

# Cryptosporidium and other Intestinal Protozoans in Children with Diarrhoea in Lagos, Nigeria

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## Citation

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## Abstract

As reports of protozoan intestinal parasites in a mega city such as Lagos, Nigeria are scanty and not updated, this study reports the prevalence of intestinal parasites, especially *Cryptosporidium* species in One hundred and five (105) children less than five years that presented with gastroenteritis in a major tertiary hospital. Standard parasitologic techniques for stool examination and special stains showed cysts and oocysts of protozoan parasites. The overall prevalence rate of intestinal parasites was 27.6%. *E.histolytica/dispar* and *Cryptosporidium* oocyst were the most prevalent (9.5% and 17.1% respectively). The age with the highest infectivity was the 4 -5 year-old children from whom the following parasites were detected: *Cryptosporidium* oocysts (27.6%), *Giardia lamblia* (4.8%) and *E. histolytica/dispar* (9.5%). The infectivity of protozoan intestinal parasite was significantly higher in the 4 -5 age-groups ( $P < 0.001$ ). The findings of this study (especially the detection of *Cryptosporidium* oocysts) underscores the need to broaden the laboratory work-up in children that presents with gastroenteritis to include special staining technique so as to properly manage the condition. No intestinal helminth was detected among these children.

## INTRODUCTION

Diarrhea is a leading cause of morbidity and mortality among young children in low-income countries<sup>12</sup>. It is also ranked among the largest numbers of reported notifiable disease in Nigeria<sup>34</sup>.

There are various aetiological agents of diarrhoea in endemic areas and these include a wide variety of bacteria, viruses and parasites. The parasitic protozoans responsible for gastroenteritis include: *Entamoeba histolytica*, *Giardia intestinalis*, *Cryptosporidium* sp etc. Of these reported gastrointestinal protozoal parasitoses, *Cryptosporidium* species is the most morbid due to its ability to cause severe diarrhea in immunocompromised and even in immunocompetent individuals<sup>56</sup>. *Cryptosporidiosis* as an emerging gastrointestinal disease can cause acute diarrhea by producing 6-8 watery stools per day<sup>7</sup>. *Cryptosporidium* and other gastrointestinal protozoan parasites have been variously reported in children presenting with diarrhea in some countries<sup>568</sup>. Generally, especially in adults, *cryptosporidiosis* has been detected in patients with several chronic health conditions that may have depressed their immunity. These include: acquired immune deficiencies such as HIV/AIDS<sup>910</sup> diabetes<sup>11</sup>, hematologic abnormalities<sup>12</sup>, hospitalized and malnourished children<sup>13</sup>. In developing

countries where the infrastructure and health systems are poor, gastroenteritis occurs frequently and this could be as a result of unclean drinking water, abysmal housing conditions and poor education.

Watery diarrhea is a characteristic presentations of patients infected with any of the parasitic protozoans such as *Entamoeba intestinalis*, *Giardia lamblia*, *Microsporidia* species and some other gastrointestinal protozoan parasite. *Cryptosporidium*, however, can by itself or in the presence of other intestinal protozoans cause severe and life threatening and even fatal diarrhea. The clinical signs ranged from abdominal cramps, fatigue, loss of appetite, nausea, vomiting, weight loss, cough, fever, headache, muscles or joints aches and malnutrition etc.

Protozoan parasitic pathogens associated with diarrhea has been severally reported in pediatric and non-pediatric patients.<sup>45678</sup> This study seeks to update available data and to highlight concurrent gastrointestinal infections involving *Cryptosporidium* in patients under 5 years of age in Lagos, Nigeria. Childhood diarrhea requires significant attention in developing countries, especially in sub Saharan Africa where these children are also burdened with malaria and respiratory disease so that the aetiological diarrhetic agents can be elucidated. The need to include *Cryptosporidium* in the

laboratory work for children presenting with diarrhea is indispensable for the appropriate management of the patients.

**MATERIALS AND METHODS**

**Study site and population:** The study was carried out in Lagos, a mega city in Nigeria. Lagos State is the commercial capital of Nigeria and has an estimated population of approximately 20 million people. The study populations were children less than five years that presented with gastroenteritis in one of the major tertiary health facilities in Lagos. Diarrhea in this study was defined as passing three or more unformed stools within a 24 - hour period. Assent was obtained from the parents or guardian prior to collection of their stools. One hundred and five (105) stool samples were collected from 43 male and 62 female children for the laboratory work-up.

**Stool collection and Examination:** Fresh stool specimen were collected in clean, grease free universal bottles and examined fresh for vegetative form of parasite by direct microscopy. Macroscopic information of the stool samples namely: consistency, presence/absence of mucus and blood were recorded before processing the stool samples for microscopy. Essentially, fresh stools specimen from diarrhetic patients were emulsified in normal saline and also in 5% lugol’s iodine preparation and observed microscopically. In addition, the modified formol-ether stool concentration technique was done for the detection of Cryptosporidium oocyst following standard protocol. Essentially, approximately 2g of the stool was added to 9ml of 10% formalin and strained through 40mm sieve tube. After about 30 minutes, 3ml of diethyl-ether was added and the sample was centrifuged at 2000 rpm for 1 minute. A drop of the sediment was transferred to a slide and examined at x10 and x40 power. Where sediment was sufficient, upto two slides were examined. The prepared slides were later examined with Ziehl-Neelsen stain as described by Garcia and Bruckner<sup>14</sup> for the detection and identification of Cryptosporidium oocyst. The nuclei of Cryptosporidium examined stained pinkish red, spherical and measured between 4 – 6µm in size.

**RESULTS**

Macroscopically, 52 (49.5%) of the stool examined were watery and had mucus; watery (33.3%); watery stool with blood and mucus (12.4%) and watery stool with blood (4.8%). Of the 62 female and 43 male children, 27.4% and 27.9% of them were infected respectively. Parasitic

protozoan were detected more in watery stool with mucus, 12/52 (23.0%) compared with watery stool alone, 5 (14.3%). The overall prevalence of parasitic infection was 27.6%.

The highest infection was in the 4–5 age groups (34.9%) and the least was in 0–1 age group (22.7%). The occurrence of protozoan parasites in the children showed that all the age-groups were infected except the < 1 year old that was not infected with Cryptosporidium. Among the patients with Cryptosporidium, 16(15.2%) had mixed infection with other enteric parasites. Cryptosporidium oocyst was observed to be more prevalent among the 2-3 and 4-5 age-groups with 15.2% and 27.9% infection rate respectively (Table 1). Five patients (4.8%) had cyst of Giardia lamblia, 10 (9.5%) for the trophozoite of E. histolytica /dispar. Multiple infections occurred more between E. histolytica/dispar and Cryptosporidium sp, 16(15.2%).

**Figure 1**

Table 1: Children that presented with gastroenteritis

Age Group (YRS)	Number Examined	Number Infected (%)	(Parasite and Species)		
			<i>E.histolytica</i> (%)	<i>G.lamblia</i> (%)	<i>Cryptosporidium</i> oocysts (%)
0 – 1	22	5(22.7)	3 (13.6)	2(9.0)	0(0.0)
2 – 3	40	9(22.5)	2 (5.0)	1(2.5)	6(15.2)
4 – 5	43	15(34.9)	5 (11.6)	2(4.6)	12(27.9)
	105	29	10	5	18

**DISCUSSION**

The rate of mortality from diarrhea diseases in the developed countries has decreased, mainly because of better therapy and institution of interventions like good sanitary conditions, and health promotion .<sup>15</sup> Nevertheless, acute diarrhea diseases continue to be one of the major causes of morbidity and mortality in the developing world such as Nigeria, where 197 in 1000 children under five die before their fifth birthday when compared with other statistical data from developed countries .<sup>16</sup>

The most important outcome of the present study is the identification of parasites associated with childhood diarrhea in Lagos, Nigeria. This study has shown a meso-endemic level of parasitic pathogens in pediatrics patients presenting with diarrhea. Belding<sup>17</sup> defined an area as mesoendemic when the parasite rate in infants and children (between 0 – 5 years) is between 10 –49 %. The fairly high incidence of these protozoa infection among the patients correlates with the results obtained by several workers.<sup>5,6,7,8,9,10</sup>

However, mesoendemicity of an infection in an area indicates the unstable nature of the infection as this situation creates room for spontaneous epidemics of the infection.

The high prevalence of infection among age group (0–5 years) in this study is within the proportion of those reported by Gascon et al.,<sup>18</sup> and Ogunlesi et al.,<sup>19</sup>. These studies had methodical limitations as *Cryptosporidium* could not be detected. Tumwine et al.<sup>20</sup> reported a 72.7% prevalence of *Cryptosporidium parvum* in Uganda using PCR – RFLP. This fact suggests that with improved technique, there is tendency to detect more of the enteric parasitic pathogens in patients with diarrhea. *Cryptosporidium* was not detected among undernourished HIV Sero-positive while a 3.8% prevalence was reported in HIV sero-negative children in Jos, Nigeria using double ELISA<sup>21</sup>. It is likely therefore, that the variation observed in the prevalence rates of gastrointestinal protozoan parasitoses especially those involving coccidian parasites such as *Cryptosporidium* species could be dependent on the diagnostic technique, geographic location of the study, patient's condition and the socio-economic status of the people.

However, we were unable to ascertain the immune status of the patients who enrolled for the study. The non-determination of the HIV status of our research participants is a limitation of this study as it would have proffered explanations on factors such as immunosuppression due to HIV/AIDs. Nevertheless, *Cryptosporidium* has also been reported in immunocompetent individuals<sup>9,10</sup>. A moderate level of protozoan gastrointestinal infection (17.3%) (Without *Cryptosporidium*) was reported in Ilesa, Nigeria, a semi-urban and rural communities<sup>10</sup>. Our finding of *Cryptosporidium* is understandable because the appropriate special stain was used and this contradicts earlier studies that did not detect the oocyst of *Cryptosporidium* as a result of deficiencies in the methods employed<sup>19,22</sup>.

This study therefore has provided current data on enteric parasitic protozoans in Lagos, Nigeria. Our reported prevalence is consistent with other studies in Africa<sup>18,19,22</sup>. Surprisingly, no intestinal helminthes was found in the stool specimens examined. Another limitation of the study was the non inclusion of bacteriological work-up to assess the level and contribution of Bacteria to childhood diarrhea.

Our findings in the age group studied brings up the burden of disease in children less than 5 years of age and that the detection of *Cryptosporidium* concurrently with other protozoans such as *Entamoeba histolytica* could complicate

the health conditions of these children. Therefore, the need for adequate and appropriate laboratory work up for children presenting with gastroenteritis to include tests for *Cryptosporidium* is hereby recommended.

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