Infection Rate of *Cryptosporidium parvum* among Diarrhea Children in Ibadan, Oyo State, Nigeria

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Abstract: Cryptosporidiosis is an important major cause of enteric parasitic infection among infants and children in developing countries with morbidity and mortality especially among immunocompetent individuals. This study was carried out to determine the prevalence of enteric cryptosporidiosis in children presenting with diarrhea in Ibadan. Two-Hundred and Fifty (250) children with diarrhea age 1month to 10years, living in urban areas (n=97) and rural area (n=153). Samples were collected, stained by modified acid stain, and examined microscopically for oocysts. The overall prevalence was 11.2% (28/250). According to age, the prevalence in age groups 1-5years was higher in female 21.9% while male was 15.4% but in age 6-10yrs the prevalence was higher in male 9.3% while female was 6.8%. There is no significant difference (p> 0.5). The prevalence was more in rural areas and in patients ward with 7.2% and 6.8% respectively as compared with urban and out-patients with 4.0% and 4.4% respectively. More so, infections was more prevalent among children in primary schools (15.0%) followed by hose in Day care centres (10.9%) while hose in Post primary schools yielded the least (4.2%) prevalence. There was significant difference as regards school related prevalence rate(p < 0.05). Cryptosporidium infection is an important cause of diarrhea in children. Clinicians and medical laboratory scientist should be encouraged to include cryptosporidium spp diagnostic techniques while dealing with diarrhea stool samples in patients especially in young children.

Keywords: Cryptosporidium, Diarrhea,Stool, Children, University College Hospital.

INTRODUCTION

Cryptosporidium spp are tiny microscopic parasites that cause cryptosporidiosis. Protozoan parasites of the genus cryptosporidium members of the phylum Apicomplexa, affects a wide variety of vertebrates hosts. In humans, cryptosporidium humans, cryptosporidium hominis (anothroponotic origin) and C. parvum (Zoonotic origin) are responsible in most areas for more than 90% of cases of cryptosporidiosis, a diarrheal disease which progression and severity are closely linked to the immune status of infected persons [1, 2]. According to Ajjampur [3], cryptosporidiosis is a major cause of diarrhea in children with or without human immuno deficiency virus (HIV) in developing countries. AIDS and protein energy malnutrition (PEM) severally impair immune system [3]. There is a high prevalence rate of cryptosporidium in young children suffering from protein energy malnutrition (PEM) [4]. Cryptosporidium is transmitted through multiple routes. The infection may be transmitted by direct person to person or contact with the infected animal by direct contact infected animal or by ingestion of contaminated food or water [5]. The oocysts are highly resistant to common household disinfectants and survive for long periods in the environment. It has major public health’s implication because infection can result from exposure to low doses of cryptosporidium oocysts [6]. Cryptosporidiosis is a diarrheal disease caused by parasites of the gene Cryptosporidium, which measure between 4-6μm in size [7, 8]. Diarrhea caused by parasite accounts for more than 3.1 million deaths each year among children less than 15 years of age, mostly in developing countries [9]. Cryptosporidiosis causes
chronic and life threatening diarrhea among immune compromised individuals as well as self limiting diarrhea in immune competent individuals [4, 10]. Infection by the parasite account for up to 6% of all diarrheal disease in immune competent person and presents in up to 24% of person with both AIDS and diarrhea world wide[11,12]. Cryptosporidiosis is normally initiated by ingestion of crytosporidiosis oocysts from contaminated drinking water, recreational water and contaminated food. Cryptosporidium oocysts are prevented in surface waters, extremely resistant to commonly used disinfectants, and generally survive for several months in aquatic environments [13]. Cryptosporidium is recognized as a major human waterborne pathogen worldwide [14]. Following ingestion, oocysts excyst in the gastrointestinal tract and release infective sporozoites, which attach to the apical membrane of the most epithelial cell. When the sporozoites mature they undergo a sexual reproduction to produce merozites which are released into the intestinal lumen. The merozoites can either infect other epithelial cell. Or mature into diarrheic stool into the environment to start another life cycle [7]. The aim of this study was to find the prevalence studies of cryptosporidium infection among children under ten years of age having diarrhea with the objective of creating awareness among the population and in caretakers of the diarrheal patients, to highlight the importance of detection of this parasite in routine stool examination and to improve and modify the treatment of these patients.

MATERIALS AND METHODS

Study area

A total of 250 stool specimens from children (under 1-10 years of age) suffering from diarrhea were examined for the presence of C. parvum oocysts. This cross-sectional study was carried out between January, 2012 and October 2013 in-out-patients and in-patients of university college hospital, Ibadan, Oyo-State Nigeria.

Participants and Specimens

The prevalence of cryptosporidium spp was determined in children with diarrhea (n=250) ages (one months to 10years of age) comprising of 114 (45.6%) males and 136 (54.4%) females. In-patients wards and out-patients ward 148(59.2%) and 102(40.8%) respectively. The samples collected for the investigation included diarrheic stool samples.

Sample Collection

Fresh fecal samples were collected in a dry, clean, leak-proof plastic container. Each sample was labelled with the child’s name, gender and age. Additional information and demographics related to each sample were obtained from the parent or guardian of all children before enrollment in the study. Matched controls were obtained from children without diarrhea in the same hospital and treated exactly in the same manner as the other specimens. Sample collection storage and transport were carried out according to the specifications of the center of Disease control [15, 16] and stained by the modified acid fast stain procedure out lined by Garcia [17].

Macroscopic Examination

The samples were examined macroscopically to note their colour, consistency (whether formed, soft or watery), presence of blood or mucus, and if blood was present, whether it was mixed with the feces and whether the specimen contained adults 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 [18]. A drop of the deposit from the concentration technique was placed on a clean-glass slide, air-dried, fixed with alcohol and stained with (Z-N staining) modified acid fast staining and examined under the microscope using oil immersion objective. Oocyst of C. parvum if present appears as red round bodies against a blue-green background.

Statistical Evaluation

Chi square (x²) was used to detect significant differences between the various groups at 5% level of significance.

A total of 153 children (61.2%) were from rural villages while 97 children 97(38.8%) were from urban areas. The prevalence of Cryptosporidium Spp. In the out-patients and in-patients wards were as follows: 11of 102 (10.8%) and 17 of 148(11.5%) respectively (Table 2). More so, infection was more prevalent among children in primary schools, 12 of 80 (15.0%) follow by Day care 5 of 46 (10.9%), home (not enrolled) 4 of 3 (10.8%), nursery 6 of 63 (9.5%) while those of post primary schools yielded the least 1 of 24 (4.2%).

Table 1: age distribution of the study subject with respect to sex

<table>
<thead>
<tr>
<th>AGE Mon/yr</th>
<th>Female</th>
<th></th>
<th></th>
<th>Male</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>n</td>
<td>P</td>
<td>N</td>
<td>n</td>
<td>P</td>
</tr>
<tr>
<td>1-12 Months</td>
<td>36</td>
<td>3</td>
<td>8.3</td>
<td>32</td>
<td>2</td>
<td>6.3</td>
</tr>
<tr>
<td>1-5 yrs</td>
<td>41</td>
<td>9</td>
<td>21.9</td>
<td>39</td>
<td>6</td>
<td>15.4</td>
</tr>
<tr>
<td>6-10 yrs</td>
<td>59</td>
<td>4</td>
<td>7.1</td>
<td>43</td>
<td>4</td>
<td>9.3</td>
</tr>
<tr>
<td>Total</td>
<td>136</td>
<td>16</td>
<td>37.3</td>
<td>114</td>
<td>12</td>
<td>10.5</td>
</tr>
</tbody>
</table>

N= number examined, n= number infected, P = percentage positive

Table 2: Total number of specimens collected and distribution according to area of residence

<table>
<thead>
<tr>
<th>Category</th>
<th>Total number</th>
<th>Cryptosporidium Spp</th>
<th>% Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ward</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In- patient</td>
<td>148</td>
<td>17</td>
<td>11.8</td>
</tr>
<tr>
<td>out-patient</td>
<td>102</td>
<td>11</td>
<td>10.9</td>
</tr>
<tr>
<td>Area of residence</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urban Centers</td>
<td>97</td>
<td>10</td>
<td>10.3</td>
</tr>
<tr>
<td>Rural villages</td>
<td>153</td>
<td>18</td>
<td>11.8</td>
</tr>
</tbody>
</table>

Table 3: School related prevalence of Cryptosporidiosis among the population studied

<table>
<thead>
<tr>
<th>Level of education</th>
<th>N</th>
<th>n</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Home (not enrolled)</td>
<td>37</td>
<td>4</td>
<td>10.8</td>
</tr>
<tr>
<td>Day care</td>
<td>46</td>
<td>5</td>
<td>10.9</td>
</tr>
<tr>
<td>Nursery</td>
<td>3</td>
<td>6</td>
<td>9.5</td>
</tr>
<tr>
<td>Primary</td>
<td>80</td>
<td>12</td>
<td>15</td>
</tr>
<tr>
<td>Post primary</td>
<td>24</td>
<td>1</td>
<td>4.2</td>
</tr>
<tr>
<td>Total</td>
<td>250</td>
<td>28</td>
<td>11.2</td>
</tr>
</tbody>
</table>

N= number examined, n= number infected

Fig. 1: Frequency of C. parvum in diarrhoeal stool (N=250)  
Fig. 2: prevalence of cryptosporidiosis among the population studied


DISCUSSION

C. parvum is an endemic, Zoonotic parasite that is highly prevalent in developing countries. Cryptosporidiosis is a serious disease in this country because it increases morbidity and mortality associated with poverty and malnutrition [19]. In developing countries, it is a leading cause of persistent diarrhea especially children. Microscopic analysis of zn stained faecal smear is the most commonly used method for screening stool specimens for C. parvum oocyst [19]. Although microscopy is considered to be a reliable diagnostic tool for detection of cryptosporidium infection, it is however, not practiced routinely in Nigeria. The result of this study shows 28(11.2%) that cryptosporidiosis is present among children in – out-patients and in patients wards of university college, hospital, Ibadan, Nigeria and is of public health significance though epidemic proportion has not been recorded in this area Njoku [20] reported a prevalence of 5.2% among residents of the state and its environs though his study was restricted within a part of the state and did not focus on children alone. The present study
which focused on children in the country recorded a higher prevalence of 11.2% among children thereby corroborating the report of Mbang and Agu [21] who reported a prevalence of 14.0% among children of ages 3months to 15yrs in Anambra state, South Eastern Nigeria. This contrasts the result of Dozie [22] who reported a total prevalence of 19.9% among children and adult population in the area. Another researcher (Nchito) [23] has also reported a higher prevalence of 18.0% among children in Luzata, Zambia. In this study, many patients were primary school children with large number. They were mostly uneducated and lived in poor hygienic conditions and environment. These children were not properly looked after and hand washing was not practiced. The crawling children who are more exposure to dirty surfaces are therefore more vulnerable to these oral- faecal infections. Similar observations were made in the study conducted by [24] in Indonesia and Gahna [24]. In the present study the prevalence of C.parvum was higher among children in the rural area 18(7.2%). This may be attributed to poor awareness of the disease and its mode of transmission coupled with rural area nature of majority of the parts of the area and the associated human habits that favour fecal-oral transmission of the pathogen. For instance, systematic observation during the study revealed that most of the children, especially in the rural communities pick, buy and eat fallen fruits and food without washing. They also tend to play out doors more often thereby predisposing themselves to contaminated foods and soil. Furthermore, the public health significance of crypto-soridiosis has equally been reported in this area [22] and other parts of the world [25, 26]. Although no significant difference was noted in sex related prevalence of infection, the observed prevalence of 11.8% and 10.5% among female and male is contrasts the result of Mbanugo and Agu [21] who reported that more males were affected than the females. This results then shows that human factors that predispose humans especially children, to infection in this part of the world is not a function of gender as noted and support by previous researchers [27, 28].

CONCLUSION

It is therefore suggested that our clinicians and medical laboratory scientist should be encouraged to include cryptosporidium diagnostic technique while dealing with diarrhea stool samples in patients especially in young children.

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