person that is not infected with HIV/AIDS of age of 12 and above without gender distinction, pregnant or not and is not suffering from any known chronic diseases (diabetes, asthma) coming for consultation at the Dschang regional annex hospital, the Saint Vincent de Paul hospital and the *Centre Catholique Notre Dame De La Sainte Servante De Marie* in Batseng'la for our group of HIV negative patients (control group).

I.2.2. Non-inclusion criteria

- Children with less than 12 years' old.

- Every person who were suffering from chronic diseases like diabetes, cancers ...

I.2.3. Exclusion criteria

-Participants who desisted during the study.

This information were obtained with the help of questionnaires

II.3 Sample size and justification

A study carried out in the West region of Cameroon by Nkenfouet *al*, 2013 [10] on the prevalence of co infection of intestinal parasites in HIV positive patients. HIV positive patients showed prevalence of opportunistic parasites of 19.02% and HIV negative participants showed prevalence of opportunistic parasites of 2% [10]

Our population size was calculated using EPI INFO (version 7.2.5.0) statcalc for sample size and power.

Two sided confidence level =95%

Power of the study=80%

Ratio (number of unexposed/number of exposed) =6.04

Percentage outcome in unexposed group=2%

Risk ratio=9.52

Odd ratio=11.5237

Percentage of exposed group=19.04

The minimal population size was estimated at 189 participants (27 HIV positive patients and 162 HIV negative participants). We carried out a cross sectional descriptive study on 305 participants (97 HIV positive patients and 208 HIV negative participants) who fulfils the inclusion criteria and agree to participate in the study during the given period of time.

I.4. Collection and transport of stool samples

A Stool container was given to HIV positive and negative to put in their stool. The container was labelled with the patient's attributed code. Patients were properly instructed on how to collect samples (they had to collect approximately 5 grams of stool and avoid mixing the stool with water or urine). Specimens were immediately transported using a box filled with ice from the collection sites in Dschang regional hospital annex, the *Saint Vincent de Paul* hospital, the 'Centre Catholic Notre Dame de la Sante Servante de Marie de Batseng'la' and DREAM centre to the laboratory of medical analyses of the Dschang Regional Hospital Annex for

analyses. The stool samples were directly conserved with formalin at 10% at -2 to -8°C (the stool specimen was totally submerging in formalin).

I.5. Analyses of samples

For HIV negative participants a confirmation of his or her HIV sero-negative status was done by practising an immune-chromatographic assay for HIV using a Determine (HIV 1&2) strip.

I.5.1. Formalin-Ether Concentration

Two grams of stools specimen were mixed with 7 ml of 10% formalin in a clean 15 ml conical centrifuge tube. The resulting mixture was filtered through a cotton gauze into a beaker and the filtrate poured back into the same tube and the debris were discarded. After adding 4 ml of ethyl acetate, the tube was closed, and shake vigorously in an inverted position for 30 seconds then we carefully removed the lead, centrifuged at 1500 rotations per minutes for 5 minutes. A cotton-tipped applicator was used to remove debris from sides of the centrifuge tube and added several drops of 10% formalin to suspend the concentrated specimen, after being sure that the specimen is well mixed. The smear was prepared using 2 to 3 drops of the specimen depending on density and it was air dried completely at room temperature.

I.5.2. Modified Zeihl-Neelsen Method

The modified Ziehl-Neelsen method uses a carbol fuchsin stain, acid alcohol decolourizer, and methylene blue counterstain. Acid-fast organisms stain red, while the background of debris stains blue. Faecal smears were made directly from the concentration deposit, it was allowed to air dry at room temperature and then fixed in methanol for 3 minutes. After staining with strong carbol fuchsin for 15-20 minutes, it was rinsed thoroughly in tap water then decolourised in acid alcohol (1% HCl in methanol) for 15 seconds later it was rinsed again thoroughly in tap water after which it was counterstained with methylene blue for 30 seconds and finally rinsed thoroughly and air dry. After staining the slides were examined at x100 objectives. For the detection of the different forms of parasites. Microscopic examinations were done independently by experienced clinical laboratory technicians and by the principal investigator; the determination and verification was done by the principal investigator.

I.5.3. Quality control

Validation (biological and technical) was done by the principal investigator and supervised by the laboratory head or any available experienced laboratory technician.

Confirmation of sufficient specimen volume and correct procedural technique was done by the principal investigator and experienced laboratory technician.

Data on antiretroviral treatment and most recent CD4+ cells count were obtained from the medical files of patients.

Data analyses were done with the help of a statistician

I.6. Data analysis

Data were entered using Microsoft Excel and analysed using Statistical Package for the Social Sciences (SPSSSTM) version 25. The Chi square (χ^2) permitted us to compare the prevalence of intestinal infections with respect to serological status, sociodemographic characters and risk factors, it permitted us to detect any relationship between two nominal qualitative variables. A multinomial logistic regression was used to calculate odd ratios and determine potential risk factors among nominal variables. This study will, make it possible to determine the prevalence of intestinal opportunistic parasitic infections and HIV and the threshold of significance of error $\alpha=5\%$ for a confidence interval 95% CI ($p<0.05$, $p>0.05$).

II. RESULTS

II.1. Presentation of studied population

Figure 1 shows that we encountered a total of 353 individuals on the field but 19 individuals were excluded and 14 refused to participate. Four parents refused that their child should participate and 11 stool samples were absent or not sufficient.

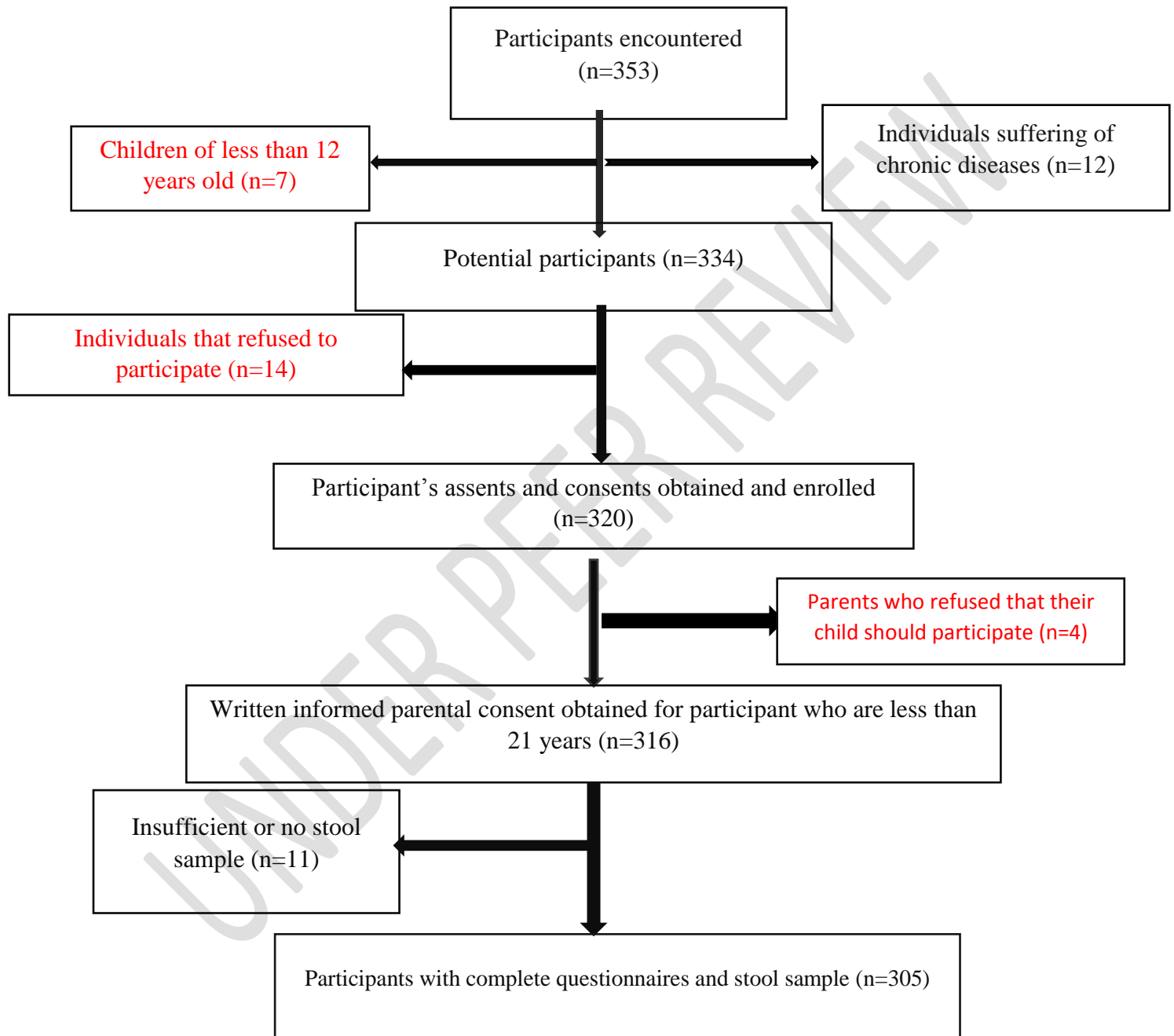


Figure 1: Synoptic diagram of studied population

II.2. Profile of our studied population

The general characteristics of the study population are summarized in Table 1. We enrolled a total of 305 (100%) participants in the study, 31.8% (97/305) were HIV positive patients and 68.2% (208/305) were HIV negative participants. We notice a predominance of females, with 72.2% (70/97) among HIV positive patients and 66.8% (139/208) among HIV negative participants. The age range of [35-65] was the most represented with 84.5% (82/97) among HIV positive patients, while [25-35] was the most represented age range with 41.3%(86/208) among HIV negative participants. Those living in rural areas were the most represented among HIV positive participants with 66.0% (64/97) but were the least represented with 40.4% (84/208) among HIV negative participants. The formal sector which was made up of bankers, teachers, and nurses was the least represented with 7.2%(7/97) among HIV positive patients. While the informal sector which was made up of traders, household, farmers, and breeders was the most represented with 86.1% (179//208) among HIV negative participants

Table 1: Distribution of the population with respect to demographic characters

Parameters		HIV-positive n (percentage%)	HIV-Negative n (percentage%)
Gender	Female	70(72.2%)	139(66.8%)
	Male	27(27.8%)	69(33.2%)
Age] 12-25]	2(2.1%)	24(11.5%)
	[25-35]	8(8.2%)	86(41.3%)
	[35-65]	82(84.5%)	81(38.9%)
	[>65	5(5.2%)	17(8.2%)
Profession	Formal sector	7(7.2%)	29(13.9%)
	Informal sector	90(92.8%)	179(86.1%)
Residence	rural	64(66.0%)	84(40,4%)
	urban	33(34.0%)	124(59,6%)

II.3. Prevalence of opportunistic parasitic infections

Of the 305 participants enrolled, 56/305(18%) were infected with at least one species of opportunistic intestinal parasite, as shown in figure 2.

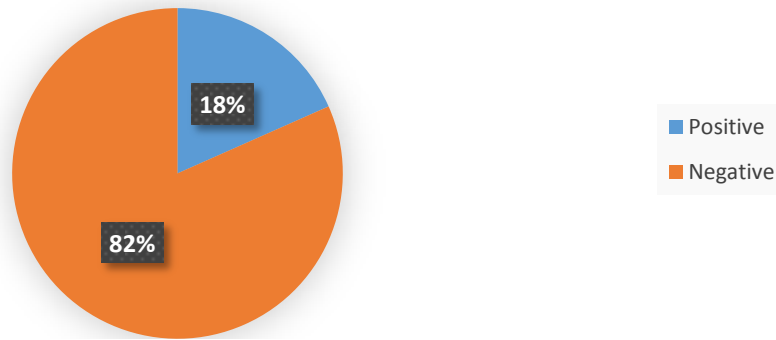


Figure 2: General prevalence of opportunistic parasitic infections among HIV positive and Negative participant

II.4. Prevalence of opportunistic parasitic infection according to HIV status

HIV negative participants were more infected with opportunistic parasitic infections with 19.2%(40/208) than HIV positive patients with 16.49%(16/97) as shown in table 2.

Table 2: General prevalence of parasitic infection according to HIV status

Status	HIV positive		HIV negative	
	N(frequency)	Prevalence (%)	N(frequency)	Prevalence (%)
Number examined	97	100.0	208	100.0
Positive	16	16.49	40	19.2

II.5. Single and mixt infections among HIV positive and negative participants

Table 3 show us that the most encountered opportunistic intestinal parasite was *Cryptosporidium parvum*, and it was more present in HIV negative participants with 13.5% (28/208) than in HIV positive patients with 10.3% (10/97). We also notice that *Cycloisospora belli* was absent in HIV negative participants. *Cyclospora cayetanensis* was the parasite that was the least present with, 1% (1/97) in HIV positive patients. Only one case of triple infection was present among the HIV negative participants. The parasites implicated in this triple infection was *Cyclospora cayetanensis*, *Cryptosporidium parvum* and *Microsporidium*. Three cases of double infections were present in HIV negative participants, but only 1 case was present in HIV positive participants. The parasites implicated in this double infection was *Cyclospora cayetanensis* and *Cryptosporidium parvum*

Table 3: Prevalence of single and mixed infections among HIV positive and negative participants

		HIV positive (N□=97)		HIV negative (N□=208)	
	Parasite specie	Frequency(n)	Prevalence (%)	Frequency(n)	Prevalence (%)
Single infections	<i>C. parvum</i> ,	10	10.3	28	13.5
	<i>C. cayetanensis</i>	2	2	2	1
	<i>C. belli</i> ,	3	3.1	0	0
	<i>Microsporidium</i>	1	1	10	4.8
Mixed infections	<i>C. cayetanensis</i> + <i>C. parvum</i> ,	1	1	3	1.44
	<i>C. cayetanensis</i> + <i>C. parvum</i> + <i>Microsporidium</i>	0	0	1	0.4

N□ = Number of positive patients

N□ =N umber of negative participants

II.6. Risk factors and sociodemographic characters

Table 4 shows that gender, occupation and residence could be identified as risk factors but none of sociodemographic character below was identified as a risk factor significantly associated with opportunistic intestinal parasitic infection.

Table 4: Multinomial logistic regression analysis of sociodemographic characters of studied population

Parameters	Number examined	Number of positive	Odds ratio	Confidence Interval at 95%	p-value
Gender					
Male	96	18	-	-	-
Female	209	38	1.019	0.533-1.948	0.955
Age					
] 12-25]	26	07	0.128	0.14-1.205	0.72
[25-35]	94	21	0.210	0.21-2.098	0.184
[35-65]	163	27	0.253	0.31-2.056	1.98
[>65	22	01			
Occupations					
Informal sector	269	50	1.17	0.437-3.285	0.806
Formal sector	36	6	-	-	-
Residence					
Urban	148	32	-	-	-
Rural	157	24	1.11	0.58-2.14	0.7

II.7. Associated risk factors of opportunistic intestinal parasitic infections

The results in table 5 shows that, washing of food before eating, washing of hands before eating, putting hands in the mouth, eating together in family in the same plate, consumption of eggs and milk are risk factors associated with opportunistic intestinal parasitic infections since their odd ratios are less than 1. These associations were only significant in the type of drinking water, where those that drink borehole water are 3.129 (CI=1.24-7.881 p=0.00) times more susceptible to get infected with opportunistic intestinal parasites than those that drink mineral water. Those that drink source water are 8.46(CI=1.56-14.34 p=0.00) times more susceptible of getting infected than those drinking mineral water. Those that drink well are 4.01(CI=3.77-4.26, p=0.00) times more susceptible to get infected. There is a significant relationship between washing of hands before eating and opportunistic intestinal parasitic infections (p=0.011). The rest of variables were not considered as associated risk factors, since there was no significant relation between them and opportunistic intestinal parasitic infections.

Table 5: Multinomial logistic regression analysis of associated Risk factors of the studied population

Parameter	Number examined	Number of positive	Odds ratio	Confidence interval at 95%	p-value
Wash food before eating					
Yes	245	42	-	-	-
No	60	14	1.376	0.571-3.314	0.477
Wash hands before eating					
Yes	75	22	-	-	-
No	230	34	0.362	0.165-0.793	0.011
Drinking water source					
Mineral	05	0	-	-	-
Borehole	132	26	3.129	1.24-7.881	0.00
Well	07	02	4.01	3.77-4.26	0.00
Tap	23	04	4.47	1.80-3.97	0.00
spring	80	11	8.46	1.56-14.34	0.00
Putting hands in the mouth					
Yes	92	20	-	-	-
No	213	36	1.182	0.628-2.227	0.604
Eating together in the same plate					
Yes	58	11	-	-	-
No	247	45	1.080	0.509-2.294	0.927
Having animals at home					
Yes	190	38	-	-	-
No	115	18	0.742	0.401-1.374	0.343
Using modern toilets					
Yes	110	19	-	-	-
Non	195	37	0.466	0.493-1.706	0.748
Washing hand after toilets					
Yes	214	35	-	-	-
No	91	21	0.652	0.335-1.197	0.167
HIV sero-status					

Positive	97	40	0.815	0.410-1.620	0.559
Negative	208	16			

III.8. Prevalence of parasitic infection according to clinical characteristic of the study population

Table 8 shows that most of HIV positive patients (59/97(60.82%)) had normal CD4+ lymphocyte count (500-1000 cells/mm³) with 22.03% (13/59) infected with intestinal opportunistic parasites. Participants with viral load lower than Low Level Detectable (LDL) were the most represented (85/97(87.63%)), but those with viral load lower than 40 copies/mL were the most infected with intestinal opportunistic parasites 28.57% (2/7) with. Most of HIV positive patients used TLD (Tenofovir-Lamivudine-Dolutegravir as ART) (80/97(82.47%)). But all those that used DRD (Dorunavir-Ritonavir-Dolutegravir) and ARAL (Atazanavir-Ritonavir-Abacavir- Lomivalone) as anti-retroviral therapy were not infected with intestinal opportunistic parasites. Patients that followed ART therapy for more than 10 years were the most infected with intestinal opportunistic parasites with 18.18% (8/44) followed by those who followed the therapy for less than 5 years with 17.94% (7/39). Generally, the occurrence of opportunistic intestinal infection was not significantly related to clinical parameters of patients (CD4⁺ count, type of ART, Viral load and duration of ART).

Table 6: Prevalence of parasitic infection according to clinical characteristic of the study population

Parameters	N	NP	Prevalence %	P value (chi ²)
CD4(cells/mm³)				
<500	22	2	9.09	0.15
500-1000	59	13	22.03	
>1000	16	1	6.25	
Viral load(copies/mL)				
<40	7	2	28.57	0.58
<LDL	85	13	15.29	
>40	5	1	20	
Type of ART				
ALD	3	0	0	0.08
ARAL	1	1	100	
ARTL	9	1	11.11	
DRD	1	1	100	
TELE	3	0	0	
TLD	80	13	16.25	
ART duration (years)				
<5	39	7	17.94	0.54

5-10	14	1	7.14
>10	44	8	18.18

NE **number** **examine,** **NP:** **number** **of** **positive**
LDL: Low Level Detectable, **TLD:** Tenofovir, Lamivudine, Dolutegravir, **ARTL:** Atazanavir, Ritonavir, Tenofovir, Lamivudine, **DRD:** Dorunavir, Ritonavir, Dolutegravir. **ARAL:** Atazanavir, Ritonavir, Abacavir, Lomivalone, **ALD:** Abacavir, Lomivalone, Dolutegravir, **TELE:** Tenofovir, Emtricitabine, Lamivudine, Elvitegravir, **ART:** Anti-Retroviral Therapy

UNDER PEER REVIEW

DISCUSSION

Patients infected with the Human Immunodeficiency Virus (HIV) often have opportunistic infections, among which coccidiosis are the most common parasitic infections that aggravate their health status[11]. Parasitological techniques (modified Ziehl Neelsen staining techniques) for the detection of these parasites are not routinely done in our hospitals, hence no or late diagnostic of these parasites. The main objective of the study was to determine the prevalence of intestinal opportunistic parasites among HIV positive patients in the Dschang health district.

Opportunistic intestinal parasites were encountered with a prevalence of 16.49% (16/97) among HIV positive patients while 19.2% (40/208) were found among HIV negative participants, the difference was not significant ($P = 0.559$). These results are different from that obtained by Nkenfouet *al*, 2013 [10] in the west region of Cameroon, where 19.04% (4/42) of HIV/AIDS patients were infected with opportunistic intestinal parasites and only 2% (7/354) of the HIV negative patients were infected with opportunistic intestinal parasites. Possible explanations for having higher prevalence of parasites among HIV negative participants when compared with HIV positive patients are that; majority (78.1%) of our participants had normal CD4- lymphocyte count ($>500\text{cells/mm}^3$). The literature reports that the prevalence of intestinal parasites is significantly higher in subjects with CD4+ cell counts below 200 cells/mm^3 , proving that CD4+ T lymphocytes are essential to fight against HIV and slow down the progression of AIDS[12]. Also, HIV infected patients are more often likely to be in contact with the health care system and thus more exposed to anti-parasitic drugs[13]. Moreover, there is evidence, in vivo as well as in vitro, that the control of cryptosporidiosis in patients on HAART are also helped by the anti-HIV protease inhibitors, which could be acting on the aspartyl protease of the parasites[13]. The prevalence of opportunistic parasitic infections in HIV positive patients was 16.49% similar to that obtained by Bissonget *al*, 2015[6] who had a prevalence of 15.5% but different from a study carried by Botero-Garcés *et al*, 2021 [14] in Antioquia, Colombia where there was only 1% of opportunistic parasitic infections in HIV positive patients. This difference may be due to the fact that sub-Saharan Africa has seasonal climate variations. Climate change exacerbate the pressures exerted on water availability, accessibility, and demand, further emphasizing the potentially increasing threat of water borne infectious diseases[15]. Based on parasitological examination of the stool specimens, 4 species of opportunistic intestinal parasites were detected in HIV positive patients. The detected parasites included: *Cryptosporidium parvum*,

Cycloisospora belli, *Cyclospora cayetanensis* and *Microsporidium*. The most frequently detected parasite was *Cryptosporidium parvum* in 10/97(10.3%) patients. These results are higher than those obtained by Kuete *et al* ,2014 [16] in Yaounde Cameroon with the prevalence of *Cryptosporidium parvum* of 6%(4/6470). This difference may be due to the difference in the site of the study, Yaounde being the political capital is more developed than Dschang[10]. These results were lower than that of Nsagha *et al* , 2017[8] and Cheforet *et al*, 2020 [9] who had prevalence of *Cryptosporidium parvum* of 44% and 23.3% respectively. These studies were carried out in the southwest region of the country, where the climate favours the rapid distribution of these protozoans[7]. The least encountered opportunistic parasite was *Cycloisospora belli* 1%(3/305). This prevalence is similar to the 0.5% obtained by Kuete *et al* ,2014[16]. This may be because none of the participants had diarrhoea. Although the incidence of *C. belli* might vary geographically, its prevalence depends on the immune and diarrhoea status of the patients[17].

The risk factors identified among the socio-demographic characteristics were: gender, occupation, and residence. Gender was identified as a risk factor associated with opportunistic intestinal parasitic infections (OD=1.019, CI= 0.533-1.948) but there was not a significant difference of the occurrence of opportunistic infections between both sexes ($p=0.955$). These results are similar to that obtained by Mbiandou *et al* , 2019 ($p=0.2161$)[18] in Cameroon and Botero-Garcés *et al* , 2021 ($p=0.06$, CI=-0.8-27.5) in Colombia[14]. This may be because males and females in our studied population practise similar activities (farming for example) that exposes them equally to these parasites[15]. Participants working in informal sectors were more infected than those working in the formal sector but this difference was not significant ($p=0.806$) like in the study of Mbiandou *et al* , 2019 ($p=0.0891$). These results may be due to the fact that those working in the informal sector (farmers, breeders, traders) are more likely to ingest cysts of protozoan from the soil, and unwashed fruits and vegetables[15]. Their line of duty as transmission of these parasites is through the ingestion of contaminated soil, water or food. Participants living in rural were more infected than those living in urban areas but, the difference was not significant ($p=0.7$, CI=0.58, 2.14, OR=1.11) similar to what was obtained by Nsagha *et al* , 2017[8] ($p=0.45$). This is most probably due to the fact that the transmission of intestinal opportunistic infections is through the ingestion of contaminated soil or water both of which were very feasible among the rural population. Also, it might be due to the fact that many rural dwellers are more in contact with their domestic animals especially goats, sheep, and dogs, thus increasing their chances of being infected with

these parasites[19].Also, it might be due to the fact that rural dwellers are involved in farming activities where they are likely to ingest cysts of protozoan from the soil, and unwashed fruits and vegetables while working[16]. The type of drinking water source was significantly associated with OIPs ($p=0.00$). This result is different from that obtained by Kuete *et al*, 2015 where consuming water from boreholes, dug wells or sachet did not influence significantly any of intestinal protozoa infections in Douala ($p>0.05$). This difference is due to the difference in the study site Douala more socio-economically developed than Dschang[7].

Clinical findings ($CD4^+$ count, viral load type of ART, duration of the ART), of HIV positive patients were not significantly related with OIPs ($p=0.15$, $p=0.58$, $p=0.08$, $p=0.54$ respectively). Similarly, in the study of Botero-Garcés *et al*, 2021 where the viral load and the $CD4^+$ count were not significantly associated with intestinal parasitic infections ($p=0.851$ and $p=0.121$ respectively). The introduction of the highly active antiretroviral therapy (HAART) as the main treatment for HIV has led to a reduction in infections frequency, including those caused by enteroparasites and has improved the clinical and laboratory outcomes of the patients[12][20]. These results are different from those of Nsagha *et al*, 2017 [8] in Cameroon and Adamu *et al*, 2013[21] in Ethiopia where intestinal parasitic infections prevalence were significantly higher in patients with $CD4^+$ T cell count below 200 cells/ μ l ($p=0.000$ and $p=0.03$ respectively). The reason for this difference is that all our HIV positive patients were enrolled at the DREAM centre, which is a clinic specialized to follow up HIV infected patients also, patients recruited there reported (through questionnaires) having no symptoms of abdominal dysfunction. Their average $CD4^+$ T cells was 724.2 cells/ μ l with the majority 90/97 (92.8%) of participants having values of $CD4^+$ T count greater than 500 cells / mm^3 of blood. Majority of our participants that is, 85/97 (87.6%) had undetectable viral load and were on ART since they were diagnosed with the disease, and had never stopped their treatment. Furthermore, in our study, all the HIV positive patients were under HAART treatment, so their viral load was controlled. A health talk was done every morning at the DREAM centre on HIV/AIDS by health personnel on the management of the disease and on ways to avoid infection with parasitic diseases.

Several limitations in our study should be highlighted. First, our HIV positive population was recruited by convenience in a time limited frame from patients attending a single health facility who were willing to inform consent. Thus, the data obtained from HIV infected patients cannot be assumed to be representative of the general population not actively receiving health care, secondly, detection of *Cryptosporidium spp.* may be under-estimated

due to limitations of the sensitivity of our assay, which is around 92%. Thus, a negative result in this assay does not rule out the possibility of *Cryptosporidium spp.* infection. Such a result may be due to intermittent excretion of the parasite, or the amount of antigen in the sample may be below the level of detection of our assay. It is possible that some parasites were not detected in this study because all the specific techniques were not used for their search. Thirdly, CD4 cells counts were not measured at the time of the stool specimen collection; instead, we have taken the most recent CD4 cells counts that were documented in the files of patients. In fact, it might have happened that the CD4 cells counts of patients may in reality be higher (for patients who started treatment) or lower (for HAART naïve patients) than the collected data by the time of stool sample collection.

CONCLUSION

The high prevalence (18%) of intestinal parasites found in this study has attracted the attention of health professionals to the need for parasitological examinations in the routine treatment of patients with HIV/ AIDS, including specific tests, given the clinical importance of these diseases in the evolution of AIDS. Also, adherence to HAART treatment has become crucial in the clinical and public health management of HIV infection.

REFERENCES

1. Sangaré I, Bamba S, Cissé M, Zida A, Bamogo R, Sirima C, et al. Prevalence of intestinal opportunistic parasites infections in the University hospital of Bobo-Dioulasso, Burkina Faso. *Infect Dis Poverty*. 2015;4:1–6.
2. Kapila A, Chaudhary S, Sharma RB, Vashist H, Sisodia SS, Gupta A. A review on: Hiv aids. *Indian J Pharm Biol Res*. 2016;4:69–73.
3. Kulkarni SV, Kairon R, Sane SS, Padmawar PS, Kale VA, Thakar MR, et al. Opportunistic parasitic infections in HIV/AIDS patients presenting with diarrhoea by the level of immunosuppression. *INDIAN J MED RES*. 2009.
4. Assis DC, Resende DV, Cabrine-Santos M, Correia D, Oliveira-Silva MB. Prevalence and genetic characterization of *Cryptosporidium spp.* and *Cystoisospora belli* in HIV-infected patients. *Rev Inst Med Trop Sao Paulo*. 2013;55:149–54.
5. Abange WB, Nkenfou CN, Gonsu Kamga H, Nguedia CA, Kamgaing N, Lozupone C, et al. Intestinal parasites infections among HIV infected children under antiretrovirals treatment in Yaounde, Cameroon. *J Trop Pediatr*. 2020;66:178–86.
6. Bissong MEA, Nguemain NF, Ng'awono TEN, Kamga FHL. Burden of intestinal parasites amongst HIV/AIDS patients attending Bamenda regional Hospital in Cameroon. *Afr J Clin Exp Microbiol*. 2015;16:97–103.

7. Lehman LG, Kangam L, Nguepi E, Mbenoun M-L, Bilong Bilong CF. Study of intestinal parasitic infections associated with HIV infection in Douala, Cameroon. *Retrovirology*. 2012;9:1–1.
8. Nsagha DS, Njunda LA, Assob NJC, Ayima CW, Tanue EA, Kibu OD, et al. Prevalence and predisposing factors to intestinal parasitic infections in HIV/AIDS patients in Fako division of Cameroon. *Am J Epidemiol Infect Dis*. 2017;5:42–9.
9. Chefor D, Ajonina MU, Nabuin N. Enteric Gastrointestinal Tract Opportunistic parasitic Infections of HIV/AIDS Patients in Limbe and its Environs South -West Region, Cameroon. 2020;:1–07.
10. Nkenfou CN, Nana CT, Payne VK. Intestinal Parasitic Infections in HIV Infected and Non-Infected Patients in a Low HIV Prevalence Region, West-Cameroon. *PLoS ONE*. 2013;8:e57914.
11. Udeh EO, Obiezue RNN, Okafor FC, Ikele CB, Okoye IC, Otuu CA. Gastrointestinal parasitic infections and immunological status of HIV/AIDS coinfecting individuals in Nigeria. *Ann Glob Health*. 2019;85.
12. Barcelos NB, Silva L de F, Dias RFG, Menezes Filho HR de, Rodrigues RM. Opportunistic and non-opportunistic intestinal parasites in HIV/AIDS patients in relation to their clinical and epidemiological status in a specialized medical service in Goiás, Brazil. *Rev Inst Med Trop São Paulo*. 2018;60.
13. Pozio E, Morales MAG. The impact of HIV-protease inhibitors on opportunistic parasites. *Trends Parasitol*. 2005;21:58–63.
14. Botero-Garcés J, Villegas-Arbeláez E, Giraldo S, Urán-Velásquez J, Arias-Agudelo L, Alzate-Ángel JC, et al. Prevalence of intestinal parasites in a cohort of HIV- infected patients from Antioquia, Colombia. *Biomédica*. 2021;41 Suppl 2:153–64.
15. GG I, Khan P, Nadia N, Cedric Y. Risk Factors Associated with Prevalence and Intensity of Gastro-Intestinal Parasitic Infections within Households in Tonga Sub-Division, West Region, Cameroon. *J Infect Dis Epidemiol*. 2020;6.
16. Kuete Yimagou E. Comparison of Intestinal Parasitic Infection among Adults with or without HIV/ AIDS in Yaoundé and Effect of HAART and CD4 Cells Counts. *J Bacteriol Parasitol*. 2014;06.
17. Occurrence of *Cryptosporidium* spp. and *Cystoisospora belli* among adult patients with diarrhoea in Maputo, Mozambique | Elsevier Enhanced Reader. <https://reader.elsevier.com/reader/sd/pii/S2405844018304249?token=D156197ACC9FE1A2114BE2927BF862D167A4FE6DDE01E1C165FBBDF1F97564E28FBBC78717B825F945BB6B05E51523AB&originRegion=eu-west-1&originCreation=20230128043124>. Accessed 28 Jan 2023.
18. Mbiandou SJ, Fosso S, Bille E, Matoh AB, Djeunga HN, Ekobo AS, et al. Prevalence of Intestinal Parasitic Infections in Relation to the HIV Status of Patients Attending the Care Units in Three Divisions in the Centre Region of Cameroon. *Int J Infect*. 2019;6.

19. Missaye A, Dagne M, Alemu A, Alemu A. Prevalence of intestinal parasites and associated risk factors among HIV/AIDS patients with pre-ART and on-ART attending dessie hospital ART clinic, Northeast Ethiopia. *AIDS Res Ther.* 2013;10:1–9.
20. Ford N, Migone C, Calmy A, Kerschberger B, Kanter S, Nsanzimana S, et al. Benefits and risks of rapid initiation of antiretroviral therapy. *AIDS Lond Engl.* 2018;32:17–23.
21. Adamu H, Wegayehu T, Petros B. High Prevalence of Diarrhoeogenic Intestinal Parasite Infections among Non-ART HIV Patients in Fitcha Hospital, Ethiopia. *PLoS ONE.* 2013;8:e72634.

UNDER PEER REVIEW