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PREVALENCE OF *Cryptosporidium* INFECTIONS BETWEEN APPARENTLY HEALTHY AND IMMUNO-COMPROMISED INDIVIDUALS AT PARKLANE HOSPITAL ENUGU

VICTOR S. NJOM [a*] AND SAMSON OBINNA NNAMANI [a]

[a] Department of Applied Biology and Biotechnology, Enugu State University of Science and Technology, PMB-1660, Enugu, Nigeria.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Cryptosporidiosis is a disease caused by a protozoan parasite of the genus *Cryptosporidium*, that affect both young animals and humans. A cross-sectional study was conducted among immune-compromised and apparently healthy subjects attending Parklane Hospital in Enugu, to determine the prevalence of *Cryptosporidium* infection and associated risk factors. Faecal and urine samples were microscopically examined to demonstrate *Cryptosporidium*. While demographic data and other risk factors were obtained by structured questionnaire interview. Only immune-compromised subjects had oocyst of *Cryptosporidium* with an overall prevalence of 2.82% and there was statistically significant difference (p=0.00213) in proportion of infection between immune-compromised and apparently healthy subjects. For type of samples, faecal material had 11(8.46%) positive cases while no *Cryptosporidium* was detected in all the urine samples. Male had the highest prevalence of 7.27%, *while* female had 5.00% C*ryptosporidium* infection. For age, the highest prevalence was in people within 15 – 25 years (9.09%) and lowest (6.25%) among 36 – 45 years old. People with educational level below primary school had highest prevalence (5.55%) though there was no association between proportion of infection and educational level of subjects (P=0.9618). Subjects who wash their hands before meal had lowest prevalence (0.68%) while those who never wash hand before meal had highest infection (16.6%), those who said that they consumed roadside food had (6,66%) infection. Subjects who used piped water had 6.25% infection, while those who buy water from other sources had 3.06% infection. Those who source water from stream/river had 3.13% infection. Sources of drinking water were associated to proportion of infection ($p<0.05$). In conclusion, cryptosporidiosis is one of the health problems of immune-compromised patients in Enugu town. These findings confirmed that risk factors such as educational level, age, and types of food and water source are epidemiological factors of *Cryptosporidium* infection in human.

Keywords: *Cryptosporidium*; zoonotic; immuno-compromised; oocyst; protozoan.

1. INTRODUCTION

Cryptosporidiosis is an infectious disease of the small intestine that leads to enteritis in animals [1], it is caused by a microscopic protozoan parasite called *cryptosporidium*, infecting a wide range of host species, including mammals, birds, reptiles and fish [2]. It causes severe diarrhoea mainly in neonatal farm

**Corresponding author: Email: victor.njom@esut.edu.ng, njomvic@yahoo.co.uk;*

animals and can be devastating in immune suppressed animal hosts, but self-limiting in competent hosts (Fayer et al. 2018). The infectious stage of the organism (oocytes) is shed in the faeces of infected animals and it is typically an acute short-term infection. It is spread through the faecal-oral route, often through contaminated water and food [3].

Cryptosporidium is one of several protozoan genera in the phylum Apicomplexa, which develops within the gastrointestinal tract of vertebrates throughout their entire life cycles [4]. Apicomplexans are obligate intracellular parasites that are characterized by the presence of special organelles located at the tips (apexes) of cells that contain materials used to penetrate the host cells and establish successful infections.

The parasite was first described in the early 20th century with *Cryptosporidium muris* and *C. parvum* being the first species described (Tyzzer 2017). *C. baileyi*, is recognized as an important cause of respiratory disease in poultry and game birds. Transmission of infection to humans and other mammals is through the ingestion of the oocysts of *C. Parvum* [4]. Upon ingestion, oocysts are activated, releasing four sporozoites, which then invade host epithelial cells in the small intestine [5,6]. The species-affecting animals (cattle) in Nigeria include *C. bovis, C. ryanae and C. andersoni* [7]. The public health challenge comes as a result of cryptosporidiosis that became apparent when *Cryptosporidium* was recognized as a common cause of acute diarrhoea in immunocompetent individuals [8].

In Nigeria, reports on cryptosporidiosis were based on microscopic detection of the oocysts in faecal samples with prevalence rates of 23.4% in Oyo State [3], 28.0% in Plateau State [9] and 33.0% in Sokoto State [10]. However, there's inadequate information of this parasite in Southeast, therefore this study aims at investigating the prevalence of *Cryptosporidium* infection among apparently healthy and immune-compromised individuals at Parklane Hospital Enugu, Enugu state.

2. MATERIALS AND METHODS

2.1 Study Design

A cross-sectional study involving microscopy and structured questionnaire was carried out at Enugu State University Teaching Hospital. The study involved both hospitalized and outpatients attending clinical consultation within the period of the study.

2.2 Sample and Data Collection

A total of 390 participants who consented were recruited for the study. The population was divided into two groups: apparently healthy group (195) and immune-compromised patients (195). A total of 260 faecal and 130 urine samples were collected from the two groups. The samples were collected using sample bottles that were pre-labelled with patients' card number and other information for easy identification. The stool samples were preserved in 75% alcohol until use while the urine samples were analysed within 12 hours of collection each day. For data on demographic and risk factors, a structured questionnaire was designed to determine some common epidemiological factors that predisposed human to *Cryptosporidium*. Questions were on demographic data (age, sex); socioeconomic background (i.e. occupational and educational status); behaviour (i.e. personal hygiene such as hand washing and food consumption etc.

2.3 Parasitological Examination

Stool sample was investigated for the presence of *Cryptosporidium* infection according to the method described by Pam et al. [9]. Briefly: approximately 0.5g of faeces was homogenized in 10 ml of distil water in a test tube; the homogenate result was filtered through a 300 µm mesh sieve into a centrifuge tube and centrifuged at 2000 rpm for 10 minutes. The supernatant was pipetted and discarded, the sediment was washed twice in distilled water for 2 minutes at 6,500 rpm, and the pellet was suspended in 15 µl of distilled water, thereafter centrifuged at 1000 rpm for 2 minutes. The resultant sediment was used to make smear on a clean glass slide, air dried and fixed in methanol for 1 minute, stained with 10% Giemsa solution for 35 -40 minutes and rinse in gentle running tap water thereafter allowed to dry and then viewed with x100 magnification (oil immersion).

2.4 Urine Examination

Urine sample (5ml) from sample bottle was discharged into a test tube and centrifuged at 1250 rpm for 10 minutes. The supernatant was discarded and the sediment (pellet) was washed by centrifugation at 6,500 rpm for 2 minutes, the resulted crude was re-suspended in Phosphate Buffered Saline (PBS) and thereafter centrifuged at 1000 rpm for 2 minutes. The end product was used to make smear on a clean glass slide which was air dried and fixed in methanol for 1 minute stained with Giemsa solution for 35 minutes and rinsed in gentle running tap water, allowed to dry and later examined with x100 magnification (oil immersion).

2.5 Data Analysis

Prevalence data were computed in percentage and association between risk faction and proportion of infection was subjected to chi-square analysis to-illustrate relationship within and across the groups studied. The association was assumed significant at $p < 0.05$. All the analysis was done using Statistical Package for Social Sciences (SPSS) 2021version.

3. RESULTS

This study found that the overall prevalence of *Cryptosporidium* during the study period was 11(2.82%). All demonstrated oocyst were from stool sample only with 11(8.46%) positive cases of *Cryptosporidium* infection. There was no positive case in apparently healthy participants and no oocyst was detected from urine samples. Immune-compromised patients (HIV/AIDS) had the highest prevalence of 11(5.64%) *Cryptosporidium*. There was significant difference ($p>0.05$) in proportion of infection between apparently healthy and immune-compromised patients (Table 1).

According to sex and age, there was no *Cryptosporidium* infection, among apparently healthy male and female. On the contrary among the immune-compromised, male had the highest prevalence with 4 (7.27%) *Cryptosporidium* infection, while female had 5.00%. In male, the highest was in age group of 26 – 35 years with 2 (11.1%) infected cases while the lowest prevalence was within the 36 – 45 years age group with 1 (6.25%) infection. The age group of 15 – 25 had 1(9.09%), while other age group had no positive case. In all there was no significant difference ($p = 0.075$) in prevalence of *Cryptosporidium* infection among the positive cases. In female, the highest prevalence was found among 15 – 25 years age group with 2 (10.5%) infected patients, while lowest was among 36 – 45 years old with 1 (2.63%) person infected. other ages recorded no positive case of *Cryptosporidium* infection as shown in Table 2.

3.1 Potential risk Factors Associated with *Cryptosporidium* Infection among Subjects

Potential risk factors that can predispose man to *Cryptosporidium* infection among subjects were assessed. For educational level, the respondents below primary school level had 5.55% infection, while those above secondary school level recorded 2.22% and subjects who attained only primary school level had the highest (11.3%). Analysis showed no statistically significant difference ($p=0.9618$) in prevalence of *Cryptosporidium* infection and educational level in spite of the difference in prevalence observed with respect to the various numbers of infections among the respondents. Another factor was behavioural risk, a low prevalence was recorded from subjects who washed their hand before meal (0.68%), while the highest prevalence was recorded from those who do not washed their hand before meal (16.6%), those who consumed road side food showed (6.66%) prevalence cases.

Table 1. Prevalence of *Cryptosporidium in* apparently healthy and immune-compromised patients in Parklane Enugu

Sources of Sample	Overall Samples	Apparently Healthy	HIV/AIDs
	No+ve (%)	**No +ve (%)**	**No. exm. +ve (%)**
Stool	260 11(4.23)	130 0(0.00)	130 11(8.46)
Urine	130 0(0.00)	65 0(0.00)	65 0(0.00)
Total	390 11(2.82)	195 0(0.00)	195 11(5.64)
P value			**0.00213**

Table 2. Age and Sex Prevalence of *Cryptosporidium* infection in Immunocompromised Patients in Park lane Enugu

Age	Apparently healthy				Immuno-compromised			
	Female		Male		Female		Male	
	No	Positive (%)	No	Positive (%)	No	Positive (%)	No	Positive (%)
0 -14	15	0(0.00)	6	0(0.00)	6	0(0.00)	8	0(0.00)
15 -25	23	0(0.00)	18	0(0.00)	11	1(9.09)	19	1(3.44)
26 -35	36	0(0.00)	28	0(0.00)	18	0(0.00)	46	2(4.34)
36 -45	18	0(0.00)	20	0(0.00)	16	1(6.25)	38	1(2.63)
46 +	13	0(0.00)	18	0(0.00)	4	0(0.00)	9	0(0.00)
	105	0(0.00)	90	0(0.00)	55	4(7.27)	140	7 (5.00)

Table 3. Potential risk factors associated with *Cryptosporidium* infection in Enugu state.

Variables	No. Exam.	No. +ve	Infection (%)	95% CL	P. value
Educational Level				**5.991**	**0.9618**
< Primary	36	2	5.55		
Primary	44	5	11.3		
≥ Secondary	180	4	2.22		
Behavioural risk				**4.815**	**2.218**
Wash hand before meal	146	1	0.54		
Don't wash before meal	24	4	16.6		
Road side food consump	90	6	6.66		
Sources of water supply				**5.317**	**0.0161**
Pipe-borne water	16	1	6.25		
Borehole	82	5	6.09		
River	64	8	12.5		
Water vendors	98	7	7.14		

There was no significant difference ($p < 0.05$) in prevalence of *Cryptosporidium* infection and the behavioural risks among respondents. The participants who use stream/river water had highest (12.5%) infection while respondents who claim to drink and use water vendors as source of water had prevalence of 7.14%. Subjects whose source of water was from piped-water supply had prevalence of 6.25%, while respondents who claim to drink and use water vendors as source of water had the prevalence of 7.14%, and those participants who use borehole water recorded 6.09% infection. There was statistically significant association ($p=0.0161$) in prevalence of cryptosporidia infection and source of water supply (Table 3).

4. DISCUSSION

Cryptosporidiosis in human occurs worldwide but prevalence vary widely depending on the geographical region, population studied and diagnostic methods used [11]. The study shows low (2.82%) prevalence of *Cryptosporidium* among subjects studied in Enugu. Our method identified *Cryptosporidium* oocyst in stool sample alone. It was observed that it is difficult to detect spores of *Cryptosporidium* in urine samples using microscopic method. Therefore, prevalence is dependent on methodology. On the other hand the prevalence was found among the People Living With Human Immune Virus (PLWHIV).

This study contradicts earlier report by Omalu et al. [12] who observed 7.29% from both apparently healthy and immune-compromised patients in Kano state, but in agreement with Muhammad, A. B., Sule, H., and Yusuf, S. [13] and Abah et al. [14] who differently observed 10% and 4.6% prevalence among HIV subjects in Kaduna and Minna Niger State respectively.

It is possible that low immune status among immune-compromised group of people was responsible for the high prevalence of *Cryptosporidium* infection observed in this study., There reports of other enteric parasitic and opportunistic infections which exacerbate immune depletion of HIV/AIDS patients and thereby complicate their conditions (Lobo et al. 2012). In addition shedding of *Cryptosporidium* spores is intermittent and it is known that patients diagnosed with cryptosporidiosis by analysis of biopsy material may have more stool that are devoid of the parasite Clarridge et al. (2010). Thus, the prevalence of *Cryptosporidium* among apparently healthy subjects and Immune-compromised individuals in Enugu would be expected to be even higher than results obtained from stool examination and other sample sources may indicate [15,16].

This present study showed that the prevalence is more in female than male counterpart. This is because female participants were more in the study population, visit hospitals for regular checkup and expose themselves more to some potential risk factors that predisposes human to Cryptosporidium infection. This report is in conformity with study of Oladele et al. [17] who observed that women are the main childcare providers and housekeepers in Nigeria and these were probably responsible for the high occurrence of both *Cryptosporidium* species among them. Women in general has more frequent contacts with children or animals than men. On the other hand the current study reported significantly higher prevalence of *Cryptosporidium* infection among age more than 15 years as compared to younger individuals [18,19]. This in line with study conducted by Tengku et al. (2018) who recorded 1.97% infection rate in Malaysia in > 15 years old. Likewise, a recent parasitological evaluation of stool samples from Czech Republic citizens (that is immunocompetent individuals and foreign students of varying age groups) had identified 34% to 56% prevalence rates of shedding spores, with the highest prevalence in the group of 46 years and above [20]. Similarly, Norhayade et al. (2008) reported a high

prevalence of 57.2% of cryptosporidiosis among adult aged more than 31 years. The high prevalence rate of the infection in this study among the > 15 years might be explained by the fact that their behavior is related to more active movement and more independent eating habits compared with children.

Information from individual case reports of cryptosporidiosis in humans and from both natural and experimental infections in animals however has provided insights about the likely modes of transmission of *Cryptosporidium* to humans (Deplazes et al. 2000). The observations that Cryptosporidium are ubiquitous in nature suggest that several modes of transmission and sources exist for human infections. The possibility of human-to-human transmission cannot be ruled out. Experimental examples of *Cryptosporidium* transmission between laboratory animals might suggest that the same infecting species could be transmitted between humans via the same pathways as in animals (Bern et al. 2005). This research was able to identify association between several significant risk factors such as, source of water, and prevalence of *Cryptosporidium* infection. Some of mild infections may have missed because only microscopy was used as our primary screening tool; PCR would have been more sensitive, but may be impractical for screening the large number of specimens analyzed in the study.

5. CONCLUSION

The present study identified an overall prevalence of 2.8% of cryptosporidium infection among the studied population. Microscopy demonstrated oocyst from stool sample only with 11(8.46%) positive cases of *Cryptosporidium* infection while no positive case was found in apparently healthy participants and no oocyst was detected from urine samples. Immune-compromised patients (HIV/AIDS) had the highest prevalence of 11(5.64%) *Cryptosporidium* infection. There was significant difference ($p>0.05$) in proportion of infection between apparently healthy and immune-compromised patients. Cryptosporidiosis is one of the health problems of immune-compromised patients in Enugu town. These findings confirmed that risk factors such as educational level, age, and types of food and water source are epidemiological factors of *Cryptosporidium* infection in human.

CONSENT

As per international standard or university standard, patient(s) written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ren X, Zhao J, Zhang L, Ning C, Jian F, Wang R, Xiao L. Cryptosporidium tyzzeri n. sp.(Apicomplexa: Cryptosporidiidae) in domestic mice (*Mus musculus*). Experimental Parasitology. 2012;130(3):274-281.
2. Helmy AY, Krücken J, Nöckler K, Samson-Himmelstjerna G, Zes-sin KH. Molecular epidemiology of Cryptosporidium in livestock animals and humans in the Ismailia province of Egyptian Veterinary Parasitology. 2017;193: 15–24.
3. O'Donoghue P. Cryptosporidium and cryptosporidiosis in man and animals. International Journal of Parasitology. 2015; 25:139 –195.
4. Fayer R, Santın M, Trout JM, Greiner E. Prevalence of species and genotypes of Cryptosporidium found in 1-2-year-old dairy cattle in the eastern United States. Veterinary of Parasitology. 2016;135, 105–12.
5. Love MS, Beasley FC, Jumani RS, Wright TM, Chatterjee AK, Huston CD, McNamara CW. A high-throughput phenotypic screen identifies clofazimine as a potential treatment for cryptosporidiosis. PLoS Neglected Tropical Diseases. 2017;11(2):e0005373.
6. Ayinmode AB, Fagbemi BO, Xiao L. Molecular characterization of Cryptosporidium spp. in native calves in Nigeria. Journal of Parasitology Reservation. 2015;107:1019–21.
7. Maikai BV, Umoh JU, Kwaga JKB, Lawal I, Maikai VA, Camae V, Xiao L. Molecular characterization of Cryptosporidium spp. in native breeds of cattle in Kaduna State, Nigeria. Veterinary Parasitology. 2016; 178, 241–5.
8. Karanis P, Plutzer J, Halim NA, Igori K, Nagasawa H, Ongerth J. Molecular characterization of Cryptosporidium from animal sources in Qinghai province of China. Parasitology Reservation. 2017;101:1575–80.
9. Pam VA, Dakul DA, Karshima NS, Bata SI, Ogbu KI, Daniel LN, Udokaninyene AD, Kemza SY, Igeh CP, Hassan AA. Survey of

Cryptosporidium species among ruminants in Jos, Plateau State, North-Central Nigeria. Journal of Veterinary Advances. 2013;3(2): 49-54.

10. Faleke OO, Yabo YA, Olaleye AO, Dabai YUM, Ibitoye EB. Point prevalence of *Cryptosporidium* oocysts in calves grazing along river Rima bank in Sokoto, Nigeria. Pakistan Journal of Biological Sciences. 2014;17(3):443-446.
11. Didier ES, Snowden KF, Shadduck JA. Biology of microsporidian species infecting mammals. Advance Parasitology. 2013;40: 283-320.
12. Omalu ICJ, Duhlinska D, Anyawu G, Pam V, Inyama P. Seroprevalence of microsporidiosis in immune-compromised patient in Kano, Nigeria. The internet Journal of Parasitic Diseases. 2006;1:2.
13. Karanis Muhammad AB, Sule H, Yusuf S. Prevalence Of Cryptosporidiosis Among HIV/*AIDS*. Bayero Journal of Medical Laboratory Science. 2022;7(1):102-110.
14. Abah MO, Ejima AA, Omalu IC, Olayemi IK, Njoku MO, Paul S. Cystoisosporiasis in Apparently Healthy subjects and HIV/AIDS patients in Minna, Niger State. Journal of Public Health and Diseases. 2018;1(1):9-14.
15. Feng Y, Ryan UM, Xiao L. Genetic diversity and population structure of Cryptosporidium. Trends Parasitology. 2018;34:997–1011.
16. Lores B, Lopez-Miragaya I, Arias C, Fenoy S, Torres J. Intestinal microsporidiosisdue to Enterocytozoon bieneusi in elderlyhuman immunodeficiency virus-negative patients from Vigo, Spain. Clinical Infectious Diseases. 2002; 34:918-921.
17. Oladele HOK., Obot E, Bello RH. Qualitative detection of serum antibodies to Human Immuno-deficiency Virus among patients in Biu, Borno State, Nigeria. Nigeria Journal of Parasitology. 20I2;32:231 -233.
18. Abba OJ, Ibrahim IS, Idoko JA. Prevalence and risk factors for HIV/AIDs among male inmates in Jos prison, Plateau State, Nigeria. Nigerian Journal of Parasitology. 2011;32(2): 181-186.
19. Pam VA, Anwuliri COE. Cryptosporidium and cryptosporidiosis in calves at Jos, Northern Nigeria. Animal Production Research. 2019; 5:70-76.
20. Sak B, Kvac M, Kucerova Z. Latent cryptosporidium infection in immunocompetent individuals- a longitudinal study. PLoS Neglected Tropical Diseses. 2011;5:1 162.