Full Length Research Paper

Prevalence and risk factors associated with Cryptosporidium species infections in Bungoma County, Kenya

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A prospective study on prevalence of Cryptosporidium and Cryptosporidiosis was carried out in Bungoma County, Kenya. A total of 712 fecal samples from children up to five years of age were collected, from four Hospitals during a 30 month period covering January 2011 to June 2013. Overall prevalence of cryptosporidiosis was 5.06%. Cryptosporidiosis was significantly associated with diarrhea (P = 0.01 OR = 1.301) and abdominal swelling and pain (P = 0.031, OR = 1.56, 95% = 1.04-2.34). Cryptosporidium infections were found to be seasonal with peaks and lows. There was a significant association between water sources for domestic use and Cryptosporidium infections (Mann Whitney test P = 0.0432). Boiling appeared to be the most effective way of water treatment to eliminate Cryptosporidium, as only one case was reported out of the 245 cases (0.04%) who reported boiling drinking water. Although a higher number of those who reported to have had exposure to livestock had Cryptosporidium than the non-exposed (23/36 and 13/36 respectively), this was not statistically significant (P >0.0634). The results suggest that prevalence of cryptosporidiosis is comparable to other regions of the world and transmission is more anthropotic than Zoonotic. Source and treatment of water for domestic use were significant risk factors, associated with the disease.

Key words: Cryptosporidium, prevalence, risk factors, gastroenteritis, co-infection.

INTRODUCTION

The protozoan parasite Cryptosporidium species is a leading cause of infectious diarrhea in humans and livestock, with fecal-oral transmission by ingestion of oocysts. Infection is generally self-limiting, followed by variable protective immunity involving humoral and cell mediated responses, except in the immune suppressed, when infection may be prolonged and fatal (Checkley et al., 2015). Cryptosporidium oocysts remain viable in water and damp soils, for prolonged periods and are resistant to disinfectants at concentrations usually used in water treatment (Korich et al., 1990). Outbreak investigations have shown diverse modes of transmission, including contact with livestock (Le chevalier et al., 1991; Sayers et al., 1996), person to person transmission in households and care settings (Cordell and Adiss, 1994), consumption of contaminated foods and drinks, including milk (Fayer et al., 2001, Gelleti et al., 1997). Cryptosporidiosis is responsible for 8 - 10% of cases of diarrheal diseases with significant effect on mortality. (Molbak et al., 1993, Tzipori & Ward, 2002, Xiao et al., 2004). A recent study on cryptosporidiosis conducted in Egypt examined 1275 children, attending two hospitals and found prevalence of 17%. The study also found that children less than 12 months of age were most likely to get cryptosporidiosis and infection was significantly associated with diarrhea,
vomiting and a need for hospitalization (Abdel-Messih et al., 2005). A recent survey on the prevalence of cryptosporidiosis among Human Immune Deficiency Virus (HIV) infected and uninfected children with persistent diarrhea at Mulago hospital in Uganda showed 73.6% (67 of 91) of HIV infected children and 5.9% (9 of 152) of HIV negative children were infected (Tumwine et al., 2005). Another recent study on cryptosporidiosis conducted in Kenya examined 4899 samples and showed an overall prevalence of 4%. The prevalence was highest in children 13-24 months of age (5.2%) and lowest among those 48-60 months of age (Wangeci et al., 2006).

The main symptoms associated with the disease were abdominal pain, vomiting and abdominal swelling (Meinhardt et al., 2001, Wangeci et al., 2006). Various methods have been applied to detect oocysts in feces but difficulties of discriminating between non-Cryptosporidial bodies, acid-fast bodies like Cryptosporidia and Cryptosporidium remain. Screening by use of wet preparations has been found to be insensitive and not particularly helpful, although it may be useful in detecting cysts or ova (Casemore et al., 1985; Morgan et al., 1996). A modified Ziehl Neelson staining method has been widely recommended and used. Its main limitation is that it has many stages involving concentration and staining, therefore unsuitable for handling large batches of specimens in routine laboratory examination. Overall, microscopic identification requires trained microscopists and involves time and labor for preparing, staining and examining (Marshall et al., 1997). As a result, immunosassays for detection of Cryptosporidium stool antigen have replaced microscopy as the routine diagnostic procedure of choice in hospitals and public health laboratories that routinely carry out this test (Garcia and Shimizu, 1997). Polymerase chain reaction (PCR) has been used to identify the various species and genotypes/strains of Cryptosporidium (Morgan et al., 1998).

There are several species of Cryptosporidium that are known to cause both human and animal infections. However, despite this fact being known, not much is known or has been done about the prevalence and risk factors associated with Cryptosporidium in many parts of Kenya, including the study area. Similarly, patients presenting diarrhea in hospitals are seldom examined for Cryptosporidium species despite it being known as one of the major causes of diarrhea. Where the test for Cryptosporidium species is specifically requested for (few laboratories offer the test), the procedure is long and time consuming (such as Modified Ziehl Neelson staining) or is expensive (P.C.R procedure). This study was carried out to determine the prevalence of Cryptosporidiosis and the risk factors associated with its transmission in Bungoma County, Kenya.

MATERIALS AND METHODS

Study site and population

This cross-sectional study was conducted in four Hospitals, Bungoma, Chwele, Kimilili and Webuye District Hospitals all in Bungoma County. The study involved as its subjects, children up to five years of age presenting gastro-enteritis with or without diarrhea in the four respective hospitals. Gastro-enteritis in this context was used to mean any stomach ailment that prompted the physician to refer the patient to the laboratory for ova/parasite examination. Two samples of stool specimens were collected from eligible patients after obtaining consent from their parents/guardians by qualified laboratory technicians. They were immediately preserved in 10% formalin before being processed.

ASSAYS

Questionnaire

Before fecal specimen collection, parents/guardians were asked to fill a patient information form and questionnaire. The patient information form provided personal details of the patient such as name, sex, age, place of residence and so on. The questionnaire on the other hand sought information relating to symptoms that prompted a hospital visit, risk factors such as presence of pets, nature of floors in their residential houses, livestock and source and treatment of water for domestic use.

Fecal concentration and Modified ZN staining

A formalin ethyl acetate concentration was performed on all the specimens before preparing a concentrated wet smear and modified Ziehl Neelson stained smears. In brief stool specimens were passed through a double layered gauze bandage before being centrifuged twice at 400gx for 10 minutes to concentrate. Smears of approximately 20 x 20 mm fixed in methanol before being stained with Carbol Fuchsin. After rinsing, the slides were decoloured in 1% acidified alcohol before counterstaining with Malachite green. The slides were then examined with X100 oil immersion and the presence or absence of Cryptosporidium was recorded.

ImmuCard assay

The ImmunoCard STAT! Crypto /Giardia rapid assay was performed on un-concentrated formalin fixed stool specimens as specified by the Manufacturer (Meridian Bioscience Inc USA). Results were viewed after 10 min. A positive control line was visible on the device each time a test was completed. A positive reaction appeared as a
grey black band visible at the Cryptosporidium/Giardia area in the test window. No reaction in the test window and a positive control line was interpreted as a negative result. Tests on samples with weak or faint reactions using the ImmunoCard assay were repeated.

Data entry, handling and analysis

The data was manually entered using SPSS data editor version 17.0 (Microsoft inc. USA) and analyzed using SPSS version 11.5 (Microsoft Inc. USA). To determine associations between infection with cryptosporidiosis, diarrhea or other symptoms, and risk factors associated with it, a Pearson’s Chi square test was used to compare samples. A P value of < 0.05 was considered statistically significant.

Ethical considerations

The study was reviewed and approved by the National Council for Science Technology and Innovation (NACOSTI) of Kenya (Research permit No. NCST/RRI/12/1/MED/223). All parents/guardians were informed of the purpose of the study and voluntary consent was sought before inclusion into the study. Results for Cryptosporidium and other intestinal parasites were handed to the caring hospital staff for appropriate action.

RESULTS

Prevalence of cryptosporidiosis

This prevalence was based on microscopic examination of Ziehl Neelson stained concentrated wet smears. Overall prevalence of Cryptosporidium was 5.06%. However the prevalence was highest during the months of June - July and October –November, which coincided with the tail end of the long and short rains respectively. Univariate analysis showed that cryptosporidiosis was seasonal with more infections likely to occur during the wet season [(OR-1.73, CI=1.23-241) P < 0.001] (Figure 1)

Cryptosporidium prevalence by ages

Prevalence of Cryptosporidium was highest in children aged between 13 - 36 months with overall prevalence of 5.83% (21/36) and least in those aged 37 - 60 months at 1.67% (6/36). There was a significant association

![Figure 1. Distribution of Cryptosporidium by seasons. There was peak prevalence in June and a smaller beak in November that coincided with ending of the long and short rains in the study area.](image)
Table 1. Cryptosporidium distribution by age.

<table>
<thead>
<tr>
<th>Age range (Months)</th>
<th>Total patients</th>
<th>Crypto case</th>
<th>% prevalence in age range</th>
<th>OR</th>
<th>95%ci</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-12</td>
<td>181</td>
<td>09</td>
<td>4.97</td>
<td>1.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>13-24</td>
<td>185</td>
<td>13</td>
<td>7.02</td>
<td>1.49</td>
<td>1.06-2.09</td>
<td>0.023*</td>
</tr>
<tr>
<td>25-36</td>
<td>132</td>
<td>08</td>
<td>6.06</td>
<td>0.54</td>
<td>0.33-0.87</td>
<td>0.011*</td>
</tr>
<tr>
<td>37-48</td>
<td>113</td>
<td>04</td>
<td>3.53</td>
<td>0.23</td>
<td>0.53-0.98</td>
<td>0.06</td>
</tr>
<tr>
<td>49-60</td>
<td>101</td>
<td>02</td>
<td>1.98</td>
<td>0.38</td>
<td>0.16-0.89</td>
<td>0.08</td>
</tr>
<tr>
<td>Total</td>
<td>712</td>
<td>36</td>
<td>5.06</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Significant.

Table 2. Symptoms associated with Cryptosporidium infections.

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Crypto present</th>
<th>Crypto absent</th>
<th>Total</th>
<th>Overall percentage</th>
<th>X$^2$</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea</td>
<td>14 (38.9%)</td>
<td>254</td>
<td>268</td>
<td>5.22%</td>
<td>0.48</td>
<td>≤0.0010</td>
</tr>
<tr>
<td>Abdominal swelling/pain</td>
<td>12 (31.6%)</td>
<td>213</td>
<td>225</td>
<td>5.13%</td>
<td>0.44</td>
<td>0.05</td>
</tr>
<tr>
<td>Dehydration</td>
<td>6 (16.7%)</td>
<td>116</td>
<td>122</td>
<td>4.92%</td>
<td>3.8</td>
<td>0.063</td>
</tr>
<tr>
<td>Vomiting/others</td>
<td>4 (1.11%)</td>
<td>93</td>
<td>97</td>
<td>4.12%</td>
<td>6.9</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td>36</td>
<td>676</td>
<td>712</td>
<td>5.06%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Water Sources and Cryptosporidium occurrence.

<table>
<thead>
<tr>
<th>Water source</th>
<th>Crypto present</th>
<th>Crypto absent</th>
<th>Total</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface/river water</td>
<td>8 (9.09%)</td>
<td>80</td>
<td>88</td>
<td>0.0432</td>
</tr>
<tr>
<td>Spring/borehole water</td>
<td>20 (3.96%)</td>
<td>485</td>
<td>505</td>
<td>0.654</td>
</tr>
<tr>
<td>Tap water</td>
<td>8 (6.72%)</td>
<td>111</td>
<td>119</td>
<td>0.058</td>
</tr>
<tr>
<td>Total</td>
<td>36 (5.06%)</td>
<td>676</td>
<td>712</td>
<td></td>
</tr>
</tbody>
</table>

between age and Cryptosporidium infection (P = 0.0345) (Table 1)

Symptoms associated with Cryptosporidium infections

Diarrhea was the most common symptom in Cryptosporidium infected patients [38.9%], followed by abdominal pain/swelling/stomach muscle cramps [33.33%. The least symptom shown by patients was vomiting at 4.12%. Cryptosporidiosis was significantly associated with diarrhea (acute/persistent/recurrent) (P = 0.01 OR = 1.301) and Abdominal swelling and pain (P = 0.031, OR = 1.56, 95% = 1.04 - 2.34). There was no significant association of cryptosporidiosis with vomiting (P = 0.334, OR = 0.831, 95%, CI = 0.657- 1.23) (Table 2).

Water source, treatment and Cryptosporidiosis prevalence

Patients who reported using surface and river water for drinking/domestic use had the highest Cryptosporidium infection, at 9.09% (8/88) while those who used spring/borehole water had the least infection, at 3.96% (20/505). The results also showed that tap water may equally be contaminated with Cryptosporidium as it had the second highest Cryptosporidium contamination (6.72%). There was a significant association between water source and Cryptosporidium infection (Mann Whitney test P = 0.0432) (Table 3).
Table 4. Water treatment and Cryptosporidium occurrence.

<table>
<thead>
<tr>
<th>Water treatment</th>
<th>Crypto present</th>
<th>Crypto absent</th>
<th>Total</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No treatment</td>
<td>29(6.56%)</td>
<td>413</td>
<td>442</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Boiling/ filtration</td>
<td>1 (0.04%)</td>
<td>244</td>
<td>245</td>
<td>0.623</td>
</tr>
<tr>
<td>Chemical treatment</td>
<td>6(24.00%)</td>
<td>19</td>
<td>25</td>
<td>0.05</td>
</tr>
<tr>
<td>Total</td>
<td>36</td>
<td>676</td>
<td>712</td>
<td></td>
</tr>
</tbody>
</table>

Table 5. Association between livestock keeping and Cryptosporidium infections.

<table>
<thead>
<tr>
<th>Livestock kept in residence</th>
<th>Crypto present</th>
<th>Crypto absent</th>
<th>Overall</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No livestock kept</td>
<td>23</td>
<td>425</td>
<td>448</td>
<td>5.13%</td>
</tr>
<tr>
<td>Total</td>
<td>36</td>
<td>676</td>
<td>712</td>
<td>5.06%</td>
</tr>
</tbody>
</table>

Table 6. Cryptosporidium co-infections with other enteric parasites.

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Prevalence</th>
<th>Number of co-infections with Cryptosporidium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascaris lumbricoides</td>
<td>9.41% (67/712)</td>
<td>0.00%</td>
</tr>
<tr>
<td>Entamoeba histolytica</td>
<td>8.9% (63/712)</td>
<td>16.6% (6/36)</td>
</tr>
<tr>
<td>Giardia lamblia</td>
<td>5.48% (39/712)</td>
<td>30.5% (11/36)</td>
</tr>
<tr>
<td>Hookworm</td>
<td>4.63% (33/712)</td>
<td>0.00%</td>
</tr>
<tr>
<td>E.coli/ E.dispar</td>
<td>3.79% (27/712)</td>
<td>27.78% (10/36)</td>
</tr>
</tbody>
</table>

diagnosed with Cryptosporidiosis, translating to a prevalence of 0.04%. Chemical treatment by chlorinating did not seem to be as effective on Cryptosporidium oocysts, with the highest prevalence of 24.00% (Table 4).

Association between Livestock keeping and Cryptosporidium infection

Cryptosporidium spp especially Cryptosporidium parvum was initially considered as an animal parasite. Therefore contact with livestock is considered a major risk factor for contraction of cryptosporidiosis. However this study found no significant difference in prevalence between those who kept livestock and those who didn’t. (Mann Whitney test = 0.0563). Again there was no significant difference in infections and type of livestock or pets kept (Table 5).

Cryptosporidium co-infections and other enteric parasites

Giardia intestinalis had the highest co-infection with Cryptosporidium spp, at 30.5%. Other parasites were Entamoeba coli/dispar at 27.78% and Entamoebahistolytica, 16.6%. Of the other enteric parasites Ascaris lumbricoides had the highest prevalence of 9.41% (67/712) while Entamoeba coli/dispar had the lowest prevalence of 2.63% (27/712) (Table 6).

Discussion

This study focused on the current state of Cryptosporidium infections in children up to five years of age, who presented gastroenteritis to the four participating hospitals and were referred to the laboratories for ova and cyst examination. This study is unlike other studies that have focused on the high risk groups such as children with HIV and/or those with persistent diarrhea only or day care centres. Cryptosporidium infections were significantly found to be seasonal with peaks and lows. The highest prevalences were in the months of June and October which marked the tail ends of the long and short rains respectively in the study area. This seasonality in infection was also found in similar studies (Wangeci et al., 2006; Pradeep et al., 2006). This peak prevalence during the end of the rain season coincided with results of
two other studies carried out in Malawi and Kolkata, India, which also had peak prevalences during the rainy season between March and October (Peng, et al., 2003; Pradeep, et al., 2006).

*Cryptosporidium* infections were found to be significantly associated with acute diarrhea, with an odds ratio (OR) of 5.056 ($P \leq 0.0010$). Other symptoms associated with the infection included, abdominal pains and swelling dehydration and to a lesser extent vomiting. These results were in agreement with studies carried out elsewhere where persistent diarrhea and abdominal pains were the commonest symptoms associated with *Cryptosporidium* (Colford et al., 1996. Morgan et al., 2002, Wangeci et al., 2006).

Patients who reported using surface and/or river water for drinking had the highest *Cryptosporidium* infection, at 9.09% (8/88), while those who used spring/borehole water had the least infection, at 3.96% (20/505). There was a significant association between water source and *Cryptosporidium* infection (Mann Whitney test $P = 0.0432$). There was a correlation between positivity of *Cryptosporidium* and type of drinking water ($r = 0.121$ and $P = 0.001$). These results were consistent with results of other similar studies (Park et al., 2007). Surface water becomes contaminated through the discharge of untreated and treated sewage and run-off of manure. Rivers receive both agricultural run-off and treated and untreated waste water. As a result *Cryptosporidium* oocysts of various genotypes are ubiquitous in surface and river waters (Park et al., 2006; Muchiri et al., 2009). Several characteristics of *Cryptosporidium* facilitate waterborne transmission. The high resistance of *Cryptosporidium* oocysts against chlorine disinfection renders this process ineffective for oocyst inactivation in drinking water. This perhaps explains why prevalence was second highest in patients who reported using piped water and those who treated water by chlorination. The oocysts are also persistent in the environment and can survive for months in surface water (Molbak et al., 1993).

Boiling appeared to be the most effective way of water treatment to eliminate *Cryptosporidium*, as only one case was reported out of the 245 cases (0.04%) who reported boiling drinking water. Similar results supporting boiling of water were obtained in Sri Lanka (Sirisena et al., 2013). Chemical treatment by chlorinating did not seem effective on the *Cryptosporidium* oocysts as already mentioned, with those who reported chemically treating water having the highest prevalence of 24% (6/25).

Although a higher number of those exposed to livestock had *Cryptosporidium* than the non-exposed (23/36 and 13/36 respectively) this was not statistically significant. This may be attributed to the fact that transmission especially of the human genotypes was mainly anthropogenic rather than zoonotic. Among those positive cases who reported to keep livestock, the prevalence was highest in males than females. This may be due to playing of male children in gardens and farms outdoor areas with soil and animals which increases the risk of parasite transmission. These results are in accordance with results of another study where men were infected at a higher rate (1.9%) than women (1.2%) (Park et al., 2006). Contact with domestic animals act as a risk factor in zoonotic infections. Zoonotic transmission has been well documented with various reports of outbreaks or cases of cryptosporidiosis in school children after exposure to calves or lambs (Casemore et al., 1985; Casemore, 1990). The higher prevalence of *Cryptosporidium* in sheep and cattle and their higher numbers of oocysts shed by infected animals, especially newborns make cattle and sheep important sources of environmental contamination with *Cryptosporidium* oocysts that are capable of infecting humans.

The overall prevalence of other enteric parasites was 29.63% (211/712). Of the other enteric parasites, *Ascaris lumbricoides* had the highest prevalence of 9.41% (67/712) while *Entamoeba coli/dispar* had the lowest prevalence of 3.79% (19/712). The prevalence of the other enteric parasites was much lower as compared to prevalences recorded in other similar studies. For example a study in Thika Sub-county, Kenya found an overall intestinal parasite prevalence of 43.7% (Ngonjo et al., 2012). A similar study on primary school children in Ethiopia found an overall prevalence of 57.3% (Workneh et al., 2014). The high prevalence of the other enteric parasites may be an indication that perhaps the primary school based de-worming campaign by the ministry of health (in collaboration with the Ministry of Education) may not be having the desired effect. There may be therefore the need to rethink the strategy since it may not be having the desired effect.

It should be noted however that the different prevalence obtained in various studies may have to a greater extent been influenced by the diagnostic methods used in detecting the parasites in the study population. Again this study was hospital based; therefore the conclusions drawn may not necessarily apply to the general population in the study area. There is therefore need to make up follow up studies in the general population in the study area to come up with a better picture and understanding of Cryptosporidiosis in Bungoma County, Kenya.

**Acknowledgements**

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