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Prevalence of Cryptosporidium Infection among HIV-1 Infected Adult Patients Attending Jos University Teaching Hospital, Jos, Nigeria

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Abstract
Cryptosporidium is a leading cause of chronic diarrhoea in HIV-1infected patients, but there is paucity of data on the prevalence of *Cryptosporidium* and factors associated with the infection. We determined the prevalence and factors associated with *Cryptosporidium* infection among HIV-1 infected adult patients at Jos University Teaching hospital, Jos, Nigeria. A cross-sectional study in which a total of 296 fecal specimens from HIV-1 infected patients with diarrhea was collected and structured questionnaire was used to collect demographic and clinical data. The fecal samples were examined for *Cryptosporidium* by formol-ether concentration and modified Ziehl-Neelsen staining technique, and the oocysts identified by microscopy. Two hundered and ninrty six feacal samples where analyse from156 (52.7%) females and 140(47.3%) males. The prevalence of *Cryptosporidium* infection among HIV-1 infected patients was 4.7%. Among females 9 (5.8%) had *Cryptosporidium* infection while (5)3.6% in males; P=0.696. The age group 21-30 years had the highest prevalence of (8)10.0%. A statistically significant association of *Cryptosporidium* infection was observed with rural type of residence, P=0.046. In conclusion the prevalence of *Cryptosporidium* infection among HIV-1 infected adult patients with diarrhea was low. This may be due to early commencement of antiretroviral drugs and the use of anti-parasitic prophylaxis among the patients. Further larger studies on the molecular identification of the species of *Cryptosporidium* are needed to determine the available zoonotic strains.
Keywords: Adults, Cryptosporidium, Diarrhea, HIV-1 infection, Jos, Prevalence.

INTRODUCTION

Cryptosporidium is an intracellular, oocyst-forming parasite which infects the gastrointestinal tract in human and animals (Ryan *et al.*, 2014). Cryptosporidiosis is common among children and causes a self-limiting diarrhoea, but could be life threatening in immune-compromised individuals (Jafari *et al.*, 2012; Shirley *et al.*, 2012). Cryptosporidium infection in humans is acquired through ingestion of food and water contaminated with animal and human faeces or from person to person (Xiao, 2010). The presence of single oocyst can cause infection and perhaps disease. The oocysts are stable and resistant to common water disinfectants likechlorine (Bouzid *et al.*, 2013). Infections in immunocompetent individuals are usually asymptomatic, but causes diarrhea in children under the age of five years and in immunosuppressed people. Symptoms such as vomiting, nausea, abdominal discomfort and low-grade fever may also be associated with Cryptosporidium infection (Adamu *et al.*, 2014). However, infection in immunocompromised individuals can be severe and fatal (Masarat *et al.*, 2012). The frequent use of antiretroviral therapy (ART) has reduced the prevalence of opportunistic infections associated with HIV infection, except in those with low CD4+ counts who are not on ART or who have drug resistant HIV strains (Maggi *et al.*, 2000; WHO, 2008).

Cryptosporidium has been implicated in prolonged and persistent diarrhea in HIV-1 infected patients with AIDS. The prevalence of Cryptosporidium in developed countries ranges from 3-4% in the general population (Lake *et al.*, 2007). Several studies have reported high prevalence of cryptosporidiosis in children and immune-compromised individuals in developing countries (Houpt *et al.*, 2005; Mor and Tzipori, 2008).

In spite of the advances made in the detection and significant demonstration of the parasite as a cause of enteric morbidity, the tests for cryptosporidium are not performed routinely in most microbiology laboratories (Wangeci *et al., 2006)*. In developing countries *Cryptosporidium* is responsible for 18-19% of cases of diarrheal disease among HIV-1 infected patients (Fayer *et al*., 2006; Tzipori and Ward, 2002), with significant morbidity and mortality (Opaluwa *et al*., 2005). The expensive nature of the rapid tests and the requirement for expert microscopy, has resulted in the paucity of published reports from developing countries. Therefore, we aimed to investigate the prevalence and factors associated with Cryptosporidium infection among HIV infected patients with diarrhoea attending a tertiary hospital in Jos, Nigeria.

METHODOLOGY

Study Area. The HIV clinic of Jos University Teaching Hospital (JUTH) provides comprehensive HIV care services for the city of Jos, which is located in Jos North Local Government Area (LGA) of Plateau State and also serves as a referral Centre for health facilities in other LGAs of the state and neighboring states in the country. A 2006 census, estimated the population of Plateau State at 3,206,531, with the capital Jos city having a population of approximately 900,000 (NPC report, 2006). Animal keeping is a common practice for domestic use and commercial purposes and these animal dungs are being flooded during raining season into streams mining ponds to contaminate the body of water. These bodies of water mostly serve as drinking water source to farmers and rural dwellers.

Study population and sample collection. In this cross-sectional study, diarrhoeal stool samples of two hundred and ninety six (296) were consecutively collected from adult HIV-1 patients attending Jos University Teaching Hospital treatment Centre from December 2015 to March 2016. The hospital was chosen as the busiest HIV clinic in Jos Metropolis.

Ethical approval was obtained from the Ethics Committee of Jos University Teaching Hospital. Consent forms were given to patients to seek their permission before being sampled and only patients that consented were enrolled for the research. A structured questionnaire was used to obtain socio-demographic and clinical variables from the patients. A fresh stool sample was collected in sterile wide mouth containers from each patient; each specimen was labeled. The consistency of the stool specimens was graded by categories (loose, watery) while waiting to be processed. The stool sample was preserved using 10% formalin for formol-ether concentration.

Formol Ether Concentration Method. A small portion of the stool, was mixed with 7ml of 10% formalin in a test tube. It was allowed to stand for 30 minutes for fixation. The suspension was strained through wire gauze. 3ml of ether was added to the filtrate, the tube was stopped and centrifuged for 10 minutes at 2000rpm for 1 minute. The fatty plug was loosened and the supernatant was decanted and a wet mount was made of the deposit to look for parasites using x10 and x40 objectives to examine the whole slide for ova and cysts (Omoruyi *et al*., 2014)

Modified Zeihl Neelsen Staining. The smear was prepared from the sediment of the formol ether concentration. The smear was allowed to air dry and fix with methanol for 3 minutes. It was then stained with heated carbol fucshin for 5 minutes and the stain washed off with water. It was decolorized with 1% acid alcohol for 10 seconds and washed off immediately with water. Finally it was counter stained with 0.5% malachite green (or methylene blue) for 30 seconds and rinsed off, and left to stand at a draining rack for the slide to dry and examined at low power (x40 objectives) and high power (x100 objectives) using oil immersion. Oocysts stain pink to red, sizes of about 4-5µm, spherical with crescent shaped internal comma-like formations (sporozoites) are seen; the bright red colour of oocyst is readily recognizable against a blue background. Stool specimen was labeled positive if oocysts with typical morphology are present.

Statistical analyses

The data was entered into excel sheet and exported into the statistical analysis software (SAS) version 9 (SAS Institute Inc., Cary, NC) where it was analyzed. The Chi-square and t-tests was used to compare categorical and continuous variables respectively. Simple distribution of study variables, frequencies and associations were determined, P value of <0.05 was considered significant.

RESULTS

Two hundred and ninety-six samples where analyzed. The prevalence of Cryptosporidiosis was 4.7% (14). Majority of the patients were female 156 (52.7%). The highest proportion of infection was in females (5.8%) compared to males (3.6%) P=0.696. The age group 21-30 years had the highest prevalence 8 (10.0%), while the least 2 (3.6%) was in age group 41-50years P=0.262. Patients with primary education recorded the highest prevalence 6 (11.0%), and patients without formal education recorded the lowest prevalence 6 (1.4%) P=0.052.

Based on occupational status, the highest prevalence was among the patients that were traders 8 (9.2%). There was no significant association between cryptosporidium infection and these demographic factors (age group, sex, occupation, toilet facility, use of animal dung and source of drinking water. Patients living in urban areas recorded highest prevalence 6(3.0%) compared to those in the rural areas 8(8.1%), (P=0.046). For sanitary practice (toilet type) used, patients who practice open defecation had the highest prevalence of 2 (16.7%), followed by water cistern 12 (4.8%). History of contact with animals or animal dung with 12 (4.1%) compared to those that did not keep animal 2 (33.3%). Details are contained in Table 1, showing the Distribution of Cryptosporidium infection among HIV-1 positive patients in relation to Socio-demographics variables.

Patients with abdominal pain had the highest prevalence 2(16.6%) compared to those without abdominal pain 12(4.2%). Those with watery stool had a frequency of 13(6.3%) while those with loose stool were 1 (1.1%). Patients with 1-2weeks duration of diarrhoea had the highest prevalence 4 (11.8%), those with duration of less than one week was 9 (4.2%), while those with unknown duration of diarrhoea was 1 (2.7%), there was no statistical significant association (P>0.05). Table 2 shows prevalence of cryptosporidiosis in relations to clinical symptoms.

Table 1. Distribution of Cryptosporidium infection among HIV-1 positive patients in relation to Socio-demographics variables

Variable	Number of Sample (%)	Number Positive (%)	P-value
Age (YEARS)			
<20	38(12.8)	0(0.0)	0.262
21-30	82(27.7)	8(10.0)	
31-40	103(35.0)	4(40.0)	
41-50	55(18.6)	2(3.6)	
>51	18(6.1)	0(0.0)	
Sex			
Male	140(47.3)	5(3.6)	0.696
Female	156(52.7)	9(5.8)	
Educational Status			
Primary	55(18.6)	6(11.0)	0.052
Secondary	66(22.3)	1(1.5)	
Tertiary	104(35.1)	6(5.8)	
No formal education	71(24.0)	1(1.4)	
Occupation			
Trader	87(29.4)	8(9.2)	0.205
Civil servant	35(11.8)	1(2.8)	
Unemployed	71(24.0)	3(4.2)	
Student	103(34.8)	2(2.0)	
Residence			
Urban	197(66.5)	6(3.0)	0.046*
Rural	99(33.4)	8(8.1)	
Toilet facility			
Water cistern	251(84.8)	12(4.8)	0.066
Pit toilet	33(11.1)	0(0.0)	
Bush	12(4.0)	2(16.7)	
Use of Animal Dung			
Yes	6(2.0)	2(33.3)	0.581
No	290(98.0	12(4.1)	
Source of drinking water			
Tap	153(51.7)	7(4.6)	0.995
Well	24(8.1)	1(4.2)	
Sachet water	113(38.2)	5(4.4)	

Table 2. Distribution of *Cryptosporidium* spp. among HIV-1 Patients in relation to Clinical symptoms

Variables	No of Samples	No of Positive (%)	P-value
Abdominal pain			0.325
Yes	12(4.0)	2(16.6)	
No	284(96.0)	12(4.2)	
Stool consistency			0.061
Watery	207(70.0)	13(6.3)	
Loose	89(30.1)	1(1.1)	
Duration of diarrhea			0.198
< I week	216(73.0)	9(4.2)	
1-2 weeks	34(11.5)	4(11.8)	
>2 weeks	9(3.0)	0(0.0)	
Unknown	37(12.5)	1(2.7)	

x^2 = Chi square (Fisher's exact for cells <5)

DISCUSSION

In the era of HIV/AIDS, diarrhoeal disease due to *Cryptosporidium* is on the increase due to the associated immune suppression (Gupta *et al*., 2008) but our study found a prevalence rate of *Cryptosporidium* diarrhoea in HIV infected patients of 4.7%. The prevalence was less than earlier reported findings between 5% to 25% in several developing countries including Jos Nigeria, (Schets *et al*., 2007; Wumba *et al*., 2010; Snelling *et al* .,2007). The low prevalence in this study also contrasts with earlier findings of 52.7% and 32.2% in Western Nigeria (Ikeh *et al*., 2007; Pam *et al*., 2013), and 12.3% in Nepal (Fayer *et al*., 2006). The high occurrence of the *Cryptosporidium* species which are mostly through anthroponotic and zoonotic transmission cycles may be due to exposure to these parasites. These occur mostly in high risk groups such as the immune-compromised individuals and people living in areas with fecal-contaminated water source and poor sanitary infrastructure. The observed variation in prevalence with earlier studies may due to the availability, increase access and changes in the threshold for the commencement of antiretroviral therapy (ART). In Nigeria, the cut off CD4+ count for commencing ARV was 200 cells/µl in 2007, 350 cells/µl in 2010, and 500 cells/µl in 2015/2016 when this study was conducted. Commencement of ART at higher CD4+ count would imply less risk for infection with Cryptosporidium species, and therefore, lower prevalence of diarrhoeal disease. It could also be as a result of the method employed for parasite detection in which only one sample was taken which may have underestimated the parasites burden since parasite shading occurs intermittently. Differences in quality of water and hygienic practices in handling food and animals may also account for the variations in prevalence. In our center, patients had regular health education regarding hygiene and were placed on Cotrimoxazole prophylaxis according to the national guidelines with consequent reduction in incidence of opportunistic infections.

In our study, the highest prevalence was observed among patients aged 21-30 years (10.0%), these may be related to the common habit of poor food hygiene, hand washing before eating and leaving the toilet, washing fruits, and also drinking water from unhygienic sources. This attitudes might have increased their risk of contacting the infection as most human Cryptosporidium infections are due to increased person-to-person contact, especially in developing countries with poor hygiene standards.

There was no significant association between cryptosporidiosis and educational status (P=0.052), in this study, although another study showed some association which might be due to the fact that *Cryptosporidium* can be found in chlorinated drinking water and recreational pools which could be ingested causing infection among the literate individuals who may have access to public treated water in urban cities (Schets *et al*., 2007).

Studies have shown that people who live in urban areas are less likely to be infected than those who leave in rural areas because of lower sanitation and hygiene standards in rural areas. In this study, patients living in urban areas recorded lower prevalence of 3.0% compared to those in the rural areas with a prevalence of 8.3%, (P = 0.046).

This agrees with the findings of Samie *et al*., (2006) but in contrast to the reports of Amatya *et al*., (2011). The high prevalence among rural dwellers could be as a result of a lower socio-economic status that impacts on quality of water supply, and living in close proximity to animals and household overcrowding (Vyas *et al*., 2012; Amatya *et al* ., 2011).

The results of this study also showed there was no statistically significant difference between the type of toilet facility used, Although an earlier finding (Fayer *et al*., 2000), reported that Cryptosporidiosis was more frequent in children from houses without a latrine or toilet facility. There was also no significant difference between source of water for drinking, (P =0.995). It is not unusual to find leaky public water supply pipes in many developing countries, including our study city, these leaky pipes are usual points of contamination of the water supply.. Furthermore, chlorine that is commonly used to treat water supplies does not kill Cryptosporidium parasite in contaminated water. There are reports worldwide of associated risk of *Cryptosporidium* infections in people who keep domestic animals at home (Bern *et al*., 2000; Pinheiro *et al*., 2011) but our study did not find a significant association with such practices, and this may be largely due to the small study sample size and the study location which was not done in the community but in a hospital setting.

Regarding the clinical variables, there was no significant association between stool consistency and the presence of parasite, (*P*=0.06), Although, documented findings show that diarrhoea as a result of *Cryptosporidium* species is associated with watery stool as clinical manifestation of the disease (Hunter *et al* ., 2004; Cama *et al*., 2008), Earlier reported studies showed that persistent diarrhea is one of the common symptoms of *Cryptosporidium* infection among HIV-infected persons and is a major sign of disease progression.

This is the first reported study in a large HIV-1 cohort in tertiary Centre in Jos, but the limitation of this study is the small sample size and we did not examine for other diarrhea-causing intestinal opportunistic parasites such as *Cyclospora* and *Isospora* species. We tested only one stool sample per patient which could have under-detected parasite since parasite shedding could be intermittent. In addition, the CD4+ count of the patients were not available to correlate their immune status with the presence of parasite.

CONCLUSION

Cryptosporidium species was identified as one of the cause of diarrhea among HIV-1 infected patients in Jos, however, the low prevalence may be due to the early commencement of ARVs and the use of cotrimoxazole prophylaxis among the HIV-1 infected patients, we recommend that this practice should be sustained and community education on drinking of safe water, good personal hygiene and healthy habits in order to prevent *Cryptosporidium* infection. Further larger studies on the molecular identification of the species of *Cryptosporidium* are needed to determine the available zoonotic strains. .

Conflict of Interest. The authors declare that there is no conflict of interest

Acknowledgement

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APPENDIX I
ETHICAL CLEARANCE

JOS UNIVERSITY TEACHING HOSPITAL
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Date: 19th October, 2015.

Oguchukwu Sandra Chigozie,
Department of Microbiology,
Faculty of Natural Sciences,
University of Jos,
Jos-Nigeria.

RE: ETHICAL CLEARANCE/APPROVAL

I am directed to refer to your application dated 16th September, 2015 on the research proposal titled:

"Detection of Cryptosporidium Species among HIV Infected Patients Attending Jos University Teaching Hospital" and your appearance before the Ethical Committee on 2nd October, 2015.

Following recommendation from the Institutional Health Research Ethics Committee, I am to inform you that Management has given approval for you to proceed on your research topic as indicated.

You are however required to obtain a separate approval for use of patients and facilities from the department(s) you intend to use for your research.

The Principal Investigator is required to send a progress report to the Ethical Committee at the expiration of three (3) months after ethical clearance to enable the Committee carry out its oversight function.

Submission of final research work should be made to the Institutional Health Research Ethical Committee through the **Secretary, Administration Department,** please.

On behalf of the Management of this Hospital, I wish you a successful research outing.

Hajia R. Danfillo
For: Chairman, MAC

INSTITUTIONAL HEALTH RESEARCH
ETHICAL COMMITTEE
DESPATCHED
19 OCT 2015
JOS UNIVERSITY TEACHING
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APPENDIX II
Plate 1. Typical Morphology of *Cryptosporidium* specie Oocyst under a microscope