ORIGINAL RESEARCH

CRYPTOSPORIDIOSIS IN IMO STATE, NIGERIA

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ABSTRACT

Background: The epidemiology and public health importance of cryptosporidiosis have not been widely reported in Nigeria. Methods: The prevalence of cryptosporidiosis was assessed in Imo State, Nigeria, between November and 0ctober 2007 using standard parasitological techniques. Stool specimen from consenting subjects registered at nine health institutions in the area were prepared using formalin/ether concentration methods, stained with modified acid fast stain and examined microscopically for oocyst of *Cryptosporidium parvum.* Results: Out of the 1,960 study participants examined, 391(19.9%; 95% confidence interval: 18.2, 21.8) were infected with oocyst. No oocysts were identified in the symptom-free control group (n=50; 95%-CI = 0, 7.1). Women (26.2%) were more likely infected than men (13.2; p<0.001). Infection was more prevalent among rural than urban dwellers (p<0.001). Children aged 0 to 14 years had the highest prevalence of *Cryptosporidium* oocyst as well as females older than 60 years (p<0.001). The prevalence was lowest in borehole users (14.1%) while those who used stream water (22.7%) and other sources (25.4%) were more likely infected (p<0.001). Immune-compromised (HIV/AIDS) participants were more likely infected (32.5%) than immune-competent participants (9.4%; p<0.001). Infection was more prevalent in watery stool samples (27.7%) than in formed stools (2.2%; p<0.001). Conclusions: These findings show that cryptosporidiosis remains a public health problem in the study area even though it is not well known. There is therefore a need to increase awareness among the general public on the public health implications of the disease as well as its prevention and control.

Key Words: Cryptosporidiosis; Prevalence; Oocyst; Immune-compromised; Immune-competent; Stool; Water sources; Infections.

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INTRODUCTION

Cryptosporidiosis is a parasitic disease affecting the microvillous regions of epithelial cells lining the digestive and respiratory organs of vertebrates (Angus, 1983; Fayer and Ungar, 1986; O' Dongue, 1995). The illness is caused by an apicomplexan protozoa of the genus *Cryptosporidium*. The specific infective agent in humans is *Cryptosporidium parvum*. Cryptosporidiosis is acute but self-limiting in immuno-competent individuals, generally abating in 8 to 20 days (Fayer and Ungar, 1986; Juranek, 1995). *Cryptosporidium* is an important cause of acute and persistent diarrhoea in young children (Soave *et al.,* 1986). It is the only water borne coccidian parasite known to infect man (Curds, 1992). First identified in laboratory mice early in the last century, it was considered unimportant and virtually ignored for the next 60 years. The first human cases of Cryptosporidiosis were reported in 1976 (Meisel *et al.*, 1976) but awareness of the public health importance of the organism really came to the fore in the 1980s, partly due to its association with HIV/AIDS infected individuals. Consequently, the concept of cryptosporidiosis changed from that of a rare largely asymptomatic infection to an important cause of enterocolitis and diarrhoea in many species, including man (Tzipori, 1983).

The illness may manifest as gastro-intestinal infection and eventually result in death in immuno-compromised individuals. In immunologically competent individuals, the illness is rarely serious, typically characterized by self-limiting diarrhoea. Potential sources of infection include pets and farm animals, association with individuals already infected, contaminated foods and water while transmission is via fecal-oral route. Numerous outbreaks of cryptosporidiosis have been reported among children in day-care centres (Alpert *et al*., 1984). Similarly hospital acquired (nosocomial) infections and a number of water-borne outbreaks have also been documented (Current, 1999).

The epidemiology and public health importance of cryptosporidiosis have not been widely reported in Nigeria. Although several studies have been carried out focussing on the prevalence of the disease in some parts of the country (Okafor and Okunji, 1994; 1996; Nwabuisi, 2001; Njoku, 2003; Banwat *et al.,* 2003; Mbanugo and Agu, 2006), yet these reports are not enough for a detailed understanding of the epidemiological profile of the disease especially in the southeastern part of Nigeria. The present study was designed to fill this gap in understanding the epidemiology of cryptosporidiosis in Imo State, south-east Nigeria. It is believed that information from the study will be useful in appreciating the public health implications of the disease and help in its prevention and control in the study area.

MATERIALS AND METHODS

Study area

This cross-sectional study was carried out between November 2006 and October 2007 in the three senatorial zones of Imo State, Nigeria: Owerri, Orlu, and Okigwe. Imo State which is located in south-eastern Nigeria lies between latitude $5^0 10^1$ and $5^0 51^1$ north, longitude $6^0 35^1$ and $7^0 28^1$ east and comprises of 27 Local Government Areas with a total land Area of 5,289.49km^2 and a total population of 3,121,824 (Imo State of Nigeria Statistical Year Book, 2000). Five rural communities and one urban/commercial town in each of the three senatorial zones were selected for this study. The mean annual rainfall is between 1800 and 2500mm per year while the average relative humidity is about 74%. Three secondary health institutions from each of the senatorial zones were enlisted as study sites. These health institutions were selected because they serve as referral centres, and are therefore a good contact point for symptomatic patients required for this study.

The sources of drinking water in the study area include streams, ponds (mostly used in dry season), boreholes, sachet water, bottled water and pipe borne water. Chemical treatment (chlorination) of municipal water supply was available in some parts of urban areas. General sewage disposal in the area and environmental sanitation favours transmission of infectious pathogens. Animal to person contact takes place, as many residents have pets and other domestic animals. In some cases, faecal materials inadvertently contaminate the sources of drinking water. Underground water pipes are known to be contaminated through breakages by runoff waters from the environment. Furthermore, the use of bush/grasslands near human habitation for such purposes as defecation is common especially in rural communities. Sometimes, children are forced by circumstances to defecate in nearby vegetable gardens which serve as sources of salads and other foods. Pit latrines were also used for defecation in the study area. Water closet system of toilets were also present and were sometimes in poor sanitary condition because of inadequate supply of pipe borne water which could constitute a potential source of oocyst.

Participants and specimens

A total of 1,960 people comprising 940 (48.0%) males and 1,020 (52.0%) females of different age groups who presented with diarrhoea and/or abdominal pains at the selected health institutions between November 2006 and October 2007 participated in the study. The samples collected for investigation included diarrhoeic samples. Patients clinically diagnosed with HIV/ AIDS were also included in the study and noted as immuno-compromised. Specimens were also collected from 50 control patients with normal stools and/or without abdominal pain and symptoms of diarrhoea.

Sample collection

Fresh stool samples uncontaminated with urine were collected from the participants for examination. The specimens were collected using a dry, sterile leak-roof plastic container, washed free of traces of antiseptics and disinfectants. About 15g of solid stool or 10g of fluid/diarrhoeal stool was collected from each subject using specimen bottles containing 10% buffered formalin and/or storage medium containing aqueous potassium dichromate (2.5%w/v final concentration). The specimens were transported to the laboratory after collection and processed within 48 hours.

Because the oocyst of *Cryptosporidium spp* in stool specimens (fresh storage media) remain infective for extended periods of time adequate precaution was taken by preserving the specimen in 10% buffered formalin (SAF) to render the oocysts nonviable. In addition, the usual safety measures (such as wearing of hand gloves, avoiding oral contact) for handling potentially infectious materials were adopted.

Macroscopic examination

The samples were examined macroscopically to note their colour, consistency (whether formed, semi-formed, soft, or watery), presence of blood and mucus, and if blood was present, whether it was mixed in the faeces and whether the specimen contained adult worms.

Specimen processing and staining of smears

Stool specimens were concentrated prior to staining and microscopic examination in order to maximize oocyst recovery. Formalin-ethyl acetate sedimentation technique was the stool concentration method used (Ukaga *et al*.,2002). A drop of the deposit from the concentration technique was placed on a glass slide, air-dried, fixed with alcohol and stained with modified acid-fast staining and examined under the microscope using oil immersion objective. Oocyst of *Cryptosporidium spp* present appears as red round bodies against a blue-green background.

Data collection

Demographic, behavioural and environmental data was collected using researcher administered questionnaires while immunological status was assessed via medical record.

Ethical considerations

The Institutional Review Board (IRB) of the Imo State University Owerri reviewed and approved this study. Informed consent was obtained from all study participants after the objectives of the study were explain to them.

Statistical analysis

The data obtained from the study were analysed using Chi-square test statistics. 95% confidence intervals (95%-CI) were calculated. The level of significance was fixed at 0.05.

RESULTS

Of the 1960 participants, 391 (19.9%; 95%-CI = 18.2 – 21.8) were infected with *Cryptosporidium* oocyst (Table 1) while the symptom-free control group of 50 participants yielded no oocyst (0%; 95%-CI = 0 – 7.1). The prevalence varied statistically significantly between the three zones with Owerri having the lowest prevalence ($p<0.001$; Table 1).

Table 1: Prevalence of *Cryptosporidiosis* according to three zones in Imo State, Nigeria.

Zone	Number examined	Number infected	Prevalence (%); 95-CI*
Orlu	600	127	21.2%; 18.0 – 24.7
Owerri	560	80	14.3%; 11.5 – 17.5
Okigwe	800	184	22.7%; 20.1 – 26.1
Total	1960	391	19.9%; 18.2 – 21.8

*95%-CI = 95% confidence interval

Overall, more females were infected than males (26.2% versus 13.2%; p<0.001; Table 2). Females older than 60 years had the highest prevalence (60.8%), followed by females in the youngest age group of 0 to 14 years (32.5%), with the lowest prevalence for females in the age group of 15 to 30 years. Males in the age group of 0 to 14 years showed the highest prevalence (26.7%), followed by those in the age group of 31 to 44 years (16.0%). Males in the age group of 45 to 60 years had the lowest prevalence (4.8%; p < 0.001 respectively).

Table 2: Age and gender specific prevalence of *Cryptosporidiosis* in Imo State, Nigeria.

Age	Gender	Number examined	Number infected	Prevalence (%)
0 – 14 years	Male	150	40	26.7%
	Female	240	78	32.5%
15 – 30 years	Male	100	21	21.0%
	Female	306	42	13.7%
31 – 44 years	Male	200	32	16.0%
	Female	100	24	24.0%
45 – 60 years	Male	230	11	4.8%
	Female	300	78	26.0%
61+ years	Male	260	20	7.7%
	Female	74	45	60.8%
Total	Male	940	124	13.2%
	Female	1020	267	26.2%

The highest prevalence (25.4%) was noted for those who drank from other drinking water sources which included roof catch rain water, Satchet water, and water from underground tanks. The lowest prevalence (14.1%) was noted amongst those who drank borehole water (p<0.001; Table 3). Of the 1064 immune-competent participants 100 (9.4%) were infected compared with 291 (32.5%; n=896) infected immune-suppressed participants (p<0.001). Of the 1015 watery/diarrhoeal stool samples 281 (27.7%) were infected with *Cryptosporidium* oocyst, followed by 102 of the 576 (17.7%) semi-formed stool samples, while 8 of the formed stools samples were infected (2.2%; n=369; p< 0.001).

Table 3: Prevalence of *Cryptosporidium* oocysts according to sources of drinking water in Imo State, Nigeria.

Source of drinking water	Number examined	Number infected	Prevalence (%)
Borehole	672	95	14.1%
Pipe borne water	568	125	22.0%
Streams	432	98	22.7%
Other sources	288	73	25.3%
Total	1960	391	19.9%

DISCUSSION

The results of the present study show that cryptosporidiosis is present in Imo State, Nigeria and is of public health significance though it has not attained epidemic proportion. Njoku (2003) in his studies on *Cryptosporidium parvum* and enteric protozoa associated with diarrhoea, in Owerri and environs, Imo State, Nigeria, reported a prevalence of 16.8% of enteric protozoan parasites, out of which 5.2% was due to *Cryptosporidium spp.* The present study showed that the prevalence in Owerri zone is now 14.3%. This implies that the pathogen is gradually attaining epidemic proportions in this area. The higher prevalence observed in Orlu and Okigwe zones, respectively, may be attributed to the rural nature of these areas and the associated human habits that favour faecal-oral transmission. For instance, systematic observation during the study revealed that most of the children in these areas pick and eat fallen fruits like mango without washing. Key informant discussion also revealed that salad materials are not thoroughly washed to get rid of contaminating microorganisms before consumption due to inadequate water supply in these areas. This finding is in agreement with a report by the Centres for Disease Control and Prevention (CDC, 1996a) which implicated *Cryptosporidium parvum* as the causative agent in many outbreaks in the US and underscored the role of contaminated water as a vehicle for transmission of this organism. The public health significance of cryptosporidiosis has been widely described by previous researchers (Fayer and Ungar, 1986; Rose, 1990; Barer and Wright, 1990; Juranek, 1995).

The observed prevalence of 26.7% and 32.5% among male and female children age 0 to 14 years contrasts the findings of Mbanugo and Agu (2006) who reported a prevalence of 14.0% amongst children aged 0 to 15 years in Anambra State, south-eastern Nigeria. The significantly higher infections observed in females than in males is probably due to the fact that females are usually more exposed to infections than men in this part of the world (Okafor and Okunji,1996; Mbanugo and Agu,2006; Chukwuocha *et al*, 2009). For instance, women in this area spend more time on farm lands cultivating vegetable than their male counterparts. They also do most of the house chores including cleaning of the toilets which in rural areas are often pit latrines. Hence women living in Imo State are often exposed to unhygienic conditions due to their living conditions.

The present study showed a higher prevalence in children than the result found by Nchito (1998) who established a prevalence of 18.0% amongst children in Luzata, Zambia. *Cryptosporidium* is associated with diarrhoea throughout the world with higher prevalence in the less developed regions with poor sanitary and environmental conditions. In the present study prevalence was highest in the age group of 0 to 14 years followed by the age group of older than 60 years. The reason may be partly due to immature immunity in the young age group and degenerating immunity in the older age group. Furthermore, young people tend to play outdoors more often than other age groups thereby predisposing themselves to contaminated soil and fruits. Previous researchers have also reported higher prevalence in young children (Fayer and Ungar 1986; Saove and Armstrong, 1986; Nchito, 1998; Nwabuisi, 2001; Mbanugo and Agu, 2006). The present study suggests that the relationship between prevalence of infection and age seems to be U-shaped rather than linear.

The prevalence of cryptosporidiosis according to sources of drinking water showed a higher prevalence for those who got their water from other unidentified sources while those who drank borehole water had the lowest prevalence. Subjects who have their daily water intake from streams also yielded a higher prevalence indicating that rivers support the propagation of infectious oocyst released from both sewage and agricultural run-off emanating from dairies and grazing lands. This finding highlights the results from a previous study by Rose (1990) who reported that sewage discharge may be a significant source of oocyst in the environment. Previously published studies also

established unreliable and contaminated water as main sources of *Cryptosporidium* infection (Alpert *et al*., 1984; Fayer and Unger 1986; Bell *et al*.,19993; McAulty *et al*.,1994;CDC, 1994a,1996b,1998).

Our study established a higher prevalence of infection in immune-compromised than immune-competent persons. This finding supports the earlier report of Ma and Tsaihong (1987) who also obtained a high prevalence (58.9%) in all diarrhoea cases among homosexuals and bisexuals with HIV/AIDS. The high prevalence in immune-compromised individuals shows that immune status plays a vital role in controlling oocyst shedding. Although the protective nature of antibodies remains uncertain in cryptosporidiosis, some indirect evidence suggests that the presence of antibodies in the intestinal lumen provides a protective effect against initial *C. parvum* infection (Rose, 1990; Barer and Wright, 1990).

Prevalence of *C. parvum* oocyst differed according to the consistency of the stool specimens. Higher prevalence rate was obtained for watery/diarrhoeal stools. Okafor and Okunji (1994) had earlier reported a prevalence of 39.5% for watery stools against 24.2% for semi-formed stool samples, respectively. Similarly, Mbanugo and Agu (2006) in their study in Anambra State, Nigeria, also obtained prevalence rates of 40.0% and 10.6% for watery and semi-formed stool samples, respectively. Absence of oocyst in the stool samples of the 50 control persons probably indicates that Cryptosporidium parvum can be strongly implicated in the incidence of diarrhoea in humans because the infected stools were mostly diarrhoea stools while non-infected stools were non-diarrhoea stools.

The findings of this study show that Cryptosporidiosis is prevalent in Imo State, Nigeria, and could constitute a considerable public health and socio-economic problem for the local population. Therefore, there is need for prompt intervention in the study area. Additional studies are needed to identify other possible foci of transmission so as to ensure its quick and early containment. Our results can aid policy makers to articulate effective and sustainable strategies which should aim to stop the pathogen from attaining epidemic proportions in the study area. It is recommended that the inclusion of diagnosis of cryptosporidiosis in routine stool examination of patients in our health institutions is necessary to predict and forestall outbreaks of infection especially in isolated areas. Long-term integrated control measures aimed primarily at improved water and food supply, domestic sanitation and overall environmental sanitation, as well as improved disposal of agricultural wastes is important.

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