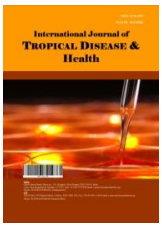

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Identification of *Cryptosporidium parvum* Oocysts among Hospitalized Children under-5years in Northeastern Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Authors UMA and TMI designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors ABS, TI and MMI managed the analyses of the study. Authors UTH, HBA and MU managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Cryptosporidium parvum is among the major pathogens causing diarrheal diseases in children. It is of major public health significance due to its low infectious dose and its oocysts are highly resistant to chlorination, common household disinfectants and survive long periods in the environment. This study was designed to evaluate the occurrence of *Cryptosporidium parvum* oocysts in stool of hospitalized children under-5 years. One hundred and fifty (150) stool samples were collected from one hundred and fifty children (Male:Female = 1:1.08, Mean Age±S.D=22.08 months ± 21.02) and were processed using the modified Ziehl-Nelson method for identification of protozoan oocysts. Out of the one hundred and fifty (150) stool samples analyzed, 16 tested positive to oocysts of *C. parvum*, which gives a parasite prevalence rate of 10.7%. This was observed to be higher among male patients (52.0%) and children between the age 32-41 months (31.3%). Parasite

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prevalence in relation to age of patients was statistically not significant (X^2=0.105, DF=1, P-value = 0.74591, p<0.05). Other intestinal protozoan parasites identified include *Entamoeba histolytica* (1.33%) and *Giardia lamblia* (2.60%).

Keywords: Cryptosporidium; Maiduguri; distribution; oocysts.

1. INTRODUCTION

In developing countries, *Cryptosporidium* is responsible for 8–19% of cases of diarrheal disease, causing a wide range of infections in vertebrate host including humans [1]. This parasite is mainly transmitted by the fecal-oral route [2]. Water, food and direct contact are sources of infection [3]. *Cryptosporidium* attacks the intestinal cell and respiratory system of vertebrate hosts [4], where it can cause a self-limited diarrheal disease. But in compromised patients, it can produce a chronic and persistent diarrhea. Cryptosporidiosis has a worldwide distribution and in most surveys is among the four major pathogens causing diarrheal diseases in children. It has major public health implications because infections can result from exposure to low doses of oocysts. The oocysts are highly resistant to chlorination, common household disinfectants and survive long periods in the environment [5]. In some countries, community outbreaks have been reported to be associated with the consumption of polluted water [4]. Five species of *Cryptosporidium* including *C. hominis* (previously known as *C. parvum,* human genotype), *C. parvum* (bovine genotype), *C. meleagridis*, *C. canis*, and *C. felis* have been found to be responsible for most human infections [6].

Cryptosporidium spp is a waterborne, obligate intracellular protozoan parasite that infects epithelial cells lining the small intestines of human and over 170 different host species causing enteric diseases [7]. There are more than ten species of *Cryptosporidium*. *C. parvum*and *C. hominis* are the two species responsible for the most cases of human cryptosporidiosis worldwide [8]. The genus Cryptosporidium was named at the beginning of the last century but was only recognized as a potential cause of the disease in 1995, when it was found to be associated with diarrheic turkey. Although Cryptosporidium was subsequently found in a broad range of farm animals, its impact was neglected until the early 1980s when it was found to be a common, serious primary cause of outbreaks of diarrhea in calves [9]. The fact that *Crsyptosporidium* was found to infect humans and could cause a life-threatening disease in immuno-deficient people, especially AIDS patients, as well as the association of *Cryptosporidium* with waterborne-related human outbreaks of diarrhea has certainly given the parasite a more widespread recognition [10], In this regard, this study seeks to examine the prevalence of *Cryptosporidium parvum* among hospitalized children under-5years. It aim to evaluate the relationship between patient demographic variables and rate of *C. parvum* infection.

2. MATERIALS AND METHODS

2.1 Study Design

This is a cross-sectional hospital-based study that tries to estimate the incidence rate of *C. parvum* infection among patients under-5years attending Mamman Shuwa Memorial Hospital, Maiduguri, in northeastern Nigeria.

2.2 Study Area

The study was conducted in Mamman Shuwa Memorial Hospital Maiduguri, the capital of Borno State. The city is located in the North-Eastern part of Nigeria which lies within latitude 11.15ºN and longitude 30.05ºE in the sudano-sahelian savannah zone with a dense population that are mostly fishermen, crop farmers, traders and herdsmen. Based on the national census conducted in 2006, Borno State has a population of 4 151 193.

2.3 Study Population

The target population for the study include in-patients and out-patients attending the Mamman Shuwa Memorial Hospital Maiduguri, 200 bed facility serving a population of over 4 million in the North-Eastern sub-region of Nigeria, comprising six States (Borno, Bauchi, Yobe, Adamawa, Taraba and Gombe) as well as a sizeable number across the borders of Cameroon, Chad and Niger Republic.

Sample collection and processing: Stool samples were randomly collected from 150 patients between the ages of 0-5years old, in a

universal container and transported to the Laboratory for analysis.

Macroscopic examination: The samples were examined macroscopically to detect the presence of any color, consistency (whether formed, semi-formed, soft, or watery), presence of blood, mucus, and adult worms.

Formal ether concentration method: One gram of fecal specimen was emulsified in 7ml of 10% formal saline contained in a screw-caped bottle. The mixture was then sieved and the filtrate transferred into centrifuge tube thereafter 3 mls of ether was added and placed in a centrifuge machine and set at low speed of 3000 rpm for about one minute.

Four (4) different layers of ether dissolved fat, fecal debris, Formal saline and sediment containing the parasites formed in the tube in ascending order. Using a Pasteur pipette, the entire layers of fluid below the fecal debris, ether and formal saline was removed and discarded; the sediment containing the oocyst was used to prepare the microscopic slides [11].

Modified Ziehl-Neelsen method: A smear from the sediment obtained by the formal ether concentration technique was made on a clean grease free slide and air-dried and fixed with methanol for 3 minutes. The smear was stained with unheated carbol fuchsin for 15 minutes and then washed off with water. It was then decolorized with 1% acid alcohol for 12 seconds and counterstained with 0.5% malachite green for 2 minutes, washed off with distilled water and the slide was kept in a draining rack to dry [11].

Microscopic examination of the slides: 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 the oocysts that appeared small, round to oval, pink red stained bodies measuring 4-6 um, or a single deeply stained red dot were considered positive [11].

2.4 Data Analysis

Data were grouped as percentages and frequencies, and presented in tabular form. Data were analyzed using Chi-square test at 95% confidence interval and 5% error margin.

3. RESULTS

Out of the one hundred and fifty (150) stool samples analyzed, Sixteen (16) tested positive to *Cryptosporidium parvum*, which gives a prevalence rate of 10.7%. Rate of infection was higher among male patients (6.0%) than female patients (4.7%) (Table 1).

A chi-square test of independence was performed to examine the relation between gender and the rate of infection with *C. parvum*. The relation between these variables was found to be *not* significant, X^2 (df =1, N=150) = 0.1296, p = 0.718836. None of the genders were more likely to be infected than the other.

Table 1. Occurrence of *Cryptosporidium parvum* according to sex of children under-5years attending Mamman Shuwa Memorial Hospital Maiduguri

Sex	Number examined	Number infected
Male	78 (52.0%)	9 (6.0%)
Female	72 (48.0%)	7 (4.7%)
Total	150 (100%)	16 (10.7%)

Table 2 shows the distribution of *Cryptosporidium parvum* infection in relation to age of patients where it was observed that children between the ages of 32-41 months recorded the highest rate of 3.33% followed by those between 22-31 months (18.7%).

Table 2. Distribution of *Cryptosporidium parvum* according to Age of children under-5years attending Mamman Shuwa Memorial Hospital Maiduguri

Age(months)	Number examined	Number infected
0-11	13	2 (1.33%)
12-21	31	2 (1.33%)
22-31	37	3 (2.0%)
32-41	32	5 (3.33%)
42-51	21	2 (1.33%)
52-61	16	2 (1.33%)
Total	150	16(10.7%)

Other parasites identified in this study include *Entamoeba histolytica* (1.33%) and *Giardia lamblia* (2.60%) (Table 3).

4. DISCUSSION

Cryptosporidium parvum is one of the coccidian parasites that lead to diarrheal disease particularly in countries where there is poor standards of hygiene. With such societal problems, infection can become persistent. In this study, *Cryptosporidium parvum* were

Table 3. Occurrence of *Cryptosporidium parvum* and other intestinal parasites identified from the stool of children under-5years attending Mamman Shuwa General Hospital, Maiduguri

Age(months)	*C. parvum*	Other intestinal parasites	
		E. histolytica	***G. lamblia***
0-11	2(1.33%)	0 (0.00%)	0(0.00%)
12-21	2(1.33%)	1 (0.66%)	0(0.00%)
22-31	3 (2.0%)	0(0.00%)	0(0.00%)
32-41	5 (3.33%)	0(0.00%)	0(0.00%)
42-51	2(1.33%)	1(0.66%)	0(0.00%)
52-61	2(1.33%)	0(0.00%)	4 (2.60%)
Total	16 (10.70%)	2 (1.33%)	4 (2.60%)

Identified among under-5years patients with an incidence rate of 10.7%. This was less than the findings of [12], where a prevalence rate of 27.6% was reported in Maiduguri. A prevalence rate of 15.0% was reported in Keffi [13].

Incidence of *C. parvum* in this study was found to be higher among males than females. Similar findings have been reported elsewhere [14,13, 15]. Contrary findings were reported by Abrahamsen et al. [16] in Kenya and in Zaria, who reported that females were more affected. Such differences can be attributed to the uneven ratio of male to females in the sample recruited for their study.

This study also has shown that *C. parvum* infection was higher among patients within 32-41 months, followed by those between 22-31 months of age. This agrees with the findings of [17]. Although the infection was detected among patients in all age groups, the highest incidence of the infection occurred among children older than two years of age. The reason for the observed difference is not clear. 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 a highest risk of being infected with the parasite because is the period when the child is growing and play actively in water and soil in its environs. This report collaborates with previous observation made by Egberongbe et al. [13], but contradicted the reports of Nwabuisi, [14] and Saneian et al. [15] who found that most cryptosporidial infections occur among patients within 0-24 months. The reason for the observed contradiction is not known.

5. CONCLUSION

The study concludes that the total incidence rate of *C. parvum* among children under-5years attending Mamman Shuwa Hospital was 10.7%. The incidence rate was higher among male patients and those between 31-41 months of age. Other intestinal parasites detected include *E. histolytica* and *G. lamblia.* However, their incidence rate is quite low.

CONSENT AND ETHICAL APPROVAL

Clearance was obtained from ethical committee of the hospital before collection of samples.

Ethical clearance was obtained from the management of the Mamman Shuwa Memorial Hospital and the consent of patients was obtained before sample and data collection.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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