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Cryptosporidiosis and Infantile Diarrhoea in Calabar, Nigeria

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The involvement of *Cryptosporidium* in infantile diarrhoea was investigated among 200 children with diarrhoea who attended the Children Emergency Ward of the University of Calabar Teaching Hospital (UCTH) and Out-Patients Department of the General Hospital, Calabar, Nigeria. Another subset of 100 apparently healthy non-diarrhoeic children of comparable age range were used as controls. Stool samples collected from the test and control groups were examined for the presence of intestinal parasites including oocyst of *Cryptosporidium* using standard parasitological procedures. *Cryptosporidium* oocyst was found in 52 (26%) of the diarrhoeic stool samples and 8 (8%) of the non diarrhoeic control samples respectively ($p<0.05$). *Cryptosporidium* occurred as a single infection in only 32 (16%) of the 200 diarrhoeic subjects examined. The highest prevalence of cryptosporidiosis occurred among subjects aged 2/12-1 year. Infection was not detected in those aged ≥8 years. Other intestinal parasites detected were *Ascaris lumbricoides* (19%), *Trichuris trichiura* (2%), Hookworm (1%) and *Entamoeba histolytica* (1%). This study has demonstrated the involvement of *Cryptosporidium* as one of the aetiologic agents of infantile diarrhoea in Calabar, Nigeria. The study has made a case for the routine screening of stool samples of children with diarrhoea for the presence of *Cryptosporidium* before administrating prompt and appropriate therapy.

Key words: Cryptosporidiosis, infants, diarrhoea, calabar, Nigeria

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INTRODUCTION

Cryptosporidiosis is an opportunistic diarrhoeal disease caused by an intracellular spore forming protozoan parasite known as *Cryptosporidia*. About 21 species of *Cryptosporidium* have been identified but the main specie responsible for human infection is *C. hominis* formerly known as *C. parvum* human genotype or *C. parvum* type 1 (Loureiro *et al*., 1989; NiChito *et al*., 1998). The infection is transmitted by ingestion of the oocyst in faecally contaminated food and water from animals to humans and through person to person contact. The probability of transmission from just a small amount of contamination is fairly high. It is often transmitted in day care setting to care givers, older children and adults who come in contact with infected children at home (Alpert *et al*., 1984) or hospital acquired (Baxby, 1983; Koch *et al*., 1985). A recent study has shown that the 50% infective dose (LD_{50}) of *C. parvum* is only 132 oocysts for healthy persons with no previous serological immunity to cryptosporidiosis (O'Donoghue *et al*., 1995)

The commonest symptom of cryptosporidiosis is diarrhea, which is usually watery stool. It is accompanied by abdominal cramps, nausea, vomiting, fever, headache and loss of appetite (Khan, 2000). The symptoms differ greatly in immunocompetent and immunocompromised individuals. In the former the infection is acute, yet a self limiting diarrhoeal illness whereas the illness is much more severe in the later. The incubation period may range from 1 to 12 days (Juranek, 1995).

Cryptosporidiosis is endemic in the tropics with high prevalence in less developed countries (Gatei *et al*., 2003) Children are more vulnerable in developing *Cryptosporidium*-associated diarrhoea (Abdel-Messih *et al*., 2005). It has been shown that children less than 12 months of age are 2.4 times more likely to be infected with *Cryptosporidium* while children aged between 12 and 23 months are 1.9 times more likely to be infected as compared with older ones (Abdel-Messih *et al*., 2005). Molbak *et al*. (1997) reported increased carriage of cryptosporidiosis in malnourished children. Studies conducted in Brazil, Peru and Guinea-Bissau have associated cryptosporidiosis with impairment of growth, physical fitness and cognitive function (Checkley *et al*., 1998; Guerrant *et al*., 1999; Molbak *et al*., 1997).

There are isolated studies on the epidemiology of cryptosporidiosis in Nigeria. In one of these studies, Useh and Jonah (1998) reported a prevalence of 4.4% for *C. parvum* amongst diarrhoeal subjects of all ages in Calabar, South Eastern Nigeria. In the same setting, Alaribe *et al*. (1998) reported a prevalence of 5.6% among diarrhoeic patients. Infection was not reported among healthy control subjects with no history of diarrhoea. Elsewhere in North Central and South Western Nigeria, higher prevalence of cryptosporidiosis of 4.8 and 14% were reported among undernourished children and diarrhoeic patients by Ikeh *et al*. (2006) and Nwabuisi (1998) respectively. Although these studies have confirmed the presence of cryptosporidiosis in Nigeria, this organism is not sought for routinely even in Teaching Hospitals like ours. It is likely that this organism may be playing a very important role particularly as an opportunistic pathogen among the HIV/AIDS patients in Nigeria. The paucity of information on the prevalence and significance of *Cryptosporidium parvum* as an agent of infantile diarrhoea necessitated this study. It is envisaged that the findings of this study may herald some radical decisions on the diagnosis and control of diarrhoea of *Cryptosporidium* origin.

MATERIALS AND METHODS

Study area: The subjects were Children who presented with diarrhoea and were attended to at the Children Emergency ward (CHER) of the University of Calabar Teaching Hospital (UCTH) and the Out-Patient Department (OPD) of the General Hospital, all in Calabar between April and September, 2006. The UCTH is a tertiary health institution which caters principally for residents of Cross River and Akwa Ibom states and others referred from other states in Nigeria and the neighboring Republic of Cameroon. The states mentioned above are located in the South-eastern rainforest belt of Nigeria which experiences a typical tropical climate. Calabar and its immediate environs lack adequate supply of portable water. The drainage system is poor. There is inadequate management of domestic waste. These may provide effective media for the transmission of parasitic diseases.

Subjects and consent: Two hundred children aged between 2 months and 8 years diagnosed of diarrhoea and 100 healthy non-diarrhoiec control subjects of the same age range who lived within the University community were examined. Diarrhoea was defined as the presence of three or more loose or watery stools within a 24 h period (Sherries, 1989). Stool samples were collected from both patients and test subjects in clean universal containers and processed within 30 min in the Parasitology Department of the UCTH.

A written permission was sought and obtained from the Ethical Committee in the two hospitals. Verbal consent was sought and obtained from parents to enable their children participate in the study.

Examination of samples: The standard method of stool examination as described by WHO (1991) was adopted for the study. These included macroscopy, microscopic examination of wet mounts in saline and Lugol's iodine and the formal ether concentration technique.

Formal ether concentration technique: All the samples were concentrated using Formal ether concentration technique. One milliliter of a well mixed stool sample was put in a tube containing 4 mL of 10% formalin. Three milliliter of the 10% formalin was again added and mixed by shaking. The suspension was sieved using a coffee strainer into a centrifuge tube. Three milliliter of diethyl ether was added and stoppered. It was then mixed vigorously for 1 min. The stopper was removed and the suspension centrifuged for 1 min at 400 g. The entire column of the fluid below the faecal debris and ether was carefully removed using a pasteur pipette and transfer into another centrifuge tube. Ten percent formalin was added to the transferred suspension to make up to 10 mL. It was then centrifuge at 1000 g for 10 min. The supernatant was decanted and the bottom of the tube tapped to re-suspend the deposit. The deposit was examined using the 10 and 40x objectives for the presence of cyst or ova of parasite.

Modified Ziehl Nelson Staining Technique for Oocyst: The modified Ziehl Nelson technique was used for the demonstration of oocyst of *Cryptosporiduim*. A methanol fixed thin smear of faeces was stained in cold carbol fuchsin for 10 min. Differentiation was done with 1% hydrochloric acid-ethanol while 0.25% methylene blue was utilized as counterstain. *Crypotosporidium* oocyst appeared as bright rose-pink spheres on a pale blue background (Current, 1989).

Data analysis: The data obtained from the study were analysed using Chi-square test to determine the influence of age and sex on the prevalence of infection.

RESULTS

Table 1 is on the distribution of Cryptosporidiosis by age of subjects examined. A prevalence of 26% was observed among the diarrhoiec subjects whereas control subjects had a prevalence of 8%. There was a statistically significant difference in prevalence rate of cryptosporidiosis between the diarrhoeic and non-diarrhoeic subjects ($\chi^2 = 13.5$, df = 1, $p<0.05$). Among the diarrhoeic group, subjects within the age group 2 months to 1 year had the highest prevalence of cryptospodiosis (38%).

The distribution of cryptospodiosis by sex of subjects examined is shown on Table 2. Female subjects had a higher prevalence rate of infection (26.9%) than the males (25%) but there was no statistical significant difference between the infection rate in both sexes ($\chi^2 = 0.09$, df 1, $p>0.05$).

The distribution of parasites detected as single infection among diarrhoeic subjects examined by age is shown on Table 3. *Cryptosprodium parvum* was found as a single infection in 16% of the total diarrhoeic subjects examined while *Ascaris lumbricoides* was found as a single infection in 5%.

Figure 1 shows the distribution of other intestinal parasites detected among diarrhoeic and the non diarrhoeic control subjects examined. *Ascaris lumbricoides* was the most frequently encountered intestinal parasite 19 and 14% in both the diarhoeric and control subjects, respectively. Other parasites detected among diarrhoeic and the non diarrhoeic control subjects were Hookworm 1 and 4%, *Trichurus trichiura* 2 and 2%, and *Entamoeba histolytia* 1 and 2%, respectively.

Table 1: Distribution of cryptosporidiosis by age of subjects examined

Age groups (years)	Diarrhoeic subjects		Control subjects	
Months	No. examined	No. (%) with infection	No. examined	No. (%) with infection
2/12-1	100	38 (38)	16	0 (0)
2-3	50	10 (20)	12	0 (0)
4-5	26	2 (7.7)	24	2 (8.3)
6-7	14	2 (14.3)	36	4 (11.1)
≥8	10	0 (0)	12	2 (16.7)
Total	200	52 (26)	100	8 (8)

0 denotes absence of infection

Table 2: Distribution of Cryptosporidiosis by sex of subjects examined

	Diarrhoeic subjects		Control subjects	
Sex	No. Examined	No. (%) with infection	No. Examined	No. (%) with infection
Male	96	24 (25)	54	4 (7.4)
Female	104	28 (26.9)	26	4 (8.7)
Total	200	52 (26)	100	8 (8)

Table 3: Distribution of parasites detected as single infection observed among diarrhoeic subjects examined by age

Parasites	Total observed	2/12 years	2-3 years	4-5 years	6-7 years	8 years
No. (%) with *Cryptosporidia parvum*	32 (16)	18 (56.3)	10 (31.3)	4 (12.5)	0 (0)	0 (0)
No. (%) with *Ascaris lum bricuides*	10 (5)	4 (40)	2 (20)	1 (10)	1 (10)	2 (20)

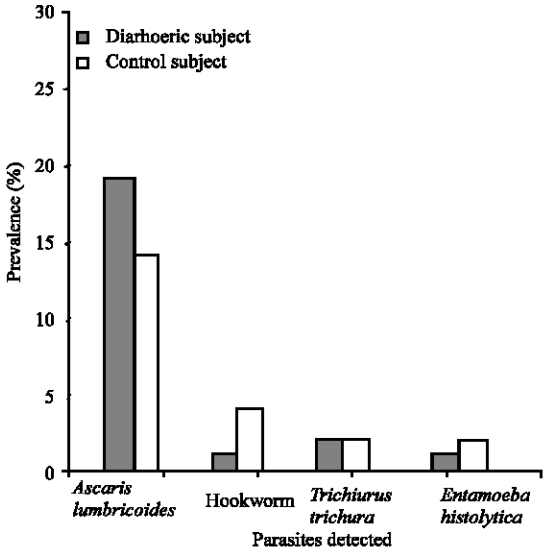

Fig. 1: Distribution of other intestinal parasites detected among diarrhoeic subjects examined

DISCUSSION

The present study has shown a very strong association between cryptosporidiosis and infantile diarrhea in Calabar, Nigeria. The prevalence rates of 26 and 8% reported for diarrhoeic and non-diarrhoeic healthy controls respectively is reasonably high. This confirms *Cryptosporidium* as one of the leading causes of diarrhoea in infants in Calabar. This rate is higher when compared to previous reports in the same area by Alaribe *et al*., (1998) (5.6%) and Useh and Jonah (1998) (4.4%). However it is within the prevalence of 3-29% summarized by Fayer and Ungar (1986) for this part of the world. The factors that may account for this high prevalence include poor personal and environmental hygiene, lack of safe and adequate drinking water, inappropriate methods of disposal of human waste, increased urbanization and poor drainage system. These factors may have created the enabling environment for the transmission and sustainable of the life cycle of the parasite.

Although, we did not confirm the HIV status of these subjects, it is likely that the lowered immunity precipitated by the infection may have been responsible for the high prevalence since *Cryptosporidium* is present in this area (8% in control subjects). Currently HIV/AIDS is a serious public health problem in Nigeria. The national prevalence rate is 4% (UNAIDS, 2004) exception in Cross River State where infection level rose from 4% in 1993-1994 to 12% in 2003 (Federal Ministry of Health, Nigeria, 2004)

The highest prevalence of cryptosporidiosis (38%) was reported among subjects aged 2/12 to 1 year. This agrees with the findings of Abdel-Messih *et al.* (2005) that younger age children aged less than 12 months were many times more likely to develop infection compared to older ones. The tendency and risk for children of this age to be exposed to the factors that leads to acquisition of infection are higher. However Cryptosporidiosis was not detected among children aged above 7 years. This is in accordance with the report of Abdel-Messih *et al.* (2005) who observed less risk factor in the disease among older children. Their poor immune status might also play a prominent role. Female subjects had a higher prevalence rate of infection (26.9%) than the males (25%) though the difference in prevalence was not statistically significant ($p>0.05$). It is possible that females were more exposed to risk factors of infection.

Ascaris lumbricoides was the most frequently encountered intestinal parasite (19%). Others were *Trichurus trichiura* (2%), *Entamoeba histolytia* (2%) and Hookworm (1%). The age of ≥8 years had the highest prevalence rate of infection with *Ascaris lumbricoides* (40%) and *Entamoeba histolytia* (20%). There was a statistically significant difference in the frequency of intestinal infection between the test and the control subjects ($p<0.05$) confirming their role in diarrhoeic causation. One of the limiting factors in this study is the fact that we did not screened our subjects for other aetiologic agents of diarrhoea like bacteria and viruses which could cause diarrhoea in those with cryptosporidiosis and others who had no oocyst detected in their stools. Other conditions such as immunosuppressive ailments like AIDS and cancer (Tanyuksel and Doganci 1995), solid organ transplantation (Campos *et al*., 2000, Gerber *et al*., 2000), malnutrition (Macfarlane and Horner-Bryce 1987), diabetes (Chan *et al*., 1989) may enhance to this infection even though these aetiologies were not sought for among our study subjects. In conclusion, this study has confirmed the involvement of Cryptosporidiosis in infantile diarrhoea in Calabar. There is a justified need for routine screening of stool samples of children with diarrhoea for *Cryptosporidium* which is not being currently done to ensure prompt and appropriate management of the disease.

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