PREVALENCE OF CRYPTOSPORIDIOSIS AMONG HIV/AIDS INFECTED INDIVIDUALS ATTENDING GAMBO SAWABA GENERAL HOSPITAL, ZARIA

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ABSTRACT

Background: Diarrhea remains one of the most important health problems globally and a leading cause of morbidity and mortality among immune compromised individuals. It accounts for over 50 million deaths (in all ages) world-wide and ranks 3rd among diseases responsible for human mortality globally**.**

Aim: This study was aimed at determining the prevalence of cryptosporidiosis in patients who presented with diarrhea attending Gambo Sawaba General Hospital Zaria, Kaduna State, Nigeria.

Methods: The study was cross-sectional study in which a total of 110 diarrheic stool samples were processed using formol ether Concentration Techniques and Stained for oocysts of *Cryptosporidium* species using modified ZN staining method while direct sample was screened for other enteric parasites.

Results: The overall prevalence rate of *cryptosporidium* oocyst in the diarrhetic HIV/AIDS patient was 10%. Other parasites isolated were *Ascaris lumbricoides* 3.6% and *Entamoeba histolytica* 4.6%. *Cryptosporidium* infection was associated with those who defecate in pit latrine with a prevalence of 9.1% at (P=0.476) and those who drank well water without boiling with a prevalence of 7.2% at (P=0.419). There was no statistical difference between *Cryptosporidium* infection and those who eat undercooked food with a prevalence of 6.4% at (P=0.680). The oocysts were detected more frequently in female with 6.3% than male participants with 3.6% prevalence and patients between age group of 25-54 years were most affected with a prevalence of 2.7%.

Conclusion: A prevalence of 10% of cases among this study group is high, hence should be considered in planning interventions aimed at optimizing management of HIV/AIDS and other immune-suppressed patients.

Key: words: Prevalence, Diarrhea, Concentration, cryptosporidiosis, HIV/AIDS

INTRODUCTION

More than 30 years have elapsed since human immunodeficiency virus type I (HIV-I) was first identified as the cause of acquired immune deficiency disease syndrome (AIDS) (Savioli*et al.*, 2006 and Barre-sinoussi*et al*., 1983). The world had experienced an HIV pandemic and the estimated number of persons living with HIV is around 34 million. From its discovery in 1981 until 2010, AIDS has killed more than 25 million people. In 2010 alone, AIDS claimed an estimated 2.4–2.9 million lives, of which more than 390,000 were children. A third of these deaths occurred in sub-Saharan Africa (the hardest-hit region), which is home to approximately 15% of the world's population (Global report, 2011). Recently, Nigeria had a HIV prevalence of 1.4% among adults aged 15-49years, while previous estimate had indicated a national HIV prevalence of 2.8% (UNAIDS, 2019).

UNAID and National Agency for the control of AIDS estimated that there are 1.9 million people living with HIV in Nigeria. This shows that Nigeria has made good progress in scaling up HIV treatment and prevention service in recent years (UNAIDS, 2019).

Cryptosporidium is a protozoan parasite, which belongs to the phylum "Apicomplexa", class "Coccidian" and order "Eimeriida". The enteric protozoan parasite, *Cryptosporidium*, was first recognized as a human parasite in 1976, when it was reported as a causative agent of diarrhea in a three-year-old child with self-limiting enterocolitis (Hommer, *et al.*, 2013andMeisel *et al*., 1976). It was not until the emergence of the HIV pandemic in the 1980's that *Cryptosporidium* became widely recognized as an important human pathogen. The first case of cryptosporidiosis in a homosexual man with AIDS was reported in 1982 (Savioli, et al., 2006 and Soave *et al.*, 1983). Since then, numerous reports worldwide have identified cryptosporidiosisas a significant disease in HIV/AIDS. There are multiple routes of transmission of cryptosporidiosis in humans, including person-to-person, waterborne, foodborne and zoonotic (Aminu*et al.*, 2014). In 1993, *Cryptosporidium* sparked great public health interest after a very large waterborne outbreak in Milwaukee, Wisconsin, which resulted in 403,000 people being affected, with 5,000 confirmed cases of cryptosporidiosis and 100 fatalities, mostly involving immunocompromised individuals (Yemisi*et al*., 2007). Currently, cryptosporidiosis is commonly reported in HIV-infected individuals and is listed as an AIDS-defining illness by the US Centers for Disease Control and Prevention (Guerrant *et al.,* 1997). The infection in individuals with HIV/AIDS is persistent and life-threatening and often involves infections of the entire gastrointestinal tract in addition to hepatobiliary and the respiratory tracts infections (Tzipori and widmer, 2008). Cryptosporidiosis is common among children and causes a self-limiting diarrhea but could be life threatening in immune-compromised individuals (Jafari *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 (Xiao, 2010). The oocysts are stable and resistant to common water disinfectants like chlorine (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, 2013). The severity of the disease is manifested in AIDS patients usually when the CD4+cell count is below 200cells/mm3 (McDonald, 2000). The World Health Organisation's guideline for drinking water classifies *Cryptosporidium* as a pathogen of significant public health importance, aided in part by the organism's low infective dose and its resistance to conventional water treatment, such as chlorination (Masarat *et al.*, 2012). Preventions measures include boiling drinking water for over a minute, point of use filter, and the use of hyperimmune bovine colostrum's as a prophylactic agent (Masarat *et al.*, 2012).

Significance of the study

The significant of this study was to determine cryptosporidiosis amongst HIV/AIDS patients which leads to diarrhea as well as increasing morbidity and mortality of the target group which is not as a result of HIV infection itself but as a result of co-infection with *cryptosporidium* parasites.

MATERIALS AND METHODS

STUDY AREA

This study was conducted at Gambo Sawaba General Hospital, Zaria. Kaduna State. Zaria is a city found in Kaduna State. Zaria LGA lies between latitude $11.11^{\circ}N$ and longitude $7.72^{\circ}E$ and has a land mass of 300 square kilometers and a population of 406,990 people as of the 2006 Nigerian census. It is bounded to the north by Sabon Gari Local Government Area, to the east by Soba LGA, to the south by Igabi LGA and to the west by Giwa Local Government Area (Okojokwu *et al.*, 2014).

STUDY DESIGN

A Cross sectional study design also referred to as prevalence study.

SAMPLE SIZE DETERMINATION

The sample size was calculated and determined using formula as follows

$n = \frac{z^2pq}{d^2}$(Lwanga and Lemeshow, 1991).

n =number of samples

z= statistics for level of confidence at 95%=1.96

p=prevalence rate of cryptosporidiosis is 7.5% (Okojokwu *et al.*, 2014).

q=1-p

d= degree of accuracy desired at 5% (0.05)

$n= (1.96)^2 \times 0.075 \times (1 - 0.075)/(0.05)^2$

n=110.00Sample

Study Population

HIV/AIDS patients who attend Gambo Sawaba General hospital presenting with diarrhea was Recruited.

Ethical Approval

An ethical approval to carry out the study was sought from Kaduna State Ministry of health.

Informed Consent

A written and verbal informed consent was sought from all subjects before inclusion into the study, Subjects who consented to participate ware recruited for the research.

Questionnaire

A structured questionnaire was used to obtained socio-demographic data from the patients.

Inclusion Criteria

HIV/AIDS positive patients presenting with diarrhea attending Gambo Sawaba General Hospital whose consent were sought ware included for this study.

Exclusion Criteria

HIV/AIDS positive patient not presenting with diarrhea or those with diarrhea, but whose consent were not sought or did not show willingness to participate in this study ware excluded for this study.

Sampling Technique

Due to the fact that most of the patients visiting the facility have already started their ART, this will greatly improve their condition and limited the number of HIV patients presenting with diarrhea. This limitation will make the choice of sampling technique to avoid bias difficult and as such diarrhea patients among HIV positive patients was recruited and enrolled consecutively as they come for follow up or related intervention when acutely sick.

Specimen Collection

Stool samples was collected from each subject into a clean Universal bottle, preserved in 5% formalin and transported to the medical laboratory Science Department Bayero University, Kano for analysis and stored at 20^0C. The participants were interviewed to collect relevant demographic data.

Sample Analysis

Macroscopy

Stool: colour, consistency and constituent were observed and recorded.

Microscopy

Direct wet preparation (Saline and iodine method)

A drop of fresh physiological saline and iodine each ware placed at each end of a clean glass slide.

A pasture pipite was used to pick a small amount of the specimen which was then mixed with the saline and iodine to make a smooth preparation. The preparation was covered with a cover slip and examined microscopically using 10x and 40x objectives for the presence of parasite cysts, larva or ova. (Arora and Arora, 2014).

Formal ether concentration technique

Small portion of stool sample was emulsified in 7mls of 10% formal saline solution in centrifuge tube thoroughly. And allow to stand for 30min for fixation. The faecal suspension was sieved, and 3ml of ether was added and shuke vigorously. The mixture was then centrifuged at 1000 rpm for 2 minutes. Using an applicator stick the debris layer, was loosened and together with supernatant ware poured away. The sediment was re-suspended by tapping the bottom of tube. A drop was placed on glass slide and examined for other parasites before smears were made with sediment for cryptosporidium oocyst. The smears were allowed to air dry before staining, protecting it from dust and flies(Arora and Arora, 2014).

Modified Zeal Nelson Staining technique

The smears (made with sediment) were stained by the modified Zeal-Nelson staining method.

The smear was fixed by heat and flood with carbol fuchsin solution and heated gently until steam rises. This was left for 5 minutes and washed with tap water. It was decolorized with 1% acid alcohol until the film becomes yellowish. It was washed with water and counterstained with malachite green for 3 minutes. It was then washed with water, dried and examined under oil immersion lens for the presence of Oocysts of *Cryptosporidium* (Arora and Arora, 2014).

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Statistical Analysis

All statistical analyses were performed using SPSS version 20.0 at 95% confidence level and provability value at ($P<0.005$). One-way Anova was used to determine parasites prevalence/ and Chi square test was used to determine levels of association between the variables used in this study.

RESULT

A total of 110 HIV patients visiting Gambo Sawaba General Hospital presented with diarrhea were investigated for Cryptosporidiosis**.** Table 1 shows the demographic and socioeconomic characteristics of the participants presented with diarrhea whose stool samples was examined. Table 2 shows the prevalence rate of the participants infected with *cryptosporidium parvum* oocysts was 11(10%) and the prevalence rate of non-infected participant was 99(90%). Table 2 showed Age range of25-34, 35-44 and 45-54 years among the study participants had the highest infection rate of 3(2.7%) and 15-24 had the infection rate of 2(1.8) and age range of 55-64 had an infection rate of 0(0.0%) with X^2= 26.025, p= 0.929. Table 4 showed that out of total number of the participants, 40(36.4%) were males and the remaining 70(67.3%) were females. *Cryptosporidiumparvum* was common among female participants 7(6.4%) when compared to the male 4(3.6%) prevalence. X^2= 0.000, p= 1.000. Table 5 showed the associated study participants 27(24%),74(62%) and 9(8%) used Borehole, well water and River water respectively.

Cryptosporidium oocyst was detected in 3(2.7%), 8(7.3%) and 0(0.0%) of participants who used Bore hole, well water and pure water respectively, X^2=0.091, p= 0.580. Majority of the participants use pit latrine 96(87.3%) while 5(4.5%) and 9(8.2%) use of bush and water closet system respectively.

Cryptosporidium oocyst was detected in 10(9.1%), 1(0.9%) and 0(0.0%) of participants who used pit latrine, bush and water closet system respectively, X^2= 1.574, p= 0.455 and Cryptosporidium oocyst was detected in 7(6.4%) of participants who eat undercooked vegetable while it was detected in 4(3.6%) participants who do not eat undercooked vegetables, X^2= 0.170, p= 0.680. Effect of contact with animal pets was assessed. The prevalence of Cryptosporidium oocyst was found to be 3(2.7%), 2(1.8%), 6(5.5%) and 0(0.0%) among participants who have contactedcat, dog, sheep, and cattle respectively.X^2= 1.335, p= 0.931. Table 6: showed that out of the 110 participants whose faecal samples were examined, 11(10%) were infected with *C. pervum*, 4(3.6%) with *Ascaris lumricoides* and 5(4.5%) with *Entamoeba histolitica* infection, X^2= 1.001, p= 0.606.

Table 1: Prevalence of *Cryptosporidium* Oocyst Among participants.

C. Pervum Oocyst	Test	prevalence	X^2	*P-value*
			0.025	0.874
Infected	11	11(10%)		
Non infected	99	99(90%)		
Total	110	110(100%)		

KEY: X^2: Chi square, %: Percentage, P-value: Provability value (P<0.05) was Significant.
NOTE: The Prevalence of *Cryptosporidium* oocyst is not significant Among Participants at p=0.874.

Table 2: Distribution of Cryptosporidiosis among Study Subjects Based on Age Group.

Variable:	Number Examined	Number Positive	Prevalence (%)	X^2	*P Value*
Age Group				26.025	0.929
15-24	20	2	(1.8)		
25-34	27	3	(2.7)		
35-44	40	3	(2.7)		
45-54	18	3	(2.7)		
55-64	5	0	(0.0)		
Total	110	11	(10.0)		

KEY: X^2: Chi square, %: Percentage, P-value: Provability value (P<0.05) was Significant.
NOTE: The Prevalence of Cryptosporidiosis is not significant Among Age Group at p=0.929

Table 3: Distribution f Cryptosporidiosis Among Study Subject Based on Sex.

Variable:	No Examine	No Positive	Prevalence (%)	X^2	*P value*
Gender				0.000	1.000
Male	40	4	36.4		
Female	70	7	63.6		
Total	110	11	100		

KEY: X^2: Chi square, %: Percentage, P-value: Provability value (<0.05) was Significant.

Table4: Distribution of *Cryptosporidium* oocyst based on demorgraphic factors.

Variable:	No. Examined	No. PositiveN	Prevalence	*F-value*	x^2	*P-value*
Water Source				0.477		0.491
Borehole	27	3	(2.8%)			
Well	74	8	(7.2%)			
Pure Water	9	0	(0.0%)			
Total	110	11	(10.0%)			
Typeof toilet used				0.512		0.476
Pit Latrine	96	10	(9.1%)			
Bush	5	1	(1.0%)			
Water Closet	9	0	(0.0%)			
Total	110	11	(10.0%)			
Animal reared				0.513		0.475
Cat	25	3	(2.7%)			
Dog	16	2	(1.8%)			
Sheep	59	6	(5.5%)			
Cattle	10	0	(0.0%)			
Total	110	11	(10.0%)			
Raw/Undercooked Vegetable				0.170		0.680
Yes	76	7	(6.4%)			
No	34	4	(3.6%)			
Total	110	11	(10.0%)			

KEY: X^2: chi square, %: percentage, p-value: provability value at ($p<0.05$) was significant.

Table 5: Distribution of other Parasites Isolated from Stool Among the Study Subjects.

Parasite type	No. Examine	No. Infected	Prevalence %
Ascaris lumbricoides	110	4	3.6
Entamoeba histolitica	110	5	4.6
Total	110	9	8.2

KEY: %: Percentage, No: Number

DISCUSSION

Diarrhea is a significant problem in HIV infected patients in Africa (Hung *et al.*,2007; Mukhopadhya *et al.*,2000). *Cryptosporidium* is a well-established cause of diarrhea among HIV infected patients worldwide with prevalence of infection ranging from 3% in developed countries to 50% in developing countries (Goldstein *et al.*, 1996). However, the role of this parasite in the occurrence of diarrhea among adult patients with HIV infection in Nigeria is just emerging (Erhabor *et al.*, 2011).

The findings of this study showed that, the overall prevalence of cryptosporidiosis was 10%. This Low prevalence from this work might be attributed to the fact that must of the study participants are receiving anti-retroviral therapy and therefore have improved immune responses. This observed findings is in contrast with the findings of Mbanugo and Agu, (2006) who reported 85% prevalence of cryptosporidiosis and other intestinal protozoa infections among HIV patient in Imo state, Nigeria.

The variation in prevalence could be due to the small sample size used. Similarly, the findings of this study showed higher prevalence of 2.7% among those aged 25-54. It could be due to levels of exposure, practices or behaviors in the community. These finding is not in keeping with the works of Sorvillo *et al.* (1994), and Muntaz *et al.* (2010) who reported that *Cryptosporidium* infection had higher prevalence in children than in adults. This may be due to decrease immune response in children than in adults. This finding shows the prevalence rate of *cryptosporidium parvum* based on sex to be 3.6% in males and 6.4% in females. This may be attributed to higher prevalence of HIV in females than males. This finding is in keeping with the work of Abdulhadi and Gwarzo (2013) and Erhabor *et al.* (2006) who also reported a high prevalence in females than in males. This finding is in contrast with the work of Muntaz *et al.* (2010) who reported higher prevalence in male than in female. This could be attributed to the lifestyle and sociocultural differences in the study areas. Similarly, a higher prevalence of 7.2% was observed in this present study based on household dependent on well water. This may be as a result of contamination of water from faeces of reared animal. This agrees with the reports that contaminated water and food serves as a major source of *Cryptosporidium* infection for humans, (Ramirez *et al.*, 2004 and Chacín-Bonilla *et al.*, 2008). Similarly, this finding shows a higher prevalence of 9.1% from houses defecating in pit latrine. This corroborates with the report of (Chacín-Bonilla *et al.*, 2008). This may be due to improper hand washing after defecating and also before meals. Similarly, the findings show an association of infection with domestic animal with a high prevalence of 5.5% in sheep. This might be due to contamination with their faeces. This finding is in line with the work of (Neumann *et al.*, 1993) that reported domestic animals as a source of infection. This might be due to the fact that most animal associated with zoonotic transmission were reared in this area. But in contrasts with the work of Bern *et al.* (2000). This could be since most animal associated with zoonotic transmission were not reared in that area.

Limitation of the Study

Formal ether concentration technique was the only technique used which probably might have destroyed the cyst due to centrifugal force as opposed to floatation technique using zinc sulphate preparation.

CONCLUSION

Cryptosporidium Oocysts have been demonstrated in faecal samples of some HIV/AIDs infected patients attending Gambo Sawaba General Hospital, Zaria. This study concludes that, there exist cryptosporidiosis in the study area with a prevalence of 10% of participants.

RECOMMENDATIONS

Bases on the results obtained from this study, the following recommendations are made.

1. Public awareness/enlightenment should be step up in order to control the spread of this disease through proper hygiene and adequate sewage disposal via complete close system to minimize contamination and outbreak of disease
2. This study also recommends further research to be conducted on molecular analysis for species identification on *cryptosporidium* infections among people living with HIV/AIDS in other part of the state.
3. It is also recommended that all individual living with HIV should have a routine diagnosis of Cryptosporidiosis.

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