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### ► To cite this version:

Assi Fiacre-Tanguy N'docho, Tuo Karim, Landry N'Guessan Tiacoh, Fulgence Kassi Kondo, Clément Koffi Attoungbré, et al.. Prevalence of Intestinal Coccidiosis Among Patients Living with the Human Immunodeficiency Virus in Abidjan (Côte d'Ivoire). *Research Journal of Parasitology*, 2021, 16 (1), pp.9-17. 10.3923/jp.2021.9.17 . hal-03816375

**HAL Id: hal-03816375**

**<https://hal.science/hal-03816375>**

Submitted on 19 Oct 2022

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# Research Journal of **Parasitology**

ISSN 1816-4943



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## Research Article

# Prevalence of Intestinal Coccidiosis Among Patients Living with the Human Immunodeficiency Virus in Abidjan (Côte d'Ivoire)

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## Abstract

**Background and Objective:** Intestinal coccidia is one of the main causes of diarrheal infections among people living with HIV (PLHIV). The occurrence of diarrhoea in PLHIV affects their health status. The purpose of this study was to determine the prevalence of intestinal coccidia in patients with HIV/AIDS and to investigate a possible correlation between these parasites and the CD4 count in patients. **Materials and Methods:** This was a cross-sectional study carried out in three health care centres of PLHIV in Abidjan. Socio-demographic, clinical and biological data were collected using a questionnaire. Stool and blood samples were collected. Parasitic coprology analysis included the direct microscopic examinations, the concentration techniques (Ritchie and kato-katz) and Ziehl Neelsen. The last one allowed the detection of the oocysts of the coccidia. **Results:** A total of 363 faecal samples were collected from 03 health care centres. The stool samples collected consisted of 47.65% of diarrhoea. The results of the microscopic analysis revealed 03 intestinal coccidia's namely *Cryptosporidium* spp. (3.86%), *Isospora* spp. (1.65%) and *Cyclospora* spp. (0.83%). The highest microscopic prevalence was recorded in *Cryptosporidium* spp. (3.86%). Intestinal coccidia was more common in females infected with type 1 of HIV. CD4 count was a significant factor in the occurrence of *Cryptosporidium* spp. ( $\chi^2 = 29.968$ , p-value = 0.0001) with a correlation coefficient of -0.2438. **Conclusion:** This study assessed the microscopic prevalence rates of intestinal coccidia, which are responsible for diarrheal disease among PLHIV. The current study also showed that the presence of these intestinal coccidia's could affect the immune system of PLHIV when the CD4 cell count is below 200 cells mm<sup>-3</sup>.

**Key words:** Gastrointestinal, coccidia, diarrhoea, PLHIV, intestinal

**Citation:** Fiacre-Tanguy, N.D.A., T. Karim, N.G.T. Landry, K.K. Fulgence and K.A. Clément *et al.*, 2021. Prevalence of intestinal coccidiosis among patients living with the human immunodeficiency virus in Abidjan (Côte d'Ivoire). Res. J. Parasitol., 16: 9-17.

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**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Diarrhoeal infections are the second main cause of death in children less than 5 years with 525,000 deaths each year<sup>1</sup>. They also affect more than 50% of people living with HIV/AIDS (PLHIV)<sup>2,3</sup>. These pathologies remain one of the leading causes of morbidity and mortality in the world and particularly in sub-Saharan Africa<sup>4</sup>. The vital prognosis of PLHIV depends on the infection level of some intestinal parasites<sup>5</sup>. One of the major intestinal parasites causing these infections belong to the group of coccidia, i.e. *Cryptosporidium parvum*, *Isospora belli* and *Cyclospora* spp.<sup>6</sup>. The main clinical signs caused by these intestinal coccidia's in PLHIV go from asymptomatic infection to severe infection characterized by chronic diarrhoea, dehydration and malabsorption<sup>7,8</sup>. In the advanced stages of the disease, a decrease in CD4+ cell counts below 200 cells mm<sup>-3</sup> observed<sup>9</sup> and the development of opportunistic infections, tumours and neurological complications are observed<sup>10</sup>. The administration of antiretroviral drugs by PLHIV allow to remarkably reduce the mortality and morbidity associated with these opportunistic infections, by the partial restoration of the immune system<sup>11</sup>.

In sub-Saharan Africa, the prevalence of these opportunistic infections, particularly the intestinal parasitosis among people living with HIV is high<sup>12</sup>, with an incidence of parasitic infections as high as 95%<sup>8,13</sup>. Among these parasitic infections, gastroenteric infections are responsible for high mortality among PLHIV<sup>5,14-16</sup>. Thus, diarrhoea has become one of the main causes of parasitological consultations among PLHIV<sup>17</sup>.

In Côte d'Ivoire, according to UN-AIDS estimations<sup>3</sup>, more than 430,000 people living with HIV and the prevalence of new cases was 0.7%, with a national prevalence of 2.4% among adults in 2019<sup>3</sup>. In this country, Abidjan is the epicentre of the disease with a prevalence of 3.4%<sup>18</sup>. In 2008, this big city displayed an incidence of 37.4% concerning diarrhoea among PLHIV<sup>19</sup>. Parasitological studies showed the involvement of intestinal coccidia in the occurrence of diarrhoea in PLHIV<sup>20,21</sup>. The observed prevalences were generally high for PLHIV with CD4 counted below 200 cells mm<sup>-3</sup><sup>21</sup>. These infected persons were vulnerable to diarrhoeal infections, which are the cause of high mortality. This study aimed to determine the prevalence of intestinal coccidia in patients with HIV/AIDS and to investigate a possible correlation between these parasites and the CD4 count in patients received in three health care centres of the Abidjan district (Côte d'Ivoire).

## MATERIALS AND METHODS

**Study framework:** The study was conducted between November, 2018 and March, 2020 in three health care centres in the district of Abidjan, namely the FSUCOM (Voluntary Screening Center, Community-based Urban Health Training) of Anonkoua-Koute, the CePReF (Voluntary Screening Center, Research and Training Care Center) of Yopougon Attie and the PPH (Voluntary Screening Center, Pneumo-physiology) of the University Hospital Center of Cocody. This was a cross-sectional, prospective, descriptive study that involved adult patients in each of the three study sites who gave their informed consent. These three study sites were chosen to be representative of the Abidjan district.

The microscopic analyses of this study were performed in the Parasitology Unit of the Pasteur Institute of Côte d'Ivoire. Immunological analyses were performed in the health care centres of our study.

**Recruitment of the patients included in the study:** Before participating in the study, the objectives and procedures of the study were explained in simpler terms accessible to patients. Consequently, each of the 363 patients enrolled in this project gave his written informed consent in French. Socio-demographic (age, sex, residence and occupation), clinical (medical history, HIV status, weight loss, vomiting, abdominal pain and fever) and clinical data were collected. Some biological data (HIV/AIDS status, CD4 mm<sup>-3</sup> count) were also collected from all patients included in the study using the questionnaire by the attending physician. Additional information's were collected with patients presenting diarrhoea cases. The physical examination was carried out by the attending physician in each health care centre. During the registration, each patient was informed on the parasitological analyses of stool and the evaluation of the CD4+ T-cell count that shall be performed on each blood sample in the purple tube. All volunteers received a sterile jar of 125 mL for the collection of their stool samples.

**Criteria for inclusion:** Participants of the study included HIV-infected adults 18 years of age and older, with or without diarrhoea, who were monitored in a health care centre in Abidjan and with their approval is written informed consent.

**Exclusion criteria:** Persons excluded from the study were those under 18 years of age, not followed in a treatment centre and those who refused to give their written informed consent.

**Collection of samples:** Patients' stools collected at each site were sent to the Parasitology Unit of the Pasteur Institute of Côte d'Ivoire (IPCI) and analyzed within two hours to identify the vegetative forms of intestinal parasites. The collected stool samples were separated into three samples: The first sample was preserved using potassium dichromate (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>), the second preserved in Sodium Chloride (NaCl 9%), while the third unpreserved sample was used for the various microscopic examinations.

**Blood sampling:** After receiving the informed consent, 4 mL of venous blood was collected from each patient in an EDTA tube (purple tube). This tube was used for the CD4 T-cell count.

**Microscopic examinations:** At the laboratory of parasitology unit of IPCI, stool samples were firstly macroscopically examined by grinding them with a clean stick to note their consistency (appearance), the presence of blood or mucus and also the possible presence of rings of *Taenia*, adults of *Ascaris* or pinworms. Then a microscopic examination of the fresh sample was carried out using physiological water. Finally, staining by the method of Zielh Neelsen was carried out to highlight parasite oocysts under an optical microscope with the x40 objective and then at x100 magnification. All the samples of the study were tested by the standard technique of Kato Katz for the detection of some helminths<sup>22</sup>.

Once the macroscopic examination is completed, using a fine clean rod, a small amount of stool is taken from the stool sample in surface and depth at different places (3-4 places depending on the appearance of the stool). This sample was diluted in a drop of physiological water (9%) deposited on a slide and covered with a cover slide. This preparation is observed under an optical microscope with the 10 and 40x objective respectively (this preparation should not be thick and should be read completely). To better identify certain parasites, a drop of Lugol was finally deposited on the edges of the lamella covering the preparation and the observation was done with the 40x objective.

**Method of ritchie modified (PBS-ether concentration):** After the direct examination of fresh stool, the method of Ritchie modified was used to concentrate the parasitic elements unnoticed in the direct examination. To do this, approximately 2 g of fresh stool was mixed with a solution of 10 mL of PBS in a clean container using a clean wand. The mixture

was filtered through a small, fine, clean strainer. The 8 mL of the filtrate is collected in a 15 mL centrifuge tube (Falcon® tube) to which 4 mL of ether is added using the 15 mL Falcon® tube scale. The Falcon® 15 mL tube is stoppered and agitated to obtain a homogeneous emulsion. This tube is centrifuged at 2000 rpm for 3 min to break the emulsion. At the end of this step, the tube presents different phases from top to bottom: An ether phase (fat), a layer of lipophilic residues, an aqueous phase (PBS solution) and the pellet. The tube is then abruptly emptied<sup>23</sup>. Using a Pasteur pipette, two to four drops of the centrifugation pellet are spread between slide and slide and examined under the microscope for cysts, eggs and parasites. A portion of the obtained pellets is then used for modified Zielh Neelsen staining.

**Microscopic detection by modified zielh-neelsen staining:**

Pellets obtained by the modified Ritchie method were put on the slide to perform thin smears. The smear slides are then air-dried and fixed with methanol for 5 min and then dipped in Zielh's Phoenix Fuchsin for 1 hr. After rinsing with water, a discolouration with sulfuric acid in a 2% solution is performed for 20 sec. This discolouration is followed by a new rinse with water, then the slides are immersed in a 5% malachite green solution for 5 min for counter-staining. After rinsing with water and drying at room temperature, the slides are examined under an optical microscope at x40 objective and then at x100 magnification with immersion oil. This simple staining technique has been used to highlight oocysts (a form of resistance) in coccidia that is sometimes difficult to detect by direct observation.

**Data mining and analysis:** Data were entered using the Epi Info software version 7.2.6. Fisher's exact test was used to test the relationship between prevalence values and a given variable.

Logistic regression was used to determine the factors associated with coccidiosis using R software (Ri3864.0.0.0 versions). For this purpose, the association between coccidiosis infections was defined as the independent variable.

Dependent variables were analyzed using univariate analysis and the strength of each association was measured with an Odds Ratio (OR). All variables with a minimum criterion of the p-value ( $p \leq 0.2$ ) were specified and included in the multivariate analysis at a 95% Confidence Interval (CI).  $p \leq 0.05$  was considered statistically significant.

## RESULTS

**Characteristic of the sample:** Parasitological survey was conducted on 363 patients infected with HIV of whom 249 were female (68.60%) and 114 male (31.40%), a sex ratio of 2.18 in favour of the female sex. The age group above 50 years was the majority age group in Table 1. Among the 363 patients investigated, 89.22% were infected with type 1 and 92.83% were under ARV treatment.

**Clinical data:** The clinical data of the patients are shown in Fig. 1. Diarrhoea (47.65%), asthenia (38.30%), anorexia (30.57%), abdominal pain (31.12%) and intermittent diarrhoea (15.15%) were the main clinical signs observed in the surveyed population.

**Prevalence's of intestinal coccidia according to sex and age:** The highest prevalence was observed in *Cryptosporidium* spp. (3.86%) in Table 2. The prevalence of *Cyclospora* spp. (0.83%) was the lowest preceded by that of *Isospora* spp. (1.65%). No *Sarcocystis* spp. was observed. Females constituted the most infested with intestinal coccidia. However, statistical analysis showed no significant relationship between sex and the prevalence of intestinal coccidia (*Cryptosporidium* spp. ( $\chi^2 = 0.125$ , p-value = 0.723), *Cyclospora* spp. ( $\chi^2 = 1.3849$ , p-value = 0.2393) et *Isospora* spp. ( $\chi^2 = 0.61517$ , p-value = 0.4328).

**Prevalence of intestinal coccidia according to the CD4+ dosage and the different treatments:** Most of the patients (86.22%) infected with the type 1 of HIV were parasitized with *Cryptosporidium* spp. (3.58%), *Isospora* spp. (1.37%) and *Cyclospora* spp. (0.55%) in Table 3. Nevertheless, no significant difference was observed between the prevalence of identified coccidia and the type of HIV

(*Cryptosporidium* spp. ( $\chi^2 = 0.641$ , p-value = 0.725), *Cyclospora* spp. ( $\chi^2 = 1.439$ , p-value = 0.487) and *Isospora* spp. ( $\chi^2 = 0.277$ , p-value = 0.870)). These three intestinal coccidia were the most observed in individuals with CD4 counts below 200 with a significant difference in *Cryptosporidium* spp. ( $\chi^2 = 29.968$ , p-value = 0.0001) with a correlation coefficient equal to -0.2438. A statistically significant association was observed between diarrhea and the prevalence of *Cryptosporidium* spp. ( $\chi^2 = 34.20$ , p-value = 0.006).

**Prevalences of other identified parasites:** Other parasites were identified in Table 4, grouped in intestinal protozoa such as *Entamoeba coli* (9.09%), *Endolimax nana* (8.26%) and *Giardia intestinalis* (2.75%) and intestinal nematodes, *Ascaris lumbricoides* (1.10%) and *Trichuris trichiura* (0.55%). A case of *Dicrocoelium dendriticum* (0.28%) which is an intestinal trematode was also identified.

Table 1: Characteristics of the study population

Characteristics	n	%
<b>Sex</b>		
Male	114	31.40
Female	249	68.60
<b>Age range</b>		
0-20	3	0.82
21-30	32	8.81
31-40	94	25.90
41-50	114	31.40
>50	119	32.78
Not defined	1	0.27
<b>Type of HIV</b>		
HIV 1	313	86.22
HIV 2	42	11.57
HIV 1 and 2	8	2.20
<b>ARV treatment</b>		
Yes	337	92.83
No	21	5.78
Not defined	5	1.37
Total	363	100.00

Table 2: Prevalences of intestinal coccidia by sex and age

Variables	<i>Cryptosporidium</i> n (%)	<i>Cyclospora</i> n (%)	<i>Isospora</i> n (%)
Positive	14 (3.86)	3 (0.83)	6 (1.65)
Negative	349 (96.14)	360 (99.17)	357 (98.35)
<b>Sex</b>			
Male	5 (1.38)	0 (0)	1 (0.28)
Female	9 (2.48)	3 (0.83)	5 (1.38)
p-value	0.77	0.55	0.66
<b>Age (years)</b>			
0-20	0 (0)	0 (0)	0 (0)
21-30	1 (0.28)	0 (0)	2 (0.55)
31-40	9 (2.48)	1 (0.28)	1 (0.28)
41-50	4 (1.10)	1 (0.28)	2 (0.55)
>50	0 (0)	1 (0.28)	1 (0.28)
NP	0 (0)	0 (0)	0 (0)
p-value	0.009	0.25	0.28
Total	14/363	3/363	6/363

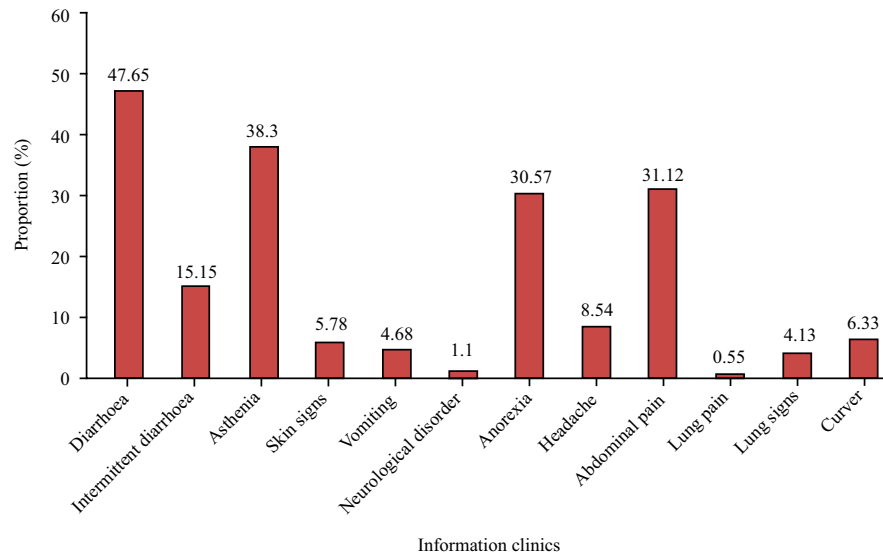


Fig. 1: Distribution of clinical information in the surveyed population

Table 3: Prevalences of intestinal coccidia as a function of CD4 count, diarrheal diseases and different treatments

Variables	<i>Cryptosporidium</i> n (%)	<i>Cyclospora</i> n (%)	<i>Isospora</i> n (%)
<b>Serology (type of HIV)</b>			
HIV 1	13 (3.58)	2 (0.55)	5 (1.37)
HIV 2	1 (0.28)	1 (0.28)	1 (0.28)
HIV 1 and 2	0 (0)	0 (0)	0 (0)
p-value	0.72	0.35	0.59
<b>CD4 count</b>			
<200	12 (3.30)	2 (0.55)	4 (1.10)
200-500	1 (0.28)	0 (0)	1 (0.28)
≥500	1 (0.28)	1 (0.28)	1 (0.28)
p-value	0.0001	0.18	0.07
<b>ARV treatment</b>			
Yes	10 (2.75)	3 (0.82)	4 (1.10)
No	4 (1.10)	0 (0)	2 (0.55)
No defined	0(0)	0 (0)	0 (0)
p-value	0.008	0.88	0.06
<b>Presence of diarrhoea</b>			
Yes	14	3	5 (1.37)
No	0 (0)	0 (0)	1 (0.28)
p-value	0.006	0.25	0.22
<b>Diarrhoeal treatment</b>			
Yes	10 (2.75)	2 (0.55)	4 (1.10)
No	4 (1.10)	1 (0.28)	1 (0.28)
No defined	0(0)	0 (0)	1 (0.28)
p-value	0.001	0.02	0.01
Total	14/363	3/363	6/363

Table 4: Frequencies of some parasites observed in PLHIV

Parasitic group	Parasitic species	n (%)
Intestinal nematode	<i>Ascaris lumbricoides</i>	4 (1.10)
	<i>Trichuris trichiura</i>	2 (0.55)
	<i>Ancylostoma duodenale</i>	1 (0.28)
	<i>Strongyloides stercoralis</i>	1 (0.28)
	<i>Dicrocoelium dendriticum</i>	1 (0.28)
Intestinal trematode	<i>Entamoeba coli</i>	33 (09.09)
Intestinal protozoa	<i>Endolimax nana</i>	30 (08.26)
	<i>Giardia intestinalis</i>	10 (2.75)

**Factors associated with intestinal coccidia:** Factors involved (Sex, Occupation, Type of HIV, ARV treatment, Health care centres, Previous diarrheal and CD4 count) that could be risk factors for *Cryptosporidium* spp., *Cyclospora* spp. and *Isospora* spp. infections are presented in Table 5.

Statistical analysis showed that sex was not a risk factor for *Cryptosporidium* spp. (OR = NA, p = NA), *Cyclospora* spp.

Table 5: Factors associated with intestinal coccidia

Explanatory variable	<i>Cryptosporidium</i> spp. n (%)						<i>Cyclospora</i> spp. n(%)						<i>Isospora</i> spp. n (%)					
	n (%)	OR	P	Univariate analyses	Multivariate analyses	P	n (%)	OR	P	Univariate analyses	Multivariate analyses	P	n (%)	OR	P	Univariate analyses	Multivariate analyses	P
<b>Sex</b>																		
Male	5 (1.38)	1.22	0.724	NA	NA	NA	0 (0)	-	0.554	NA	NA	NA	1 (0.28)		0.446	NA	NA	NA
Female	9 (2.48)						3 (0.83)						5 (1.38)					
<b>Occupation</b>																		
Public servant	3 (7.31)	-	0.99	NA	NA	NA	0 (0)	-	1	NA	NA	NA	1 (2.43)	0.1	0.094*	2.25	0.53	
No public servant	11 (3.52)						3 (0.96)						3 (0.96)					
Learner	0 (0)						0 (0)						2 (20)					
<b>Type of HIV</b>																		
HIV 1	13 (3.58)	0.460	0.439	NA	NA	NA	2 (0.55)	1.95	0.488	NA	NA	NA	5 (1.37)	1.03	0.968	NA	NA	NA
HIV 2	1 (0.28)						1 (0.28)						1 (0.28)					
HIV 1 et 2	0 (0)						0 (0)						0 (0)					
<b>ARV treatment</b>																		
Yes	10 (2.75)	-	0.55	NA	NA	NA	3 (0.82)	NA	NA	NA	NA	NA	4 (1.10)	-	1	NA	NA	NA
No	4 (1.10)						0 (0)						2 (0.55)					
<b>Health care centres</b>																		
CPC AK	4 (2.80)	0.651	0.709	NA	NA	NA	2 (1.40)	-	0.217	NA	NA	NA	3 (2.09)	0.5797	0.6675	NA	NA	NA
CPC CP	3 (1.84)						0 (0)						2 (1.22)					
CPC PPH	7 (12.28)						1 (1.75)						1 (1.75)					
<b>Previous diarrheal</b>																		
Yes	11 (3.52)	1.871	0.4	NA	NA	NA	1 (0.41)	0.246	0.3	NA	NA	NA	6 (2.54)	-	0.18*	2.18	0.51	
No	3 (7.31)						2 (1.68)						0 (0)					
<b>CD4 count</b>																		
<200	12 (3.30)	0.04	0.0001*	0.887	0.001**	0.001**	2 (0.55)	0.279	0.3	NA	NA	NA	4 (1.10)	0.136	0.059*	0.964	0.072	
200-500	1 (0.28)						0 (0)						1 (0.28)					
≥500	1 (0.28)						1 (0.28)						1 (0.28)					



(OR = NA, p = NA) and *Isospora* spp. (OR = NA, p = NA) infections.

The same finding was observed for factors such as occupation, type of HIV, ARV treatment, health care centre and previous diarrheal for *Cryptosporidium* spp., *Cyclospora* spp. and *Isospora* spp. infections (Table 5).

CD4 count was not associated with *Cyclospora* spp. (OR = NA, p = NA) and *Isospora* spp. (OR = 0.964, p = 0.072) infections but it was associated with *Cryptosporidium* spp. infection (OR = 0.887, p = 0.001).

## DISCUSSION

In the current study, *Cryptosporidium* spp. was the most frequent parasite identified among the intestinal coccidia with a prevalence of 3.86%. This prevalence was lower compared to Kassi and Collaborators observations who reported a prevalence of 11.8% in *Cryptosporidium* spp. among persons living with HIV at the Treichville University Hospital Center which was 11.8%. The difference could be due to the duration time (05 years) of the study conducted by Kassi *et al.*<sup>22</sup> compared to the current study. The high prevalence observed by these authors could also be due to the choice of the place for stool samples collection i.e. infectious and tropical diseases department which generally receive patients suffering from chronic diseases. This percentage of *Cryptosporidium* spp. (3.86%) was also lower than the results of studies conducted among PLHIV hospitalized in Kinshasa in the Democratic Republic of Congo (DRC) in 2010 (12.6%).

No coccidia of the genus *Sarcocystis* was collected in the present study. This could be explained by the fact that parasite is not common in Sub-Saharan Africa. Indeed, it is an intracellular, cosmopolitan protozoan, common in Southeast Asia<sup>24</sup>.

In current results, the prevalences of the three intestinal coccidia are highest in female patients (*Cryptosporidium* spp. (2.48%), *Isospora* spp. (1.38%) and *Cyclospora* spp. (0.83%)) compared to males (*Cryptosporidium* spp. (1.38%), *Isospora* spp. (0.28%) and *Cyclospora* spp. (0%)). But these prevalences do not differ significantly by sex ( $p > 0.05$ ). These results are contrary to those of Kassi *et al.*<sup>21</sup> in a 2017 study in Abidjan where they showed that male PLHIV were the most infested with *Cryptosporidium* spp. Results from previous studies conducted in DRC among PLHIV also showed no significant relationship between sex ( $p\text{-value} > 0.05$ ) and coccidial prevalence.

The study revealed that individuals with CD4 counts below 200 cells  $\text{mm}^{-3}$  were the most parasitized to *Cryptosporidium* spp. (3.30%), *Isospora* spp. (1.10%) and

*Cyclospora* spp. (0.55%). This presence of intestinal coccidia could be explained on the one hand by the decrease of the patient's immunity and on the other hand by the absence of antiparasitic treatment against diarrheal diseases. This hypothesis is supported by Campo *et al.*<sup>25</sup> who showed that immune reconstitution with antiretroviral combination therapy is the most effective way to prevent or control opportunistic diseases such as cryptosporidiosis in PLHIV.

A prevalence of 3.30% *Cryptosporidium* spp. was obtained in patients with CD4 counts below 200 cells  $\text{mm}^{-3}$ . This corresponds to a proportion of 85.50% of patients infected with *Cryptosporidium* spp. The prevalence of patients infested with *Cryptosporidium* spp. with CD4 counts below 200 cells  $\text{mm}^{-3}$  (85.5%) shows a significant difference ( $p\text{-value} = 0.0001$ ) with the degree of immunosuppression as measured by CD4 cell counts. These results were similar to the studies by Agholi *et al.*<sup>26</sup> in PLHIV which also showed a statistically significant difference between *Cryptosporidium* spp. and CD4 cell count  $< 200$  cells  $\text{mm}^{-3}$ .

The study showed a correlation between CD4 count and the presence of *Cryptosporidium* spp. ( $\chi^2 = 29.968$ ,  $p\text{-value} = 0.0001$ ,  $r = -0.2438$ ). These results are similar to those of Bissong *et al.*<sup>27</sup> who showed a correlation between opportunistic parasites and CD4 count, specifically for *Cryptosporidium parvum* in people living with HIV/AIDS at the Bamenda Regional Hospital in Cameroon.

Current results also showed prevalences of other intestinal parasites in PLHIV with respectively 9.09% for *Entamoeba coli* and 8.26% for *Endolimax nana*. These prevalences were lower than those obtained in a retrospective study in health facilities in Côte d'Ivoire covering five years (2011-2015) among PLHIV, which were 1.5% for *Entamoeba coli* and 0.4% for *Endolimax nana*<sup>22</sup>.

The current study showed the presence of a case of intestinal trematode with the species *Dicrocoelium dendriticum* with a percentage of 0.28%. It should be noted that this is a parasite whose presence is unusual in stool samples. Its presence could be explained by the ingestion of infested ants by the PLHIV.

The identification of intestinal helminths such as *Ascaris lumbricoides*, four times *Trichuris trichiura* two times and *Ancylostoma duodenale* and *Strongyloides stercoralis* one-time inpatient could be evidence of inadequate hygiene practice of PLHIV. It should be noted that these intestinal helminths are faecal-oral transmissible<sup>28</sup>.

The present study showed that CD4 count was a significant factor (OR = 0.887,  $p \leq 0.05$ ) in the occurrence of *Cryptosporidium* spp. This result is similar to that of Wondmienieh *et al.*<sup>29</sup> in their analysis of a series of

independent studies (meta-analysis) on opportunistic pathologies in PHAs, in particular Cryptosporidiosis, where they showed that CD4 T-cell count was a factor associated with the occurrence of *Cryptosporidium* spp. Contrary to current results, Wondmienieh *et al.*<sup>29</sup> also showed that a history of diarrhoea was significantly associated with intestinal parasitic infections.

## CONCLUSION

Intestinal coccidia is not negligible opportunistic parasites in the causes of diarrhoea among PLWHA in the management centres in Abidjan (Côte d'Ivoire). This survey revealed that among the intestinal coccidiosis studied, *Cryptosporidium* spp. (3.86%) was the most frequent. CD4 T-cell count was the factor associated with *Cryptosporidium* infection (OR = 0.887,  $p \leq 0.05$ ). It is imperative that in the presence of diarrheal disease, a systematic diagnosis of these intestinal parasitoses by appropriate techniques be carried out in particular in PLHIV for better management.

## SIGNIFICANCE STATEMENTS

This study showed the important role of protozoa in the development of diarrheal diseases in people living with HIV and a correlation between the decrease of CD4 count (below 200) and the presence of protozoa such as *Cryptosporidium* spp. which may be beneficial for the control of diarrheal infections in people living with HIV (PLHIV). This study will help researchers discover critical areas in the diagnosis of diarrheal disease in PLHIV. Thus, a new theory on the control of diarrheal infections in PLHIV could be developed.

## ACKNOWLEDGMENT

We would like to thank all the partner structures that have allowed the realization of this study and also the technicians of the Pasteur Institute of Côte d'Ivoire.

## REFERENCES

1. Djafar, M.A., S. Alido, K. Moumouni, M. Garba, S. Aboubacar and M.R.A. Ahidan, 2018. Knowledge and attitudes of hospital physicians in Niamey on the prevention and management of acute diarrhea in children under five. *J. Pediatr. Childcare*, 31: 235-240.
2. Zemene, T. and M.B. Shiferaw, 2018. Prevalence of intestinal parasitic infections in children under the age of 5 years attending the debre birhan referral hospital, North Shoa, Ethiopia. *BMC Res. Notes*, Vol. 11. 10.1186/s13104-018-3166-3.
3. Seytre, B., 2019. Côte d'Ivoire: Textbooks misinform on HIV. *Bull. Soc. Pathol. Exot.*, 112: 90-95.
4. Koné, B., M. Doumbia, I. Sy, K. Dongo and Y. Agbo-Houenou *et al.*, 2014. Study of diarrhea in peri-urban areas in Abidjan using the ecohealth approach. *VertigO-Environ. Sci.*, Vol. 19. 10.4000/vertigo.14976.
5. Tonen-Wolyec, S., S. Batina-Agasa, J. Muwonga, F.F. N'kulu, R.S.M. Bouassa and L. Bélec, 2018. Evaluation of the practicability and virological performance of finger-stick whole-blood HIV self-testing in french-speaking Sub-Saharan Africa. *PLoS ONE*, Vol. 13. 10.1371/journal.pone.0189475.
6. Nkenfou, C.N., C.T. Nana and V.K. Payne, 2013. Intestinal parasitic infections in HIV infected and non-infected patients in a low HIV prevalence region, West-Cameroon. *PLoS One*, Vol. 8, No. 2. 10.1371/journal.pone.0057914.
7. Stark, D., J.L.N. Barratt, S. van Hal, D. Marriott, J. Harkness and J.T. Ellis, 2009. Clinical significance of enteric protozoa in the immunosuppressed human population. *Clin. Microbial. Rev.*, 22: 634-650.
8. Babatunde, S.K., A.K. Salami, J.P. Fabiyi, O.O. Agbede and O.O. Desalu, 2010. Prevalence of intestinal parasitic infestation in HIV seropositive and seronegative patients in ilorin, Nigeria. *Ann. Afr. Med.*, 9: 123-128.
9. Chas, J., A. Hema, L. Slama, N.F. Kabore and F.X. Lescure *et al.*, 2015. The day-hospital of the university hospital, Bobo dioulasso: An example of optimized HIV management in Southern Burkina Faso. *PLoS ONE*, Vol. 10. 10.1371/journal.pone.0125588.
10. Zhang, C., Q. Ren and W. Chang, 2020. Epidemiological features and risk factors for acquiring hepatitis B, hepatitis C and syphilis in HIV-infected patients in Shaanxi province, Northwest China. *Int. J. Environ. Res. Public Health*, Vol. 17. 10.3390/ijerph17061990.
11. Anazi, A.R.A., 2009. Gastrointestinal opportunistic infections in human immunodeficiency virus disease. *Saudi J. Gastroenterol.*, 15: 95-99.
12. Tanon, A., A. Jaquet, D.K. Ekouevi, J. Akakpo and I. Adoubi *et al.*, 2012. The spectrum of cancers in west Africa: Associations with human immunodeficiency virus. *PLoS ONE*, Vol. 7. 10.1371/journal.pone.0048108.
13. Adamu, H. and B. Petros, 2010. Intestinal protozoan infections among HIV positive persons with and without antiretroviral treatment (ART) in selected ART centers in Adama, Afar and Dire-Dawa, Ethiopia. *Ethiopian J. Health Dev.*, Vol. 23. 10.4314/ejhd.v23i2.53230.

14. Khalil, S., B.R. Mirdha, S. Sinha, A. Panda, Y. Singh, A. Joseph and M. Deb, 2015. Intestinal parasitosis in relation to anti-retroviral therapy, CD4<sup>+</sup> T-cell count and diarrhea in HIV patients. *Korean J. Parasitol.*, 53: 705-712.
15. Idris, N.S., P.G. Dwipoerwantoro, A. Kurniawan and M. Said, 2010. Intestinal parasitic infection of immunocompromised children with diarrhoea: Clinical profile and therapeutic response. *J. Infect. Dev. Ctries.*, 4: 309-317.
16. Abdel-Hafeez, E.H., A.K. Ahmad, B.A. Ali and F.A. Moslam, 2012. Opportunistic parasites among immunosuppressed children in Minia district, Egypt. *Korean J. Parasitol.*, 50: 57-62.
17. Bhajjee, F., C. Subramony, S.J. Tang and D.J. Pepper, 2011. Human immunodeficiency virus-associated gastrointestinal disease: Common endoscopic biopsy diagnoses. *Pathol. Res. Int.*, Vol. 26. 10.4061/2011/247923.
18. Inghels, M., 2020. Practices and factors associated with recent HIV testing in the general population, Côte d'Ivoire. Results of the ANRS 12323 DOD-CI study. *Bull. Soc. Exot. Pathol.*, 113: 268-277.
19. Inghels, M., A.K. Kouassi, S. Niangoran, A. Bekelynyck and S. Carillon *et al.*, 2020. Cascade of provider-initiated human immunodeficiency virus testing and counselling at specific life events (pregnancy, sexually transmitted infections, marriage) in Côte d'Ivoire. *Sexually Transmitted Dis.*, 47: 54-61.
20. Wang, Z.D., Q. Liu, H.H. Liu, S. Li, L. Zhang, Y.K. Zhao and X.Q. Zhu, 2018. Prevalence of *Cryptosporidium*, microsporidia and *Isospora* infection in HIV-infected people: A global systematic review and meta-analysis. *Parasites Vectors*, Vol. 11. 10.1186/s13071-017-2558-x.
21. Kassi, K.F., P.C.M. Kiki-Barro, A. Konaté, K.E. Angora and A. Vanga-Bosson *et al.*, 2017. Hospital prevalence of cryptosporidiosis and intestinal microsporidiosis in people living with HIV from 2011 to 2015 at treichville teaching hospital of, Abidjan, Côte d'Ivoire. *Revintscméd-RISM*, 19: 363-368.
22. Zendejas-Heredia, P.A., V. Colella, S.F. Hii and R.J. Traub, 2021. Comparison of the egg recovery rates and limit of detection for soil-transmitted helminths using the Kato-Katz thick smear, faecal flotation and quantitative real-time PCR in human stool. *PLOS Neglected Trop. Dis.*, Vol. 15. 10.1371/journal.pntd.0009395.
23. Sow, D., Y. Dieng, D. Haouchine, K. Niang and T. Niang *et al.*, 2017. Comparison of para-selles bailenger/Kop-color fumouze, para-selles-iodésine/Kop-color II fumouze diagnostic kits with conventional microscopic methods in identifying intestinal parasitic diseases in Senegal. *J. Parasitic Dis.*, 41: 814-822.
24. Wumba, R., B. Longo-Mbenza, M. Mandina, T.O. Wobin and S. Biligui *et al.*, 2010. Intestinal parasites infections in hospitalized AIDS patients in Kinshasa, democratic republic of Congo. *Parasite*, 17: 321-328.
25. Campo, J., J.D. Romero, J. Castilla, S. García, C. Rodríguez and A. Bascones, 2002. Oral candidiasis as a clinical marker related to viral load, CD4 lymphocyte count and CD4 lymphocyte percentage in HIV-infected patients. *J. Oral Pathol. Med.*, 31: 5-10.
26. Agholi, M., G.R. Hatam and M.H. Motazedian, 2013. HIV/AIDS-associated opportunistic protozoal diarrhea. *AIDS Res. Hum. Retroviruses*, 29: 35-41.
27. Bissong, M.E.A., N.F. Nguemain, T.E.N. Ng'awono and F.H.L. Kamga, 2015. Burden of intestinal parasites amongst HIV/AIDS patients attending bamenda regional hospital in Cameroon. *Afr. J. Clin. Exp. Microbiol.*, 16: 97-103.
28. Strunz, E.C., D.G. Addiss, M.E. Stocks, S. Ogden, J. Utzinger and M.C. Freeman, 2014. Water, sanitation, hygiene and soil-transmitted Helminth infection: A systematic review and meta-analysis. *PLOS Med.*, Vol. 11. 10.1371/journal.pmed.1001620.
29. Wondmieni, A., G. Gedefaw, B. Alemnew, A. Getie, M. Bimerew and A. Demis, 2020. Intestinal parasitic infections and associated factors among people living with HIV/AIDS in Ethiopia: A systematic review and meta-analysis. *PLOS ONE*, Vol. 15. 10.1186/s41043-017-0092-2.