

Prevalence of Cryptosporidiosis among HIV Seropositive Patients in a Tertiary Health Institution, Nigeria

Dr. Yunusa, Thairu ^{MBBS, MSc., FMCPath}

Dr. Kolade-Yunusa, Hadijat Oluseyi ^{MBBS, FMCRad}

¹ Consultant clinical microbiologist and immunologist Lecturer / Honourary Consultant, Department Of Microbiology And Parasitology, University Of Abuja/ University Of Abuja Teaching Hospital Gwagwalada, Abuja Federal Capital Territory, Nigeria.

² Consultant Radiologist, Department Of Radiology, University Of Abuja Teaching Hospital Gwagwalada, Abuja Federal Capital Territory, Nigeria.

Abstract:

Introduction: Cryptosporidiosis is a neglected disease. At this critical moment in the fight against HIV/AIDS, when the world has made incredible progress in reducing the HIV/AIDS scourge, the fight against opportunistic infections should be encourage especially war against *Cryptosporidium parvum*. Cryptosporidiosis causes prolong or persistent diarrhoea in HIV/AIDS patients and the cost of rehydration and management of such patients is huge, therefore this research set to determine the prevalence of Cryptosporidiosis among HIV seropositive patients attending a tertiary health centre in Nigeria.

Methodology: This is a prospective descriptive cross – sectional study and 182 consecutive adults and children of both sexes between one year and sixty-five years who were HIV seropositive and receiving treatment at Prof Sadiq Wali treatment center in Aminu Kano Teaching Hospital (AKTH), Kano.

Result: The mean age of subjects studied was 26 years \pm 14.7. There were 74 males (40.7%) and 108 females (59.3%). Out of the 74 males investigated for *C. parvum* 21 (28.3%) were positive for the parasite representing 36% of the total seropositive population sampled. Thirty seven (37, 34.2%) females tested positive with the parasite representing 63.7%. Fifty six (56, 56.5%) of the fifty eight *C. parvum* ELISA seropositive were obtained from subjects who presented with mucoid diarrhoea. An isolate rate of 55.0% was obtained from those with CD4 counts of less than 200 cell/cmm, 12 (15.6% *C. parvum* ELISA seropositive subjects were obtained from those with CD4 counts of 200-500 cell/cmm. This was statically significant with $p=0.001$, Pearson Chi-Square= 35.167, positive Spearman Correlation coefficient of 0.340.

Conclusion: The prevalence of Cryptosporidiosis in this study was 31.9%. Improved health education and prompt referral to tertiary health institution will improve the quality of life of people living with HIV.

Keywords: Prevalence, Cryptosporidiosis, HIV seropositive, HIV treatment centre, Nigeria.

I. Introduction

Cryptosporidiosis is an opportunistic infection caused by *Cryptosporidium* species.¹ *Cryptosporidium parvum* is a protozoan parasite that causes gastrointestinal illness and is transmitted by ingestion of oocysts excreted in human or animal faeces and modes of transmission include person to person, animal to person, by exposure to contaminated surfaces, and by ingestion of contaminated food or water.^{1,2,3} Protozoan parasites of genus *Cryptosporidium* were first identified in the stomach of mice by.² The species name *Cryptosporidium parvum* was proposed in 1912 to describe parasites identified in murine intestines.⁴ The first human cases were described only in 1976.⁵ In the early 1980s, large numbers of cases were noted to be associated with the emerging epidemic of acquired immunodeficiency syndrome (AIDS).⁶ *Cryptosporidium parvum*, although, a common parasite in animals, was relatively unknown as a human pathogen before the Milwaukee outbreak in 1993, where an estimated 403,000 residents of the greater Milwaukee, Wisconsin, area with population of approximately 1.61 million people became ill when an ineffective filtration process led to the inadequate removal of *Cryptosporidium* oocysts in one of two municipal water treatment plants.^{1,2} The signs and symptoms of the disease in both patients are the same but the course and severity of the disease in these groups of patients are largely different. It causes a self-limited diarrhoea syndrome in immunocompetent persons while in immunocompromised patients, it causes much more serious clinical signs, being manifested as prolonged debilitating diarrhoea, weight loss, fever, and abdominal pain, with occasional spread to the trachea.⁷

Cryptosporidium species are recognized globally as important causes of diarrhoea in children⁸ and adults and is a leading cause of persistent diarrhoea in developing countries.⁹ In Nigeria; it is a significant cause of diarrhoea. In a study by¹⁰ where a total of 354 patients were examined for the presence of *C. parvum* infection,

diarrhoea was present in 129 of the patients and with 225 of these patients having nodiarrhoea. Among those with diarrhoea, 21 had *C. parvum* while 11 who had nodiarrhoea but had *C. parvum*.¹⁰

The following groups have elevated risk of being exposed to *Cryptosporidium* infection.^{7,9}

- People who swim regularly in pools with insufficient sanitation
- Child care workers
- Parents of infected children
- People who take care of other people with cryptosporidiosis
- International travelers
- People who handle infected cattle
- People exposed to human feces through sexual contact
- People including swimmers, who swallow water from contaminated sources.

Several studies have the implications of Cryptosporidiosis in HIV/AIDS and immunosuppressed individuals and have documented a correlation between CD4 count of <200 cells/cmm and symptomatic cryptosporidiosis. A study by¹¹ in India showed that children with diarrhoea, had a higher isolation rate of cryptosporidiosis that ranged from 1.1% to 18.9%. These lymphocytes are part of the body's immune defensive system and play a key role in cell mediated immunity.¹²

In another study conducted in Tanzanian where several opportunistic parasite such as Cryptosporidiosis, microsporidiosis, and cyclosporiasis were analysed in HIV patients, *Cryptosporidium* was the most frequently occurring parasite with *Cyclospora* being the least.¹³

Intestinal parasites are endemic in many regions of the world where HIV/AIDS syndrome is also prevalent of which the Sub-Saharan Africa is among the regions where those intestinal parasitic infections are entrenched.¹ In humans, the disease results in sickness and severe diarrhoea and can be life threatening in either young, elderly or the immunosuppressed individuals, particularly those with HIV infection.⁸

Among HIV patients with diarrhoea, *Cryptosporidium* was found in as many as of children with chronic diarrhoea in developing countries. However, infection was not more frequent in HIV patients during the Milwaukee outbreak.¹⁴ The prevalence of Cryptosporidiosis also varies. In Nigeria, 2.9% was reported in the south-south,¹⁵ 25.0% prevalence reported in Northwestern Nigeria,¹⁶ 4.8% among malnourished children aged 0- 5 years in Jos, Northcentral¹⁷ and 15.1% reported among children aged 0- 14 years in Northcentral Nigeria.¹⁸ 52.7% was obtained in Osun State.^{19,20} found prevalence of *C. parvum* infection ranging from 3% in developed countries to 50.0% in developing countries whereas²¹ reported zero prevalence in Honduras.

In developed countries with low rates of environmental contamination where potent ART is widely available, cryptosporidiosis occurs at an incidence of <1 per 100 person-years among persons with AIDS.²² In less developed countries like Nigeria however, which has high rate of promiscuous defecation, use of human faeces as manure, inadequate water supply, poor sanitary facilities and inadequate ART supplies, Cryptosporidiosis may be difficult to control.^{22,23}

The health and economic impact of acute, chronic, persistent infection in HIV patients or outbreak of infection in the general public is enormous and the global burden of this disease is high considering the fact that there is increased incidence of war and armed conflicts across the world than ever before. Therefore the aim of this study was to determine the prevalence of Cryptosporidiosis among HIV seropositive patients presenting at the Prof Sadiq Wali HIV treatment center in Aminu Kano Teaching Hospital (AKTH), Kano, Nigeria.

II. Materials And Methods

Study background

The study was carried out at the Prof Sadiq Wali treatment center in Aminu Kano Teaching Hospital (AKTH) (HIV treatment center in Kano). AKTH is a 600 bedded tertiary Health institutions serving Kano State and majority of the states in the North West Geopolitical Zone of Nigeria. Kano State is located at latitude 9° 55' N and longitude 8° 53' E and at a laltitude of about 1300 metres (M) (about 4,100 ft) above the sea level. The temperature in the state is generally cold, and the annual rainfall is about 1,300 mm, with an estimated large land mass of about 130,913 square kilometres and a population of 12,178,712 people with population density of 403 per square kilometres, based on 2006 National population census.²⁴ The main occupation of the people is farming while most of the urban populations are traders with polygamous family setting. The adult literacy rate was estimated at 29.1% and 16.5% for males and females respectively.

Study population: Consecutive adult and children male and female between one years and sixty-five years who were HIV seropositive and receiving antiretroviral therapy at the Prof Sadiq Wali treatment center in Aminu Kano Teaching Hospital (AKTH) (HIV treatment center in Kano), presented with symptom of diarrhoea, abdominal discomfort, and nausea consented to participate in the study and were enrolled.

Inclusion Criteria:

- i. Adult and children of both sexes aged one to sixty-five years with symptom of diarrhoea.
- ii. HIV seropositive patients
- iii. Patients on HIV chemotherapy

Exclusion Criteria:

- i. Patient that discontents to be part of the study.
- ii. Patients not on HIV chemotherapy.
- iii. Patients with Chron's disease or irritable bowel syndrome.
- iv. Patient with other concomitted immunosuppressive disorders.
- v. Patients on antibiotics or antiparasitic agents prior to presentation.

Study design

This was a prospective descriptive cross – sectional study which spanned over eight months.

Study Sample Size

The minimum sample size was calculated using the following formula²⁵

$$N = \frac{Z^2 p q}{d^2}$$

Using local prevalence of 2.9%.

P= Prevalence rate of C. parvum is 2.9%.¹⁵

N= 173.0

To take care of invalid sample, 2.5% of the minimum sample size of 173.0 was added to the minimum sample size, thus:

5% of 173.0= 4.3

Hence, 173.0 +4.3 =177.4, approximated to 182.

Sampling Method

Diagnosis was achieved using clinical and laboratory methods to ascertain the HIV status of the patients and using the World Health organisation clinical classification of HIV/AIDs to classify the patients. Stool sample was collected from the patients for analysis. The purpose of this work was explained to the participants before they fill in the consent form. An interviewer-administered, structured questionnaire was used as the study tool. This form contained bio-demographic data, diagnosis and laboratory result.

Laboratory Procedure

Specimen Collection, Transportation And Processing

Stool specimens were collected in universal transparent container with screw cap lid. Blood specimen was also collected in EDTA bottle. The materials for blood specimen collection were: surgical gloves; pressure cuff, 70% alcohol, 2% iodine tincture, sterile swab, sterile needle and syringes and the material for stool collection were universal transparent container.²⁶

Samples were transported to the laboratory. On arrival in the laboratory, safety precautions were observed throughout the period of processing the specimens. The specimens were appropriately labelled and registered with the patient's study number.

Macroscopy: Stool samples were each examined for colour and consistency. Evidence of blood in the stool will be noted and was centrifuge.

Microscopy: A drop of stool was placed on the slide and covered with a cover slip and viewed under high power field of a microscope with iodine background for the presence of ova and parasites. Another faecal smear was also made with a drop of stool and allowed to dry in the air, and stained with Acid-fast stain for evidence of Cryptosporidium cyst which will appear pink or reddish oval shape.^{26,27}

Determination of HIV: All the patients that enrolled for the study were screened at the counseling and testing units. Finger pricks were used to aseptically collect blood samples for HIV serology, using HIV rapid test kits. They were either used singly or in combination with other test kits. The positive results were repeated with other test kits to confirm their HIV positive status. The former gave room for participants to supply information on the type of medication (if any), source of the drugs, and duration of administration and hygiene ethics.

Formaline ether concentration technique for stool examination

The modified formol-ether concentration method by use of sedimentation technique as described by³⁷ was used so that individuals with light infections that could otherwise not be detected by direct saline wet mount method could as much as possible be detected. Materials used included stool specimen, Binocular microscope, laboratory coat, hand gloves, 10% formol water, screw cap bottles, centrifuge tubes, 40mm sieve gauge, diethyl ether (ethyl acetate), slides and cover slips.²⁶

Procedure: Four (4) cm³ of 10% formol water was transferred into a screw bottle with the aid of a Pasteur pipette. Using an applicator stick, an estimated 1g (pea-size) of stool was emulsified in the formol water in the bottle. Another 4ml of formol water was added. The bottle was capped and shaken vigorously to mix. A centrifuge was taken and a cone of 40mm sieve gauge was inserted on its mouth. The emulsified stool in the screw cap bottle was filtered through the sieve gauge into the centrifuge tube. The volumes of the tubes centrifuged were leveled up by adding 3-5ml diethyl ether. The tubes were corked with cotton wool and taken into the centrifuge that was set at 2000 revolution per minute (rev/min) for 5-minute to concentrate the parasite present in the 10 tubes at a time therefore, 10 specimens each were prepared in the above manner at each time of centrifugation. After centrifugation, the centrifuge tubes were brought out and each was carefully inverted to discard the ether, fiscal debris and the formol solution. The bottom of each of the tubes was tapped to re-suspend the sediment. The above procedure was repeated for a second time before the specimen was examined under the microscope. Using Pasteur pipette, a drop of the sediment was made on a clean grease free slide. The drop was then stained with lugol's iodine solution to identify the cysts, ova and larvae of parasites as previously done.²⁰

Microscopic Examination (Modified Ziehl-Neelsen)

Stool samples were collected and smeared on a clean glass slides, followed by fixation with 95% absolute methanol and stained using the modified Ziehl-Neelsen stain and air dried. Afterwards the smear was further stained with cold carbol fuchsin and allowed to stand for 10 minutes after which it was washed off with clean tap water. The smear was decolorized with 3% hydrochloric acid (HCl) in 95% ethanol, rinsed off and counterstained with 0.25% weight per volume malachite green for 30s. The smear was, again, washed off with clean tap water and air dry. The slide was then observed microscopically for oocysts. A confirmed positive specimen was used as quality control slide and for comparative purposes.^{28,29,30}

Determination of Cryptosporidium Antigen via ELISA

The kit produced by DIAGNOSTIC AUTOMATION, INC. CALABASAS, USA. Break off to expose the wells needed (number of samples plus 2 for controls) and place in holder. Two (2) drops of negative control was added to well no 1, 2 drops of positive control added to well no 2. Two (2) drops of the stool supernatant was added to each test well. Then incubated for 30 minutes at room temperature (15-25°C), then washed. Two 2 drops of blue solution of reagent 1 was added to each well. It was incubated for another 5 minutes, then washed. Two (2) drops of Reagent 2 (red solution) was added to each well, and incubated for 5 minutes, then washed. Two (2) drops of Chromogen was also added to each well and incubated for 5 minutes. Finally, drops of Stop solution was added to each well and mixed by gently tapping the side of the strip holder with index finger and the result read visually or at 450/620-650nm. Interpretation of result was done using ELISA Reader. Zero reader on air. Wells read at 450/620-650nm. Absorbance reading of 0.15 OD units and above indicated that the sample contained Cryptosporidium antigen and so such results were recorded as REACTIVE.

Absorbance reading less than 0.15 OD units indicated that the samples did not contain detectable level of Cryptosporidium antigen and so recorded as Non-reactive.

Data Analysis: Data were analysed using SPSS 19.0 software. The chi square-test and Fischer exact test was used to perform and establish any statistical difference. Probability values of <0.05 was considered as statistically significant.

Ethical Considerations: The study was approved by the Ethical Committee of Aminu Kano Teaching Hospital, Kano.

III. Results

This study was carried out among 182 children between the ages of one month and seventy years. Samples were collected from both children and adult who were HIV seropositive and none withdrew after consenting to the study. There were 74 males (40.7%) and 108 females (59.3%) and the male to female ratio (M:F) was 1:1. The mean age of children studied was 26 ± 14.7years, with the highest proportion within the age range of 21-30 years accounting for 58.0% of the study population and the lowest proportion being 61-70 years accounting for 4.0%. However, this distribution was statistically significant (P =0.001, Table 1).

From the 182 subjects, the prevalence of cryptosporidiosis among HIV seropositive clients attending Sadiq Wall centre was 31.9% (a total of 58 subject were positive via ELISA methods). Of the 58 isolates, 10 tested positive from the 0 – 10 years age group giving a prevalence rate of 40.0%; 11 (28.2%) from the 11-20 years age group; 17 (29.3%) from the 21-30 years age group; 12 (41.3%) from the 31-40 years age group; 6 (40.0%) from the 41-50 years age group and 1 each from the 51-60 years and 61-70 years age groups, giving a prevalence of 8.3% and 2.5% respectively. (Table 1). Out of the 74 males investigated 21 (28.3%) were positive with *C. parvum* parasite representing 36% of the total seropositive population while 37 (34.2%) females tested positive with *C. parvum* representing 63.7%, this was not statistically significant with Chi-Square test of 0.699(p= 0.403, Table 2)

Among the 182 individual studied 61 (33.5%) and 52 (28.6%) were artisans and traders, 23 (12.6%) were civil servants and 22 (12.1%) were full time house wives. 16 (8.8%) were students and 8 (4.4%) were retired individuals; Of the *C. parvum* seropositivity in this study, 43 ELISA seropositives were individuals who were artisans, giving a prevalence rate of 70.4%, 5 were civil servants with prevalence rate of 21.7% while another 5 ELISA seropositives of the patients were fulltime housewives with a prevalence rate of 22.7%. Two and 3 ELISA positive results were obtained from students and retired individuals giving a prevalence rate of 18.7% and 25.0% respectively. No positive isolate was obtained from traders. These seropositive rates were not statistically significant (p>0.05, Table 3).

In terms of symptom of diarrhoea, 99 (54.4%) of the 182 recruited patients for the study presented with nonmucoid diarrhoea, 60 (33.0%) HIV seropositive subjects presented with no symptom of diarrhoea and 23 (12.6%) presented with bloody diarrhoea. 56 (56.5%) of the fifty-eight *C. parvum* ELISA seropositives were obtained from subjects who presented with nonmucoid diarrhoea while two (3.3%) of the ELISA seropositives patients were subjects who had no diarrhoea and none of the individuals who presented with bloody diarrhoea tested positive with *C. parvum* using ELISA. This was statistically significant (p=0.001, Table 4). Those who drank from the municipal pipe water supplies were 72.7% with positive *C. parvum* isolation rate, while none of those who drank well water tested positive for *C. parvum*. Three subject and 22 (42.3%) of the subjects who tested positive for *C. parvum* derived their drinking water from boreholes and satchet water sources respectively. This was not statistically significant (p=0.091, Table 5).

In relation to the CD4 counts, 69 (37.9%) of the 182 recruited for this study had CD4 counts of less than 200cells/cmm, 77 (42.3%) HIV seropositive subjects had CD4 counts of between 200 and 500cells/cmm while 36 representing (19.8%) had CD4 count of greater than 500cells/cmm. Of this, 38 (55.0%) *C. parvum* ELISA seropositives were obtained from subjects with CD4 counts of less than 200cells/cmm; 12 (15.6%) of the *C. parvum* ELISA seropositives were obtained from subjects with CD4 counts of 200-500cells/cmm while 8 (22.1%) other *C. parvum* ELISA seropositives were obtained from subjects with CD4 counts of greater than 500 cells/cmm. These differences were statistically significant (p=0.001, Pearson Chi-Square= 35.167, df= 2, positive Spearman Correlation= 0.340, Table 6). The distribution of age in relation to the CD4 cells was analysed using the average cells count, the lowest average CD4 cell was distributed in the vulnerable age groups, 220 cell/cmm within 0-10 age group and 203 within the 61-70 age group, 554 cells/cmm in the 21-30 age group while the average cells of 519 and 460 cell/cmm were observed in the 41-50 and 11-20 age group respectively. This was statistically significant (p=0.001, Table 1).

In comparing the two methods used in the identification of the *C. parvum* parasite among the HIV seropositive patients, 58 isolates were reported using the ELISA method from the 182 samples analysed this gave a prevalence of 31.8% while a lower isolation rate was observed using the Ziehl neelsen acid-fast method with 49 specimen reported positive with a prevalence rate of 25.2%.

Table 1: Age distribution of Cryptosporidium Parvum among the HIV seropositive patients in Kano.

Age group (Years)	Total	Percent (%)	C. Parvum positive	Percent (%)	Average CD4 count (cell/cmm)
0 – 10	25	13.7	10	40.0	220
11 – 20	39	21.4	11	28.2	460
21 – 30	58	31.9	17	29.3	554
31 – 40	29	15.9	12	41.3	380
41 – 50	15	8.2	06	40.0	519
51 – 60	12	6.6	01	8.3	306
61 - 70	4	2.2	01	2.5	203
Total	182	100.0	58	31.9	

P =0.001 df=6

Table 2: Gender Distribution of Cryptosporidium parvum among HIV seropositive patients Kano.

Gender	Total	Percent (%)	C. parvum	Percent (%)
Male	74	40.7	21	28.3
Female	108	59.3	37	34.2
Total	182	100.0	58	31.9

p= 0.403 $\chi^2 = .699$ df= 1

Table 3: Occupational distribution of Cryptosporidium parvum among HIV seropositive patients in Kano

Occupation	Total	Percent (%)	C. Parvum	Percent (%)
Artisan	61	33.5	43	70.4
Traders	52	28.6	0	0.0
Civil Servants	23	12.6	5	51.7
Fulltime housewives	22	12.1	5	22.7
Students	16	8.8	2	18.7
Retired	8	4.4	3	25.0
Total	182	100.0	58	31.9

Table 4: Distribution of Cryptosporidiosis among HIVseropositive patients with diarrhoea in Kano.

Symptom	Frequency	Percent (%)	C. parvum	Percent (%)
Diarrhoea	99	54.4	56	56.5
Bloody diarrhoea	23	12.6	0	0.0
No diarrhoea	60	33.0	2	3.3
Total	182	100.0	58	31.9

Table 5: Source of water supply withCryptosporidium parvum among HIV seropositive in Kano.

Source of Water	Total (%)	Percent positive	C. parvum	Percent (%)
Surf water	44	24.2	17	38.6
Piped	22	12.0	16	72.7
Well	52	28.6	0	0.0
Borehole	12	6.6	3	25.0
Satched	52	28.6	22	42.3
Treated water	0	0.0	0	0.0
Total	182	100.0	58	31.9

Table 6: Distribution of Cryptosporidiosisamong HIV seropositive patients in relation with CD4⁺ count in Kano.

CD4 Count	Frequency	Percent (%)	C. parvum	Percent (%)
< 200	69	37.9	38	55.0
200-500	77	42.3	12	15.6
>500	36	19.8	8	22.1
Total	182	100.0	58	31.9

IV. Discussion

The prevalence of Cryptosporidiosis among HIV seropositive presenting at the Prof Sadiq Wali HIV treatment center in Aminu Kano Teaching Hospital (AKTH) was 31.9%. This figure in the current study varies with findings in other parts of Nigeria,^{16,17} and that in other parts of the world.²¹

The overall prevalence of 31.9% of C. parvum in this study is higher than studies in Nigeria and other part of the world. In Nigeria the prevalence was higher than 0.0 % prevalence among 161 HIV patients reported by³¹ in southeastern Nigeria, 2.9% among 105 HIV-infected adults reported by¹⁵ in the south-south, 25.0% prevalence reported by¹⁶ in northwestern Nigeria and 4.8% among malnourished children reported by¹⁷ in

Northcentral Nigeria. The prevalence in this study is higher than that reported by²¹ in Honduras, who did not detect *C. parvum* among the 133 HIV infected patients. The differences may be due to the fact that the prevalence of *C. parvum* varies remarkably among regions of the world as well as among communities depending on the level of contamination of piped and drinking water with human and animal excreta. In this study, the municipal water supply from waterboard was inefficient accompanied by high rate of pipe water damages which allowed for contamination water from outside the pipe to enter the pipe because of reduced pressure inside the pipe. Above 70.0% of the population depend on pipe water as their main source of water supply. The high prevalence rate might also be due to poor sanitation and inadequate sanitary facilities, couple with indiscriminate defecation of faecal matters which facilitate contamination of edible vegetable food and drinking water. In this study patients on antiparasitic agents were excluded unlike the study reported in southeastern and south-south Nigeria but those two reports show apparent rarity of *C. parvum* in the southern part of Nigeria.

In Nigeria the prevalence obtained in this study is lower than a study done in south-west Nigeria, where the value of 52.7% was obtained.¹⁹ Other lower prevalence reported by²⁰ *C. parvum* infection was up to 50.0% in developing countries. These differences observed may be due to the methodology of parasite detection. In this study, ELISA method was mainly employed while in the research¹⁹ in south-west Nigeria Ziehl Neelsen was used. In this study higher isolation rate was observed with ELISA method than with Ziehl Neelsen method. Although the latter was the gold standard it has several drawbacks such as interobserver error, colour blindness and turnaround time which were eliminated in this study by the ELISA method. This finding was however contrary to the finding by Yemisi and colleagues¹⁹ who got a much higher isolation rate of (52.7%) using Ziehl neelsen method compared to the isolation rate in this study using ELISA method (31.9%).

C. parvum is an opportunistic parasite acquired via drinking contaminated water, the municipal water supply 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 sickled cell patients. Chlorinated piped water will help eliminate the organism. No organism was isolated from HIV patients who depend on treated water as a source of their drinking water.

From this study, more than 60% of those who tested positive with *C. parvum* were within the 11-20, 21-30, and 31-40 years age groups. This findings was contrary with established knowledge that infection with *C. parvum* occurs at extreme of age, but the presence of HIV virus in those individual might have weaken the immunity at any age and this was statistically significant. This finding was consistent with findings¹⁶ in Northwest and¹⁵ in south-south. In both studies *C. parvum* infection was found mainly between those aged 16 and 45 years. There was a relationship between the average CD4 count and the development of cryptosporidiosis, children between the 0-10 age group had low average CD4 count of 220cell/cmm and recorded an isolation rate of 40.0% but those in the 21-30 age groups who had cell count of 554cell/cmm recorded an isolation rate of 29.3%. The higher isolation rate in children might be due to both the age limit and reduced CD4 count. This is consistent with finding by¹⁷ in northcentral and whose study was limited to under five while study in south south¹⁵ and southeast³¹ excluded children in their study.

This study recorded high prevalence rate among female than their male counterparts, although this was not statistically significant. This was consistence with other¹⁶ but at variance with some other reports^{19,20}. This higher prevalence of females (63.7%) may be attributed to the fact that polygamous family settings is highly encouraged with higher number of females infected with HIV thereby increasing the chances of more females acquiring opportunistic infection such as *C. parvum* parasite.

In this study, the predominant occupation of people living with HIV investigated in this study was artisans and traders. This was not statistically significant. This findings was consistent with other finding and support the fact that *C. parvum* is more likely isolated from low socioeconomic status like artisans. Traders in the area of study are generally wealthier than an average citizen of the state and more likely to be refining in what they eat and drink. Although the trader may be the least inform person in the area.

In this study, larger proportion of those investigated had nonmucooid and bloody diarrhoea while the rest had no diarrhoea (33.0%). *C. parvum* was a significant cause of diarrhoea in these HIV positive patients since virtually all the individuals who tested positive for *C. parvum* had diarrhoea (56 vs 58). The prevalent type of diarrhoea seen in this study was nonmucooid diarrhoea. *C. parvum* was not a cause of bloody diarrhoea in HIV positive patients in this study. The isolation rate in this study was higher but consistent with other studies. The mechanism by which *C. parvum* cause diarrhoea is not know and it is unlikely to involve inflammatory responses leading to bloody diarrhoea but the extent of diarrhoea might depend on the part of intestine involved.

In this study, there was a relationship between HIV seropositivity, CD4 counts and *C. parvum*. HIV patients whose CD4 counts were higher than 500cells/cmm and had Cryptosporidiosis were negligible (8 out of 58) compare to those whose CD4 counts were less than 200cells/cmm (65.6%). This was statistically significant. This compared favorably with previous reported studies^{12,15}. There was a positive correlation between CD4

count <200cells/comm and infection with *C. parvum*. 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 findings¹⁵ and In the reports from Northwest¹⁶ the CD4 count was not determined.

V. Conclusion

C. parvum infection rate was 31.9%, higher infection rate in females, in the younger age group and Artisans. The predominant symptom was nonmucoid diarrhoea and there is a strong association between CD4 count and infection of *C. parvum*. Improved health education and prompt referral to tertiary health institution will improve the quality of life of people living with HIV and protect the patient from *C. parvum* infection.

Recommendation

1. That all individuals living with HIV should have a routine examination for *C. parvum* infection, this should also include laboratory examination.
2. HIV patients should be encouraged to chlorinate their drinking water.

Acknowledgment

No any financial support received in any form

No conflict of interest

Acknowledge the contribution of my research assistance: Gabriel Adelus.

References

- [1]. Michael JG, Ana-Maria C and Kelly P. Intestinal Protozoa. In: C. Gordon and Z. Alimuddin, eds. *Manson's Tropical Diseases*, 22nd ed. United Kingdom Saunders Elsevier, 2009: 1397-1406.
- [2]. Miller K. *Encyclopedia and Dictionary of Medicine, Nursing and Allied Health*, 2nd ed. UK Saunders imprint, 2003: 375.
- [3]. Casemore DP. Human cryptosporidiosis. In: DS Reeves, AM Geddes, eds. *Recent advances in infection*, no 2. Edinburgh Churchill Livingstone, 1989: 209-36.
- [4]. Tyzzer EE. *Cryptosporidium parvum* a coccidium found in the small intestine of the mouse. *Arch Protistenkunde.*, 1912, 26: 394 – 398.
- [5]. Slavin D. *Cryptosporidium meleagridis* (spnov). *J. Comp. Pathol.* 1955, 65: 262 – 266.
- [6]. Current WL, Reese NC. and Ernst JV. Human cryptosporidiosis in immunocompetent and immunodeficient persons: Studies of an outbreak and experimental transmission. *N Engl J Med.* 308: 1993, 1252-1257.
- [7]. Chen XM, Keithly JS, Paya CV. and LaRusso NF. "Cryptosporidiosis,". *The New England Journal of Medicine.* 2002, 22: 1723 – 1731.
- [8]. Siobhan MM, and Saul T. Cryptosporidiosis in Children in Sub-Saharan Africa: A Lingering Challenge. *Clin Infect Dis*, 2008, 47: 915 – 921.
- [9]. Lorenza P, and Donato M. Global Distribution, Public Health and Clinical Impact of the Protozoan Pathogen *Cryptosporidium*. *Interdisciplinary Perspectives on Infectious Diseases*, 2010, 10: 1155 – 1194.
- [10]. Igbe MA, Ajayi J, Anyanwu G, Igwillio C, Ameh C. and Nwoke N. *Cryptosporidium parvum* In Patients With And Without Diarrhoea In Abuja, Nigeria. *The Internet Journal of Health*, 2012, 13: 112.
- [11]. Ajjampur SS, Sankaran P and Kang G. *Cryptosporidium* species in HIV-infected individuals in India. *National Medical Journal India*, 2008, 21(4): 178 – 84..
- [12]. Dwivedi KK, Prasad G, Saini S, Mahajan S, Lal S. and Baveja UK. Enteric opportunistic parasites among HIV infected individuals: associated risk factors and immune status. *Jpn J Infec Dis.* 2007, 60(2-3): 76-81.
- [13]. Cegielski JP, Ortega YR, McKee S, Madden JF, Gaido L, Schwartz DA, Manji K, Jorgensen AF, Miller SE, Pulipaka UP, Msengi AE, Mwakyusa DH, Sterling CR. And Reller LB. *Cryptosporidium* enterocytozoon, and *Cyclospora* infections in pediatric and adult patients with diarrhea in Tanzania. *Clin Infect Dis.*, 1999, 28(2): 314 – 21.
- [14]. White AC Jr. *Cryptosporidium* specie. In: G. Mandell, J. Bennet and E. Dolin (eds). *Principle and Practice of infectious diseases*, 7th ed. Philadelphia Churchill livingston 2010: 3547.
- [15]. Erhabor O, Obunge O. and Awah I. Cryptosporidiosis among HIV-infected persons in the Niger Delta of Nigeria. *Niger J. Med.*, 2011, 20 (3):372-375
- [16]. Kumurya and Gwarzo. Prevalence of Cryptosporidiosis among patient with Human immunodeficiency virus (HIV)/ acquired immunodeficiency syndrome (AIDS) in Northwestern Nigeria. *Journal of AIDS and HIV Research*, 2013, 5(8).301-305.
- [17]. Banwat EB, Egah DZ, Onile BA, Angyo IA and Audu ES. Prevalence of *Cryptosporidium* infection among undernourished children in Jos, Central Nigeria. *Niger Postgrad Med. J.* 2003; 10: 84-87.
- [18]. Nwabuisi C. Childhood cryptosporidiosis and intestinal parasitosis in association with diarrhoea in Kwara State, Nigeria. *West Afr. J. Med.*, 2001; 20:165-168.
- [19]. Yemisi OA, Rofiat OL, Samuel ST, Sunday AF. and Oluwaseyi AA. Cryptosporidiosis in HIV infected patients with diarrhoea in Osun State Southwestern Nigeria. *Eur. J. Gen. M.*, 2007; 4(3):119-122.
- [20]. Goldstein ST, Juranek DD. and Ravenholt O. Cryptosporidiosis: an outbreak associated with drinking water despite state-of-the art water treatment. *Ann. Intern. Med.*, 1996; 124: 459- 468.
- [21]. Kaminsky RG., Soto RJ, Campa A, Baum MK. Intestinal parasitic infections and eosinophilia in an human immunodeficiency virus positive population in Honduras. *MemInstOswaldo Cruz.* 2004, 99: 773-778.
- [22]. Habib AG, Onyemelukwe GC, Kangave D. Clinical presentation of HIV infection in Northern Nigeria and its relationship to CD4 + T-cell count. *Nigerian Medical Practitioner.* 1998, 35: 3-8.
- [23]. Khumalo NB, Luo NP, Chintu C, Sunkutu R, Sakala-Kazembe F. Gut parasites in HIV-seropositive Zambian adults with diarrhoea. *East Afr Med J*, 1994; 71(6): 379 – 83.
- [24]. The National Population Commission nigeriamuse@gmail.com last updated 10th Nov. 2007 accessed 20th May, 2012.

- [25]. Araoye OA. Research methodology with statistics for health and social sciences. 2nd ed. Ibadan, Nathadex Publishers, 2004: 120.
- [26]. Washington CW, Elmer WK and Williams MJ. Konemans Colour Atlas and Textbook of Diagnostic Microbiology. 6th ed. Baltimore, Lippincott Williams & Wilkins 2006: 431-452.
- [27]. Freeman R. Bacteriology of normally sterile body fluids. In: P.M Hawkey and D.A Lewis (eds). Medical Bacteriology: Practical Approach. England, Oxford University Press 1989: 21-40.
- [28]. Ryan KJ and Ray CG. Cryptosporidiosis, In: Sherri's Medical Microbiology 5thed. McGrawHill 2010: 102-108.
- [29]. Shore Garcia L. Practical guide to diagnostic parasitology 14th ed. ASM press 1999: 349.
- [30]. Casemore DP, Armstrong M, Sands RL. Laboratory diagnosis of Cryptosporidiosis. J Clin Pathol. 1985; 38: 1337-41.
- [31]. Nwokediuko SC, Bojuwoye BJ. And Onyenekwe B. Apparent rarity of cryptosporidiosis in human immunodeficiency virus (HIV)-related diarrhea in Enugu, south-Eastern, Nigeria. Nig Postgrad Med J., 2002, 9: 70-3.