Cryptosporidium Infection Among Patients Presenting With Diarrhoea In Kaduna State, Nigeria

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Abstract: This study was aimed at determining the prevalence of cryptosporidiosis in patients who presented with diarrhea in three major hospitals in Kaduna State, Nigeria. 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 modified Ziehl-Neelsen staining method. The prevalence of cryptosporidiosis was 5.0%. Children under 5 years were found to have significantly (χ^2 = 3.943, p = 0.047) higher prevalence (7.6%) than older patients (3.5%). Symptoms including abdominal pain (χ^2 = 9.416, p = 0.002), fever (χ^2 = 6.643, p = 0.010) and duration of diarrhoea (χ^2 = 8.562, p = 0.036) 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 (χ^2 = 3.943, p = 0.047), where rural dwellers had 7.0% (18/257) prevalence and urban dweller had 3.5% (12/343); animal contact (χ^2 = 6.558, p = 0.010), patients who admitted having contact with animals had 7.6% (27/357) prevalence as against 1.2% (3/248); and method of water treatment (χ^2 = 18.844, $p < 0.001$), 7.9% (30/379) of subjects who did not treat their water before consumption had cryptosporidiosis. These findings therefore highlighted the presence of *Cryptosporidium* species infection among humans in Kaduna State. The study highlighted the significance of animal contact and methods of water treatment as possible risk factors.
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1. Introduction

Cryptosporidiosis is a gastrointestinal illness characterized by watery diarrhea in a wide variety of mammals, birds and reptiles (Fayer *et al.,* 2000; Xiao *et al.,* 2004). The disease is caused by *Cryptosporidium* species, an apicomplexan protozoan parasite. *Cryptosporidium* is classified as an emerging pathogen by the Centres for Disease Control and Prevention (CDC) (Guerrant, 1997). Based on molecular (genotyping) tools, five species of *Cryptosporidium,* namely *Cryptosporidium parvum, Cryptosporidium hominis, Cryptosporidium* meleagridis, *Cryptosporidium canis* and *Cryptosporidium felis* have been demonstrated to be responsible for most cryptosporidiosis in human. *Cryptosporidium parvum* and *Cryptosporidium hominis* appear to be the most commonly implicated species in clinical infections (Sulaiman *et al.,* 2005).

Cryptosporidium parvum is an obligate intracellular parasite that infects the epithelial lining of luminal surfaces of gastrointestinal and respiratory tracts in a wide array of hosts. The parasite is ingested as oocysts which undergoes excystation to sporozoite that parasitise the host. Infection can occur in oesophagus and any portion of gastrointestinal tract can be involved; it usually starts in the lower small intestine. Other areas include the gall bladder, bile ducts, pancreas and respiratory tract. The infection provokes symptoms such as abdominal cramps, diarrhea, vomiting, loss of appetite, low grade fever, generalized malaise and nausea (Armson *et al.,* 2003). While the infection can resolve without intervention in immunocompetent individuals, cryptosporidiosis is increasingly becoming a major public health threat as an opportunistic infection in immunosuppressed and immunocompromised individuals, especially in HIV/AIDS (Hunter and Nichols, 2002; Nyamwange *et al.,* 2012).

In immunocompetent individuals, the parasite is localized in the distal small intestine and proximal colon, but occurs throughout the gut, biliary and respiratory tracts in immunocompromised hosts. Defects in innate, humoral or cellular immunity in a patient infected with *Cryptosporidium* results in severe or prolonged illness. The life threatening potential of *Cryptosporidium parvum* in immunocompromised and immunosuppressed individuals has increased the importance of cryptosporidiosis as a global public health problem (Moghaddam, 2007).

Khan *et al.* (2004) reported that *Cryptosporidium* can be transmitted through diverse routes which include anthroponotic (person-to-person), zoonotic (animal-to-human through contact with infected

animal or animal dung) or by ingestion of contaminated food or water. Gatei *et al.* (2006) said the resistance of *Cryptosporidium* oocysts to common household disinfectants, chlorination used for water treatment and long period of survival in the environment have major public health consequence because the parasite has low infective dose.

This study was aimed at finding the prevalence of *Cryptosporidium* species infection and the associated risk factors in patients who present with diarrhea in Kaduna metropolis, Nigeria.

2. Material And Methods

2.1 Study design

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

2.2 Study site and population

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.

2.3 Inclusion criteria

All patients that presented with diarrhoea in the hospitals and who gave their consent to participate in the study were recruited.

2.4 Exclusion criteria

Non-diarrhoeic patients who were willing and diarrhoeic patients who turned down or refused consent were not included in the study.

2.5 Collection of stool samples and demographic data

A total of 600 freshly voided diarrhoeic stool samples were collected in wide-mouthed sample containers. Ten percent (10%) formalin (twice the volume of the stool) was added to each container for preservation before being transported to the postgraduate laboratory of 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.

2.6 Microscopic screening for oocysts

Microscopy being a more reliable diagnostic method than immunologic methods for detection of *Cryptosporidium* species oocysts (Morgan *et al.,* 1998) was used. Formol-ether concentration technique was used to concentrate the oocysts followed by modified Ziehl-Neelsen staining for detection of *Cryptosporidium* oocysts in stool specimens according to Casemore (1991). Oocysts sizes were measured using graticule. Pinkish red spherules measuring 4 – 6 μm in oil immersion field were considered positive.

Data analyses

Statistical analysis was done using the Statistical Package for the Social Science (SPSS Inc., IBM Company, USA). 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 ≤ 0.05 were considered significant.

3. Results And Discussion

3.1 Demographic characteristics of the study population

Table 1: Effect of demographics on prevalence of cryptosporidiosis in patients with diarrhoea

Demographics	No. tested	No. positive (%)	χ^2	p	Odds ratio	95% CI
Age (years)						
≤ 5	256	18(7.6)	3.943	0.047*	2.127	0.998 – 4.545
> 5	344	12(3.5)				
Gender						
Male	357	15(4.2)	1.215	0.270	0.659	0.313 – 1.389
Female	243	15(6.2)				
Education						
Primary	273	15(5.5)	3.141	0.370		
Secondary	132	3(2.3)				
Tertiary	84	6(7.1)				
None	111	6(5.4)				

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

A total of 600 patients who presented with diarrhoea were recruited for this study. The demographic characteristics are as shown in Table 1. The prevalence of cryptosporidiosis in the study

population was 5.0% (30/600). The association between demographics of the study population and occurrence of *Cryptosporidium* oocysts in the diarrhoeic stool samples was assessed using chi square. Age was significantly associated with occurrence of oocysts in stool ($\chi^2 = 3.943$, $p = 0.047$). Age group ≤ 5 years had 7.6% (18/256) positive cases with odds ratio of 2.127 and 95% confidence interval (95% CI) of 0.998 – 4.545. Similarly, assessment of the relationship between gender and occurrence of oocysts revealed that there was no significant association ($\chi^2 = 1.215$, $p = 0.270$) between gender and oocysts occurrence in stool though 6.2% (15/243) of female had cryptosporidiosis as against 4.2% (15/357) in male. The odds ratio was observed to be 0.659; 95% CI: 0.313 – 1.389 (Table 1).

3.2 Association between cryptosporidiosis and health complaints

Table 2 depicts the relationship, if any, between symptoms of cryptosporidiosis and prevalence of *Cryptosporidium* oocysts in diarrhoeic stool samples. All the 30 (9.7%) of the *Cryptosporidium* oocysts positive samples were collected from patients who complained of abdominal pain. Abdominal pain was shown to be significantly associated ($\chi^2 = 9.416$, $p = 0.002$) with cryptosporidiosis. The odds ratio was 0.903; 95% CI: 0.871 – 0.937. Fever was also significantly associated ($\chi^2 = 6.643$, $p = 0.010$) with cryptosporidiosis. All the 30 (9.0%) *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 ($p > 0.05$) with occurrence of *Cryptosporidium* oocysts while duration of diarrhoea and stool characteristics were significantly associated ($p < 0.05$) with presence of oocysts in stool. Chi square test showed that duration of diarrhoea was significantly associated with cryptosporidiosis ($\chi^2 = 8.562$, $p = 0.036$). Out of the 269 patients that had diarrhoea that lasted for more than one week to two weeks (> 1 ≤ 2 weeks), 21 (7.8%) had *Cryptosporidium* oocysts in their stool; 2.8% (9/319) of the patients who had diarrhoea that lasted for ≤1 week had oocysts in their stool. On assessment of the stool characteristics, it was found that 6.5% (30/461) 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 ($\chi^2 = 9.825$, $p = 0.002$).

Table 2: Symptoms presented by patients presenting with cryptosporidial diarrhoea

Symptoms	No. tested	No. +ve (%)	χ^2	p-value	Odds ratio	95% CI
Abdominal pain						
Present	465	30(6.5)	9.416	0.002**	0.903	0.871 – 1.937
Absent	135	0(0.0)				
Fever						
Present	498	30(6.0)	6.643	0.010*	0.910	0.879 – 0.941
Absent	102	0(0.0)				
Vomiting						
Present	219	12(5.5)	0.171	0.679	1.174	0.549 – 2.512
Absent	381	18(4.7)				
Stool characteristics						
Mucoid	461	30(6.5)	9.825	0.002**	0.902	0.869 – 1.930
Bloody mucoid	139	0(0.0)				
Stool consistency						
Watery	281	18(6.4)	2.287	0.130	1.784	0.836 – 3.809
Loose	319	12(3.8)				
Duration of diarrhoea						
≤ 1 week	319	9(2.8)	8.562	0.036*		
> 1 ≤ 2 weeks	269	21(7.8)				
> 2 weeks	3	0(0.0)				
Unknown	9	0(0.0)				

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

3.3 Association between cryptosporidiosis and possible risk factors

Possible risk factors of cryptosporidiosis were studied and presented in Table 3. Place of residence ($\chi^2 = 1.945$, $p = 0.163$) and sources of water ($\chi^2 = 5.403$, $p = 0.067$) were not associated with the infection. Contact of patient with animal or animal dung was significantly associated ($\chi^2 = 6.558$, $p = 0.010$) with *Cryptosporidium* infection. Three hundred and fifty seven (357) patients admitted to having

contact with animal or their dung, out of which 7.6% (15/357) had *Cryptosporidium* infection. On the other hand, 1.2% of patients who had no animal contact were infected with cryptosporidiosis. The method of water treatment was significantly associated (χ^2 = 18.844, p < 0.001) with *Cryptosporidium* infection. Study subjects who agreed that they sieved or boiled their water prior to drinking had no cryptosporidiosis (0.0%) while all the positive cases (7.9%) recorded in this study occurred among those that carried out no treatment of water prior to drinking.

Table 3: Association of risk factors with prevalence of cryptosporidiosis

Risk factors	No. tested	No. positive (%)	χ^2	p	Odds ratio	95% CI
Residence						
Rural	257	18(7.0)	3.943	0.047*	2.127	0.998 – 4.545
Urban	343	12(3.5)				
Animal contact						
Yes	357	27(7.6)	6.558	0.010*	4.320	1.285 – 14.523
No	243	3(1.2)				
Water source						
Tap	321	15(4.7)	5.403	0.067		
Well	214	15(7.0)				
River	65	0(0.0)				
Method of water treatment						
Sieving	182	0(0.0)	18.844	<0.001**		
No treatment	379	30(7.9)				
Boiling	39	0(0.0)				

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

4. Discussion

The findings of this study showed that prevalence of cryptosporidiosis among patients who presented with diarrhoea was 7.5%. This observed prevalence is lower than the prevalence rates of 10 – 52.7% reported in previous studies in Nigeria (Mbanugo and Agu, 2005; Adesiji *et al.,* 2007; Akinbo *et al.,* 2010). The wide variation in the reported and observed prevalence of cryptosporidiosis may be attributed to geographical locations and the type of method used for the detection of oocysts. Adesiji *et al.* (2007) noted that prevalence of cryptosporidiosis tends to vary from one locality to another and from one country to another though this variation is predicated on the extent of contamination of water, food and animal contact; all these are considered important factors for dissemination of the coccidian parasite. Sample size could also play prominent role in variation of the prevalence since the study of Muntaz *et al.* (2010) with 200 samples (18 cases) had a prevalence of 9.0%. Akinbo *et al.* (2010) observed prevalence of 6.0% 36 (36/600) while our findings in this study showed 5.0% (30/600) prevalence.

Prevalence of cryptosporidiosis was higher among patients in age group ≤ 5 years than it is in the > 5 years (5.2%). This findings revealed that there was statistically significant (χ^2 = 3.943, p = 0.047) association between age and cryptosporidiosis with children in the age group ≤ 5 years more prone to contract the infection than their older counterparts. The odds ratio (OR) of 2.127 (95% CI = 0.998 – 4.545) indicates that children in the age group ≤ 5 years are twice more predisposed or liable to contract cryptosporidiosis than those who are older. This may be due to the fact that children in the ≤ 5 years group have been found to be more vulnerable when exposed to contaminated environment, food and water. Immunity is less than optimal at both ends of life, that is, ≤ 5 years and in the elderly. Newborns appeared to have less T-cell functions and antibodies are acquired by the transfer of IgG from their mothers through the placenta. This maternal IgG decay overtime with little remaining by 3 – 6 months of age; and the risk of infection in children is higher after the age of 6 months (Levinson and Jawetz, 2003). These findings are in tandem with the works of Sorvillo *et al.* (1994), Muntaz *et al.* (2010) and Nyamwange *et al.,* (2012) which showed that *Cryptosporidium* infection had higher prevalence in children than in adults. Apart from the fact that children have higher susceptibility to infections attributable to immature immune system, the possibility of faecal-oral transmission of the infections is higher in children aged ≤ 5 years since they are more likely to practice coprophagy and more unlikely to practice good hygienic habits (Nwabuisi, 2001).

Concerning gender distribution of positive *Cryptosporidium* cases, the study revealed 6.3% males and 7.5% females. The relation was insignificant (χ^2 = 1.215, p = 0.270). Similarly, in the work of Gatei *et al.* (2006), they found that infection rates did not vary

with gender distribution; conversely, Muntaz *et al.* (2010) reported higher prevalence rate of cryptosporidiosis in males than in females.

No statistical association ($\chi^2 = 3.141$; $p = 0.370$) existed between *Cryptosporidium* infection and educational status of the patients or their care-givers (for patients who have not attained school age).

Abdominal pain was significantly associated with the presence of *Cryptosporidium* oocysts ($\chi^2 = 9.416$; $p = 0.002$). This implies that only 2 out of a thousand cases of patients with cryptosporidiosis may not be associated with abdominal pain. There was no strong association as the odds of infection were insignificant (OR = 0.903; 95% C.I. = 0.871 – 0.937). The manifestation of abdominal pain could be attributed to the production of chemokines and cytokines by the cells of the infected intestines. The infection could also up-regulate the expression of cyclooxygenase-2, production of prostanglandins by the epithelial cells and productions of neuropeptides by the inflammatory cells (Lauret *et al.,* 1998; Robinson *et al.,* 2003; Gookin *et al.,* 2004; Hernandez *et al.,* 2007).

All the patients with cryptosporidiosis had fever as well. Though this finding contradicts the reports of El-Helaly *et al.* (2012) and Nyamwange (2012) whose studies showed no association between *Cryptosporidium* infection and fever. The significant association ($\chi^2 = 6.643$; $p < 0.001$) of fever with cryptosporidiosis could be secretion of cytokines, interleukin-1 (IL-1) by the host cells in response to the presence of the parasite which induced fever.

Stool consistency showed no association ($\chi^2 = 2.287$; $p = 0.130$) with cryptosporidiosis. Perusal of infection rate in watery stool in comparison with loose stool revealed higher prevalence (6.4%) of cryptosporidiosis among patients with watery stool than patients with loose stool (3.8%). This may be due to the fact that the infection is characterised by defects in intestinal permeability. Increased permeability may result in decreased absorption of fluids and electrolytes as well as solute fluxes into the gut. Studies of AIDS patients with cryptosporidiosis have demonstrated a direct correlation between the severity of disease and altered intestinal permeability as measured by ratios of excretion in the urine of lactulose and mannitol (Goodgame *et al.,* 1995; Lima *et al.,* 1997; Sharpstone *et al.,* 1999). Similar defects have been noted by Zhang *et al.* (2000) in children with cryptosporidiosis. *Cryptosporidium* infection directly induces defects in intestinal epithelial cell barrier function *in vitro*. The prevalence of cryptosporidiosis among patients with watery stool (6.4%) in this study was low compared to the prevalence of 39.66% reported by Mbanugo and Agu (2006) and 40.7% by El-Helaly *et al.* (2012). This observed disparity in prevalence could be attributed to the population under study; while this research focused on patients of all age groups that presented to the hospital with diarrhoea, the works of Mbanugo and Agu (2006) and El-Helaly and colleagues (2006) restricted their studies to 0 – 15 years and 1 – 5 years respectively.

Duration of diarrhoea was significantly associated ($\chi^2 = 8.562$; $p = 0.036$) with the presence of cryptosporidiosis among the study population. The odds of infection was higher and in favour of patients who had diarrhoea for more than one week but less than 2 weeks ($>1 \leq 2$ weeks) compared to those patients with unknown duration of the diarrhoea, less than (or equal to) one week or greater than two weeks. This finding is in tandem with the findings reported by Barboni *et al.* (2008). The persistence of diarrhoea for more than one week (>1 weeks) implies loss of nutrient from the body of the patients for the period of duration of the diarrhoea. Previous researchers noted that *Cryptosporidium* infection is among the more common causes of persistent diarrhoea in developing countries causing approximately one-third (⅓) of cases (Sodemann *et al.,* 1999; Tumwine *et al.,* 2003; Gatei *et al.,* 2006). The long duration of the diarrhoea could also result in malabsorption, and premature death (Lima *et al.,* 2000; Amadi *et al.,* 2001, 2002; Behera *et al.,* 2008). It has been observed by Lima *et al*. (2000) that an episode of persistent diarrhoea caused by *Cryptosporidium* is a marker or indicator for the onset of increased risk of recurrent episodes of diarrhoea.

Furthermore, children who are less than one year (< 1 year) could stand the risk of poor physical and cognitive development. Urban *et al.* (1996) carried out a long-term follow-up study of children with onset of cryptosporidiosis before age 1 year which suggested an association with poorer physical fitness and poorer cognitive development that persists for years. In another study by Guerrant *et al.* (1999), long-term effects of early childhood malnutrition were noted, but there was no significant association with *Cryptosporidium* compared with other pathogens. However, total duration of diarrhoea is an important predictor of malnutrition. Hence, Checkley *et al.* (2008) concluded that *Cryptosporidium* is more commonly associated with persistent diarrhoea than other causes. The variation is duration of diarrhoea could be attributed to the species and subtypes of *Cryptosporidium* that infected the study subjects. The use of molecular tools in previous research works has demonstrated clinical differences between *Cryptosporidium* species and subtypes. In most studies, *Cryptosporidium hominis* is associated with more severe disease, that is, more dehydration, longer duration and more oocysts shed while

Cryptosporidium meleagridis has milder disease (Hunter *et al.,* 2004; Ajjampur *et al.,* 2007; Cama *et al.,* 2008).

5. Conclusion

The present study has demonstrated that *Cryptosporidium* species is prevalent in the study population. Children under 5 years had higher prevalence cryptosporidiosis than the older patients. Symptoms including abdominal pain, fever, and duration of diarrhoea were found to be associated with cryptosporidiosis. Among the risk factors studied, place of residence, contact with animals and method of water treatment were significantly associated with *Cryptosporidium* infection. It is therefore pertinent that contacts with animals and their dung be minimized. Water should be boiled or filtered before drinking. Further studies are recommended to determine the molecular profile of the different genotypes and subgenotypes of *Cryptosporidium* in circulation in Kaduna State.

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