SERO-PREVALENCE OF CRYPTOSPORIDIOSIS AMONG HIV/AIDS INFECTED INDIVIDUALS IN KANO, NORTH WESTERN NIGERIA

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ABSTRACT
Background: *Cryptosporidium* sp 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 10% in developed countries.
Aim: The study was aimed at determining the sero-prevalence of cryptosporidiosis among HIV/AIDS infected individuals in Kano.
Methods: A structured questionnaire was used to collect data from 90 participants. A total of 90 blood samples were collected from the participants Serological Analysis of blood was done using Human *Cryptosporidium parvum* (CP) ELISA detection test Kit. (Human *Cryptosporidium parvum*) from Melsin Medical Co., Limited, China LOT NUMBER: 20191023), and CD4 Cells count was performed. Data obtained was evaluated using SPSS version 20.0 package
Results: Out of the 90 participants, 14(15.55%) were found to be positive with *Cryptosporidium parvum* and 76(84.4%) were negative with an overall prevalence of 15.55% *Cryptosporidium* infection was more in males with a percentage distribution of 53.3%, than female participants with percentage distribution of 46.7%. Patients between age group 26-35 years were more affected with a percentage distribution of 43.3% and having a high percentage distribution of 41.1% among those who attended secondary school. 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 prevalence of ART duration was found to be higher in the range of 6-10days at (p=0.999). The association of *Cryptosporidium parvum* and CD4+ count of the participants was determined using chi square test in which it was statistically insignificant (p= 0.409). The higher the CD4+ count the lesser the risk for *Cryptosporidium parvum* infection hence no association since about 80% of the participant had CD4+ count higher.
Conclusion: This study reveals an overall sero-prevalence of 15.6% of *C. parvum* among HIV/AIDS patients and there is no association between CD4+ count and infection of *Cryptosporidium parvum.*
Key Words: Seroprevalence, *Cryptosporidium,* HIV/AIDS, Kano, ELISA, Human

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 the 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. 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, 1976). A review of the first 7 cases of cryptosporidiosis led to the conclusion that it was predominantly a disease of the immunocompromised, (Tzipori, 2008) although later it was also found to be widely prevalent among immunocompetent children (Guerrant, 1997). Over the previous decade, the job of *Cryptosporidium* as an operator of 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. 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 *et al.*, 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 (Smith *et al.*, 1998).

Cryptosporidium species are increasingly being recognized as an important pathogen causing diarrhea 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 in Africa (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 the Milwaukee, Wisconsin, USA, in which an estimated 403,000 people were infected (Mackenzie *et al.,* 1995).

MATERIALS AND METHODS

Study area

This study was conducted at Aminu Kano Teaching Hospital Kano. Kano state is located at the north-western region of Nigeria and lies between latitude $11°N$ and longitude $8°E$ with a total land area of 20,760sq kilometre. It borders Katsina State to North-west, Jigawa State to North-east, Bauchi State to South-east and Kaduna State to South-west.

Research design

This study is a cross-sectional study.

Sample size determination

The sample size was calculated and determined using formula as follows

$$n = \frac{z^2pq}{d^2}$$ (Cochran, 1963)

n=number of samples

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

p=prevalence rate of cryptosporidiosis is 5.6% (Alaribe *et al.,* 1998).

q=1-p

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

$$n = \frac{3.8416x0.04x0.96}{0.0025}$$

n=82.2

Attrition rate = 10% $\quad n = \frac{82.2x10}{100}$

n=8.2

n= 82.2+8.2= 90.2

Study Population

HIV/AIDS patients who attended Aminu Kano Teaching Hospital presenting with diarrhoea were Recruited for the study.

Inclusion Criteria

All HIV/AIDS patients that were positive and who gives their consent to take part in the study were recruited.

Exclusion Criteria

i. HIV/AIDS positive patients that do not give their consent.

ii. Other immuno-compromised patients.

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 approval to conduct this research was obtained from Health Research Ethical Committee of Aminu Kano Teaching Hospital.

Informed Consent

Written and verbal informed consent was obtained from the participants.

Data Collection

Ninety (90) freshly blood samples were collected in EDTA containers. The samples were collected and stored in a refrigerator at 2-8 degree Celsius as directed by ELISA kit manufacturer before being subjected to ELISA for processing at Aminu Kano Teaching Hospital Microbiology Laboratory where the samples were analyzed.

Sample analysis

Detection of Cryptosporidiosis sero-prevalence was carried out using ELISA kit. The detection of Cryptosporidiosis sero-prevalence in the samples was assessed using a commercially available ELISA kit for blood sample. The procedure was carried out according to the manufacturer's instruction.

ELISA Protocol

Procedure: (According to the manufacturer's instructions. All reagents were prepared before starting assay procedure.

All reagents were added in duplicate to the Micro Elisa Strip plate.

Fifty microliters (50µl) of Positive control and Negative control were added to the Positive and Negative well and 10µl of testing sample was also added. Sample diluent of 40µl was added to testing sample well; Blank well was left empty. 100µl 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 apirated and washed four times. Each well was washed by filling with Wash Solution (400µl) using a squirt bottle, manifold dispenser or autowasher. After the last wash, any remaining Wash Solution was removed by aspirating or decanting. The plate was inverted and blotted against clean paper towels. Chromogen solution A 50µl and chromogen solution B 50µl 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 color was changed from blue to yellow. Optical Density was read at 450nm using a microtiter plate.

CD4+ COUNT

Protocol:

The blood sample was placed on a roller mixer for at least 15min for proper mixing. A 20 µL of CD4+ easy count monoclonal antibody was added to the partec test tubes. A 20 µL of blood sample was added and incubated for 15mins in the dark at room temperature (20-30) by mixing at interval. Afterwards, 800 µL of CD4+ easy count-no lyse buffer was added to the tubes and it was shaked gently. The blood samples were analyzed on a cyflowpartec device.

STATISTICAL ANALYSIS

Data obtained was evaluated using SPSS version 20.0 package. Frequency distribution and percentage prevalence of all data were generated. Chi-square test was performed to check for association between dependent variables and independent variables.

RESULTS

Table 1 shows the sero prevalence of *Cryptosporidium parvum* among HIV/AIDS patients**.** Out of the 90 participants recruited for this study only 14(15.55%) were positive and 76(84.4%) were negative, with an overall prevalence of 15.55.

Table 2 shows association of cryptosporidiosis among HIV/AIDS sero positive patients in relation with CD4+ count With regards to association to CD4 count, the participants were grouped into 3 groups. Ten (10) (11.1%) of the 90 recruited for this study had a CD4 counts between the range of 20-200, 65 (72.2%) had a CD4 count between 300-600cells/mm³ while 15 representing (16.7%) had a CD4 counts between 700-1500. *C. parvum* ELISA seropositive were obtained from patients with CD4 count of l between the range of<20-200cells/mm³; 9 (64.3%) of the *C. parvum* ELISA seropositive were obtained from subjects with CD4 counts of 300-600cells/mm³ while 4 (28.6%) other *C. parvum* ELISA seropositive were obtained from subjects with CD4 counts of 700-1500cells/mm³. There is no statistical significance difference ($p > 0.05$) therefore, there is no association of Cryptosporidiosis among HIV/AIDS sero positive patients in relation with CD4+ count. Table 3 shows distribution of socio-demographic data associated with cryptosporidiosis.

Out of the 90 participants in this study, seven 7(7.77%) each for both males and females were found to be infected with *Cryptosporidium*. The Age category of 26–35 was found to have the highest infection 7(7.7%) with 46 – 55 having the least infection 4(4.4%). Those from Hausa ethnic group were mostly infected 6(6.6%) while Fulani 4(4.4%) and other 4(4.4%) ethnic group were least infected. *Cryptosporidium* is highly prevalent among secondary school children 8(8.8%). There is no any statistically significant difference for the distribution of cryptosporidiosis for age, gender, religion, ethnic group and educational status.

Table 1: Sero prevalence of *Cryptosporidium parvum* among HIV/AIDS infected individuals.

Result	**Frequency (n)**	**Prevalence (%)**
Positive	14	(15.55)
Negative	76	(84.4)
Total	90	100

Key: n = frequency. % = percentage

Table 2: Distribution of Cryptosporidiosis among HIV sero positive patients in relation with CD4+ count

CD4 count	**Frequency**	***C. Pavum***	***χ2***	***P value***
20–200	10	1(7.1%)	1.789	0.409
300-600	65	9(64.2%)		
700-1500	15	4(28.5%)		
Total	**90**	**14(100%)**		

Key: $\chi 2$ =chi square test, $P < 0.05$ is significant

Table 3: Distribution of socio-demographic data associated with cryptosporidiosis.

Characteristics	Positive	Negative	*χ2*	*P value*
Age				
15-25	0(0%)	7(7.7%)	3.469	0.325
26-35	7(7.7%)	32(35.5%)		
36-45	3(3.3%)	26(28.8%)		
46-55	4(4.4%)	11(12.2%)		
Total	**14(15.55%)**	**76(84.2%)**		
Gender				
Male	7(7.77%)	41(45.5%)	0.074	0.786
Female	7(7.77%)	35(38.8%)		
Total	**14(15.55%)**	**76(85.3%)**		
Religion				
Islam	10(11.0%)	58(64.4%)	0.607	0.738
Christianity	4(4.4%)	17(18.8%)		
Others	0(0%)	1(1.1%)		
Total	**14(15.55%)**	**76(84.3%)**		
Ethnic group				
Hausa	6(6.6%)	39(43.3%)	0.607	0.738
Fulani	4(4.4%)	22(24.4%)		
Others	4(4.4%)	15(16.6%)		
Total	**14(15.55 %)**	**76(84.3%)**		
Educational status				
Uneducated	1(1.0%)	9(9.9%)	3.027	0.388
Primary	1(1.0%)	19(21.1%)		
Secondary	8(8.8%)	29(32.2%)		
Tertiary	4(4.4%)	19(21.1%)		
Total	**14(15.55%)**	**76(84.3%)**		

Key *χ2* =chi square test, P < 0.05 is significant

DISCUSSION

Cryptospodiosis in patient with the acquired immune deficiency syndrome (AIDS) who also have severely impaired immunity maybe 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 ART and poor hygiene (Arora and Arora, 2009; Ayinmode *et al*., 2014).

Out of the 90 participant recruited in this study, an overall prevalence of 15.55% were positive with *Crysptosporidium* using ELISA which is in line with the study by Soave and colleagues (1997) who found out 20.8% of individuals with HIV to have *Cryptosporidium* antibodies. This is however in contrast to previous reports which showed higher prevalence by Abdulhadi and Gwarzo (2013) 31.8%. This study shows the prevalence rate distribution of *Cryptosporidium parvum* based on sex among HIV positive participants to be 7(7.77%) for males and 7(7.77%) for females which could be attributed to the fact that females are more prone to HIV infections than males, so despite the males were recruited more, or may be due to playing of male children in the gardens and farms outdoor area with soil and animals, which increase the risk of parasite transmission. This is in agreement with a study by Molbak *et al*. (1994) and Park *et al*. (2006).

In this finding, the percentage distribution (43.3%) of those who tested positive for *C. parvum* were within 26-35year age group. This agrees with findings in North-west and in South-west by Erhabor *et al*. (2011) who reported 41.3%. This finding was contrary with established knowledge that infection with *C. parvum* occurs at extreme of age and this was statistically significant.

The occurrence of the infection in participants from households using water closet may be due to improper hand washing after defecating and also before meals and other unhygienic habits such as finger sucking, fingernail nibbling amongst others. This collaborates with the report of Chacín-Bonilla *et al.* (2008) with a ($p > 0.569$).

Cryptosporidium parvum is an opportunistic parasite acquired via drinking contaminated water, the municipal water in the study area despite the high level of education might have contributed to higher prevalence rate. The provision of adequate, clean and uncontaminated piped water cannot be overemphasize both to the general public and most especially the vulnerable groups of the society such as HIV patients, malnourished and sickle cell patients. Chlorinated piped water will help eliminate the organism. This agrees with the reports that contaminated water serves as a major source of *Cryptosporidium* infection for humans Ramirez *et al. (*2004) with a p value of ($p > 0.829$) and Chacín-Bonilla *et al.* (2008) with a p value of ($p > 0.173$).

In this study, there was no relationship between HIV seropositivity, CD4+ counts and *C .parvum*. HIV patients whose CD4 counts were higher and had Cryptosporidiosis fell under the range of 300-600cells/cmm³ (64.2%). This was statistically significant. This compared favorably with previous reported studies Dwivedi *et al*. (2007) and Erhabor *et al.* (2011), to be 55.0%. There was a positive correlation between CD4count 300-600cells/cmm³ and infection with *C. parvum* 65(64.2%). HIV destroys the cell mediated immune system which is provided by the CD4 lymphocytic cell, these lymphocytes when significantly destroyed below 200 predisposes the patients to opportunistic infection and invariably more chance of acquisition of *C. parvum* infection. This is consistent with other findings by Erhabor *et al*. (2011) and in the reports from Northwest Kumurya and Gwarzo (2013) the CD4+ count was not determined.

CONCLUSION

This study reveals an overall prevalence of *C. parvum* among 90 HIV/AIDS patients attending Aminu Kano Teaching Hospital to be 15.55 %. And 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.

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