



Sero-Prevalence of *Cryptosporidiosis* among People Living with HIV/AIDS in North-western Nigeria

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Abstract

Background: *Cryptosporidium* species are obligate, intracellular, protozoan parasites belonging to the phylum Apicomplexa, which is a substantial threat to HIV/AIDS-infected individuals with an estimated risk of infection of around 52% in developing countries. **Aim:** The study was aimed at determining the Sero-Prevalence of *Cryptosporidiosis* among people living with HIV/AIDS in North-western Nigeria. **Methods:** Structured questionnaires were administered to 90 participants who were selected by inclusion criteria sampling technique with a total of 90 blood samples collected from them. Serological analysis of the blood samples was carried out using the Human *Cryptosporidium parvum* (CP) ELISA detection test Kit. (Melsin Medical Co., Limited, China LOT NUMBER: 20191023), and CD4 cell count was performed. **Results:** Out of the 100 participants, 19(19%) were found to be positive for *Cryptosporidium parvum* and 81(81%) were negative with an overall prevalence of 19%. There was no statistical significance between *Cryptosporidium* infection and those who used pit latrine ($p=0.347$), eating outside ($p=0.494$), reared animals ($p=0.838$) and the type of water source ($p=0.641$). The association between *Cryptosporidium parvum* and the CD4⁺ count of the participants was determined using a statistically insignificant chi-square test ($p= 0.409$). The higher the CD4⁺ count, the lesser the risk for *Cryptosporidium parvum* infection. **Conclusion:** This study reveals an overall Sero-Prevalence of 19% of *C. parvum* among HIV/AIDS patients and there is no association between CD4⁺ count and infection of *Cryptosporidium parvum*.

Keywords: Seroprevalence, *Cryptosporidium*, HIV/AIDS, Kano, ELISA, Human

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Introduction

Cryptosporidium species are obligate, intracellular, protozoan parasites belonging to the phylum Apicomplexa (Fayer *et al.*, 2000). Currently, there are 22 known species of *Cryptosporidium* that infect vertebrate hosts reported in scientific literature (Fayer, 2010) of which the zoonotic *Cryptosporidium parvum* and the anthroponotic *Cryptosporidium hominis* are the major causes of human *Cryptosporidiosis* throughout the world (Chen and LaRusso, 2002). First discovered by Ernest Edward Tyzzer in the year 1907 in the gastric mucosa of mice (Tyzzer, 1907), *Cryptosporidium* remained largely unrecognized as a human pathogen until the first reported case in 1976 in an immuno-competent child (Nime *et al.*, 1976).

A review of the first 7 cases of *Cryptosporidiosis* led to the conclusion that it was predominantly a disease of the immunocompromised, although it was also reported to be widely prevalent among immunocompetent children (Guerrant, 1997; Tzipori, 2008). Fluid loss in AIDS patients with *Cryptosporidiosis* is often extreme; 3-6 liters of watery stool per day and as much mucus as 17 litres of watery stool per day (Amadi *et al.*, 2001). In 75% of cases, diarrhoea is accompanied by crampy abdominal pain with approximately 25% of patients having nausea and vomiting (Arora and Arora 2009).

Over the previous decade, the job of *Cryptosporidium* as an operator of the human

looseness of the bowels has been re-imagined from that of an uncommon pioneer to a pathogen with around-the-world dissemination and the potential for critical dismalness and furthermore, mortality (Manabe *et al.*, 2009). Of late, the solid relationship between instances of *Cryptosporidiosis* and immunodeficient people, (for example, those with AIDS) brought *Cryptosporidium* to the bleeding edge as a universal human pathogen (Smith and Rose, 1998). Directly, the expanding populace of immunocompromised people and different episodes through disease by water-borne *Cryptosporidium* oocysts (regularly in drinking water) has set a much more noteworthy accentuation on this pathogen. Until this point in time, there is no demonstrated powerful medical treatment for this microbe and to some degree because of its powerlessness to develop well in vitro, the human safe reaction to *Cryptosporidium* contamination remains ineffectively comprehended (Alaribe *et al.*, 1998; Smith and Rose, 1998; Abubakar *et al.*, 2007).

Cryptosporidium species are increasingly being recognized as an important pathogen causing diarrhoea in children, with the highest associated morbidity and mortality, especially among children in developing countries

(Snelling *et al.*, 2007). The highest prevalence of *Cryptosporidiosis* has been documented in children aged 6-12 months (Tzipori, 2008; Perch *et al.*, 2001). The ability of *Cryptosporidium* to cause large-scale explosive outbreaks has been well documented. It was implicated in the largest waterborne outbreak of acute gastroenteritis in Milwaukee, Wisconsin, USA, in which an estimated 403,000 people were infected (Mackenzie *et al.*, 1995). In north-western Nigeria, a prevalence of 4.0% was reported among HIV patients (Kumurya and Gwarzo, 2013).

Materials and Methods

Study Area

The study was carried out in six (6) states of North-western Nigeria (Kano, Kaduna, Katsina, Jigawa, Sokoto and Zamfara). One hospital was selected from each state in the geopolitical zones Kano-Aminu Kano Teaching Hospital (AKTH), Kaduna-Ahmadu Bello University Teaching Hospital (ABUTH), Katsina-Federal Medical Center (FMC, Katsina), Jigawa-Federal Medical Center (FMC, B/kudu), Sokoto-Usman Danfodio University Teaching Hospital (UDUTH) and Zamfara- Federal Medical Center (FMC, Gusau).

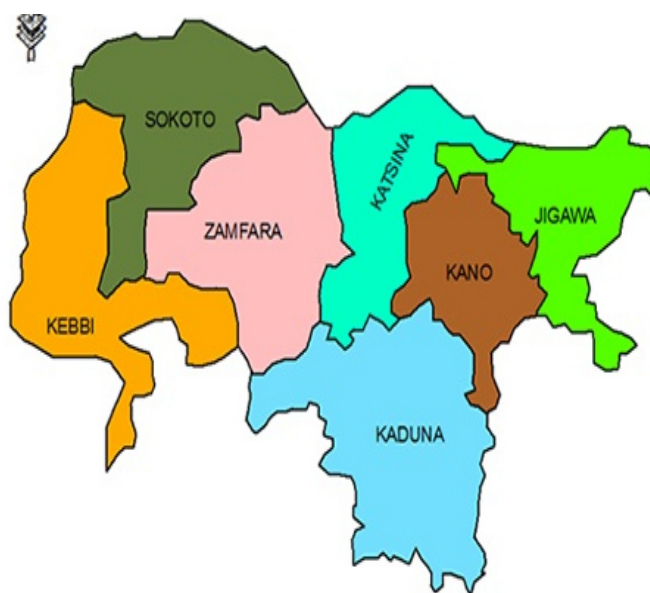


Figure 1: Map of North-western Nigeria showing the study area (NPC, 2016)

Research Design

This study is a cross-sectional study.

Study Population

People living with HIV/AIDS who presented with diarrhoea attending the selected hospitals in North-western Nigeria were recruited for the study

Inclusion Criteria

- ✧ All HIV-infected patients attending the selected hospitals within the study period, presenting with diarrhoea that gave informed consent
- ✧ Control subjects (HIV Negative individuals presenting with diarrhoea)

Exclusion Criteria

- ✧ Other immunocompromised individuals attended the same hospitals in the study areas other than the selected hospitals.
- ✧ All other patients other than HIV Patients
- ✧ HIV-positive patients not presenting with diarrhoea

Structured Questionnaire

A structured questionnaire was used to collect data and patients' information on age, sex, feeding, water source, educational background, sanitation and symptoms.

Ethical Consideration

Ethical clearance to conduct the research was sought for and obtained from the health Ethics Committees of the various selected hospitals (Ethical Approval from AKTH; Ethical Approval from Kano state government; Ethical Approval from ABUTH; Ethical Approval from UDUTH; Ethical Approval from FMC, KATSINA; Ethical Approval from FMC, B/KUDU; Ethical Approval from FMC, GUSAU; Ethical Approval from Sokoto state government). The participant's informed consent/assent was sought in writing through the administration of well-explained informed consent/assent forms to all the subjects used in the study. Sociodemographic, socioeconomic and educational data of

participants was obtained using a well-structured administered questionnaire.

Sample Collection and Processing

Proportionate Sample Distribution

The 100 Samples were distributed proportionately in the study area based on the current HIV/AIDS prevalence of 2019 (NACA, 2019) as follows;

Kano State 23, Kaduna State 32, Katsina State 10, Jigawa State 10, Sokoto State 11 and Zamfara State 14

Blood Sampling

Three ml of venous blood sample was collected in an EDTA vacutainer from each subject. Within six hours of collection, the sample was centrifuged at $800 - 1600 \times g$ for 20 minutes at room temperature to separate the plasma. Separated plasma was transferred into a sterile polypropylene screw cap tube and stored frozen at -80°C till further use (Stevens *et al.*,2008)

Sample Analysis

Detection of *Cryptosporidiosis Sero-Prevalence* was carried out using an ELISA kit. The detection of *Cryptosporidiosis Sero-Prevalence* in the samples was assessed using a commercially available ELISA kit for a blood sample. The procedure was carried out according to the manufacturer's instructions.

ELISA Protocol

All reagents were prepared before starting the assay procedure. All reagents were added in duplicate to the Micro Elisa Strip plate. Exactly $50\mu\text{L}$ of Positive control and Negative control were added to the Positive and Negative well and $10\mu\text{L}$ of testing sample was also added. A sample diluent of $40\mu\text{L}$ was added to the testing sample well; the Blank well was left empty. $100\mu\text{L}$ of HRP-conjugate reagent was added to each well, it was then covered with an adhesive strip and incubated for 60 minutes at 37°C . Each well was aspirated and washed four times. Each well

was washed by filling with Wash Solution (400µL) using a squirt bottle, manifold dispenser or auto washer. After the last wash, any remaining Wash Solution was removed by aspirating or decanting. The plate was inverted and blotted against clean paper towels. Exactly 50 µL each of *chromogen* A and B were added to each well, it was then mixed and incubated for 15 minutes at 37°C and 50µL of stop solution was added. The colour changed from blue to yellow. Optical Density was read at 450nm using a *microtiter* plate (Asimita *et al.*, 2016).

CD4 T- lymphocytes count measurement

Protocol:

The blood samples were placed on a roller mixer for at least 15 minutes for proper mixing. Twenty microliters of CD4+ easy-count monoclonal antibody were added

to the parties' test tubes. Exactly 20 µL of blood sample was added and incubated for 15 minutes in the dark at room temperature by mixing at intervals. Immediately, afterwards, 800 µL of CD4+ easy count-no lyse buffer was added to the tubes and was gently shaken. The mixtures were analyzed on a cyflow partec device (Ajjampur *et al.*, 2008).

Statistical Analysis

Data was analyzed using the software Statistical Package for Social Sciences (SPSS) version 21.0 The data obtained were subjected to normality testing to check if the data is normal or otherwise using *Kolmogorov*. Hence the data was normal, and then a parametric test was done to check for statistical significance differences with the chi-square test in continuous and categorical variables, probability values of P<0.05 were considered statistically significant

Result

Table 1: Northwest Zone Distribution of *Cryptosporidium parvum* Antibody by ELISA Methods among the Study Subjects

North-west Geo-political zones	Number Examined	Number Positive	Prevalence (%)	χ^2	P- Value
KANO STATE	23	6	5.4	26.025	0.929
KADUNA STATE	32	6	5.4		
KATSINA STATE	10	3	3.7		
JIGAWA STATE	10	1	0.9		
SOKOTO STATE	11	0	0.0		
ZAMFARA	14	3	3.7		
TOTAL	100	19	19		

Key: χ^2 : chi-square, %: Percentage, P-value: probability value at (p<0.05) was significant.

Table 2: Sero-Prevalence of *Cryptosporidium parvum* among PLWH Presenting with Diarrhoea

Result	Frequency (n)	Prevalence (%)
Positive	19	(19.0)
Negative	81	(81.0)
Total	100	100

Key: n = frequency. % = percentage

Figure 1 shows the seroprevalence of *Cryptosporidium parvum* among HIV/AIDS-infected individuals based on

gender, whereby females had the highest number of infections while males had the least number of infections

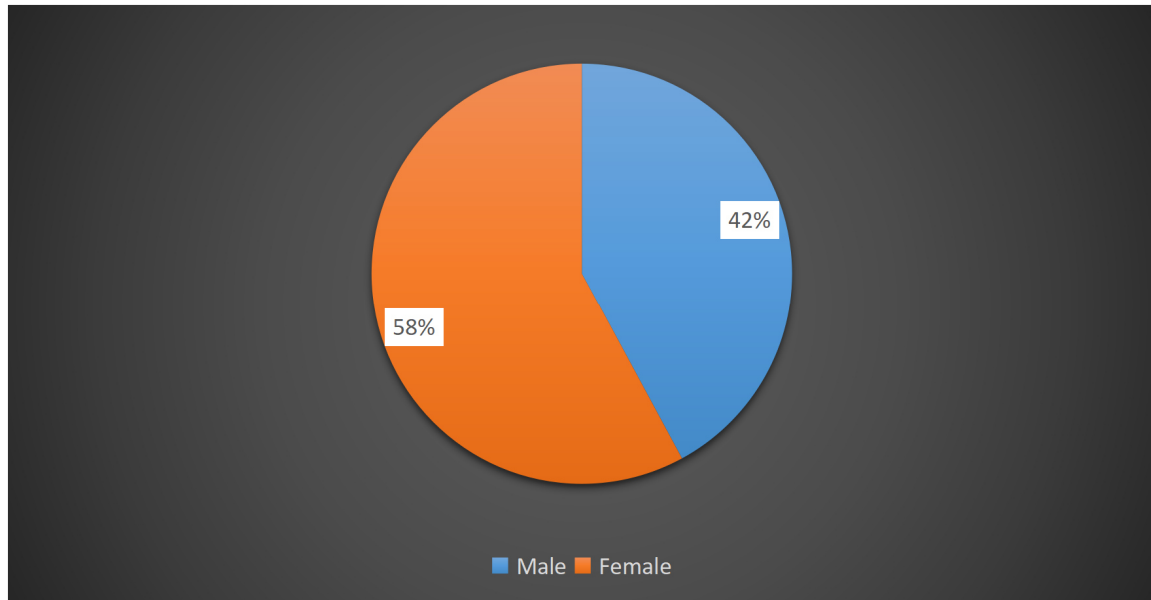


Figure 1: Sero-Prevalence of *Cryptosporidium parvum* among HIV/AIDS-infected individuals based on gender

Table 3: Shows the association of *Cryptosporidiosis* among seropositive HIV/AIDS patients in relation to CD4+ count. Based on CD4+ count, the participants were grouped into 3 groups. Of the 100 recruited participants, 10 (11.1%) had a CD4+ counts between the range of 20-200cells/mm³, 65 (72.2%) had a CD4 count between 300-/600cells mm³ while 25 representing (16.7%) had a CD4+ counts between 700-1500. The *C. parvum* ELISA seropositive from patients with CD4 count between the

range of <20-200cells/mm³; was 14 (73.7%), *C. parvum* ELISA seropositive from subjects with CD4 counts of 300-600cells/mm³ was 4 (21.0%), while *C. parvum* ELISA seropositive from subjects with CD4 counts of 700-1500cells/mm³ was 1 (5.3%). There is no statistical significance difference ($p > 0.05$) although, there is an association of *Cryptosporidiosis* among HIV/AIDS seropositive patients in relation to CD4+ count

Table 3: Association of Seropositive *Cryptosporidiosis* among HIV/AIDS-Infected Individuals in Relation to CD4+ count

CD4+ count	Frequency	<i>C. parvum</i> infection (%)	F-value	P-value
20-200	10	14(73.7)	1.789	0.409
300-600	65	4(21.0)		
700-1500	25	1(5.3)		
Total	100	19(100)		

Key: F-value=ANOVA value, %= percentage, p-value= provability value at ($p < 0.05$) was significant.

Table 4 shows the distribution of *Cryptosporidiosis* based on associated risk factors (Borehole, well water and River water) with participants recording 3(2.7%), 8(7.3%) and 0(0.0%) who used Borehole, well water and River water respectively, $\chi^2=0.091$, $p=0.580$. Majority of the participants used pit latrine 86(77.3%) while 5(4.5%) and 9(8.2%) used bush and water closet system with *Cryptosporidiosis* detected in 10(9.1%), 1(0.9%) and 0(0.0%) respectively, $\chi^2= 1.574$,

$p= 0.455$ and participants who eat undercooked vegetable, *Cryptosporidiosis* was detected in 7(6.4%) while participants who do not eat undercooked vegetables recorded 4(3.6%), $\chi^2= 0.170$, $p= 0.680$. The effect of contact with animal pets was also assessed and *Cryptosporidiosis* was found to be 3(2.7%), 2(1.8%), 6(5.5%) and 0(0.0%) in participants who had contact with Cat, Dog, Sheep and cattle respectively. $\chi^2= 1.335$, $p= 0.931$.

Table 3: Distribution of *Cryptosporidium parvum* Antibodies Based on Associated Factors

Variable:	No. Examined	No. Positive N	Prevalence (%)	F-value χ^2	P-value
Water Source				0.477	0.491
Borehole	27	6	(2.8)		
Well	64	8	(7.2)		
Pure Water	9	5	(0.0)		
Total	100	19	(10.0)		
Type of toilet used				0.512	0.476
Pit Latrine	86	10	(9.1)		
Bush	5	5	(1.0)		
Water Closet	9	4	(0.0)		
Total	100	19	(10.0)		
Animal reared				0.513	0.475
Cat	24	6	(2.7)		
Dog	17	2	(1.8)		
Sheep	49	8	(5.5)		
Cattle	10	3	(0.0)		
Total	100	19	(10.0)		
Raw/Undercooked Vegetable				0.170	0.680
Yes	65	12	(6.4)		
No	35	7	(3.6)		
Total	100	19	(10.0)		

Key: χ^2 =chi square, %= percentage, p-value= probability value at ($p<0.05$) was significant.

Discussion

Cryptosporidiosis in a patient with acquired immune deficiency syndrome (AIDS) who also has severely impaired immunity may be a devastating disease. Not only can it cause chronic severe and intractable diarrhoea that greatly reduces the patient's quality of life, but

in many patients, it significantly shortens their life expectancy due to low CD4+ cell counts, lack of access to antiretroviral treatment (ART) and poor hygiene (Chacín-Bonilla *et al.*, 2008; Ayinmode *et al.*, 2014). The overall prevalence of 19% seropositive for *Cryptosporidium* using an ELISA Test kit is

similar to the study of Soave *et al.* (2007) in their study, who reported a prevalence of 20.8% of individuals infected with HIV/AIDS having *Cryptosporidium* antibodies. These discrepancies might be in part due to differences in the methodology employed during the study, differences in geographical settings, and the sample size. This study also shows the seroprevalence rate of *Cryptosporidium* infection based on gender among HIV-positive participants to be 42% in males and 58% in females which may be explained by the difference in the number of female participants recruited were higher than the male participants, though there is no significant difference statistically ($P=0.786$). This is not in agreement with a study by Xiao *et al.* (2012), that shows a higher prevalence among males than females in HIV-positive patients, (because most of the participants were males). In this study, a relationship between CD4+ counts and *Cryptosporidium* seropositivity was observed (Table 2). This was compared favourably with previous studies by Erhabor *et al.* (2011), who reported that there was an inverse correlation between CD4 count and infection with *Cryptosporidium*. Since it is known that HIV destroys the cell-mediated immune system, which is provided by the CD4 lymphocytic cell, these lymphocytes when significantly destroyed below 200 cells/mm³ predispose the individual to opportunistic infection and invariably more chance of acquisition of *Cryptosporidium* infection. This is consistent with other findings by Erhabor *et al.* (2011).

Conclusion

This study reveals an overall *Sero-Prevalence* of 19% for *C. parvum* among 100 HIV/AIDS patients attending the selected hospital in the study area. This study also shows the *Sero-Prevalence* rate of *Cryptosporidium* infection based on gender among HIV-positive participants to be 42% in males and 58% in females. The distribution of *Cryptosporidiosis* based on associated risk factors (Borehole, well water and River water) with participants recording 3(2.7%), 8(7.3%) and 0(0.0%) who used Borehole, well water

and River water respectively. Also, there is a strong association between CD4 count and infection of *C. parvum*. Improved health education will improve the quality of life of people living with HIV and protect the patient from *C. parvum* infection.

Implication of the Study

Cryptosporidiosis in immunocompetent hosts is usually a self-limited diarrheal illness, but in immunosuppressed individuals, such as those with human immunodeficiency virus (HIV) infection, the disease can be severe and life-threatening. In HIV-infected individuals, *Cryptosporidium* is the most frequent diarrhoea-causing microbe, which usually causes chronic bulky and intermittent diarrhoea with liquid non-bloody stools, accompanied by abdominal pain and as well as noticeable loss of weight. Fluid loss in AIDS patients is often extreme, with 3-6 litres of watery stool per day and as much mucus as 17 litres per day.

Recommendations

1. There is a need for policymakers to cooperate with *Cryptosporidium* screen tests as routine in the management of HIV/AIDS patients for better management and epidemiological data
2. There is a need for continued efforts in this area, especially more studies in the area of vaccine development against this disease, since no vaccines have been discovered yet, and there are new strains wild type, that are continuously being discovered as seen in this study.

Conflict of interest

There is no conflict of interest between the authors of this manuscript

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