ORIGINAL ARTICLE

Cryptosporidiosis in Human Immunodeficiency Virus Positive Patients and Associated Risk Factors in Maiduguri, Northeastern, Nigeria

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ABSTRACT

Background: *Cryptosporidium* is an intestinal parasite that causes diarrhoea in immune-compromised individuals. It is associated with high morbidity and potentially high mortality rates among the immune-compromised, resulting in serious economic threats. This study aims to evaluate the prevalence and risk factors of Cryptosporidiosis among people living with HIV (PLWH) in Maiduguri, Nigeria. **Methods:** This cross-sectional study was conducted at the Microbiology Department, University of Maiduguri Teaching Hospital, Nigeria. Non-probability consecutive sampling technique was used, and a total of 269 stool samples were collected from in and outpatients attending the antiretroviral (ARV) clinic in UMTH. Each sample was collected according to standard protocol. *Cryptosporidium species* were detected and identified using Modified Ziehl-Neelsen (MZN) staining, and polymerase chain reaction–restricted fragment length polymorphism (PCR-RFLP) of the small subunit (SSU) rRNA genes. Risk factors predisposing to Cryptosporidiosis were also assessed and the relationship was statistically determined. **Results:** Among the 269 enrolled patients, the prevalence of Cryptosporidiosis was 13.4% using Modified ZhielNelseen stain (MZN), 17.1% using enzymes linked immunosorbent assay (ELISA), and 19.3% using polymerase chain reaction (PCR). Cryptosporidiosis was found to be statistically associated with Cluster of Differentiation 4 (CD4) count <200 cells/mm3, male gender, poor toilet facility, and low personal income. **Conclusion:** *Cryptosporidium* is a common cause of diarrhea among people living with HIV in Maiduguri.

Keywords: Cryptosporidium, Maiduguri, HIV

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Introduction

Cryptosporidium is an enteric protozoon that causes infectious diarrhea. It is, estimated to be second only to rotavirus as the leading cause of moderate-to-severe diarrhoea.[1] Cryptosporidiosis is a neglected public health problem affecting immune-compromised individuals and children under five years of age.[2] Although about 26 species were described, over 90% of human infections are caused by *Cryptosporidium parvum* and *Cryptosporidium hominis*.[2] Other species less commonly associated with human disease include *Cryptosporidium meleagridis, Cryptosporidium cuniculus, Cryptosporidium felis, and Cryptosporidium canis. Cryptosporidium hominis* is more prevalent in North and South America, Australia, and Africa, whereas *C. parvum* causes more human infections in the United States of America (USA) and Europe, especially in the United Kingdom (UK).[3]

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Although the clinical presentations of *C. parvum* and *C. hominis* infections are very similar, several variants have been reported.[3] The disease presents as self-limiting diarrhoea in immune-competent adults and as fulminant life-threatening diarrhoea in immune-compromised adults and young children.[1,4] *Cryptosporidium* infection has been associated with a longer duration of diarrhoea and 2-3 times higher mortality in young children.[2] Researchers have demonstrated differences in clinical manifestations among *Cryptosporidium* species and subtypes in people living with HIV, with *C. hominis* associated mainly with diarrhoea, nausea, vomiting, and malaise. However, *C. parvum*, *C. meleagridis*, *C. canis,* and *C. felis* are associated with diarrhea only. In addition, it was shown that *C. hominis* infection was related to non-intestinal sequelae (joint pain, eye pain, recurrent headache, and fatigue), which were not reported among people infected with *C. parvum*.[3,4]

The worldwide infection and illness caused by *Cryptosporidium spp.* have been reported in more than 40 countries on six continents. The burden of cryptosporidiosis is significantly underestimated worldwide. An estimated 750,000 cases occur annually in the USA and, only 1% of this estimated figure in the USA is reported.[5] *Cryptosporidium* infection accounts for 3.1 million deaths each year among children less than 15 years of age globally and is responsible for 30 - 50% of the deaths in children under five years of age in the developing countries.[6]

Ninety percent of human infections are caused by *C. hominis* (anthroponotic origin) and *C. parvum* (zoonotic origin).[7] Diseases with cryptosporidiosis in developed regions are more common among children than adults.

There is no preference for cryptosporidiosis in impoverished areas as the infection universally affects people of all ages.[8] The prevalence of cryptosporidiosis varies in different parts of the world. In Asia, a prevalence of 9.2% was reported.[7] Molecular methods have enabled researchers to understand better the relationship between environmental factors, disease transmission dynamics, and public health interventions. Studies from the southern part of the African continent revealed prevalence rates of 1.2 -21%.[7] The prevalence of cryptosporidiosis among people living with HIV is often high, and the prevalence of 43.9% was reported by Etsehiwot *et al.* in a study done in southern Ethiopia.[9] In some African countries, the prevalence of *Cryptosporidium* in people living with HIV was reported to be higher than 70.0%, such as 73.6% in Uganda, 79.0% in Nigeria, and 75.6% in South Africa according to a retrospective epidemiological study done by Liu *et al.*[10] In a study done in Kumasi Ghana (West Africa) the prevalence of *Cryptosporidium* was 5.2%.[11]

In a study done by Olopede*et al.* in Ile Ife, Osun State, the prevalence of *Cryptosporidium* in people living with HIV was 4.4%[12], Nassar *et al*. reported the prevalence of 38.3% in Awo Ogbomosho.[13] The prevalence rate of *Cryptosporidium* in children with diarrhoea in Jos was found to be 4.8%[10] The prevalence of 9% was found among people living with HIV in Benin Edo state according to Akinbo *et al*.[14] The prevalence of 6.3% was found according to Ukwah et al in a study done in Ebonyi and Nsukka Southeast Nigeria.[15] According to Kanya *et al.,* the prevalence of *cryptosporidium* in Kebbi state is 13.8%.[16] The prevalence of 27.8% was found in Zaria, north-central Nigeria.[17]

Risk factors for cryptosporidiosis are related to the host, environment, and the species of the parasite. Immune-compromised adults, including those with HIV/AIDS or on immunosuppressive drugs, people with diabetes, and men having sex with men, are at increased risk.[8] Cryptosporidiosis in people living with HIV can lead to high morbidity and extra-intestinal spread.[18]Factors leading to the acquisition of infection in children are low socioeconomic status, crowded living conditions, age < 2 years, male gender, presence of animals (pigs, cats, and dogs) in the household, storage of cooked food, diarrhea in the family, drinking non-potable water, rainy season, low birth weight, stunted growth, and lack of breastfeeding.[19] Cryptosporidiosis in childhood has been associated with complications such as growth retardation, cognitive deficits, and a higher risk of mortality.[20] Cryptosporidiosis is a neglected global disease and an opportunistic infection with high morbidity and mortality, especially in immunocompromised individuals.[21]

This study was conducted to assess the prevalence of cryptosporidiosis in people living with HIV and associated risk factors in Maiduguri, Nigeria.

Methods

We conducted a cross-sectional study conducted at the University of Maiduguri Teaching Hospital, Borno State, Northeastern Nigeria. A total of 269 consenting

adults living with HIV who complained of diarrhea (passage of loose or watery stool at the frequency of 3 or more per day) attending the ARV clinic or are admitted into the wards were recruited into the study. The sample size was calculated using the Fischers formula. Adults living with HIV on immunosuppressant and anti-helminthics were excluded from the study.

A self-administered questionnaire was used to collect relevant demographic information, clinical information, and socio-economic status of the patient. Questions on risk factors predisposing to cryptosporidiosis like housing structure, occupation, water supply, toilet facility, and contact with animals were included.

This study was conducted at the Department of Medical Microbiology and Parasitology, UMTH, Borno State, Nigeria.

Sample collection

Stool samples were collected from each participant who has been diagnosed with HIV and has complained of diarrhea. The stool samples were collected into a dry clean universal container and were transported to the laboratory. The sample was examined macroscopically and direct smear samples of the stool were made with both saline and iodine and was examined microscopically using 10x and 40x objectives, for ova and cyst of parasites.

Stool concentration and Modified Ziehl Neelsen staining

Stool samples were concentrated using the formal ether concentration method. Sediments were smeared and a modified Ziehl Neelsen stain was done to detect the oocyst of *Cryptosporidium spp.*

The smear of the concentrated stool was made on a clean dry and grease-free slide and after drying, fixed with methanol for 2-5 minutes. The slide was flooded with concentrated carbofulchsin and stained for 15 minutes. It was then thoroughly washed under running tap water and decolorized using 1% hydrochloric acid for 10-20 seconds. After rinsing with tap water, it was then counter-stained using the 0.5% malachite green for 30 seconds. The slide was afterward air-dried and was observed using the light microscope with a 40x objective for the presence of Cryptosporidium oocyst which was viewed using 100x oil immersion.

Nested PCR amplification and extension

A nested PCR targeting the small subunit of the 18s-rRNA gene was performed at the University of Maiduguri biotechnology center, with the use of Initial primers Cr18PA: (5′ -TTC TAG AGC TAA TAC ATG CG-3′) and Cr18PB: (5′-CCCATT TCC TTC GAA ACA GGA-3′) amplified a 1.3 kb fragment of 18S-rRNA gene. The inner primer Cr18NA: 5′-GGA AGG GTT GTA TTT ATT AGA TAA AG-3′ and Cr18NB: 5′-CTCATA AGG TGC TGA AGG AGT A-3′amplified 826-864 bp fragment of former amplified sequence.

The reaction mixture of the PCR consists of 3µl, 1X PCR buffer (50mM KCL, 20 mM Tris-HCL, 2.5 mM Mgcl2, pH8.4) and 1µl, of 0.1 µg/ml BSA, 2µl of the initial primers, and 1µl of 0.3mM concentration of each of dNTPs, 0.5µl of recombinant Taq polymerase and two µl of the purified DNA and2µl of 1.5 mM $Mgcl_2$.

The reaction mixture was prepared as described above for the second round of amplification, except for the primers and DNA template. Again, one µl of the first PCR product as a source of DNA and the inner primers was used. Primary amplification was carried out in 35 cycles, each consisting of 95ºC for 30 seconds, 45ºC for 30 seconds, and 68ºC for 30 seconds, with an initial denaturation at 95ºC for 3 min and a final extension at 68ºC for 5 min. Thirty-five cycles were used for secondary amplification, with identical temperatures and times with an annealing temperature of 48 ºC. All PCRs were run in a Thechnethermo cycler.

Ethical Standards

The study was approved by the ethical committee of UMTH and all procedures in this work complied with the ethical standards (REF ADM/TH/497/VOL1 2020) In addition to this, informed consent was obtained from the participants.

Statistical Analysis

The information obtained from the patient questionnaire and other relevant data from this study was entered into an Excel sheet. Data was then imported from the Excel sheet into the statistical package for social sciences (SPSS). Data was then analyzed using (SPSS) (version 23, USA). The data was thereafter presented as tables, frequencies, and proportions. A parametric test was done to check for statistical significance with the chi-square test for the continuous and categorical variables, probability values of <0.05 were considered statistically significant.

Results

Demography

A total of 269 study participants were included in this study. Of this, more than half (70.3%) were females.

The age group 38-47 years had the highest number of participants 37.4% then the least age group that participated was≥58 years (4.8%). Of the total study participants, 33.8% were housewives and 5.9% were farmers, 29.4% had no formal/primary school certificate (Table 1). Out of these, 13.4% were positive using MZN, and 19.3% using PCR

The highest prevalence of *Cryptosporidium* infection was observed in males (25%) compared to females (16.9%). The age group 18-27 years had the highest prevalence of (23.5%), while the lowest prevalence of (11.6%) was found in the age group 48-57 years, as shown in Table 1.

Table 1: Socio-demographic Characteristics of study participants

Variable	Frequency n=269	PCR Negative (%)	PCR Positive (%)
Age (years)			
18 – 27	28(10.4)	21(9.6)	7 (13.5)
28 – 37	85(31.5)	65(29.8)	20 (38.5)
38 – 47	101(37.4)	83(38.1)	18 (34.6)
48 – 57	43(15.9)	38(17.4)	5(9.6)
≥58	13(4.8)	11(5.1)	2(3.8)
Gender			
Male	80(29.7)	60(27.6)	20(38.5)
Female	189(70.3)	157(72.4)	32(61.5)
Level of education			
None/primary	79(29.4)	60(27.6)	19(36.5)
Secondary	147(54.6)	121(55.8)	26(50.0)
Tertiary	43(16.0)	36(16.6)	7(13.5)
Occupation			
Business	82(30.5)	70(32.3)	12(23.1)
Civil servant	68(25.3)	55(25.3)	13(25)
Farmer	16(5.9)	11(5.1)	5(9.6)
Housewife	91(33.8)	71	20(38.5)
Student	12(4.5)	10	2(3.8)

Socio-demographic characteristics and their association with Cryptosporidiosis

One hundred and eighty-nine of the study populations were females and eighty were males. Gender was not statistically associated with Cryptosporidiosis (x^2=2.347, p-value 0.126). Occupation, type of toilet facility, and contact with animals were not significantly associated with Cryptosporidiosis. (p=0.544, 0.488,0.866) respectively. Only CD4 count was statistically associated with Cryptosporidiosis.

Source of water and cryptosporidiosis

The majority of the study participants used boreholes as their source of water. One hundred and twenty-eight of the study participants used boreholes and this was not significantly associated with Cryptosporidiosis (p=0.249) as shown in Table 2.

Toilet facility and association with Cryptosporidiosis

One hundred and forty-six of the study participants used a pit latrine, it was found that the pit latrine is not found to be statistically associated with cryptosporidiosis (p=0.488)

Contact with animals and Cryptosporidiosis

The majority of the study participants had a history of contact with domestic animals e.g. chickens, cats, cows, and sheep. It was found not to be significantly associated with cryptosporidiosis (p=0.866)

Other findings include the Duration of diarrhea, consistency of stool frequency of stool, and mucoid stool were found not to be associated with cryptosporidiosis. (p-value = 0.256, 0.810, and 0.742 respectively).

Table 2: Socio-demographic characteristics of study participants

Risk factors	Crypto negative	Crypto positive	X^2	*p*-Value
Sample size	217	52	-	-
Gender				
Male	60 20		2.347	0.126
Female	157 32			
Occupation				
Farmer	11	5		
Civil servant	55	13		
Housewife	71	20	3.083	0.544
Student	10	2		
Business	70	20		
Source of water				
Tap	89	26	1.384	0.239
Borehole	28	26		
Toilet facility				
Pit	146	31		
Water system	70	21	1.433	0.488
CD4 count				
Less than 200	62	23	4.759	0.029**
Greater than 200	155	29		
Contact with animals				
Yes	173	42	0.290	0.866
No	44	10		
Eating away from home				
Yes	151	38	0.245	0.621
No	66	14		
Drinking away from home				
Yes	37	40	1.023	0.312
No	180	12		
Monthly income				
Less than 18000	136	33	0.011	0.916
Greater than 18000	81	19		

* p value < 0.05 = Statistically significant

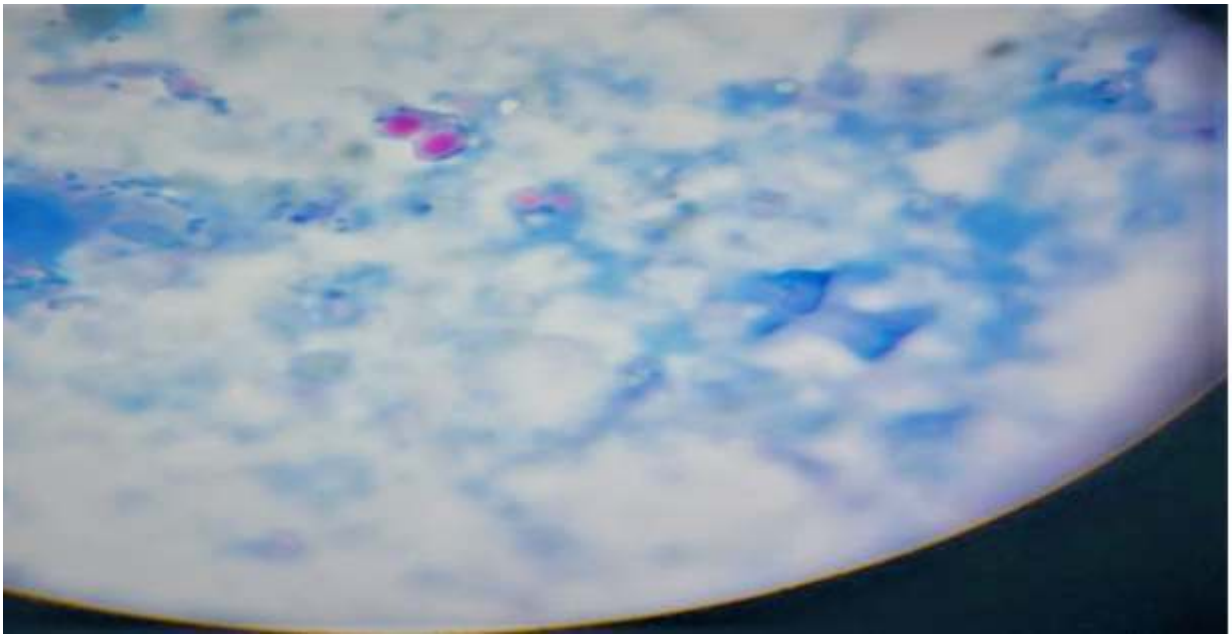

Fig 1: *Cryptosporidium* oocysts stained pinkish-red (examined at 100x) were observed as a thick-walled spherical structure of approximately 2-8µm in diameter.

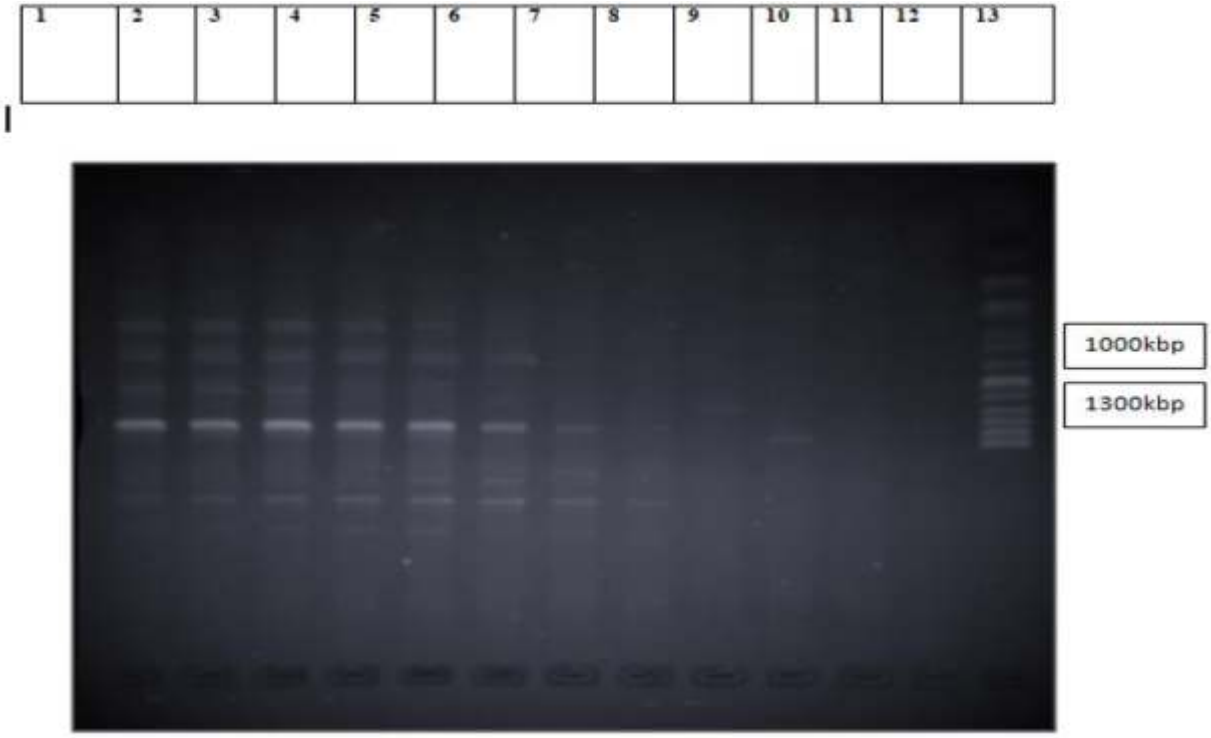

Fig 2: PCR amplification products SSU rRNA gene using 1500kbp Lane 1-13; Lane 13; 1.5kbp molecular maker. Lane 12 Negative control for *Cryptosporidium spp,* Lane 1-7 clinical samples positive for *Cryptosporidium spp,* Lane 8-11 clinical samples negative for *Cryptosporidium spp.*

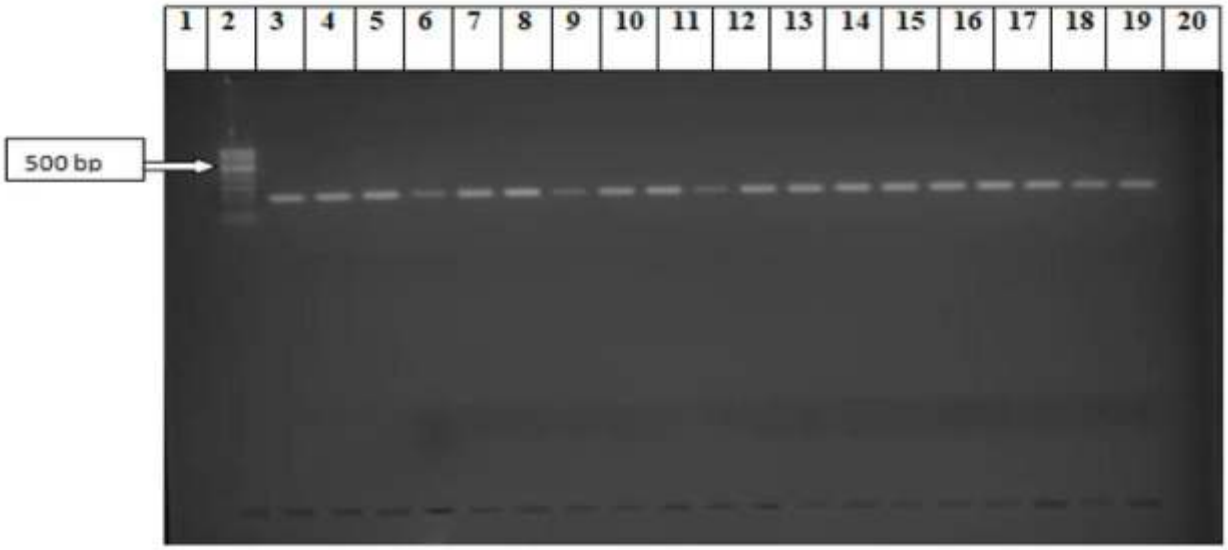

Fig 3: PCR gel electrophoresis showing the inner primer Cry18NA product. 1; molecular marker, 2-20 positive samples between 826 – 837 bp.

Discussion

This study shows a prevalence of 19.6% Cryptosporidiosis amongst HIV-positive patients in Maiduguri. This prevalence is similar to earlier reported findings among HIV-infected patients in Michika, northeast Nigeria,[21] and in a study done in Jos North central Nigeria, the prevalence of Cryptosporidiosis was 27% in HIV-infected patients.[22] This study recorded a higher prevalence compared to a study by Olopede *et al*.[23] This disparity could be as a result of the nature of the stool sample used, it has been shown that diarrheic samples yield more *Cryptosporidium* oocyst than non-diarrheic stool.[24] Many of the participants were residents of the internally displaced persons (IDP) camps with poor water and sanitation hygiene, poor access to affordable and quality education, and inadequate healthcare services, among others. This setting promotes feaco-oral transmission of infection, and may partly explain the high prevalence of *Cryptosporidium* infection observed in the study. Immune-suppressed individuals showed a two-fold increase in prevalence rate when compared with immune-competent individuals.[25]

This study recorded a higher prevalence in females; This observation agrees with the report by Solomon Ngutar *et al*.[28]which females were more infected than males, however, this finding is not in keeping with that of Nassar *et al*.[26] and Kanya *et al*.[27] The higher prevalence in females may reflect the increased exposure to sources of contamination than males or another probability will be their healthcare seeking behavior. Moreover, in rural communities of developing countries, females may be more susceptible to diarrheal diseases as they are the primary caregivers for children and are therefore frequently in contact with their stool, diapers, and lack of clean water which could potentially lead to infection as a result of poor hygiene.[29] Several reports indicate that the infection may not always have a positive association with gender since, in many communities, both genders are equally exposed to similar risks of infection. In addition, both sexes engage in the same recreational activities and are likely to be similarly exposed to the infection.[26] The prevalence of Cryptosporidiosis was found to be higher among less educated subjects with no formal education and primary education. The reason for this observation may be because better-educated individuals have access to more resources, better access to safe drinking water, and better quality of life compared to the less educated. Also, better-educated individuals can make better-informed decisions about their health. Less educated individuals are rural dwellers, that have problems with access to clean and portable drinking water, which predisposes them to

infection with Cryptosporidium and other intestinal parasites. This finding is in keeping with what was found in a study in Sokoto State, Nigeria.[29]

Infection was detected most frequently amongst patients in the age group 18-27 years. This result is similar to the findings in a study done in Malaysia[30] which showed the highest prevalence to be seen in ages more than 30 years. However, this may be because the incidence of HIV is highest among individuals aged 15-35 years[31] and contrary to the finding of Ukwah *et al.*[32] with a finding of highest prevalence greater than 50 years.

This study also assessed the risk factors predisposing to the development of cryptosporidiosis among these patients. Several factors were investigated to determine their association with cryptosporidiosis. It is interesting to note that CD4 count was found to be significantly associated with cryptosporidiosis among the factors assessed. This further establishes the fact that cryptosporidiosis is related to a CD4 count of <200 cells per mm^3. CD4 cells play a significant role in the immune response to gastrointestinal pathogens. It has been shown that low CD4 counts are associated with an increased risk of infection with enteric pathogens and chronic diarrhoea.[33] This finding is in keeping with a meta-analysis done by Ukwah *et al.*[34] in Nigeria.[35] In contrast, Olopede*et al.*[23] reported that CD4 count is not a known risk factor for cryptosporidiosis. Immunity plays a significant role in the survival and proliferation of intestinal parasites in HIV-positive patients. Cryptosporidiosis is common in immune-compromised persons in low- and middle-income countries, especially HIV-positive patients. In an intercontinental study, the prevalence of Cryptosporidiosis in HIV-infected persons with low CD4 count ranges from 5.6% to 25.7% in Africa, 3.7% to 45% in Asia, 5.6% to 41.6% in South America, and 2.6% to 15.1% in Europe.[35] In our study, it was noticed that all the HIV infected patients with cryptosporidiosis had no protease inhibitor added to their regimen. This is similar to the findings in a study done in Ebonyi, South-east Nigeria.[33] It is, therefore, possible that the non-inclusion of HIV-protease inhibitors in their treatment regimen could be an additional factor because it is known to prevent the occurrence of *Cryptosporidium* infection.[36] Another reason for the occurrence of cryptosporidium infection in these patients could also be attributable to non-adherence to treatment as is quite common in Nigeria.[37]

Other risk factors assessed were level of education, source of water, eating, drinking water away from home, contact with animals, and viral load, and were all found not to be statistically significant; however, a contrary result was obtained from Southwest Uganda where all these factors are associated with Cryptosporidiosis.[31] Due to the insurgency bedeviling the North-eastern region of Nigeria with Maiduguri as the epicenter, numerous international non-governmental organizations have promoted healthcare practices and policies within the region, which has significantly improved the outcome of these risk factors. The participants' economic status was also considered a risk factor; it was found that the prevalence is high among participants with low socioeconomic status, which is similar to a study done in Iraq.[32] Poverty and living in poor neighborhoods and internally displaced person camps reflect a painful reality about the absence of essential services and infrastructure. These challenges are seen in both Nigeria (Northeast) and Iraq, where these studies were conducted. *Cryptosporidium* infection has a low prevalence among socio-economically advantaged individuals.[33]

Conclusion

This study found a prevalence of 19.6% of Cryptosporidiosis among people living with HIV and CD4 count <200 cells/mm^3 as an associated risk factor for *Cryptosporidium* infection among people living with HIV in Maiduguri.

Recommendation

This study recommends that:

Health education for people at risk of cryptosporidiosis to interrupt transmission of this opportunistic parasite is of paramount importance. To provide potable drinking water with particular emphasis on people with compromised immune status. It is also recommended to include protease inhibitors among the drugs used as HAART for

people living with HIV to reduce the burden of intestinal parasites.

Conflict Of Interest: We declare no competing interest

Ethical Consideration: The study was approved by the ethical committee of UMTH and all procedures in this work complied with the ethical standards. (UMTH REC 609) In addition to this, informed consent was obtained from the participants.

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Authors Contribution

MUK, KBA, ASB, and YM conceived and designed the project, and NZ, MD, and MUK coordinated the patient recruitment, sample collection, and laboratory analysis. YMY, BBD, and ABS coordinated the statistical analysis and interpretation. The entire work was supervised by GBG and SBZ. All the authors contributed significantly during the manuscript drafting, and revision and accept responsibility for the integrity of the data and accuracy of analyses.

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Data Availability

The datasets generated during this study are available with the corresponding author on request.

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