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PREVALENCE OF *CRYPTOSPORIDIUM* OOCYSTS AMONG CHILDREN WITH ACUTE GASTROENTERITIS IN ZARIA, NIGERIA

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ABSTRACT
This research was conducted to determine the prevalence of Cryptosporidium oocysts among children with acute gastroenteritis in Zaria, Nigeria by Kinyoun Modified Carbol-Fuchsin Staining (Modified Ziehl-Neelsen Staining) Technique. The results for the screening of Cryptosporidium oocysts showed that out of 372 stool samples investigated, 17 samples were positive for the oocysts. Among 17 positive samples, 8 were among 199 male while 9 were among 173 female children studied. The statistical association between microscopic detection of oocyst and gender was not significant (χ^2 = 0.297, df = 1, p=0.586). However, there was no statistically significant association between the prevalence of Cryptosporidium oocyst and the age of children observed (χ^2 = 7.268, df = 9, p = 0.609). The results also showed no significant association between the prevalence of Cryptosporidium oocysts and the house hold animals (χ^2 = 1.489, df = 4, p=0.829). There was also no statistically significant association between the prevalence of Cryptosporidium oocysts and the source of drinking water (χ^2 = 6.367, df = 4, p=0.173). Similarly, there was not statistically significant association between the prevalence of Cryptosporidium oocysts and the type of toilet among the study population (χ^2 = 1.128, df = 2, p=0.569).
Keywords: Children, Cryptosporidium, Gastroenteritis, Oocysts, Prevalence, Zaria

INTRODUCTION

Cryptosporidium, a coccidian protozoan parasite, is an important causative agent of human and animal gastrointestinal illness globally (Huang *et al*., 2009). Unlike other intestinal pathogens, *Cryptosporidium* can infect several different hosts, can survive in most environments for long periods of time due to its "hardy cyst" (Keusch *et al*., 1995). Cryptosporidiosis, a disease caused by *Cryptosporidium,* is self-limiting gastroenteritis in the general population. The infection generally is more common among the under fives than among children more than 5 years (Shah *et al*., 2003; Huang *et al*., 2009). Worldwide infection and illness caused by *Cryptosporidium* spp has been reported in more than 40 countries on six continents (Percival *et al*., 2004). Infection is common among children than adults in developed regions and nearly universal in impoverished areas (Kosek *et al.* 2001). The transmission form is a robust, environmentally resistant oocyst, excreted in the stool, which can exist for long periods of time in the environment (Kosek *et al.,* 2001). The infectious dose has been shown in human infectivity studies to be low, with infection occurring following challenge of subjects without evidence of prior infection with 30 oocysts (Caccio *et al*., 2003). Infection with *Cryptosporidium* spp. has been known to occur from as low as 10-100 oocysts, while only 1g of faeces from infected animals in the laboratory contained 10^6 oocysts (Koompapong *et al*., 2009). But it was earlier reported that a single oocyst is sufficient to produce infection and disease in susceptible hosts (Pereira *et al.,* 2002).

MATERIALS AND METHODS

Study Population

This research work was conducted in some selected Primary Health Care (PHC) centres Zaria Hajiya Gambo Sawaba General Hospital, Zaria, Zaria Clinic and Medical Centre (ZCMC) Tudun Wada, all in Zaria Local Government Area. Zaria is about 300 km^2 (100 sq mi) located at 11°04′N 7°42′E of Kaduna State, Northern Nigeria. As of 2006 census, Zaria was populated with about 408,198 people (NPCN, 2009). The study population consists of three hundred and forty (340) infants and young children 0-60 months with gastroenteritis and thirty two (32) age-matched control who attended the selected PHC centres, clinics and hospitals in the study area. The population comprised of one hundred and ninety nine (199) males and one hundred and seventy three (173) females.

Sample collection questionnaires were designed by the researcher to seek for information on demographic and risk factors of the disease such as source of drinking water, breast feeding practices, presence of pets, type of toilet etc.

Microscopic Detection of *Cryptosporidium* oocysts by Kinyoun Modified Carbol-fuchsin Staining (Modified Ziehl-Neelsen staining) Technique

Sample Concentration

One gram of faecal specimen (The faecal specimen was thoroughly mixed before sampling) was emulsified in 4ml of 10% formol water contained in a screw-caped bottle. This was followed by addition of 4ml of 10% formol water. The mixture was then sieved and the filtrate transferred into a centrifuge tube thereafter.

Four milliliter (4 ml) of ethyl ether was then added into the tube, stoppered and mixed for about 1minute.

The stopper was then loose and the tube placed in a centrifuge machine and set at low speed of 1000 rpm for about one minute.

Four (4) different columns of ether and dissolved fat, feacal debris, Formal water and sediment containing the parasites formed in the tube in ascending order. Using a Pasteur pipette, the entire column of fluid below the faecal debris and ether was carefully removed and transferred into another centrifuge tube. Formol water was then added to make the volume up to 12 ml. The mixture was then centrifuged at 3000 rpm for 10 minutes. The sediment containing the oocysts was used to prepare the microscopic slides (Cheesbrough, 2009).

Staining of Slides

A smear from the sediment obtained by the formol ether concentration technique was air-dried and fixed with methanol for 3 minutes thereafter. The smears were then stained with unheated carbol fuchsin for 15 minutes and then washed off with water. They were then decolorized with 1% acid alcohol for 12 seconds and washed off with water thereafter. All the smears were counterstained with 0.5% malachite green for 30 second, washed off with distilled water and kept in a draining rack to dry (Cheesbrough, 2009).

Microscopic Examination of the Slides

The microscope was calibrated using graticule to enable differentiation and confirmation of *Cryptosporidium* ocysts from other coccidian oocysts. The prepared slides were examined microscopically for oocysts, using a low power magnification to detect the presence of the oocysts and the oil immersion objective to identify them. Oocysts that appeared small, round to oval, pink red stained bodies measuring 4–6µm,or a single deeply stained red dot were considered positive (Cheesbrough, 2009).

RESULTS

The results for the screening of *Cryptosporidium* oocysts by Kinyoun Modified Carbol-Fuchsin Staining (Modified Ziehl-Neelsen Staining) Technique under oil immersion showed that out of 372 (340 diarrheic and 32 age marched control) stool samples investigated, 17 were positive for the oocysts. Among 17 positive samples, 8 were among 199 male while 9 were among 173 female children studied (Table 1) but no ocyst was detected among control samples. The statistical association between the prevalence of *Cryptosporidium* oocyst and gender was not significant (χ^2 = 0.297, df = 1, p=0.586). However, there was statistically no significant association between microscopic detection of *Cryptosporidium* oocyst and the age of the children (χ^2 = 7.268, df = 9, p = 0.609). *Cryptosporidium* oocysts were detected with highest prevalence among 49-54 months age group (16.7%: 1/6) followed by 31-36 months age group (11.1%: 2/18) (Table 1).

The results also showed no significant association between the prevalence of *Cryptosporidium* oocysts and the households animals (χ^2 = 1.489, df = 4, p=0.829). *Cryptosporidium* oocysts were detected with nearly similar percentage prevalence among children irrespective of presence or absence of animals. Nevertheless, highest incidences of oocysts were recorded among children from house hold with only cats (6%: 7/112) (Table 2). No oocyst was detected in houses with both cats and dogs. There was also no statistically significant association between the prevalence of *Cryptosporidium* oocysts and the source of drinking water (χ^2 = 6.367, df = 4, p=0.173). Highest prevalence of the infection were recorded among children from households dependent on well water (7%: 15/214) followed by stream (5%: 2/44) (Table 2). No oocyst was detected among children from houses dependent on other sources of drinking water. Similarly, there was no statistically significant association between the prevalence of *Cryptosporidium* oocysts and the type of toilet among the study population (χ^2 = 1.128, df = 2, p=0.569). Highest prevalence of the infection was recorded among children from households that defecate in the open field (25%: 1/4) (Table 2). There was no observed difference in the prevalence of the infection among children from other households using other type of toilets (Table 2).

Table 1: Prevalence of *Cryptosporidium* Oocysts in Relation to Gender and Age among Children with Acute Gastroenteritis in Zaria, Nigeria.

Population	No. Examined		No. Positive		%Prevalence		p-value
	Diarrhoeic	Control	Diarrhoeic	Control	Diarrhoeic	Control	
Sex							
Male	181	18	8	0	4.4	0	0.586
Female	159	14	9	0	5.7	0	
Total	340	32	17	0	5.0	0	
Age (Months)							
0-6	78	2	5	0	6.4	0	
7-12	111	10	5	0	4.5	0	
13-18	35	6	1	0	2.8	0	
19-24	44	5	3	0	6.8	0	
25-30	28	4	0	0	0.0	0	0.609
31-36	18	2	2	0	11.1	0	
37-42	3	2	0	0	0.0	0	
43-48	8	0	0	0	0.0	0	
49-54	6	0	1	0	16.7	0	
55-60	9	1	0	0	0.0	0	
Total	340	32	17	0	5.0	0	

Key: Control = Non-diarrhoeic

Table 2: Prevalence of *Cryptosporidium* Oocysts in Relation to the Presence of Households Pet, Source of Water and the Type of Toilets among Children with Acute Gastroenteritis in Zaria.

Variable	No. Examined		No. Positive		%Prevalence		p-value
	Study	Control	Study	Control	Study	Control	
Presence of Pets							
Cats	112	3	7	0	6	0	
Dogs	22	1	1	0	5	0	
Cats and dogs	3	1	0	0	0	0	
Other animals	111	15	6	0	5	0	0.829
Not any animal	92	12	3	0	3	0	
Source of Water							
Well	214	20	15	0	7	0	0.173
Stream	44	3	2	0	5	0	
Pipe borne	78	8	0	0	0	0	
Bore-hole	3	1	0	0	0	0	
Type of Toilet							
Pit latrine	316	29	15	0	5	0	
Water cistern	20	3	1	0	5	0	0.569
In the field	4	0	1	0	25	0	

DISCUSSION

The prevalence of 4.6% (17/372) obtained in this study by microscopy using Kinyoun Modified Carbol-fuchsin Staining (Modified Ziehl-Neelsen staining) Technique was similar to the findings of Reinthaler *et al*. (1987) in Ogun and Banwat *et al*. (2003) in Jos, Nigeria. The result was also similar to the reports from other parts of the world such as Jordan (Nimri and Batchoun, 1994), Iran (Saneian *et al.,* 2010) and Turkey (Elung and Koltas, 2011). This observation confirms the existence of cryptosporidiosis in humans in the study area as reported earlier (Kwaga *et al*., 1988). The prevalence obtained in this study is in contrast with the higher prevalence reported from Zaria (Kwaga *et al*., 1988) and other parts of Nigeria such as Jos (Ikeh *et al.,* 2007) and Imo State Nigeria (Ikechukwu *et al.,* 2011). The observed contradiction particularly with Kwaga *et al.* (1988) and Ikeh *et al.* (2007) could be attributed to small number of samples collected in their research. The higher prevalence reported from Imo State (Ikechukwu *et al.,* 2011) could be attributed to the inclusion of immunocompromised individuals in the samples whom are known to be highly vulnerable to opportunistic infection such as cryptosporidiosis.

The distribution of *Cryptosporidium* oocysts with respect to gender was slightly higher in females than in males even though the statistical association was no significant. This observation is consistent with earlier observation made in this regard by Kwaga *et al.* (1988) in Zaria but contradicted the reports of higher prevalence in males than females by other researchers (Nwabuisi, 2001; Egberongbe *et al*., 2010; Saneian et al., 2010). The reason for the observed difference is not clear.

The prevalence of *Cryptosporidium* oocysts with respect to age was statistically no significant. This supported the assertion that unlike rotavirus diarrhoea which is confined to the first 18 months of life, cryptosporidiosis is a disease of all ages (Tzipori and Ward, 2002; Mor and Tzipori, 2008). Although the infection is of all ages, highest prevalence of the infection occurred among children older than two years of age. It is noted from this results that incidences of the disease increase with increase in age of the patients and children of 4 years and above are at highest risk of being infected with the parasite. This report collaborates with previous observation made in this regard by Egberongbe *et al*. (2010). But contradicted the reports of Nwabuisi, (2001), and Saneian *et al.* (2010), that most of cryptosporidial infections occur within 0-24months. The reason for the observed contradiction is not known.

Similar to the reports of Mor and STzipori (2008), and Ayinmode *et al*. (2012) there was statistically insignificant association between the detection of *Cryptosporidium* oocysts and the presence of house hold pets in this study. Contrary to these reports, domesticated animals have been reported as reservoir of *Cryptosporidium* oocysts and hence source of human infection (DuPont *et al.,* 1995; Keusch *et al.,* 1995). This observed contradiction agreed with the report of Ajjampur *et al.* (2007) that the epidemiology of cryptosporidiosis in humans is not completely understood due to the existence of multiple transmission routes such as person-to-person, animal-to-person, waterborne, food-borne, and possible airborne transmission. This need to be further investigated.

The observation made from this study showed insignificant association between the detection of *Cryptosporidium* oocysts and the source of drinking water. Though statistically insignificant, the highest number of oocysts was detected among children from houses dependent on well water. Wells in this study area are mostly shallow and can easily be contaminated with human and animal excreta which could serve as the reservoir of the oocysts. This agrees with the reports that contaminated water represents the major source of *Cryptosporidium* infection for humans (Nimri and Batchoun, 1994; DuPont *et al.,* 1995; Keusch *et al.,* 1995; Ramirez *et al*., 2004; Chacín-Bonilla *et al.,* 2008; Garvey and McKeown, 2009; Egberongbe *et al*., 2010).

The detection of *Cryptosporidium* oocysts in relation to the type of toilet was statistically insignificant. Nevertheless, highest prevalence occurred among children from houses defecating in the open field. This collaborates with the report of Chacín-Bonilla *et al.* (2008). The occurrence of the infection among children from households using water closet and pit latrine may be due to absence of or improper hand washing after defaecating and/or before meals and other unhygienic habits such as finger sucking, finger-nail nibbling and food-picking from the ground or floors.

CONCLUSION

The finding of this study indicates that *Cryptosporidium* oocyst is prevalent and aetiologic agent of diarrhoea among children with acute gastroenteritis in Zaria, Nigeria.

Recommendations

All the oocysts detected in this study were from diarrheoic samples, further studies are therefore recommended to determine the exact association between the agent and the diarrheoa.

The need for adequate and appropriate diagnosis for children presenting with gastroenteritis to include tests for *Cryptosporidium* is hereby recommended.

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