Clinical Features and Risk Factors Associated with Cryptosporidiosis in Diarrhoiec Patients in Kaduna State, Nigeria

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Abstract: Background: Prevalence of cryptosporidiosis in developing countries vary widely implying that the infection can be more common than surveys of stool oocysts excretion demonstrate. Diarrhoea caused by *Cryptosporidium* species in childhood may be associated with subsequent impaired physical and cognitive development. **Objective:** This study was carried out to determine the prevalence and risk factors with the presence of *Cryptosporidium* species oocysts in human stool. **Methods:** The study is a cross-sectional survey carried out in Kaduna State. The study was a prospective cross-sectional study, a total of 600 diarrhoeic stool samples were collected and screened for oocysts of *Cryptosporidium* species using Sheather's sugar flotation method. **Results:** The prevalence of cryptosporidiosis was 4.5%. Children under 5 years were found to have significantly ($\chi^2 = 4.761$, $p = 0.029$) higher prevalence (6.6%) than older patients (2.9%). Symptoms including abdominal pain (Fisher's exact test = 0.001), fever (Fisher's exact test = 0.016) and stool characteristics (Fisher's exact test = 0.002) were also demonstrated to be significantly associated with cryptosporidiosis. *Cryptosporidium* infection in Kaduna State also had significant association ($p \leq 0.05$) with place of residence ($\chi^2 = 6.559$, $p = 0.010$), where rural dwellers had 7.0% (18/257) prevalence and urban dweller had 2.6% (9/343); animal contact ($\chi^2 = 12.848$, $p < 0.001$), patients who admitted having contact with animals had 7.0% (25/357) prevalence as against 0.8% (2/248); and method of water treatment ($\chi^2 = 16.486$, $p < 0.001$), 7.1% (27/379) of subjects who did not treat their water before consumption had cryptosporidiosis. **Conclusion:** These findings show that cryptosporidiosis is prevalent in Kaduna State, Nigeria and pose a threat to public health. Important factors in dissemination of the parasite are age of patient, place of residence, animal contact and method of treatment of water. It was also found that clinical manifestation of the infection include abdominal pain, fever and stool characteristics.
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1. Introduction

Cryptosporidium species is a protozoan parasite that causes gastrointestinal infection called cryptosporidiosis leading to diarrhoea in both humans and animals [1]. Diarrhoea caused by *Cryptosporidium* species in childhood may be associated with subsequent impaired physical and cognitive development [2]. Though the disease had worldwide distribution, reports suggested that the prevalence in developing countries vary widely implying that the infection can be more common than surveys of stool oocysts excretion demonstrate [3,4].

Various risk factors have also been reported to be associated with transmission of cryptosporidiosis. These include contact with animals, source of drinking water, age, level of education amongst others.

This study was designed to investigate the prevalence of *Cryptosporidium* infection in diarrhoiec stool samples from patients in Kaduna State and to determine the risk factors that may be associated with cryptosporidiosis.

2. Material and Methods

This study was a prospective cross-sectional descriptive, health facility-based study carried out between January – December, 2013.

This study was carried out among patients presenting with diarrhea who consented to take part in the study. Prior to stool sample collection, ethical

approval was sought for and issued by the ethical committee of the Kaduna State Ministry of Health. Samples were then collected from patients presenting with diarrhoea at the Yusuf Dantsoho Memorial Hospital, Hajiya Gambo Sawaba General Hospital and General Hospital Kafanchan, all in Kaduna State.

All patients that presented with diarrhoea in the hospitals and who gave their consent to participate in the study were recruited. Non-diarrhoeic patients who were willing and diarrhoeic patients who turned down or refused consent were not included in the study.

A total of 600 fresh diarrhoeic stool samples were collected in wide-mouthed containers. Ten percent (10%) formalin was added to each container and transported to the laboratory in the Department of Microbiology, Ahmadu Bello University, Zaria, Nigeria where the samples were processed. Demographic data and patients' information on residence, housing and sanitation was collected via structured questionnaire.

Cryptosporidium oocysts were concentrated using Sheather's sugar flotation. About 2 ml of the formalin-fixed stool suspension was strained through two layers of gauze into a conical tube. Eight milliliters (8 ml) of Sheather's sugar solution was added, and the suspension was mixed thoroughly by inversion. The tube was capped and centrifuged at 500 x g for 10 min. After centrifugation, debris at the surface of the suspension was removed and placed on a glass slide and covered with a cover slip. Each slide was examined for *Cryptosporidium* oocysts by microscopy using the high-power dry field (x40 objective). Results were recorded as the average number of oocysts observed per high-power dry field.

Statistical analysis was done using the Statistical Package for the Social Science (SPSS Inc., IBM Company, USA) for Windows® version 23. Data were summarised using frequency tables. Univariate association between *Cryptosporidium* species infection and possible risk factors were assessed using Pearson's Chi-square (χ^2) test. The odds ratio (OR) and the corresponding 95% confidence interval (95% Cl) were calculated to measure the strength of association between variables and occurrence of *Cryptosporidium* oocysts. P-values $\leq$ 0.05 were considered significant.

3. Results

A total of 600 patients who presented with diarrhoea were recruited for this study. The demographic characteristics are as shown in Table 1. Binary logistic regression was used to test the association between the demographics and the infection. The prevalence of cryptosporidiosis in the study population was 4.5% (27/600). Age was significantly associated with occurrence of oocysts in stool (χ^2 = 4.761, p = 0.029). Age group $\leq$ 5 years had 6.6% (17/256) positive cases with odds ratio of 2.376 and 95% confidence interval (95% CI) of 1.069 – 5.280. Similarly, assessment of the relationship between gender and occurrence of oocysts revealed that there was no significant association (χ^2 = 0.686, p = 0.407) between gender and oocysts occurrence in stool though 5.3% (13/243) of female had cryptosporidiosis as against 4.0% (14/357) in male. The odds ratio was observed to be 0.722; 95% CI: 0.335 – 1.565. The level of education of the patients or their care-givers showed no significant association (χ^2 = 3.777, *P* = 0.287) with cryptosporidiosis but those without formal education had odds ratio of 3.066 (95%CI = 0.583 – 16.121) over those that have attained secondary education (Table 1).

Table 1: Prevalence of cryptosporidiosis in patients with diarrhea based on demographics

Demographics	No. tested	No. positive (%)	χ^2	P	Odds ratio	95% CI
Age (years)						
≤ 5 [Ref]	256	17(6.6)	4.761	0.029*	2.376	1.069 – 5.280
> 5	344	10(2.9)				
Gender						
Male	357	14(4.0)	0.686	0.407	0.722	0.335 – 1.656
Female [Ref]	243	13(5.3)				
Education						
Primary	273	15(5.5)	3.777	0.287	0.811	0.288 – 2.289
Secondary	132	2(1.6)			3.066	0.583 – 16.121
Tertiary	84	5(6.0)			0.745	0.209 – 2.663
None [Ref]	111	5(4.5)				

Table 2 presents the relationship between symptoms of cryptosporidiosis exhibited by the patients and prevalence of cryptosporidiosis. Abdominal pain was significantly associated with cryptosporidiosis (Fisher's exact test = 0.001). All the cryptosporidiosis positive patients [5.8% 927/465)]

complained of abdominal pain. Fever was also significantly associated (Fisher's exact test = 0.016) with cryptosporidiosis. All the 27 (5.4%) *Cryptosporidium* positive samples were obtained from patients that had fever while no positive sample was seen amongst non-febrile patients. Vomiting and stool consistency were not associated (p = 0.250) with occurrence of *Cryptosporidium* oocysts; although the comparison showed that 5.0% (11/219) of patients that reported vomiting had cryptosporidiosis while 3.1% (12/381) of those that did not report vomiting presented with *Cryptosporidium* infection. Individuals that presented with vomiting had odds ratio of 1.626 (95%CI = 0.705 – 3.750). Duration of diarrhoea and stool characteristics was not significantly associated (χ^2 = 7.624, p = 0.054) with presence of oocysts in stool. Out of the 269 patients that had diarrhoea that lasted for more than one week to two weeks (> 1 ≤ 2 weeks), 19 (7.1%) had *Cryptosporidium* oocysts in their stool; 2.5% (8/319) of the patients who had diarrhoea that lasted for ≤1 week had oocysts in their stool. The duration of diarrhoea was not significantly associated (χ^2 = 7.624, p = 0.054) with *Cryptosporidium* infection although it showed odds ratio of 2.955 (95%CI = 1.272 – 6.862). On assessment of the stool characteristics, it was found that 5.9% (27/461) of the mucoid stool samples had *Cryptosporidium* oocysts as against none in the 139 bloody mucoid stool samples indicating significant association of stool characteristics with presence of oocysts (χ^2 = 7.624, p = 0.054).

Symptoms	No. tested	No. +ve (%)	χ^2	p-value	Odds ratio	95% CI
Abdominal pain [F]						
Present [Ref]	465	27(5.8)		0.001**		
Absent	135	0(0.0)				
Fever [F]						
Present	498	27(5.4)		0.016*		
Absent	102	0(0.0)				
Vomiting						
Present [Ref]	219	11(5.0)	1.324	0.250	1.626	0.705 – 3.750
Absent	381	12(3.1)				
Stool characteristics [F]						
Mucoid	461	27(5.9)		0.002**		
Bloody mucoid	139	0(0.0)				
Stool consistency						
Watery [Ref]	281	16(5.7)	2.287	0.130	1.784	0.836 – 3.809
Loose	319	11(3.4)				
Duration of diarrhoea						
≤ 1 week	319	8(2.5)	7.624	0.054	2.955	1.272 – 6.862
> 1 ≤ 2 weeks [Ref]	269	19(7.1)				
> 2 weeks	3	0(0.0)				
Unknown	9	0(0.0)				

* = p ≤ 0.05, ** = p ≤ 0.01, χ^2 = chi square, CI = confidence interval, p = level of significance.
[F] = Fisher's exact test; [Ref] = Reference variable

Possible risk factors of cryptosporidiosis were studied and presented in Table 3. Chi square test of association between patients' place of residence and cryptosporidiosis was significantly associated (χ^2 = 6.559, p = 0.010) with adds ratio of 2.795 and 95%CI of 1.234 – 6.328. The prevalence of cryptosporidiosis among rural dwellers was 7.0% (18/257) as against 2.6% (9/343) in urban settlers. Also, contact with animals showed significant association (χ^2 = 12.848, p < 0.001) with cryptosporidiosis. The odds ratio between those with animal contact and patients without animal contact was 9.074 (95%CI = 2.129 – 38.675). Patients' source of water exhibited no association (p = 0.071) with *Cryptosporidium* infection but the prevalence of cryptosporidiosis among those that use well water was 6.5% (14/214), while tap or pipe-borne water users had prevalence of 4.0% (13/321).

Table 3: Risk factors associated with prevalence of cryptosporidiosis

Risk factors	No. tested	No. positive (%)	χ^2	p	Odds ratio	95% CI
Residence						
Rural [Ref]	257	18(7.0)	6.559	0.010*	2.795	1.234 – 6.328
Urban	343	9(2.6)				
Animal contact [F]						
Yes [Ref]	357	25(7.0)	12.848	< 0.001**	9.074	2.129 – 38.675
No	243	2(0.8)				
Water source						
Tap	321	13(4.0)	5.291	0.071	0.774	0.514 – 1.166
Well [Ref]	214	14(6.5)				
River	65	0(0.0)				
Method of water treatment						
Sieving	182	0(0.0)	18.486	<0.001**		
No treatment [Ref]	379	27(7.1)				
Boiling	39	0(0.0)				

* = $p \leq 0.05$, ** = $p \leq 0.01$, χ^2 = chi square, CI = confidence interval, p = level of significance.

4. Discussions

The prevalence of cryptosporidiosis in this study was 4.5%. These results were similar to previous study of Okojokwu *et al*. [5] in which they reported 5.0% prevalence of cryptosporidiosis among patients that presented with diarrhoea; although in that study, formol-ether concentration of *Cryptosporidium* oocysts from stool followed by modified Ziehl-Nelseen staining was used while in the present study, Sheather's sugar flotation of oocysts was employed. Cryptosporidiosis is widely prevalent all over the world with variable prevalence ranging from 1.6% in Egypt to as high as 94% in Kuwait [6].

The significantly lower prevalence of cryptosporidiosis among children 5 years and below could be attributed to their poorer hygienic practices. At that age bracket, especially ages 2 to 5, exposure to infection by *Cryptosporidium* species could be high because they lack adequate knowledge of hygiene, eat without washing their hands, play with soil, and wastewater such as sewage. Children in this age group also are more exposed to faecal-oral contact 7,8].

No significant variation in cryptosporidiosis prevalence among gender was observed in this study. This suggests that the infection is not gender bias. In some clines, the prevalence of infection was reported to be higher in males than in females [9,10]. The findings of this study are in consonance with the reports of Lu *et al*. [11] that boys and girls had similar prevalence.

The present study established that abdominal pain is significantly associated with cryptosporidiosis. All the 27 patients in whom *Cryptosporidium* species oocysts were detected in their stool complained of abdominal pain. These individuals represent 5.8% of the 465 patients that complained of abdominal pain. In agreement with our findings, Youssef *et al*. [12] reported abdominal pain was associated with cryptosporidiosis, but in contrast to our findings they said there was significant association between *Cryptosporidium* infection and other symptoms such as fever and vomiting. It can therefore be inferred from the work of Gatei *et al*. [13] (where they showed *Cryptosporidium* hominis was associated with diarrhoea, nausea, vomiting and malaise while *C. parvum, C. mleleagridis, C. canis* and *C. felis* were associated with diarrhoea only) that the clinical manifestations are partially attributable to the different species of *Cryptosporidium* and subtypes.

As regards prevalence of positive cases based on place of residence, rural dwellers had 7.0% prevalence and 2.6% in urban settlers. Residence in rural area appears to be a contributing factor to cryptosporidiosis. This is due to increased exposure to zoonotic infection, low socioeconomic status and close contact with soil in rural areas [12].

In tandem with these findings, Iqbal and colleagues [14] said contact with infected animals could be a possible risk factor for the transmission of cryptosporidiosis. On the other hand, however, reports of the studies carried out by Khashba *et al*. [15] and Youssef *et al*. [12] have failed to find any animal association.

Our data indicated a potential risk of transmission of *Cryptosporidium* oocysts through untreated water, sieving and/or boiling of water could eliminate *Cryptosporidium* oocysts from water and is therefore advocated.

5. Conclusion

The findings of this study have shown that cryptosporidiosis is prevalent in Kaduna State, Nigeria and could pose a threat to public health. Important factors in dissemination of the parasite are age, place of residence, animal contact and methods of water treatment. It was also found that clinical

manifestation of the infection includes abdominal pain, fever and stool characteristics.

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