***Cryptosporidium* infection among HIV positive and HIV negative out-patients attending selected HIV care hospitals in Gboko, Benue state, Nigeria**

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Abstract

Globally, *Cryptosporidium* infection continues to be of significant public health importance and one major causal organism of diarrhoea in both immunocompetent and immunocompromised individuals. This cross-sectional study was carried out to survey the prevalence of *Cryptosporidium* infection among HIV positive and HIV negative subjects attending selected hospitals in Gboko Township. A total of 300 stool samples comprising 200 from HIV positive and 100 from HIV negative subjects were collected and analyzed using formol ether oocyst concentration technique and modified Ziehl Neelsen technique. Other information concerning demography and risk factors were obtained with the aid of structured questionnaire. Data was analyzed using chi-square test and P≤0.05 were considered significant. Overall, 122 samples comprising 88 from HIV positive patients and 34 from HIV negative patients were positive for *Cryptosporidium* oocysts with an overall infection prevalence of 40.7%. Males recorded the highest prevalence of 42.9% while age group 50-59 had 50.0%. Patients who presented with watery stool had the highest *Cryptosporidium* infection rate of 81.8% while those who do not wash their hands and others who only wash occasionally after going to the toilet revealed the highest prevalence of 43.8%. Civil servants as well as patients who normally drink water from river/streams had higher prevalence of 45.8% and 60.0% respectively (P>0.05) when

compared with their counterpart. *Cryptosporidium* infection was significantly associated with the consistency of the stool samples ($P < 0.001$). Hence, watery stool is strongly implicated as a sign of *Cryptosporidium* infection among HIV positive and HIV negative subjects in Gboko. Thus, improved sanitation and good personal hygiene should be adopted to avoid infection.

Keywords: *Cryptosporidium* infection, Prevalence, out-patients, Gboko, Benue State.

Introduction

Cryptosporidiosis due to *Cryptosporidium spp* is considered to be one of the most worrisome opportunistic infections which further complicates AIDs disease. Cryptosporidiosis is a major cause of intestinal parasitic infection among people in developing countries especially in immunodeficient individuals (Nassar *et al*., 2017). Human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS) is a deadly disease that severely impairs the immune system. Over the years, HIV/AIDs have continued to pose serious threat to world health (WHO 2023).

The damaging effects exercised on the body immune system by the virus weakens its defense mechanism thereby making it a free entrance for so many opportunistic infectious agents worldwide. Parasitic infections affect the nutritional status of patients culminating to alterations that promote reduction in the efficiency of the immune response thus favoring the occurrence of other numerous pathogens (Haliu *et al*., 2015). Opportunistic infections pose major health issues among HIV positive patients especially during the advance stage of the disease when immune-suppression becomes more severe (Jegede *et al*., 2014). In underdeveloped countries, HIV/AIDS patients continue to suffer the health implications of intestinal protozoa due to their accompanying low CD4 count. Numerous protozoa parasites have been implicated in the morbidity and mortality in HIV/AIDS patients most frequent of which include *Cryptosporidium spp*, *Entamoeba histolytica*, *Microsporidia spp*, and *Isospora belli* (Ajayi *et al*., 2021). *Cryptosporidium species* are important gastrointestinal parasitic pathogens as well as aetiologic agents of diarrhoea illness in humans and animals and have been recognized as the most common opportunistic waterborne pathogen in HIV infected patients (Banjo *et al*., 2013). Initially thought to be caused by single species, molecular studies have shown human illness

to be caused by no less than 20 different species, among which *C.parvum* and *C. hominis* are most frequently reported (Tombang *et al*., 2019) *Cryptosporidium species* are protozoan parasites and are known to inhabit the small intestine and colon of the host with the developing stages associated with the luminal surface of the intestinal epithelial cells where it remains intracellular (Shrihari *et al*., 2011). According to Shinkafi and Muhammad (2017), low dosage of 10-30 *Cryptosporidium* oocysts can cause human illness when ingested were they become intracellular but extracytoplasmic and resistant to treatment

Cryptosporidium is a well-established cause of diarrhea among HIV infected patients worldwide with prevalence of infection ranging from 3 % in advance countries to 50% in resource poor countries (Adesiji *et al*., 2007). Reports have indicated that diarrhoea occur in about 30-60% of HIV infected patients in developed countries and 90% in developing countries. *Cryptosporidium* species which causes cryptosporidiosis are unique pathogenic parasites which affects the gastrointestinal tract of both the immunocompetent and the immunocompromised individuals. The parasite is also found in colon, rectum and oesophagus (Karshima *et al*., 2013).

Different variables like aetiological and environmental factors, new strain of microbe and drug resistance results to new infections, hence increasing the host susceptibility to *Cryptosporidium* infections (Ajayi *et al*., 2021). In immune suppressed individuals, *Cryptosporidium* is the most frequent microbial cause of diarrhoea which leads to chronic, bulky and intermittent diarrhoea with liquid, non-bloody stools accompanied by pain, abdominal colic, vomiting, dehydration, nausea and amnesia (Tamomh *et al*., 2021; Adesiji *et al*., 2007). While infection in immunocompetent host is restricted to the intestine, the infection in immunocompromised host occurs throughout the gastrointestinal tract and within the epithelial cells of the biliary tract, pancreatic duct and the air ways (Gerace *et al.,* 2019). Prolong infection among AIDs patient places them at greater risk of developing extra-intestinal cryptosporidiosis where the infection spread to other parts of the body like biliary, pancreatic and respiratory tracts causing cholangiohepatitis, cholecystitis, choledochitis or pancreatitis. In chronically infected gut, there is the gradual but

profound disorganization of the mucosa which includes disrupted epithelial surface, fibrosis, crypt abscessation and cellular infiltration (Kumar *et al.,* 2018). Despite the seemingly ubiquitous nature of cryptosporidiosis, little attention has been paid to it, prompting WHO in 2004 to list it among globally "neglected diseases" which is commonly linked to poverty in most underdeveloped countries (Tombang *et al*., 2019).

Cryptosporidium oocysts are highly infective and can be easily transmitted from an infective source to a healthy source through the faecal oral route or via food, water, soil and on surfaces that have been contaminated by feacal matter from infected individuals or animals (Shinkafi and Muhammad, 2017; Tamomh *et al*., 2021). Communal eating habit, contaminated vegetables and fruits, poor sanitation, poor education, poor hygiene practices, poor housing, ignorance, oral and anal sex, poverty, climate, wilderness travel with ingestion of contaminated food and water, exposure to infected animals as well as close contact with infection sources such as nursery schools, prisons and mental homes as well as day care centers have also been implicated in the transmission of these protozoa parasite (Petri and Singh, 2006; Arora and Arora, 2009). Other important predisposing factors for transmission include deficient immunologic factors such as immunoglobulin A (IgA) and T-cell responses (Atu *et al*., 2014)

In Benue State, north central Nigeria, numerous hospital and community based studies conducted over the years has reported various opportunistic parasitic pathogens affecting the general populace. Poor sanitary conditions, lack of good personal hygiene and decreased immunity due to other diseases such as HIV/AIDS observed in Benue state and as documented by the federal ministry of health (FMOH) in 1999 has further aid the easy transmission of opportunistic infections in the state. However, epidemiological data is grossly inadequate on one of the major opportunistic infections; Cryptosporidiosis in Gboko Local Government Area of the State. This research work thus, seeks to determine the prevalence of *Cryptosporidium* infection among HIV/AIDS positive and negative subjects in Gboko local government area of Benue state.

Materials and methods

Study Area: The study was carried out on patients attending Nongu u kristu u Sudan hen tiv (NKST) hospital, Mkar, Comprehensive Health Care Centre, three brothers' transport (TBT) hospital and General hospital Gboko. Gboko Local Government Area which is also the traditional seat of the Tiv nation, is one among the 23 Local Government Areas in Benue State and was created in 1970 when the Tiv division was split into Gboko, Katsina-Ala and Makurdi. Gboko is situated in the north eastern part of the state and covers a land mass of about 12,744 square kilometers. It lies between latitudes 63º and 81º north of the equator and longitudes 8º and 10º east of the Greenwich meridian. The landscape is guinea savannah, with a typical vegetation and climate which is tropical with a gentle and undulating land mass. Geographical boundaries shows that Gboko is bounded to Tarkaa on the north, Konshisha on the south, Gwer and Buruku on the west.

Study Design: The study was cross-sectional hospital-based research conducted in NKST hospital Mkar, Comprehensive health care center, TBT and General hospitals in Gboko Local Government Area of Benue State, Nigeria between November 2015 and January 2016.

Study Site Permission: Permission to conduct this study in the four hospitals sampled was obtained by writing to the head of the various hospitals. Subsequently, permission was also received from the unit heads of the laboratory and HIV/AIDS in the four hospitals.

Informed Consent: Informed consent was sought from the patients after a clear explanation of the study was given to them. Consent was also obtained from care givers or relatives of patients who were incapable of consenting. Information generated from patients in the study was kept confidential and used strictly for the purpose of this research. Patient identities were not disclosed and treated with great confidentiality.

Selection of Hospitals: The hospitals were selected for this study based on their status as renowned HIV care hospitals in the study area as well as on the bases of government, private and missionary hospital status.

Inclusion/Exclusion Criteria: All out patients who gave consent and were able to produce stool specimen at the time of hospital visitation were included in the study.

However, patients who did not consent and those who could not provide stool samples were excluded from the study.

Sample Collection: Patients were given properly labelled, grease free, sterile wide mouthed plastic specimen bottles and instructed to collect their stool samples into the bottles. The samples were then carefully arranged in Giostyle cool box containing icepacks and transported immediately to the laboratory of the Biological Sciences Department, Benue State University, Makurdi, for parasitological analysis. Patients were also given structured Questionnaires to obtain other vital demographic information. The questionnaires were numbered to tally with the number indicated on the sample containers given to each patient.

Sampling Procedure and Sample Size: Patients attending these hospitals within the study period between November 2015 and January 2016 were eligible for the study. Participants were briefed on the relevance of the survey. Counselling was done and consented participants willingly received sample containers in which they added their faecal specimen for laboratory analysis. Distribution of the sample containers was subject to availability of patients. Hence, 104 samples were collected from NKST hospital Mkar, 130 samples from Comprehensive health care center, 40 samples from TBT and 26 samples from General hospital in Gboko. A total of 300 patients were sampled for this study. Out of these, 200 samples were collected from HIV positive subjects while 100 samples were collected from HIV negative subjects that visited Clinics and hospitals for counselling and testing, check-up and receipt of anti-retroviral drugs. The status of the HIV negative patients was confirmed by the various hospitals' medical laboratory scientists.

Laboratory Analysis: The methods involved in the laboratory analysis were formol ether oocyst concentration technique and modified Ziehl Neelsen staining method. The procedures were performed as described by Cheesbrough, (2009).

Result Analysis: Data collected from the stool analysis and the questionnaire was subjected to statistical analysis using SPSS version 20. Prevalence rates were calculated and expressed as percentages (%) for all the variables. Chi square test (χ^2) was used to test for difference in prevalence rates among the variables and p- values < 0.05 were

considered significant while p- values > 0.05 were regarded as not significant.

Results

Out of the 300 stool samples examined, 122 were positive for cryptosporidiosis with a prevalence of 40.7%. The socio-demographic characteristics of the participants is usually key in prevalence studies. These present the base upon which various factors are considered as possible cause(s) of parasitic infection under study. For this study, the various socio-demographic characteristics of the patients that were examined are as outlined in "table 1" below:

Table 1: Socio-demographic characteristics of the study patients.

Variables	**No Examined**
HIV Status	
HIV Positive	200
HIV negative	100
Sex	
Male	105
Female	195
Age (years)	
0-9	32
10-19	33
20-29	78
30-39	75
40-49	44
50-59	28
>60	10
Occupation	
Civil servants	24
Farmers	116
Business	73

Students	74
Others	13
Stool consistency	
Formed	116
Semi-formed	84
Unformed	56
Watery	44
Source of drinking water	
Tap	102
Well	183
River/stream	15
Do you wash your hands after going to toilet	
Yes	147
No	64
Occasionally	89

According to their HIV status, the infection was found to be more prevalent among HIV positive subjects with 44.0% while HIV negative subjects had a lower rate 34.0 %. Gender wise, Males recorded the highest prevalence of 42.9% compared to the 39.5% by females. However, the result was not statistically significant (p=0.571). *Cryptosporidium* infection was found to be highest between the ages of 50-59 years (50.0%) and lowest between the age range of 20-29 with 34.6% (Table 2).

Table 2: Prevalence of *Cryptosporidium* infection among patients according to their HIV status, Sex and Age

Variables	No Examined	No Infected (%)	X^2	P-value
HIV Status				
HIV positive	200	88(44.0)	2.763	0.096
HIV negative	100	34(34.0)		
Sex				
Male	105	45(42.9)	0.321	0.571
Female	195	77(39.5)		
Age (in years)				
0-9	32	13(40.6)	2.971	0.813
10-19	33	13(39.4)		
20-29	78	27(34.6)		
30-39	75	34(45.3)		
40-39	44	17(38.6)		
50-59	28	14(50.0)		
>60	10	4(40.0)		

With regard to their Occupation, *Cryptosporidium* infection was found to be highest among the civil servants with a prevalence rate of 45.8 %. According to stool consistency, patients with watery stool presented highest prevalence rate of 81.1%. There was a significant association between stool consistency and *Cryptosporidium* infection (p=0.000). Base on their source of drinking water, subjects who drank from river/streams recorded the highest infection prevalence of 60.0%. Patients who did not wash their hands after going to the toilet revealed higher prevalence of 43.8% similar to those who wash occasionally (Table 3).

Table 3: Prevalence of *Cryptosporidium* infection among patients according to their occupation, stool consistency, source of drinking water and hand washing habits

Variables	No Examined	No Infected (%)	X^2	P-value
Occupation				
Civil servants	24	11(45.8)		
Farmers	116	46(39.7)		
Business	73	32(43.8)		
Students	74	25(37.8)	0.890	0.926
Others	13	5(38.5)		
Stool consistency				
Formed	116	20(17.2)		
Semi-formed	84	36(42.9)		
Unformed	56	8(53.6)		
Watery	44	36(81.8)	61.294	0.000
Source of drinking water				
Tap	102	47(46.1)		
Well	183	66(36.1)		
River/stream	15	9(60.0)	5.167	0.075
Do you wash your hands after going to toilet				
Yes	147	55(37.4)		
No	64	28(43.8)		
Occasionally	89	39(43.8)	1.263	0.532

Discussion

The overall prevalence of 40.7% *Cryptosporidium* infection recorded in this study is high and shows that *Cryptosporidium* infection is endemic in Gboko. The result of this research is higher than the findings of Tamomh *et al*. (2021), Tombang *et al*. (2019), Nassar *et al*. (2017), Atu *et al*. (2014), Karshima *et al*. (2013) and Shrihari *et al.* (2011) that reported prevalence rates of 17.3%, 8.9%, 38.3%, 14.1%, 27.0% and 32.69% in Sudan, Cameroon, Osun state, Benue state, Plateau state, Nigeria and India respectively. This overall prevalence is however lower than the 62.5% *Cryptosporidium* infection prevalence in Wamakko local government area of Sokoto State reported by Shinkafi and Muhammad (2017). The observed disparity can be attributed to the large gap in sample size used in these studies. The prevalence 44.0% observed in HIV positive subjects though not statistically significant was higher than the 34.0% observed in HIV negative subjects. This can be explained by the low-level immunity due to reduced CD_4 T helper cells associated with these immunosuppressed individuals which makes them susceptible to parasitic infections. This HIV positive rate is however lower than that reported by Adesiji *et al.* (2007) 79% in Osun state while in contrast, the 34% HIV negative prevalence rate is higher than the 6% reported by Jegede *et al*. (2014) in Kano, Nigeria. The result is also lower than the 46.8% reported by Nassar *et al*. (2017) who used Enzyme Linked Immunosorbent Assay (ELISA) diagnostic method. The variations in prevalence's may be due to variation in location, levels of water contamination and foodstuffs as well as contact with animals which are important factors in dissemination of the parasite (Karshima *et al.,* 2013). The high prevalence recorded by this study may be connected with the poor environmental sanitation and personal hygiene practices of this patients. The differences in prevalence are also hugely connected to the sensitivity of the diagnostic method used. Research has proven molecular methods such as ELISA, PCR etc. to be more sensitive and reliable in the diagnosis of *Cryptosporidium* infection (Atu *et al.,* 2014).

This explains the difference seen in the result of this research with other studies such as Nassar *et al*. (2017) and Atu *et al*. (2014) who used ELISA method and Rida Quick immunochromatographic methods in their research respectively. The implication of this result is that patients due to their reduced immunity will constantly be ravaged by parasitic diseases as a result of their low resistance. Additionally, patients social and mental wellbeing suffers due to constant sickness and consequently loss in self confidence.

This study confirmed the fact that *Cryptosporidium* infection presents with diarrhoea among infected individuals as seen in the high prevalence of 81.8% among patients with watery stool (P=0.000). This is similar to the work of Tamomh *et al*. (2021) and Jegede *et al.* (2014) who also reported a higher prevalence of 27.1% and 45% among patients with watery stool and a significant (P=0.003) and (P=0.00) respectively. This characteristic watery diarrhea in chronically infected patients can be as a result of the gradual but profound disorganization of the mucosa which includes disrupted epithelial surface, fibrosis, crypt abscessation and cellular infiltration (Tzipori and Ward, 2002). The implication of this finding is that these patients suffer from dehydration due to loss of water and sodium chloride, potassium depletion as well as metabolic acidosis which is due to the loss of bicarbonate. Prolong dehydration of these patients will be fatal as it can cause decreased blood volume (hypovolaemia), cardiovascular collapse and eventually death (WHO 1992). With regards to age, this infection was highest between the ages of 50-59 and lowest between 20-29 with prevalence's of 50.0% and 34.6%. This result contradicts the work of Ajayi *et al.* (2021) that documented higher prevalence within the age group 30-39 (30.8%) and lower prevalence within the age bracket ≥50 (26.4%). This result also differs from the work of Tamomh *et al*. (2021) who also documented a higher prevalence 10.7% of cryptosporidiosis in children under five years. This prevalence could be attributed to the fact that the older one becomes, the weaker the immune system and coupled with the effect of HIV/AIDS on the body, susceptibility to infections becomes inevitable. By implication, this result means older people are at greater risk of parasitic invasion due to the combine effects of decreased immunity and parasites activities. Older people thus need to take proper care of their body by focusing on their personal hygiene, diet, exercise and frequent

medical checkup. On the bases of Sex, higher prevalence was recorded in males 42.9% than females 39.5% though it was statistically insignificant. A possible explanation may be the common practice of men eating in commercial food joints which are mostly unhygienic thus resulting to food contamination. This finding agrees with the work of Karshima *et al*. (2013) that reported higher prevalence of cryptosporidiosis in males than females in Nigeria but contradicts the report of Jegede *et al*. (2014) and Ajayi *et al*. (2021) that reported higher prevalence of 14% and 31.6% respectively in females than males in Kano and Jos, Plateau States, Nigeria. The implication of this result is that higher number of men are currently sick and hence resulting in far reaching consequences of decrease in a country's overall production. By nature men provide bulk of the physical strength needed to run any setting as well as being bread winners in most homes. Thus, much efforts should be put into maintaining the health and wellbeing of men.

Occupationally, civil servants had a slightly higher prevalence of 45.8% while those in the business category were the second most infected with a prevalence of 43.8%. This could be as a result of the numerous contacts made during work were embracing and shaking of hands takes place. Similarly, medical personnel's such as laboratory scientists, nurses, doctors and cleaners can become infected at work while attending to infected persons. This result deviates from studies conducted by Tombang *et al*. (2019) and Haliu *et al*. (2015) who both reported a higher prevalence rate among farmers with an insignificant P=0.150 and P=0.15 respectively. Farmers in this study were only the third most infected group as oppose to studies reported in Cameroon and Ethiopia by Tombang *et al.* (2019) and Haliu *et al.* (2015) respectively. On the other hand, subjects who usually drink water from rivers/streams recorded the highest infection rate of 60.0% although it was not statistically significant. Reason been that rivers/streams are raw forms of water which have not been pass through any form of artificial purification thus is exposed to easy contamination by animals, humans and run-off from surrounding farms. The highest prevalence observed among patients who usually consume water from rivers/streams in our study contradicts earlier studies conducted by Jegede *et al*. (2014) that revealed higher infection rate of 13.2% among patients who normally drink tap water and Tombang *et al*. (2019) that also reported higher prevalence 7.14% among patients who usually drink tap

water P=0.02. Although statistically insignificant, *Cryptosporidium* infection was also jointly highest 43.8% among patients who do not wash their hands or only wash occasionally after going to the toilet. This can be as a result of their hands been contaminated in the course of washing or cleaning their anus, as such resulting to faecal oral route transmission. Such a behavior can also led to the contamination of food, water, door knobs, utensils and other objects around the home which can eventually cause transmission of the parasite to unsuspecting individuals. This result is in line with the findings of Atu *et al.* (2014) that also reported higher prevalence of 68.9% (p-value= 0.002) among patients who only wash their hands occasionally after visiting the toilet. Researches have indicated that when properly used, soft water in conjunction with soap eliminate dirt and contaminants of any kind (Jing *et al.,* 2020). The implication of this finding is that of a clear lack of hand hygiene among majority of patients in the study area. This means more sick people frequenting hospital visits due to feacal-oral transmission of pathogenic organisms. It is therefore necessary in maintaining good hand hygiene as a preventive measure against parasite transmission.

Conclusion: *Cryptosporidium* oocysts were demonstrated in the stool samples of both HIV positive and HIV negative subjects with a higher infection prevalence of 44.0% recorded among HIV positive individuals while 34% was recorded among HIV negative patients and an overall infection prevalence of 40.7% in Gboko LGA. Among all the factors considered to have influenced *Cryptosporidium* infection in patients, only stool consistency was found to be statistically significant with P-value=0.000. This research work thus, affirm the endemicity of this parasite in the area and the fact that the infection presents as watery diarrhoea in most patients.

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Conflict of interests
The authors declare that they have no conflict of interest.

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