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

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Cryptosporidiosis is a zoonotic disease caused by infection with protozoan parasite of the genus *Cryptosporidium*. It is associated with moderate to severe diarrhoea which may eventually lead to death. The parasite impedes growth and development of affected children. 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 samples were examined for oocysts of *Cryptosporidium* species using Formol Ethyl-Acetate sedimentation and modified Ziehl Neelsen's staining techniques. The overall prevalence of the parasite was 14.4% (79/550). Enugu State University of Science and Technology Teaching Hospital recorded the highest prevalence 32(25.6%) of *Cryptosporidium* species while St. Patrick Hospital recorded the least 1(1.1%). Public

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hospitals recorded more infections than private hospitals. The age group ≤ 5years 43(15.8%) recorded the highest prevalence. Males 52(17.1%) were more infected than females 27(10.98%), which was not significant (P>0.05). Children whose parents/caregivers had only primary education 11(26.8%), has the highest prevalence while those whose parents/caregivers had tertiary education 17(8.9%), has the least. Children whose parents/caregivers were farming has the highest prevalence 9(25.0%), while children of civil servants, 40(12.4%) has the least which was not significant (P>0.05). Children living in multifamily residences 48(15.2%) had higher prevalence than those living in private homes, 2(6.3%). Other protozoan parasites identified in the study were *Entamoeba histolytica* 88(16.1%) and *Giardia lamblia* 69(12.5%). There were co-infections of *Cryptosporidium* and *Entamoeba histolytica* 27(4.9%), *Cryptosporidium* and *Giardia lamblia* 21(4.3%) which was not significant (P>0.05). Intervention through health education on good hygiene habits, and 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 study conducted between November, 2021 and August, 2022. The randomly selected hospitals include: Public hospitals; University of Nigeria Teaching hospital (UNTH) Ituku-Ozalla, Enugu State University of Science and Technology Teaching Hospital (ESUTTH) Parklane GRA, and Poly General Hospital (PGH) Asata. Private Hospitals; Mother of Christ Specialist Hospital Ogui (MCSHO), and St Patrick hospital (SPH) Independent Layout.

2.3 Inclusion and Exclusion Criteria

Children both male and female 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 the 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

A total of 550 stool samples were collected for the study. 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. The analysis was conducted at the laboratory of the department of Parasitology and Entomology, Nnamdi Azikiwe University, Awka Nigeria.

2.6 Examination of the Stool for *Cryptosporidium* Oocysts

Physical examination was conducted on the stool samples. The collected stool-samples were concentrated using the formol-ethyl-acetate concentration technique and Cryptosporidium oocysts were detected using the modified Ziehl Neelson staining practice according to the procedure described by Cheesbrough [18]. Cryptosporidium oocysts were confirmed using the oil immersion objective of the microscope as pinkish-red spherules on a blue background.

2.7 Determination of Risk Factors for Cryptosporidiosis

Semi-structured pretested questionnaires were used to collect demographic information of the study participants such as name, age, gender, clinical symptoms and 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

A *Cryptosporidium* 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 not significant (p=9.48; P>0.05). Prevalence was higher in public hospitals 9(4.7%) than in privately owned hospitals. [Table 1]. Children ≤ 5years has the highest prevalence, 43(15.8%) while the least was among the age group 6-10years, 28(12.7%), although not significant (p=5.99; P>0.05). Infection was higher in males, 52(17.1%) than in females, 27(11.0%) which was not significant (p=3.84; P>0.05) [Table 2].

Children whose parents/caregivers had only primary education 11(26.8%), had the highest prevalence while those whose parents/caregivers had tertiary education 17(8.9%), 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. Children living in multifamily residences 48(15.2%) had higher prevalence than those living in privately owned homes, 2(6.3%). 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. [Table 3].

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

Hospital type	Number examined	Number infected (%)	P value
Public			
ESUTTH	125	32(25.6)	
UNTH	119	11(9.2)	9.48
PGH	115	27(23.5)	
Total	359	70(19.5)	
Private			
MCSHO	101	8(8.0)	
SPH	90	1(1.1)	
Total	191	9(4.7)	
Grand Total	550	79(14.4)	

Table 2. Prevalence of *Cryptosporidium* in relation to age and gender of participants

Age category	Examined	Infected (%)	P value
≤ 5yrs	273	43(15.8)	
6-10yrs	220	28(12.7)	5.99
11-15yrs	57	8(14.0)	
Total	550	79(14.4)	
Gender			
Male	304	52(17.1)	3.84
Female	246	27(11.0)	
Total	550	79(14.4)	

Table 3. 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)	

Risk Factors	Parameters	Frequency (n=550)	Number positive (%)	P value
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)	

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 4]. 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%) [Table 5].

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 slightly low prevalence in this study may be because most inhabitants of the study area has access to good drinking water, and modern toilet facilities, thus reducing the occurrence of parasitic infections. Also, the prevalence rate recorded may also be determined by the 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%). 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 more to some risk factors than females. Males also stay outdoor than females and can eat outside the home. These could be a contributory factor. However, Tombang et al. [22] reported a slightly higher prevalence of cryptosporidiosis in females (5.36%) than in males (3.57%) in Cameroon.

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. 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].

Children who drink rain/stream were at higher risk of infection than those who drink municipal tap water, sachet and bottled water. Sachet and bottled water are mostly treated and sealed to avoid contamination but not so with rain/stream water. Faecal matter can enter the stream through wastewater overflow and irrigation sources. 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 the edibles, sources of water and handling processes as the beverages were not pasteurized or sealed. There were also possibilities of mechanical transmission pattern involving flies that pick parasites immature stages 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.

Children whose parents/guardian run poultry farms around homes had higher prevalence. According to Khan et al. [9], there were higher cases of *Cryptosporidium* infection among those keeping domestic animals. This could be due to unhygienic practices which may involve cleaning animal houses 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.

Table 4. 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 5. Co-infection of *Cryptosporidium* infection and other protozoan parasites

Hospital	Number examined	Single Infection of *Cryptosporidium* (%)	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)

5. CONCLUSION

The findings of this study revealed that *Cryptosporidium* is prevalent in children of 0-15years in the study area. Poor hygiene, lack of clean drinking water and improper faecal disposal are among the risk factors in the study area. Intervention by means of health education on good hygiene in schools and homes is highly recommended. Also, thorough washing of hands often with soap and water, and the use of alcohol-based sanitizer to reduce infection rate should be practiced. Future studies should focus on establishing the species and subtypes circulating among the human and animal populations. Also, information to understand the molecular epidemiology of the parasite is necessary.

CONSENT

Written informed consent was obtained from parents 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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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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