Original Research Article

Cryptosporidiosis and Associated Risk Factors Among Children Attending Paediatric Hospitals in Enugu Metropolis, Enugu State, Nigeria

ABSTRACT

Cryptosporidiosis is a zoonotic disease caused by infection with protozoan parasite of the genus *Cryptosporidium*. It is associated with moderate to severe diarrhoea and increased mortality in Africa, and negatively affects child growth and development. A hospital based cross-sectional study was conducted to assess cryptosporidiosis and the associated risk factors among children aged 0-15years attending selected paediatric hospitals in Enugu Metropolis between November 2021 and August 2022. A total of five hundred and fifty stool specimens were examined for oocysts of *Cryptosporidium* species using Formol Ethyl-Acetate sedimentation and modified Ziehl Neelsen's staining techniques. The overall prevalence was 14.4% (79/550). Enugu State University of Science and Technology Teaching Hospital (ESUTTH) had the highest prevalence of crypto while St. Pat 32(25.6%) had the least 1(1.1%); also, private hospitals were less infected than public hospitals which was statistically significant (P<0.05). The age group ≤ 5years 43(15.8%) has the highest prevalence. Males 52(17.1%) were more infected than females 27(10.98%), which was statistically significant (P<0.05). Children whose parents had only primary education 11(26.8%) had the highest cryptosporidiosis case (P<0.05). There were no significant differences in the occupation and the type of residence of parents/caregivers (P>0.05). Possession of house pets 23(25.3%), drinking streams/rain water 27(26.7), eating meals from outside the home 47(20.6%), persons who practice open bush defaecation 23(26.4%), and household with poultry farm 43(25.6%) were significantly associated with cryptosporidiosis (P<0.05). Other protozoan parasites identified were *Entamoeba histolytica* 88(16.1%) and *Giardia lamblia* 69(12.5%) although not statistically significant (P>0.05). There was co-infection of *Cryptosporidium* and *Entamoeba histolytica* 27(4.9%), *Cryptosporidium* and *Giardia lamblia* 21(4.3%) which were statistically significant (P<0.05). Intervention by way of education of the public on good hygiene habits, inclusion of examination of stool samples for *Cryptosporidium* infection at health institutions, provision of clean water and proper disposal of faeces will help reduce cryptosporidiosis among children.

Keywords: Cryptosporidiosis, Children, Risk factors, Enugu Metropolis, Hospital

1. INTRODUCTION

Cryptosporidiosis is a parasitic disease caused by *Cryptosporidium*, a protozoan parasite in the Phylum Apicomplexa [1]. It causes diarrhoea which is a leading cause of morbidity and mortality among children in developing countries [2]. There are various aetiological agents of diarrhoea which include a wide variety of bacteria, viruses and parasites including *Entamoeba histolytica*, *Giardia lamblia*, *Cryptosporidium* species amongst others [3]. They result in sporadic outbreak of self-limiting diarrhoea in healthy persons, and chronic life-threatening illnesses in immune compromised patients. It also results to malnutrition, weight loss, stunted growth and cognitive impairment in young children [4]. *Cryptosporidium* species are highly successful parasites due to their large host range, high oocyst output from infected individuals, waterborne transmission route and low infectious dose which could be as low as one oocyst can cause infection [5, 6]. Other studies also suggest that flies may play a role in its mechanical transmission [7].

Cryptosporidium species has been revealed to be second only to Rotavirus as a contributor to moderate to severe diarrhoeal disease among children in the first five years of life and causes 30 to 50% of deaths in infants and children [5, 8, 9]. Infection with cryptosporidiosis may be acquired through direct contact

with infected persons or transmitted by animals, and ingestion of contaminated food and water [10]. Oocysts of *Cryptosporidium* species are resistant to harsh environmental conditions and not eliminated by chlorination and may persist in post treatment water supplies [1].

Cryptosporidium infection in children was estimated to be associated with 44.8 million diarrhoeal episodes and 48,300 deaths globally of these, Africa accounts for 75% of the diarrhoeal episodes and 88% of the deaths and is greatest in sub-Saharan Africa, especially Nigeria and Democratic republic of Congo (DRC) where about 48% of the under-5 associated deaths occur [11]. The clinical signs of cryptosporidiosis ranged from abdominal cramps, fatigue, loss of appetite, nausea, vomiting, weight loss, cough, fever, headache, muscles or joint aches and malnutrition [3]. People who are more at risk for infection are children who attend day care centers, including diaper-aged children, child care workers, parents of infected children, older adults (ages 75years and older), Doctors who take care of infected patients, children in an internally displaced persons (IDPs) camp who lack portable water supply and good sanitation [5, 12].

There is no fully effective drug treatment as the treatment is often symptomatic and supportive [13, 14]. The lack of specific treatment options for human cryptosporidiosis means that prevention of infection is paramount [15]. Practice of good hygiene by washing hands often with soap and water and the use of protective equipment when handling faeces especially on farms and day care centers.

There is paucity of data on *Cryptosporidium* infection among children in Enugu and its environs. Majority of the previous studies focused on diarrhoeal disease caused by other parasites and bacteria. Information on Cryptosporidiosis and predisposing factors can aid effective intervention. Hence, the aim of this study was to determine the prevalence of cryptosporidiosis and the associated risk factors in children attending selected paediatric hospitals in the study area.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted in selected paediatric hospitals in Enugu Metropolis. Enugu is the capital city of Enugu State, Nigeria. Both public and private primary, secondary and tertiary institutions are present in the area. The area is predominantly rural and agrarian, with a substantial proportion of its working population engaging in farming. Some inhabitants in the Metropolis use free spaces in their residence to plant crops or practice poultry farming. Recent data by Water, Sanitation and Hygiene (WASH) National Outcome Routine Mapping [16] shows that only 38% of the state's population has access to decent sanitation, with 40% still defecating in the open. About 28% of the population is without access to basic water supply services, relying on contaminated streams and rivers, unlined and unprotected wells to meet their needs. The residents include civil servants, students of various levels of education, business men and women, artisans and other people in various occupations of life.

2.2 Study Design

The research was a hospital based cross-sectional conducted between November 2021 and August 2022. The randomly selected hospitals include: University of Nigeria Teaching hospital (UNTH) Ituku-Ozalla, Enugu State University of Science and Technology Teaching Hospital (ESUTTH) Parklane GRA; Mother of Christ Specialist Hospital Ogui (MCSHO), Poly General Hospital (PGH) Asata, and St Patrick hospital (SPH) Independent Layout.

2.3 Inclusion and Exclusion Criteria

Children within the age 0-15years, whose parents/guardians gave consent after clear explanation of the study objective were enrolled in the study. Those children who did not meet these criteria were excluded. Only participants consulted or hospitalized in the paediatric sections were included.

2.4 Sample Size Calculation
Sample size was calculated from the Lorenz formula using the 7.6% prevalence of cryptosporidiosis from a previous study in Enugu State Nigeria [17]. A minimum sample size of 108 was calculated. However, a total of 550 children were selected.

2.5 Stool Specimen Collection, Handling and Storage
The parents/guardians of children were given a well labelled wide neck container to provide stool sample. The specimens were produced by the study participants early the next day and was received the immediately. The stool samples were preserved in the refrigerator at 4^{o}C while waiting for analysis at the end of each day's collection.

2.6 Examination of the stool for *Cryptosporidium* oocysts
Stool examination and identification of *Cryptosporidium* oocysts were done according to Cheesbrough [18]. Stool specimens were concentrated using formol ethyl-acetate method technique while detection of *Cryptosporidium* oocysts in the concentrated stool was done using the modified Ziehl Neelsen's staining technique.

2.7 Determination of Risk Factors for Cryptosporidiosis
Pretested questionnaires were used to collect demographic information such as name, age, and gender, clinical symptoms including diarrhoea, its duration and also the risk factors of cryptosporidiosis**.**

2.8 Data Analysis
Data were entered into Microsoft Excel version 2007 and were subjected to descriptive statistics. Chi-square at 95% significant level ($p<0.05$) was conducted to test relationship between variables.

3. RESULTS
Of the 550 children examined for *Cryptosporidium* oocysts, a prevalence of 79(14.4%) was recorded. Highest infection was recorded in ESUTTH, 32(25.6%), while the least was in SPH, 1(1.1%). The prevalence of *Cryptosporidium* among the selected hospitals was statistically significant ($P<0.05$) [Table 1]. Prevalence was higher in public hospitals 9(4.7%) than in privately owned hospitals which is statistically significant ($P<0.05$). [Table 2]. Children ≤ 5years has the highest prevalence, 43(15.8%) while the least was among the age group 6-10years, 28(12.7%), although not statistically significant ($P>0.05$) [Table 3]. Infection was higher in males, 52(17.1%) than in females, 27(11.0%) which was statistically significant ($P<0.05$) [Table 4].

Children whose parents/caregivers had only primary education 11(26.8%), had the highest prevalence while those whose parents/caregivers who had tertiary education 17 (8.9%), ($P<0.05$) has the least. Children whose parents/caregivers were farming had the highest prevalence of cryptosporidiosis, 9(25.0%) while children of the civil servants, 40(12.4%) had the least which was significant ($P>0.05$). Children living in multifamily residences 48(15.2%) had higher prevalence than those living in privately owned homes, 2(6.3%) ($P>0.05$). Also, children who have pets in their houses 23(25.3%); who had stream and rain water as their sole sources of drinking water 27(26.7%); who take local beverages 54(15.7%); who eat meals from outside the home 47(20.6%); who practice solely open bush defecation 23(26.4%); who had poultry in their houses 43 (25.6%); were infected more with cryptosporidiosis than their counterparts who have opposite attributes. The results were statistically significant ($P<0.05$) [Table 5].

Aside *Cryptosporidium* oocysts which was 79(14.4%), other parasites encountered were cysts of *Entamoeba histolytica* 88(16.1%) and cysts of *Giardia lamblia* 69(12.5%) [Table 6]. Co-infection of these parasites with *Cryptosporidium* was also recorded. The children had higher cases of *Cryptosporidium* infection only, 31 (5.7%), *Cryptosporidium* and cyst of *E. histolytica*, 27 (4.9%), *Cryptosporidium* and cysts of *Giardia* sp., 21 (3.8%), which was statistically significant ($P<0.05$) [Table 7].

Table 1: Prevalence of *Cryptosporidium* in children from different hospital

Hospital	Number examined	Number positive (%)
ESUTTH	125	32(25.6)
UNTH	119	11(9.2)
PGH	115	27(23.5)
MCSHO	101	8(8.0)
SPH	90	1(1.1)
Total	550	79(14.4)

(χ^2 39.39> χ^2 9.48; P<0.05).

Table 2: Prevalence of *Cryptosporidium* in public and private hospitals

Hospital	Number examined	Number positive (%)
Public	359	70 (19.5)
Private	191	9 (4.7)
Total	550	79 (14.4)

(χ^2 22.2 > χ^2 3.84; P<0.05)

Table 3: Prevalence of *Cryptosporidium* in age groups

Age (years)	Number examined	Number positive (%)
≤ 5yrs	273	43(15.8)
6-10yrs	220	28(12.7)
11-15yrs	57	8(14.0)
Total	550	79(14.4)

(χ^2 0.911 < χ^2 5.99; P>0.05)

Table 4: Prevalence of *Cryptosporidium* in gender groups

Gender	No examined	Number positive (%)
Male	304	52(17.1)
Female	246	27(11.0)
Total	550	79(14.4)

(χ^2 4.2 > χ^2 3.84; P<0.05)

Table 5: Risk factors of cryptosporidiosis among the study participants

Risk Factors	Parameters	Frequency (n=550	Number positive (%)	P value
Level of Education of Parents	Primary	41	11(26.8)	
	High School	318	51(16.0)	0.005
	Tertiary	191	17(8.9)	
Occupation of Parents	Farming	36	9(25.0)	
	Trading	191	30(15.7)	0.099
	Civil Servant	323	40(12.4)	
Type of residence	Private Home	32	2(6.3)	
	Self Contained	203	29(14.3)	0.385
	Multifamily residence	315	48(15.2)	

Possession of house pet	Yes	91	23(25.3)	0.001
	No	459	56(12.2)	
Sources of drinking water	Municipal Tap	239	33(13.8)	
	Rain/stream	101	27(26.7)	0.0001
	Bottled/Sachet	210	19(9.1)	
Local Beverage intake	Yes	345	54(15.7)	
	No	205	25(12.2)	0.264
Meals from outside the home	Yes	228	47(20.6)	
	No	322	32(9.9)	<0.0001
Type of Toilet	Water Closet	296	30(10.1)	
	Pit Toilet	167	26(15.6)	<0.0001
	Bush	87	23(26.4)	
Presence of Household Poultry	Yes	168	43(25.6)	<0.0001
	No	382	36(9.4)	

Table 6: Prevalence of other protozoan parasites identified

Hospital	Number examined	Number positive for *Cryptosporidium* (%)	Number positive for *E. histolytica* (%)	Number positive for *G. lamblia* (%)	Total (%)
ESUTTH	125	32(5.8)	29(5.3)	14(2.5)	75(13.6)
UNTH	119	11(2.0)	16(2.9)	19(3.5)	46(8.4)
PGH	115	27(4.9)	26(4.7)	21(3.8)	74(13.4)
MCSHO	101	8(1.5)	12(2.2)	9(1.6)	29(5.3)
SPH	90	1(0.2)	5(1.0)	6(1.1)	12(2.2)
Total	550	79(14.4)	88(16.1)	69(12.5)	236(43)

(χ^2 13.3 > χ^2 9.48; P>0.05)

Table 7: Co-infection of *Cryptosporidium* infection and other protozoan parasites

Hospital	Number examined	Number positive for *Cryptosporidium* only (%)	Co-infection of *Cryptosporidium* and *E. histolytica* (%)	Co-infection of *Cryptosporidium and G. lamblia*	Total (%)
ESUTTH	125	16(2.9)	9(1.6)	7(1.3)	32(5.8)
UNTH	119	2(0.4)	6(1.1)	3(0.5)	11(2.0)
PGH	115	7(1.3)	11(2.0)	9(1.6)	27(4.9)
MCSHO	101	5(0.9)	1(0.2)	2(0.4)	8(1.5)
SPH	90	1(0.2)	-	-	1(0.2)
Total	550	31(5.7)	27(4.9)	21(3.8)	79(14.4)

(χ^2 85.98 > χ^2 9.48; P<0.05)

4. DISCUSSION

A *Cryptosporidium* prevalence of 14.4%. was recorded in this study. The observation is less than 21.8% reported by Obiukwu *et al*. [19], but higher than 7.6% by Onyemelukwe *et al*. [17] both in Enugu State. The prevalence in this study was probably because Enugu metropolis is a city with improved access to drinkable water, toilet facilities, less faecal contamination rates, thus limiting the occurrence of parasitic infections and cryptosporidiosis. Also, the prevalence recorded may be explained by methodology used. Abdullahi *et al*. [20] in Abuja recorded the prevalence of 13.7% and 26.3% using mZN technique (microscopy) and Enzyme-linked immunosorbent assay (ELISA) respectively. It may be difficult to detect the parasite in stool samples, which contain few or distorted oocysts, leading to a false negativity of the mZN microscopy and also due to scarcity of parasite [20].

In this study, age group 0-5years were most infected (15.8%). Other studies have also observed highest prevalence in the same age group in Lagos, Enugu and Abuja [3, 19, 20] This age group may be vulnerable because of deficient immunity in younger children. This age group is also more susceptible to diarrhoeal infections because basic hygiene activities are not observed [5].

Cryptosporidiosis infection was higher in males (17.1%) than in females (11.0%) which was statistically significant. This compares favourably with 26.8% by Obiukwu *et al*. [19] in Enugu State, 12.0% by Abdelhakam *et al*. [21] in Sudan and 37.5% by Addullahi *et al*. [20] in Abuja. In the study area, males were exposed to a lot of activities like swimming in the river than the females. They also stay outdoor than females and can eat outside the home. These could be contributory factors. However, Tombang *et al*. [22] reported a slightly higher prevalence of cryptosporidiosis in females (5.36%) than in males (3.57%) in Cameroon.

The distribution of *Cryptosporidium* oocyst with regards to the educational status of the parents/caregivers showed that children whose parents had only primary education (26.8%) had the highest prevalence. This could be as a result of ignorance to the preventive measures of cryptosporidiosis such as implementation of good hygiene. Similarly, Tombang *et al*. [22] in Cameroon recorded 80% prevalence for children whose parents were uneducated. Also, Abdullahi *et al*. [20] made similar observation in Abuja Nigeria. Also, there is higher prevalence in children who live in multifamily residence. Children whose parents live in close quarters tend to ignore the importance of implementing proper hygiene and sanitation and these practices might increase the general environmental contamination as well as the risk of *Cryptosporidium* infection for all other persons living in the same setting.

Possessions of house pets were among the significant risk factors of cryptosporidiosis. Some of the children were from areas where birds, cats and dogs are commonly wandering freely which may be a route for subsequent zoonotic spreading of oocyst, contaminating the soil and water with their feaces. Also, the isolation of *C. canis* from both dogs and children from the same household in Peru highlighted the possibility of human infections from dogs [23].

Findings from this study also revealed that children who use rain/stream for drinking purposes were at higher risk of infection than those who use municipal tap, sachet and bottled water which was statistically significant. This could be as a result of the contamination level of the sources of drinking water. Sachet and bottled water are mostly treated and sealed to avoid contamination but not so in rain/stream water. Faecal matter can enter the stream through wastewater overflow and even agricultural effluent. Also, rain water may become contaminated before use when the container is left open.

People who take locally made beverages like “Kunu”, “Soya-milk”, “Zobo” and Tiger nut drink and those who take meals outside of home were more infected. This may be as a result of methods used in preparing these edibles, sources of water and handling processes as the beverages were not pasteurized or sealed to avoid contamination. Infected food handlers/vendors may also be a factor. There is also possibility of transmission pattern involving flies that can pick the *Cryptosporidium* oocysts from faeces transferring it to food. Higher prevalence recorded as a result of poor sanitary facilities in the case of open bush defecation and pit toilet could be attributed to improper disposal of human and animal waste, which could lead to contamination of food and drinking water sources.

Children who have poultry farms in their homes had higher prevalence. According to Khan *et al*. [9], there are higher cases of *Cryptosporidium* infection in those living with domestic animals. This could be as a result of unhygienic practices that involve cleaning the animal farm without personal protective equipment and not washing hands properly before touching food items [23]. Other protozoan parasites which include, cysts of *E. histolytica* and cysts of *G. lamblia* were also identified in this study either singly or as coinfection with *Cryptosporidium*. Unhygienic food and water consumed by children, poor sanitary practices in homes and schools and the lack of knowledge or non-compliance to preventive measures of these protozoan parasites by parents/caregivers may be contributory factors.

5. CONCLUSION

The findings of this study revealed that *Cryptosporidium* is an important protozoan etiologic agent for children attending paediatric hospitals in Enugu metropolis. Intervention by means of health education to the public to practice good hygiene in schools and homes is recommended. Thorough washing of hands often with soap and water, and the use of alcohol-based sanitizer to reduce infection rate should be practiced.

CONSENT

Written informed consent was obtained from parents and caregivers of the children before the study.

ETHICAL APROVAL

Ethical approval was obtained from the Ethical Committee, Mother of Christ specialist hospital, Ogui (MOCSP/CERT/17), while study permission was obtained from the Ministry of Health, Enugu (MH/MSD/REC21/233).

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