

EPIDEMIOLOGY OF CRYPTOSPORIDIOSIS IN HIV POSITIVE PATIENTS TREATED IN MAJOR TERTIARY/SECONDARY HOSPITALS IN RIVERS STATE, SOUTHERN NIGERIA

Authors:

Solomon N Ukibe¹, Ifeoma M Ekejindu¹, Godsplan U John², Iloduba N. Aghanya¹, Simon N Ushie¹, Ifeoma S. Anagor¹, Ngozi G Uzoewulu¹, and Ugochukwu U. Nwaogwugwu³.

Author Affiliations:

1. Department of Medical Microbiology and Parasitology, Nnamdi Azikiwe University Teaching Hospital, Nnewi, PMB 5025 Nnewi, Anambra State Nigeria.
2. Department of Medical Laboratory Science, Faculty of Health Sciences & Technology, Nnamdi Azikiwe University, Nnewi campus
3. Department of Medicine, Siberian State University, Tomski, Russia.

Corresponding Author:

Solomon Nwabueze Ukibe

Email: soloukibe@yahoo.com

Tel: 08035000346

ABSTRACT

Background: Cryptosporidiosis is one of the protozoan, opportunistic infections that are common in HIV positive patients and is characterized by gastroenteritis marked with spurious diarrhea that may lead to death if not treated.

Aim and Objectives: To evaluate the epidemiology of Cryptosporidiosis in HIV positive patients undergoing treatment at University of Port Harcourt Teaching Hospital (UPTH) and Zonal Hospital Ahoada (ZHA), Rivers State, Southern Nigeria.

Materials and Methods: 250 HIV positive patients were randomly recruited for the study consisting of 101 males and 149 females. Demographic data/information was obtained using a well-structured self-administered questionnaire. Stool samples were collected from each of the patients by standard methods. Modified Ziehl Neelsen (MZN) technique was employed to stain thick stool smears which were microscopically examined under high power magnification for the diagnosis of Cryptosporidium species oocysts.

Results: Out of the 250 samples, 19 were positive giving a prevalent rate of 7.6%. The age group with the highest prevalence (2.4%) was 30-40 years followed by 26-30 years (1.6%). The prevalent rate in females (5.6%) was significantly more than that of males (2%)

($P < 0.05$). Farmers were significantly more affected (3.2%) than other occupational groups ($P < 0.05$). Patients whose source of domestic water was bore hole had the highest percentage (89.5%) of infection. Personal hygiene (hand washing) had a significant effect on infection rate. Diarrhea was the most frequent presentation of the disease.

Conclusion and recommendation: Cryptosporidiosis was prevalent in HIV positive patients who presented in UPTH and ZHA in Rivers State, Southern Nigeria. Strict environmental sanitation and provision of portable water are public health measures recommended to reduce the prevalence of this disease especially in HIV/AIDS patients.

Key words: *Cryptosporidiosis, epidemiology, HIV, Rivers, Nigeria.*

INTRODUCTION

Cryptosporidiosis is one of the commonest opportunistic infections in Human Immunodeficiency Virus (HIV) positive patients Worldwide. It is believed to be one of the commonest causes of chronic diarrhea in immune-suppressed individuals throughout the World^{1,2,3}. Both man and animals are infected by *Cryptosporidium* species including *Cryptosporidium hominis* and *Cryptosporidium parvum* and infection is usually by the faeco-oral route when the infective oocysts are ingested in food, water and other contaminated surfaces⁴

The prevalence of Cryptosporidiosis has varied from one locality of study to another. Whereas a study⁵ in the Southeastern State of Enugu did not report any incidence of Cryptosporidiosis in 2002, Erhabor et al⁶ reported a prevalence rate of 2.9% Cryptosporidiosis in 2011 with female patients being the most affected. Studies in Southwest Nigeria^{7,8} reported prevalence rates ranging from 2.3% to 2.6% with the highest rates reported in HIV positive patients with diarrhea. Other epidemiological studies outside Nigeria^{9,10} have shown similar rates with HIV positive patients bearing the highest burden of disease. Epidemiological factors which play significant roles in the transmission of cryptosporidiosis include the source of domestic water supply, food hygiene and life style habits including practice of oral sex and other unhealthy habits⁴.

The present study was therefore designed to evaluate the prevalence and epidemiology of Cryptosporidiosis in HIV positive patients treated in a tertiary hospital and secondary hospital in Rivers State, Southern part of Nigeria.

MATERIALS AND METHODS

Study area: The study was conducted at the University of Port Harcourt Teaching Hospital (UPTH) Choba, Port Harcourt and the Zonal Hospital, Ahoada (ZHA) all in Rivers State, Southern Nigeria. Rivers State is one of the 36 states of the Nigerian Federation located in the geopolitical zone known as South-South Nigeria. It has an estimated population of 5.2M people according to 2006 census¹¹ Its neighboring states include Abia, Imo, Delta and Edo states.

Study Population: A total of 250 subjects were recruited for the study consisting of 135 subjects from UPTH and 115 subjects from ZHA respectively. All the subjects were screened for HIV using the conventional methods and grouped as follows:

- i) HIV positive subjects on Highly Active Antiretroviral Therapy (HAART) and not diarrheic
- ii) HIV positive subjects not yet on ART
- iii) HIV positive subjects who had diarrhea

Study Design: This was a cross sectional study and random sampling method was used.

The minimum sample size was calculated using the formula:

$$N = Z^2 \times P(1-P)/D^2 \text{ (Naing et al 2008)}$$

Where N = Minimum sample size for blood and stool

P = Prevalent rate for Cryptosporidiosis in HIV positive patients with diarrhea in Niger Delta = 2.9% (Erhabor et al, 2011)

D = Desired level of significance = 95% (0.05)

$$Z = \text{Confidence interval } 95\% = 1.96$$

$$\begin{aligned} \text{Ie } N &= 1.96^2 \times 2.9/100 \times (1-2.9)/100 \div 0.05^2 \\ &= 37 \end{aligned}$$

Questionnaire: A well-structured, self-administered questionnaire was used to obtain demographic data

Ethical Clearance: This was obtained from the Ethics Department of the University of Port Harcourt Teaching Hospital (UPTH) and from the Nigerian Institute for Medical Research, Yaba, Lagos, Nigeria)

Informed consent: This was obtained from all the participants and those who did not want to participate were allowed to opt out.

Inclusion Criteria:

- All HIV positive patients aged 15-65 years both on HAART and not
- All HIV positive patients who had diarrhea

Exclusion Criteria:

- Children below 15 years of age
- Subjects above 65 years of age

Statistical Analysis: Chi square (X^2) test was used to analyze the data obtained and the level of significance used was $P \leq 0.05$

Collection of Stool samples/Analysis

One gram of stool sample was collected from each of the 250 patients into a clean wide mouthed sterile bottle. A stool smear was made from each fresh faecal sample on a clean frosted end, grease-free glass slide for Modified Ziehl Nielsen (MZN) staining technique. The remaining sample was fixed with 10% formol saline and preserved in a refrigerator at 2° C for further use.

Modified Ziehl Nielsen (MZN) Staining Technique

- a) The thick stool smears were fixed with absolute alcohol(methanol) for 5 minutes and air dried at room temperature
- b) Carbol fuchsin with phenol was applied for 15 minutes
- c) The stain was washed with tap water
- d) The stain was decolourized with 1% acid alcohol (1% sulphuric acid in methanol) for 10 minutes
- e) Counter staining with 0.4% methylene blue solution for 1 min
- f) Slide was washed with tap water and air dried

Microscopy/Diagnosis

The slide was viewed with oil immersion microscope at x400 magnification. Oocysts of Cryptosporidium species appear as pink to red spherical to oval bodies in blue background.

RESULTS

The overall prevalence of Cryptosporidiosis in the study area was 7.6%. The age group most affected was 36-40 years (2.4%) followed by 26-30 years (1.6%). Prevalence significantly varied according to age (P < 0.05, Table 1). Female patients were significantly more affected (5.6%) than males (2%) (P < 0.05).(Table 2). The occupational group most affected was farmers (3.2%), followed by traders/business men (2%). A significant difference existed between the occupational groups. (P = 0.000; P < 0.05) (Table 3). Patients whose water source was borehole suffered most from Cryptosporidiosis while the patients who obtained their domestic water from river or well suffered the least (Fig 1). Personal hygiene (hand washing) significantly affected the incidence of the disease (P < 0.05) while diarrhea was the most single important presenting symptom of the disease.

Table 1: Showing Age distribution of Cryptosporidiosis in HIV positive patients treated at a tertiary and secondary Hospital in Rivers State, Southern Nigeria

Age group (years)	Total no	No positive	Prevalence (%)
15-20	9	0	0
21-25	33	5	2
26-30	42	4	1.6
31-35	47	1	0.4
36-40	54	6	2.4
41-45	20	1	0.4
46-50	35	2	0.8
Total	250	19	7.6

$X^2 = 135.493, P = 0.000, P < 0.05$

Table 2: Gender distribution of Cryptosporidiosis in HIV positive patients treated at a Tertiary and Secondary hospital in Rivers State, Southern Nigeria

Age gp (yrs)	Total no	Male	No positive	Prevalence (%)	Female	No positive	Prevalence (%)
15-20	10	4	0	0	6	0	0
21-25	35	12	2	0.8	21	3	1.2
26-30	43	19	1	0.4	26	3	1.2
31-35	49	20	0	0	29	1	0.4
36-40	54	22	2	0.8	33	4	1.6
41-45	22	9	0	0	13	1	0.4
46-50	37	15	0	0	22	2	0.8
Total	250	101	5	2	149	14	5.6

$X^2 = 30.335, P = 0.000, P < 0.05$

Table 3: Occupational distribution of Cryptosporidiosis in HIV positive Patients treated at a Tertiary and Secondary hospital in Rivers State, Southern Nigeria

Occupation	Total no	No Positive	Prevalence (%)
Civil Servants	42	2	0.8
Traders	102	5	2
Students	43	1	0.4
Farmers	51	8	3.2
Com Drivers	7	1	0.4
Applicants	5	2	0.8
Total	250	19	7.6

$X^2 = 34.782$, $P = 0.000$, $P < 0.05$

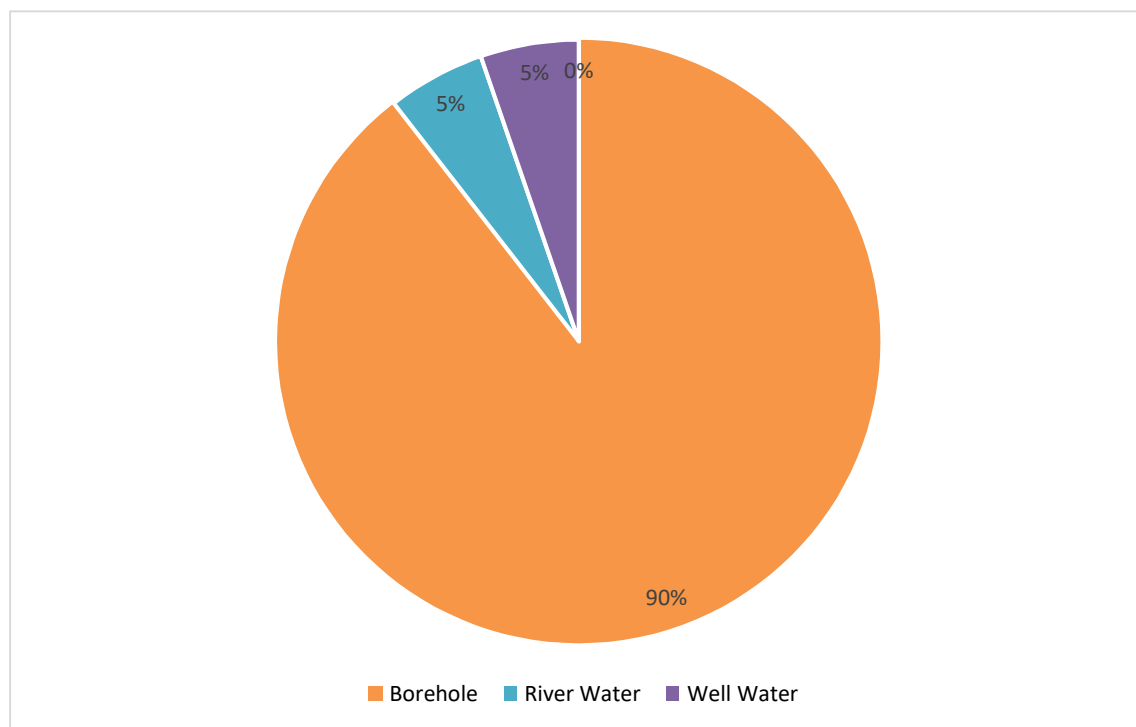


Fig 1: Pie chart showing Distribution of Cryptosporidiosis Cases According to domestic water supply

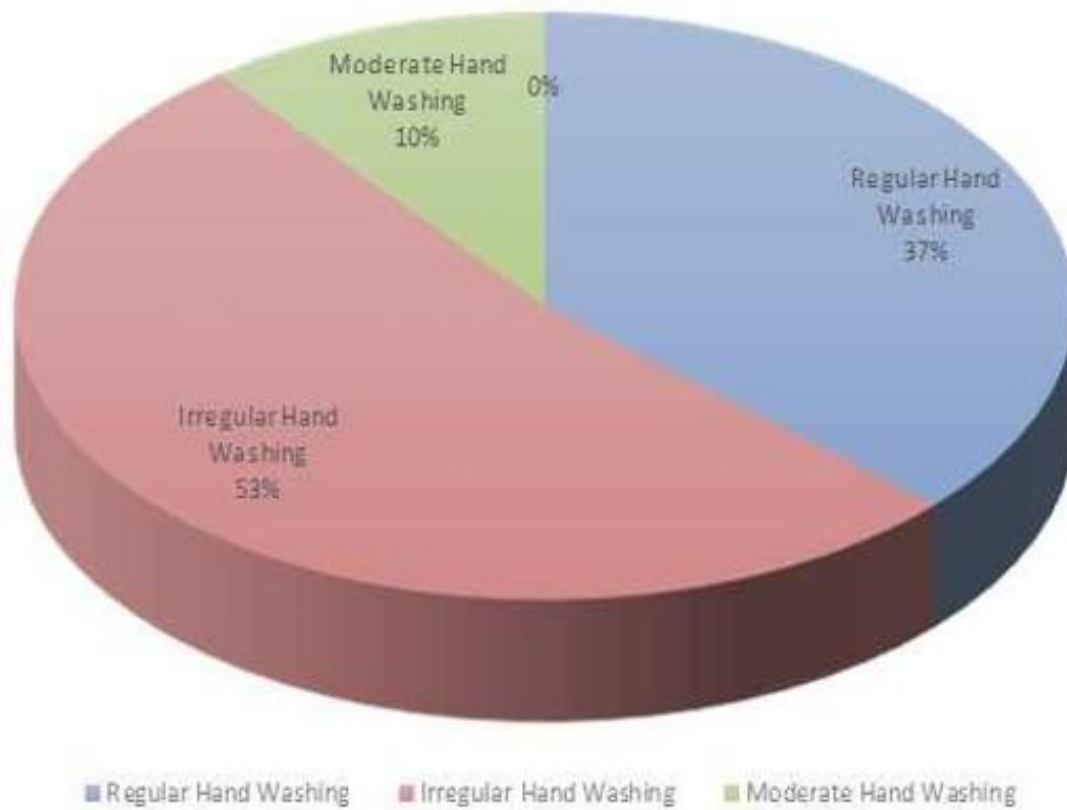


Fig 2: Pie chart showing Distribution of Cryptosporidiosis Cases According to personal Hygiene (hand washing)

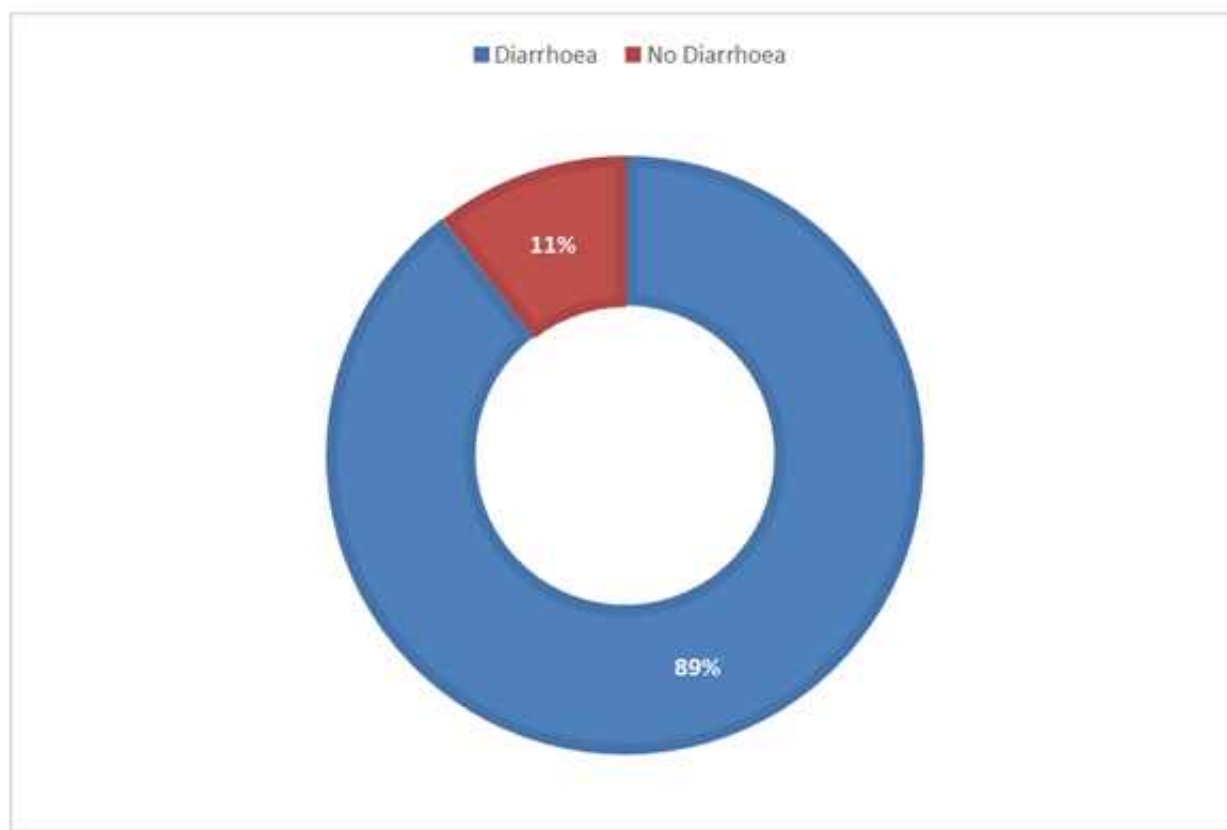


Fig 3: Pie Chart Showing Association of Cryptosporidiosis cases in HIV Patients with diarrhea

DISCUSSION

The present study (Table 1) showed that the overall prevalent rate of Cryptosporidiosis in HIV infected patients in Rivers state was 7.6% with female patients being significantly more affected than their male counterparts ($P < 0.05$). A similar but community based study in Port Harcourt reported a higher prevalence rate¹⁸. Previous studies conducted in the other geopolitical zones of Nigeria have reported varying prevalent rates ranging from zero % (as reported in Enugu, Southeastern Nigeria) to 80% (as reported in Wamako, Sokoto State, North Western Nigeria)^{5,6,7,8,12,13,14}. In all these studies, the prevalent rates seem to have been influenced by the location of study, the sample size, the age range of subjects recruited and environmental factors to mention but a few. Studies conducted outside Nigeria^{4,9,10,15,16} reported prevalent rates ranging from 1.5% to 11% and as observed in the local studies, the reported rates seemed to have been affected by the study population, the age group involved and other environmental factors that depended on the locality of the study.

The study (Table 1) also showed that people aged 36-40 years had the highest infection rates followed by people aged 21-26 years. The prevalent rate was significantly different in the various age groups ($P < 0.05$). Age normally affects the prevalence of many diseases with the extremes of life (children and elderly people) being most affected. In the present study however, children aged below 15 years and elderly people above 65 years were excluded. Some previous studies^{7,12,14,18} have collaborated this finding with some of them reporting greater prevalence in children than in adults¹⁸. Children are believed to have weaker and immature immune status. This is made worse when they are HIV positive. They also have poorer toilet habits and hygiene and may come in close contact with domestic animals more frequently¹⁸.

Conversely, children are more exposed to Cryptosporidium infection than adults.

Furthermore, this study (Table 2) revealed that female patients were significantly more affected by Cryptosporidium infection than the male counterparts ($P < 0.05$). Similar findings have been reported previously^{14,19}.

However, a study by Egberengbe et al in 2010¹² reported that more males were infected with Cryptosporidium species than females. In the said study, most of the victims used bush toilet method and fetched their domestic water from ponds and local rivers. It is worthy to note that in all the studies, more females were recruited than males and this could partly account for the difference in prevalent rates.

The study (Table 3) further showed that among the occupational groups investigated, farmers had the highest prevalent rate (3.2%) followed by traders/businessmen (2%). There was a significant difference in prevalence between the various occupational groups ($P < 0.05$). This may be probably due to the fact that farmers are more likely to come into close contact with animals which carry the oocysts of Cryptosporidium species especially those of them that engage in animal/poultry farming. A previous study in Port Harcourt by Tariuwa et al¹⁸ collaborated this finding. Furthermore, this study (Fig 1) showed that majority (89.5%) of the patients infected by Cryptosporidium species obtained their domestic water supply from bore hole ie pipe borne water. This finding raises a lot of public health concern. Bore hole is a common source of domestic water in this environment. The finding suggests the likelihood of contamination of drinking water by infected people carrying the Cryptosporidium oocysts.

This calls for public health experts to institute investigation to find sources of contamination of domestic water supply in the study area. Surprisingly, people whose water source was rivers and streams had the least number of infection. Considering the fact that Rivers State is a riverine area, it is possible that some local indigenes may practice open defaecation and disposal of wastes thus increasing the chances of contamination of streams with *Cryptosporidium* oocysts. To guide against this, the Centre for Disease Control (CDC) has proposed the prohibition of people suffering from Cryptosporidiosis from swimming in public water bodies/pools to prevent them from washing their anal and genital areas into the water thereby contaminating the water and spreading the infection

20

This study further showed that majority of the cases of Cryptosporidiosis occurred in patients who did not observe strict personal hygiene by washing their hands regularly especially after using toilet facilities (Fig 2). Regular hands washing with soap has been associated with reduction of the incidence of many infectious diseases including Covid-19. The higher prevalence of Cryptosporidiosis reported in primary school children¹⁴ may be associated with poor hygiene especially in places where water is not readily available. The place of hygiene may also explain the fact that majority of the Cryptosporidiosis cases (89%) presented with diarrhea (Fig 3). This is marked in HIV/AIDS patients who have not been placed on antiretroviral therapy (ART). Similar findings have been reported in previous studies^{12,13,18,19}, thus diarrhea is a prominent symptom of HIV/AIDS disease. It is also the major cause of sudden death of the victims as a result of severe fluid and electrolyte imbalance and cardiovascular collapse.

CONCLUSION

Cryptosporidium species was a prominent protozoan cause of gastroenteritis in HIV/AIDS patients who presented in major hospitals in Rivers State, Southern Nigeria and it affected both male and females groups and cut across most occupational groups. Environmental sanitation and provision of safe portable water can go a long way to reduce the incidence especially in HIV/AIDS patients who were the targets of the present study.

Acknowledgement

The authors are grateful to the staff of the Medical Microbiology Laboratory of University of Port Harcourt Teaching Hospital, Nigeria for their cooperation and also the Medical Research Institute, Yaba, Lagos, Nigeria.

REFERENCES

1. Blansherd C, Francis N, Garzard B. Investigation of Chronic diarrhea in Acquired Immune Deficiency Syndrome: A Prospective Study of 155 patients: *Gut* 1996; 39:824-832.
2. Webber R, Ledergender B, Zbinden R, Altwegg M, Ifyffer GE, Spycher MA, Briner J, Kaiser L, Oparavil M, Meyenberg C et al. Enteric Infections and Diarrhea in Human Immune Deficiency Virus infected persons: Prospective Community based Study: Swiss HIV Cohort study. *Arch. Intern Med.* 1999; 159:1473-1480
3. Cotte L, Rabodonivina M, Piens MA, Perreard M, Mojon M, Trepo C. Prevalence of Intestinal Protozoans in French patients infected with HIV. *J. Acq Immune Def. Synd.* 1993;6:1024-1029.
4. Nwoke BEB Public Health Parasitology 2018 Edition. Cryptosporidiosis Chapter 17:245-255
5. Nwokediuko SC, SC, Bojuwoye BJ, Onyenekwe B. *Niger Postgraduate Med J.* 2002 June 9(2):70-73
6. Erhabor O, Obunge O, Awah I. Cryptosporidiosis among HIV infected persons in the Niger Delta of Nigeria. *Niger J. Med* July-Sept 2011; 20(3):372-375.
7. FF Reinthaler, K Hermentin, F Mascher, G Kler, W Sixl Cryptosporidiosis in Ogun State, Southwestern Nigeria. *Trop Med. Parasitol* 1987 March 38(1): 51-52.
8. Adesiji YO, Lawal RO, Taiwo SS, Fayemiwo SA, Adeyemo OA. Cryptosporidiosis in HIV infected patients with diarrhea in Osun state, Southwestern Nigeria. *Eur J. Gen. Med* 2007; 4(3):119-122
9. Yal Lim, Rohola M, Sim BLM, Jamaiah I, Nuroayah M. Prevalence of Cryptosporidiosis in HIV infected patients in Kajang Hospital, Selangor. *Southeast Asian J. Trop Med. Pub Health*
10. Raccurt CP, Phillip B, Rose NP. Human Cryptosporidiosis and *Cryptosporidium* species in Haiti. *Trop. Med. Int. Health* 2006 June 11(6):929-934.
11. Nigerian National Population Commission (NPC) 2006.
12. Egberongbe HO, Agbolade OM, Adesetan TO, Magbekoye OO, and Olubode AM. Cryptosporidiosis among Children in relation to toilet facilities in Ijebu and Ramo areas, Southwest Nigeria. *J. Med. Med. Sci;* i(10): 485-489.
13. Kurmurya AS, and Kwarzo MY. Cryptosporidiosis in HIV infected patients with diarrhea in Kano State, North western Nigeria. *J. AIDS HIV Res* 2013 Aug 5(8): 301-305
14. Shinkafi SA, And Mohammed Z. Prevalence of *Cryptosporidium* oocysts among Primary school Children in Wamako Local Government of Sokoto State, Nigeria. *Nig J. Basic Applied Sci* June 2017; 25(1):11-16.
15. Omolabi KF, Odeniran PO, Soliman ME. *J. Parasitol Dis* 2021 Aug 18:1-13
16. Mohebal M, Yimam Y, Worreta A. Cryptosporidiosis infection among people living with HIV/AIDS in Ethiopia: A systemic review and metanalysis. *Path. Glob. Health.* 2020 May 18: 114(4): 183-193.
17. Wang RJ, Li JQ, Jun Quian Li, Yuan-Caichen, Zhang Loag-Xian, LI- Hua Xiaao. Widespread Occurrence of *Cryptosporidium* infection in patients with HIV/AIDS: Epidemiological, Clinical features, diagnosis and Therapy. *Acta Trop.* 2018 Nov (187):257-268.
18. Tariuwa HO, Ajogi I, Ejembi CL, Awah IJ, Green PA, Fadipe EO, and Odoba MB. Incidence of *Cryptosporidium* infection in Port Harcourt, Rivers state, Nigeria based on regular contacts with domestic animals. *Nig Vet. J.* 2007; 28(3):1-5
19. Anejo-Okpopi AJ, Ejiogu UE, Gomerep S. Okojokuw OJ, Okechalu JO, Oguchukwu CS, Lar MP. Prevalence of Cryptosporidiosis among HIV infected adult patients attending Jos University Teaching Hospital, Jos, Nigeria. *UJMR* June 2017; 2(1):166-172.
20. Centre for Diseases Control 2001 Atlanta, USA