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Prevalence of Cryptosporidiosis in diarrhoeal stools of children under-five years seen in Ahmadu Bello University Teaching Hospital Zaria, Nigeria

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Abstract: *Introduction:* Human cryptosporidiosis is a zoonotic disease and is increasingly recognized as a major public health problem. It is associated with significant effects on growth, physical and cognitive functions and excess mortality especially among children.
Aim: To determine the prevalence of *Cryptosporidium* oocyst excretion in children less than 5years with diarrhoea in ABUTH Zaria.
Methods: Children aged 0 to 59 months managed in paediatrics wards of ABUTH for diarrhoea were studied between July 2008 and June 2009. Stool specimens obtained from these subjects were analysed for *Cryptosporidium* oocysts using the modified ZN staining technique.
Results: A total of 185 children were enrolled. There were 78 (42.2%) boys and 107(57.8%) girls. A total of 33 children studied excreted oocysts in their stools, giving a prevalence of Cryptosporidium oocysts of 17.8%. The highest rate (21.7%) was observed in children aged between 13 and 36 months, and no oocysts were observed in stools of neonates. Oocyst excretion was observed to be commoner in the rainy season.
Conclusion: *Cryptosporidium* is a common cause of diarrhoea among under-five children in our environment. It was commoner after infancy and in the rainy season.
Recommendation: Routine screening for *Cryptosporidium* should be part of evaluation of diarrhoeal illness especially in children beyond the neonatal age group.

Key words: Cryptosporidiosis; diarrhoea; under-five; children

Introduction

Cryptosporidium spp. is one of the leading causes of severe diarrhoea[1]. It is said to be the third or fourth commonest cause of diarrhoeal disease, and its prevalence is much higher in developing countries than elsewhere[2,3]. It has been described as an emerging infectious threat[1], with dire consequences including prolonged hospitalization, adverse effect on the weight gain of children[4], severe effects on growth and nutritional status[5,6] diminished cognitive function[7] and may ultimately lead to death[8].

It is a zoonotic disease with mainly bovine and human reservoirs[3]. Several reports from parts of the developing world suggest cryptosporidiosis is endemic and is one of the most common causes of persistent diarrhoea among children, being associated with more than a quarter of all diarrhoeal illness[9,10]. Prospective community studies of childhood diarrhoea conducted in Guinea Bissau, found prevalence of Cryptosporidiumoocysts in diarrhoeal stools of under-five children to range between 6.0% and 7.7%[6,8]. A prevalence of 8.1% was reported among under-five children in Addis Ababa[11]. Reports from northern Ghana showed that *Cryptosporidium* oocysts was found only sporadically in both patients and controls[12]. In Nigeria, the prevalence of *Cryptosporidium* oocystsin stool samples in Enugu varied between 12.5%and 29%[13,14]. The prevalence was 10.8% in Port Harcourt[15] and 14% in Ilorin[16]. In one report from south west Nigeria, *Cryptosporidium* oocyst was not found in stool samples of patients studied[17], but a more recent study showed that *Cryptosporidium* is the most prevalent intestinal pathogenic parasite in Lagos, especially among diarrhoeal cases[18].

While several workers had previously identified Cryptosporidiosis to be an important cause of diarrhoeal illness in parts of Nigeria[13-16,18] there are no studies focussed on the prevalence of *Cryptosporidium* oocysts excretion in diarrhoeal stools of under-five children in this community. This study was therefore undertaken with a view to

determining the prevalence of Cryptosporidium oocysts excretion in diarrhoeal stools of children less than five years presenting to ABUTH Zaria. This will assist in formulating recommendations for the management and prevention of the condition in our environment.

Subjects and methods

Children between the ages of zero and fifty-nine months admitted to the paediatric wards of the Ahmadu Bello University Teaching Hospital (ABUTH) Zaria with diarrhoea or who developed diarrhoea while on admission in the course of their hospital stay were consecutively recruited between July 2008 and June 2009. For the purpose of this study, diarrhoea was taken to be an increase in frequency and fluidity of the stool of the patient.[19] Children whose parents or caregivers declined consent for the study were excluded. Relevant demographic details of all children enrolled for the study were obtained and recorded into a specifically designed proforma.

Stool sample was collected in dry clean container on the day of recruitment from each study subject. This was transported on the same day to the microbiology laboratory of ABUTH within 30 minutes of collection for microbiologic evaluation. To maximize recovery of oocysts, stool samples were concentrated prior to microscopic examination using the formalin-ethyl acetate sedimentation stool concentration method for clinical laboratories[20]. A smear was made from the stool sediment on a clean slide, air dried and fixed with methanol, Staining was with unheated carbolfuchsin and counterstained with 0.4% methylene blue. The smear was then carefully examined microscopically for Cryptosporidiumoocysts[20]. An experienced parasitologist rechecked the smears to ensure quality control.

Data obtained from the study was analyzed using the computer SPSS version 15.0. Results are presented in figures, tables and graphs as appropriate. Student's t-test was used to compare the means of normally distributed continuous variable while differences between proportions were evaluated by the Chi-square test and Yate's correction was applied as appropriate. A p-value of less than 0.05 was considered to be statistically significant in comparative analyses.

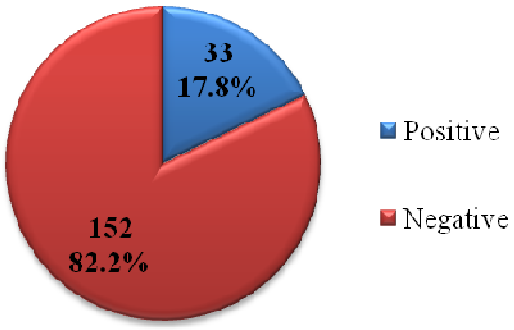
Case Management of children: All children recruited for the study were routinely additionally investigated as appropriate based on their other presenting symptoms and signs to establish the existence or otherwise of concomitant disease according to the standard of care in the hospital. All the children recruited for the study were then managed accordingly in the Diarrhoeal Therapy Unit (DTU) for diarrhoeal disease or in any of the paediatric units when there was a concomitant disease. The mothers and caregivers were taught the basic principles of personal and environmental hygiene, home management of diarrhoeal disease including prevention and correction of dehydration using ORS, and maintenance or improvement of nutrition.

Ethical Consideration: Approval of the Scientific and Ethical committee of the Ahmadu Bello University Teaching Hospital Zaria was obtained before the commencement of the study. Informed consent of each of the children's parents or caregivers was obtained before recruitment into the study.

Results

A total of one hundred and eighty five children (185) with diarrhoea aged 0 to 59 months, who consented for the study were enrolled and all completed the study. The prevalence of *Cryptosporidium* oocysts excretion among the 185 under five children who had diarrhoea studied was 17.8%. (Figure 1).

Fig 1: Prevalence of Cryptosporidium among the studied children with diarrhoea



Among the children studied, the gender distribution reflected a slight preponderance of females with a male to female ratio of 0.73:1. The mean age of the children was 19.8 ± 6.0 months. The mean weight and length of the studied children were 9.1kg ± 2.4 and 73.3cm ± 11.5 respectively. Those admitted with diarrhoea constituted 83.8% of the total while the rest developed diarrhoea in the course of their hospital stay. There was no statistically significant difference in gender between those admitted with diarrhoea and those who developed diarrhoea in the course of their hospital stay.

Majority of the children (55.7%), as shown in Table 1, were toddlers aged between 13 and 36 months. Sixty three children (30.8 %) were infants, while nineteen (10.3%) were aged above thirty six months. Table 1 also shows that male children predominated beyond the age of thirty six months. The age difference between gender was not statistically significant ($c^2 = 2.768$, df = 2, p = 0.251).

Age prevalence

Table 2 shows the frequency distribution of Cryptosporidium infection in diarrhoeal stools of children studied by age category. The highest *Cryptosporidium* prevalence rate (9.75%) was observed in toddlers

Discussion

This study has revealed the presence of *Cryptosporidiumoocyts* in the stools of children aged less than five years with diarrhoea in ABUTH. The 17.8% prevalence of *Cryptosporidium* infection in the study population may indicate that the organism is a significant pathogen in children less than five years with diarrhoea in this environment. This prevalence is within the observed range of up to a quarter of all diarrhoeal illness in the developing world in paediatric population being associated with cryptosporidiosis[9-10] and similar to prevalence rates of 14% in Ilorin,[16] but much lower than 29% reported among school children in Enugu[14]. The rate of detection of *Cryptosporidium* in this study was higher than the reported rates of 12.5% in Enugu,[13] 10.8% in Port Harcourt[15] and much higher than 6.0% and 7.7% reported in Guinea-Bissau[6,8] and 8.1% reported in under -five children in Addis Ababa[11] as well as rate reported in south west Nigeria.[18]

The reasons for these differences are possibly due to different methods used for detection of cryptosporidiosis and differences in the study population. While several community-based studies report lower prevalence rates of cryptosporidiosis in children with diarrhea in the range between 6.0% and 8.1%[6,11] prevalence rates reported among children recruited from hospitals tended to be generally higher.[16]

Although children aged 13 to 36 months were found to shed oocyts most in the study, however there was no statistically significant difference between the various age groups in oocysts excretion. ($c^2 = 1.464$, df = 2, p = 0.481). The peak age of oocysts shedding in this study was 22 months. This is in contrast to studies that found that *Cryptosporidium* infectionpeaks at 10 months.[9] This study however agrees with other studies that showed that young children less than 15 years,[13]and in particular children 0-2 years of age[15]appear to be more susceptible to *Cryptosporidium.* In this study, it appears that oocysts excretion in stools appear to taper off as the children approach sixty months. Similar finding was reported in a study that showed that oocysts excretion subsided after 36 months[9]and were not encountered after the age of four years.[15-16]

Perhaps, a plausible reason for the later peak in age of infection in this study is the possibility that age 22 months coincides with the age at cessation of breast milk and a complete transition to family diet in this community. It is also the age about which most children begin to freely explore their environment, coming in contact with animal dropings, contaminated foodand objects that they readily put in the mouths.

Boys in this study were as affected as girls, with no statistically significant difference in their rates of cryptosporidium oocyst excretion in stools. This is in line with other studies reflecting equal exposure and susceptibility in the environment.

While cryptosporidiosis appears to be present in this community all year round, prevalence was found to increaseduring the rainyseason(69.7%). This clear seasonal variation in the rate of *Cryptosporidium* oocyst excretion in this study was of statistical significance($c^2 = 4.509$, p = 0.0337). The same seasonal variationhas also been observed in other studies from Uganda,[9] Enugu[14] and the USA[1]. Seasonal variation observed in this study might be related to increased agricultural activities during rainy season in our community that promote contact with animals and their wastes used for agricultural activities. This affects the number of oocysts in the environment and promotes exposure to oocysts. Furthermore, factors such as humidity and temperature during the rainy season may enhance oocysts survival in that season.

This study shows that the important clinical features associated with Cryptosporidiosis are persistent diarrhea, watery stools, history of diarrheal illness in the past year, stool frequency more than four times a day and dehydration. This substantiates the literature where it has been said that symptomatic *cryptosporidium* infection is characterised by watery stools, persistent diarrhoea[5,9] and a tendency to reoccur.

Conclusion

This study has clearly demonstrated the presence of *Cryptosporidium* as an important pathogen among under-five children presenting to ABUTH with diarrhoea. It has also been able to identify some children more likely to excrete *Cryptosporidium* oocysts in diarrhoeal stools. Based on these findings, it is recommended that evaluation of cases of diarrhoeal illnesses in children less than five years presenting to the hospital should include screening for *Cryptosporidium* oocysts, especially in children beyond the neonatal age.

Prevention of *Cryptosporidium* infections is by avoidance of contamination of food and water sources by oocysts through protection of water sources. Implementation of multiple barriers to safeguard public drinking water sources can prevent cryptosporidiosis. However, effective control of diarrhoea caused by *cryptosporidium* remains largely elusive in sub-Saharan Africa and other developing regions.[10]

Authors' contribution
Musa, S: Concept and design of study, data collection, analysis and interpretation, writing of the initial draft.
Yakubu AM: Critical review for important intellectual input to draft, final approval of the manuscript.
Olayinka AT: Design of laboratory method, contribution of intellectual input to draft, approval of manuscript.
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between the 13 to 36 months age bracket. Only three (1.6%) of the children aged more than 36 months had *Cryptosporidium* Oocyts in their stools. However, these differences, when subjected to statistical analyses, were found not to be significant. (c^2 = 1.464, df = 2, p = 0.481).

Table 1: Characteristics of children with diarrhoea studied for *Cryptosporidium* oocysts in stools at ABUTH, Zaria.

Characteristic	Frequency (%)
Gender	
Male	78 (42.2)
Female	107 (57.8)
Period of development of diarrhoea*	
Admitted with Diarrhoea	155 (83.8)
Males	65 (41.9)
Females	90 (58.1)
Developed diarrhoea on admission	30 (16.2)
Males	13 (43.3)
Females	17 (56.7)

(No difference in gender between those admitted with diarrhoea and those who developed diarrhoea in the course of their hospital stay, c^2 = 0.020, df = 1, p = 0.887)

Table 2: Distribution of *Cryptosporidium* infection by age category

Category of children	*Cryptosporidium* oocysts		Total
	Positive	Negative	
Infant	8 (12.7)	47(87.3)	63 (100.0)
Toddler	22 (21.7)	81 (78.6)	103 (100.0)
Pre-school	3 (15.8)	16 (84.2)	19 (100.0)
Total	33 (17.8)	152 (82.2)	185 (100.0)

(No statistically significant difference in rate of oocyst excretion between age groups studied, c^2 = 1.464, df = 2, p = 0.481)

Sex prevalence

Table 3 shows the distribution of *Cryptosporidium* oocyst excretion in diarrhoeal stools of children studied by gender. The highest *Cryptosporidium* prevalence rate (10.3%) was observed in girls, while the prevalence in boys was 7.6%. These differences were found not to be statistically significant. (c^2 = 0.001, df = 1, p = 0.973).

Table 3: Distribution of *Cryptosporidium* infection by gender

Gender	ZN Positive	ZN Negative	Total
Males	14 (7.6)	64 (34.6)	78 (42.2)
Females	19 (10.3)	88 (47.6)	107 (57.8)
Total	33 (17.8)	152 (82.2)	185 (100.0)

(No statistically significant difference in rate of oocyst excretion between gender of children studied, c^2 = 0.001, df = 1, p = 0.973)

Seasonal prevalence

Ninety eight children were seen in the rainy season and 87 in the dry season. More children (69.7%) excreted oocysts during the rainy season (fig 2). The difference in excretion of oocysts between rainy and dry seasons is statistically significant. (c^2 = 4.509, p = 0.0337).

Types of diarrhoea

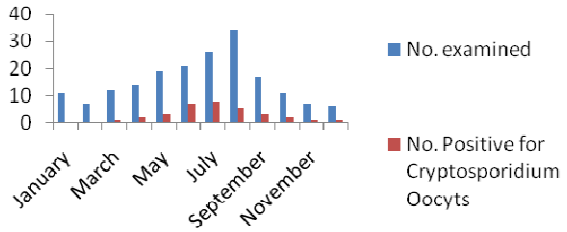
The type of diarrhoea seen among the studied population shows that acute watery diarrhoea occured in 132 (71%) of all children presenting with diarrhoea, while persistent diarrhoea and acute bloody diarrhoea were seen in 39 (21%) and 14 (8%) of the children, respectively.

Clinical correlates of *cryptosporidium* oocysts excretion
It was found out that oocysts excretion was more likely in children with persistent diarrhoeal illness (p = 0.0001, Fisher's exact test), watery stools (p = 0.0002, Fisher's exact test), previous history of diarrhoeal illness in the past year (p = 0.0005, Fisher's exact test), high frequency stools (p = 0.0001, Fisher's exact test) and dehydration (p = 0.0065, Fisher's exact test) as shown in Table 4.

Table 4: Clinical correlates of *cryptosporidium* oocysts in children studied

Variable	*Cryptosporidium* Oocyts		p value, Fisher's exact test	Logistic Regression Analysis OR (95% CI)
	Positive	Negative		
Diarrhoea duration				
Acute	103 (55.7)	8 (4.3)	0.0001	6.569 (2.764 – 15.613)
Persistent	49 (26.5)	25 (13.5)		
Type of stools				
Watery	32 (17.3)	101 (54.6)	0.0002	16.158 (2.146 – 121.645)
Dysentry	1 (0.5)	51 (27.6)		
Previous history of diarrhoea				
Yes	30 (16.2)	91 (49.2)	0.0005	6.703 (1.959 – 22.942)
No	3 (1.6)	61 (33.0)		
Stool frequency				
Low	6 (3.2)	98 (53.0)	0.0001	8.167 (3.1744 – 21.010
High	27(14.6)	54 (29.2)		
Hydration status				
Dehydrated	31 (16.8)	110 (70.3)	0.0065	5.918 (1.356 – 25.829)
Not dehydrated	2 (1.1)	42 (11.9)		

Fig 2: Seasonal variation of detection of *Cryptosporidium* oocysts in stools of children with diarrhoea studied at ABUTH, Zaria



(There was a statistically significant difference in rate of oocyst excretion between rainy and dry seasons, c^2 = 4.509, p = 0.0337)

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