CRYPTOSPORIDIOSIS: PREVALENCE, GENOTYPE ANALYSIS, AND SYMPTOMS ASSOCIATED WITH INFECTIONS IN CHILDREN IN KENYA

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Abstract. Cryptosporidium parasites are leading causes of enteric disease, especially in children. A prospective survey on the prevalence of cryptosporidiosis in children less than five years of age was undertaken at six microbiology laboratories in Kenya on fecal samples submitted for routine parasite and ova investigations. Analysis of 4,899 samples over a two-year study period showed an overall prevalence of cryptosporidiosis of 4% that was highest between November to February. Investigations on the nature of enteric diseases prompting ova and cyst examination requests showed 66.4% had acute diarrhea, 9% had persistent diarrhea, and 21% had recurrent diarrhea. The main symptoms were abdominal pain (51.1%), vomiting (51.6%), and abdominal swelling (11%). The prevalence of cryptosporidiosis was highest among children 13–24 months of age (5.2%) and least among those 48–60 months of age (2%). No significant differences were observed by sex but vomiting was slightly higher in males than in females (65% males and 52% females; \(P = 0.07\)). Cryptosporidiosis was significantly associated with persistent diarrhea (\(P = 0.0001\), odds ratio [OR] = 2.193, 95% confidence interval [CI] = 1.463–3.29), vomiting (\(P = 0.0273\), OR = 1.401, 95% CI = 1.04–1.893), and abdominal swelling (\(P = 0.0311\), OR = 1.56, 95% CI = 1.04–2.34). Genotype analysis based on polymerase chain reaction–restriction fragment length polymorphism of the 18S rRNA gene fragment showed that 87% (153 of 175) of the Cryptosporidium isolates were \(C.\) hominis, 9% (15 of 175) were \(C.\) parvum, and remaining 4% were \(C.\) canis, \(C.\) felis, \(C.\) meleagridis, and \(C.\) muris. The most common protozoa in coinfected patients were Entamoeba histolytica/E. dispar, \(E.\) coli, and Giardia intestinalis (6%, 5%, and 2%, respectively). Our results show that Cryptosporidium is among the most common protozoan parasites in children with enteric diseases and that anthropoontic species are the leading cause of human cryptosporidiosis in Kenya, which suggests that human-to-human transmission is the main mode of spread.

INTRODUCTION

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 Cryptosporidium oocysts.1 The oocysts are highly resistant to chlorination, common household disinfectants and survive long periods in the environment.2 In developing countries, Cryptosporidium is responsible for 8–19% of cases of diarrheal disease.3,4 with a significant effect on mortality.5 A recent study on cryptosporidiosis conducted in Egypt examined 1,275 children attending two hospitals and found a prevalence of 17%.6 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. A recent survey on the prevalence of cryptosporidiosis among human immunodeficiency virus (HIV)-infected and uninfected children with persistent diarrhea at Mulago Hospital in Uganda showed that 73.6% (67 of 91) of HIV-infected children had cryptosporidiosis and 5.9% (9 of 152) of HIV-negative children were infected.7 Genotype analysis in the Ugandan study showed that 73.7% of the infections were \(C.\) hominis, 18.4% were \(C.\) parvum, 3.9% were mixed infections with both species, and 3.9% were \(C.\) meleagridis. In Malawi, molecular epidemiologic studies of cryptosporidiosis in children showed that 41 of 43 were infected with \(C.\) hominis and only 2 with \(C.\) parvum. In this study, the peak prevalence was among children less than 18 months of age with a mean age of 10 months.8 Our previous studies on the occurrence of Cryptosporidium genotypes infecting children in Kenya suggested that \(C.\) hominis was the dominant species, and \(C.\) parvum, \(C.\) meleagridis, and \(C.\) muris were identified in HIV-infected persons.9,10 Elsewhere, a prevalence of as high as 32% has been reported among children in Guatemala, with a significant variation between female (44%) and male (17%) children.11 A longitudinal study of Cryptosporidium infections in children in northeastern Brazil that examined 1,476 episodes of diarrhea showed a cryptosporidiosis prevalence of 7.4%.12 The most common symptoms were persistent diarrhea (16.5%) and acute diarrhea (8.4%), and the least common symptom was no diarrhea (4%). In the same region, another study showed that an episode of cryptosporidiosis in children less than one year of age had a strong association with increased acute diarrheal disease burden lasting more than 21 months coupled with long-term decline in growth.13

In spite of advances made in the detection and demonstration of the significance of the parasite as a cause of enteric morbidity, tests for Cryptosporidium sp. are still not performed in routine ova and parasite requests in most microbiology laboratories. The current survey was based at six microbiology laboratories in Kenya working in collaboration with the Center for Microbiology Research at the Kenya Medical Research Institute (CMR-KEMRI) with an objective to assess the occurrence of Cryptosporidium parasites in children less than five years of age during routine ova and parasite diagnosis. The survey also attempted to describe some of the factors associated with these infections. Molecular genotyping of Cryptosporidium parasites was undertaken to profile the current range of species circulating in the region.
MATERIALS AND METHODS

Study samples and on site examinations. The six microbiology laboratories participating in the survey were Narok District Hospital, Thika District Hospital, Machakos Provincial General Hospital, Getrude’s Gardens Children Hospital, Aga Khan Hospital, and Kenyatta National and Referral Hospital. Examinations were undertaken twice a week in Thika and Machakos and once a week in the other centers for logistic reasons. The hospitals were selected based on their proximity to CMR-KEMRI laboratories and their diverse patients’ catchments areas within the region. The laboratories routinely perform ova and parasite tests on fecal samples. We undertook to expand on this examination to include acid-fast staining for coccidian parasites that may be associated with enteric disease. The patient’s age, sex, and home location were noted. Details of the symptoms prompting the examination request were also noted. The gross appearance of the fecal samples was recorded before processing. Portions of the stool samples were aliquoted for routine examination and for Ziehl Neelsen (ZN) staining. Fecal samples for routine ova and parasite examination were emulsified in 2.5% sodium acetate formalin and processed for concentration method. Thin smears of the concentrated pellet were prepared on glass slides and air-dried before modified ZN staining. Each slide was scanned at 400× magnification and confirmed under oil emersion. Results from the ZN staining were availed for inclusion in the patients’ examination results at each of the laboratories. The remaining samples were preserved in 2.5% potassium dichromate or 75% ethanol and transported to CMR-KEMRI laboratories for DNA extraction and genotyping. No samples from any previous studies on Cryptosporidium at CMR-KEMRI were included in this study.

Nested polymerase chain reaction (PCR) amplification and restriction fragment length polymorphism analysis. All samples positive for Cryptosporidium by ZN staining were preserved in either 2.5% potassium dichromate or 75% ethanol. Approximately 200 μL of fecal suspension was washed three times in distilled water before extraction. Genomic DNA was then extracted using the QIAamp kit (Qiagen, Crawley, West Sussex, United Kingdom) according to the manufacturer’s instructions.

Cryptosporidium genotyping targeted an 18S ribosomal rRNA gene fragment using a nested PCR as previously described.14 The fragment is highly specific for the Cryptosporidium genus. The different species were identified after restriction digestion of the secondary PCR product using two endonucleases. For the restriction, 10 μL of secondary PCR product for each reaction was digested in a total volume of 40 μL consisting of 15 units of Sph I and 2 μL of restriction buffer in the first reaction and Asn I (Boehringer Mannheim, Livingston, United Kingdom) in the second reaction at the same concentration. The digestion products were separated by electrophoresis in a 2% agarose gel, stained with ethidium bromide, and viewed under ultraviolet light.

Data entry, handling, and analysis description. The data were manually entered using Epi-Info for Windows version 3.22 (Centers for Diseases Control and Prevention [CDC], Atlanta, GA) and analyzed using SAS version 9 software (SAS Institute Inc., Cary, NC). To determine associations between infection with cryptosporidiosis, diarrhea, or other symptoms, a Pearson chi-square test was used to compare proportions for independent samples. Normality was assumed in all analysis and a P value < 0.05 was considered statistically significant.

Ethical considerations. The study was reviewed and approved by the National Ethical Review Committee of Kenya. All guardians of participating children were informed of the expanded examination and voluntary consent was sought before inclusion. Results were included in the patients’ reports for appropriate management by the caring clinicians through the routine laboratory reporting system.

RESULTS

Prevalence by centers. A total of 4,899 samples from 4,899 patients were examined over a period of 24 months. Cryptosporidium was detected in 183 samples (prevalence = 4%). Prevalence by the center studied was 7% (7 of 99) in Narok District Hospital, 4% (45 of 1,118) in Thika District Hospital, 4% (69 of 1,648) in Machakos Provincial General Hospital, 4% (30 of 690) in Kenyatta National and Referral Hospital, 3.5% (17 of 480) in Aga Khan Hospital, and 2% (15 of 863) in Gertrude’s Gardens Children Hospital. When analyzed by children’s residence, 46% of those with cryptosporidiosis were living in peri-urban areas, 14.2% in towns, and 40% in rural areas.

Prevalence by seasons. Cryptosporidium infections showed bimodal transmission with the highest infection rates of 6.1–8.2% between November to February (Figure 1), the typical driest season in Kenya that follows the short rains. A lower peak was observed in June–July with infections rates of 3.4–4.4%. This is the dry and cold season that follows the long rainy period of April–May. The long rainy period had the lowest prevalence of infection (mean = 1.5%).

Prevalence by age. Children examined in the study ranged in age from less than 1 month to 60 months. Most children examined were 0–12 months of age; 3.4% (62 of 1,809) had cryptosporidiosis and 5.2% (59 of 1,135) of those examined were 0–12 months of age; 3.4% (62 of 1,809) had cryptosporidiosis and 5.2% (59 of 1,135) of those 13–24 months of age were infected. This group had the highest rate of infection. A Cryptosporidium prevalence of 3% (25 of 804) was observed in children 25–36 months of age and a prevalence of 4.2% (27 of 644) was observed in those 37–48 months of age. Children 49–60 months of age had the lowest prevalence of infection (2.1% [10 of 472]), as shown in Table 1.
Acute diarrhea. A summary of the associations of cryptosporidiosis with symptoms is shown in Table 2. A total of 3,253 children had acute diarrhea as the presenting complaint. Of these, 1,833 were males and 1,420 were females. Cryptosporidiosis prevalence was 4.3% (78 of 1,833) among male children and 3.2% (46 of 1,420) among female children. There was no significant difference in cryptosporidiosis infection rates by sex (P = 0.69, odds ratio [OR] = 1.066, 95% confidence interval [CI] = 0.777–1.462).

Persistent diarrhea. A total of 417 children presented with persistent diarrhea lasting more than 14 days, of whom 7.2% (30 of 417) had cryptosporidiosis. Among them, 235 were males with an infection rate of 7.2% (17 of 235) and 182 were females with an infection rate of 7.1% (13 of 182). Cryptosporidiosis was significantly associated with persistent diarrhea (P < 0.0001, OR = 2.193, 95% CI = 1.463–3.288).

Recurrent diarrhea. A total of 1,020 children had recurrent diarrhea of whom 3.2% had cryptosporidiosis. Among these, 588 of 1,020 were males with an infection rate of 3.4% (20 of 588) and 432 of 1,020 were females with an infection rate of 3%. There was no significant association of cryptosporidiosis with recurrent diarrhea (P = 0.344, OR = 0.831, 95% CI = 0.567–1.22).

Vomiting. A total of 2,526 children had episodes of vomiting of whom 4.3% (109 of 2,526) had cryptosporidiosis. Of those children, 1,431 of 2,526 were males with an infection rate of 5% (72 of 1,431) and 1,095 of 2,526 were females with an infection rate of 3.4% (37 of 1,095). Cryptosporidiosis was significantly associated with vomiting (P = 0.027, OR = 1.4, 95% CI = 1.04–1.89). Overall, male children with cryptosporidiosis had vomiting as a symptom more frequently than female children (P = 0.07, OR = 1.75, 95% CI = 0.954–3.197), although this was not statistically significant.

Abdominal pain. A total of 2,504 patients had abdominal pain of whom 3.7% had cryptosporidiosis. Cryptosporidiosis prevalence was 3.9% (55 of 1,418) among 1,418 of 2,504 males and 3.4% (37 of 1,086) among 1,086 of 2,504 females. There was no significant association between cryptosporidiosis infection and abdominal pain (P = 0.817, OR = 0.966, 95% CI = 0.7188–1.2975).

Abdominal swelling. A total of 537 children had abdominal swelling of which 5.4% had cryptosporidiosis. The cryptosporidiosis infection rate was 4.7% (16 of 344) in males 6.7% (13 of 193) in females. Cryptosporidiosis infection was significantly associated with abdominal swelling, (P = 0.031, OR = 1.56, 95% CI = 1.04–2.34).

Coinfections. The most common parasitic coinfections with cryptosporidiosis were Entamoeba histolytica/E. dispar, E. coli, and Giardia intestinalis (6%, 5%, and 2%, respectively). Ascaris lumbricoides was the most frequent nematode identified (prevalence = 5%). Other parasites identified (prevalence < 1%) included hookworms, Trichuris trichiura, and Schistosoma mansoni.

Cryptosporidium species. Of 183 Cryptosporidium-positive specimens identified by microscopy, 175 were amplified by the 18S ribosomal RNA PCR. Eight samples were not amplified or had nonspecific amplification whose genotypes could not be determined by the subsequent digestion. Results from restriction endonuclease digestion of the PCR product confirmed that 87% (153 of 175) Cryptosporidium isolates were C. hominis and 9% (15 of 175) were C. parvum. The remaining 4% of the samples were C. canis (3 isolates), C. felis (2 isolates), C. muris (1 isolate), and C. meleagridis (1 isolate). There were no discernable differences in the distribution pattern of zoonotic species by region, with C. hominis being the most prevalent species in all areas.

**DISCUSSION**

Our study focused on the current state of Cryptosporidium infections in children less than five years of age coming to hospitals in Kenya for ova and parasite examinations. This study is unlike other studies undertaken in Africa that have focused on the high-risk groups such as children with HIV and/or those with persistent diarrhea only, or day care outbreaks. Our two-year period, 4,899 samples were examined from microbiology laboratories in six different hospitals with patient’s catchments that included all socioeconomic settings in this region, ranging from the government district and provincial hospitals serving those in the peri-urban and rural areas to private and more expensive hospital institutions within Nairobi.

Our results show that Cryptosporidium is one of the most common enteric parasites associated with diarrhea. However, tests for this parasite are not routinely performed during ova and parasite diagnosis. Being a cross-sectional survey, only one sample per patient was examined, resulting in an overall prevalence of 4%, a rate that has been observed elsewhere based on studies using ZN staining. Higher rates of infections reported in other studies on HIV-negative children include 17% in Egypt, 13% and 9% in Tanzania, and 5.9% in
However, in all these surveys, detection of Cryptosporidium used commercial kits based on direct-fluorescent monoclonal antibody tests (Merifluor; Meridian Diagnostics, Cincinnati, OH) immunofluorescence microscopy, or a Cryptosporidium (TechLab Inc., Blacksburg, VA) enzyme-linked immunosorbent assay kit. Our study at the initial detection stage used routine methods currently available in the collaborative laboratories for parasite identification. Our results indicate that based on the single stool examination, Cryptosporidium infections are as common as those of the parasites routinely investigated, including E. histolytica/E. dispar, and nearly three times more common than G. lamblia.

Of significance is the seasonal occurrence of Cryptosporidium infections with peak transmission seasons of November–February. The peak transmission coincided with the hot and dry season and is similar to that observed elsewhere including Peru, the United States, and Guatemala. However, data from the meteorologic center in Nairobi indicated that 2003–2004 was unusually wet, especially between November and January. However, it could not be confirmed if this wet season was similar in all areas within the survey region. A similar study in Malawi reported a peak prevalence between October and March, but this is the rainy season in this region. A high prevalence of cryptosporidiosis in the hot and dry periods was also reported in Gaza with a significant decrease at the onset of the cooler, wet season. This may be attributed to water shortages that are common in these regions during the dry season, which result in poor hygiene or alternative water sources, including wells that may harbor higher concentrations of oocysts.

Although infections did not vary with sex, they were highest among children 13–24 months of age. This is in contrast to reports on cryptosporidiosis in children in Egypt, where infection was most common among children less than 12 months of age. However, high infection rates have been reported in children 13–24 months of age. A study on cryptosporidiosis in children in Gaza showed the highest levels of infection among children 1–2 years of age and a prevalence of 14.2%. Another study in Alder Hey Children’s Hospital in the United Kingdom reported a cryptosporidiosis prevalence of 29.2% in children 19 months of age. It is not clear why there are differences in susceptibility by age of children, but they may be due to the prevailing Cryptosporidium species endemic in specific areas. Understanding the transmission dynamics warrants further molecular investigation of the Cryptosporidium species and subtypes in circulation.

Cryptosporidiosis causes serious morbidity in young children. A study examining long-term effects of the disease in infants suggested that early infection with Cryptosporidium was associated with physical functional defects 4–7 years later. This is in addition to the effects of persistent and sometimes chronic diarrhea associated with cryptosporidiosis. Our study is in agreement with previous reports showing that cryptosporidiosis is significantly associated with persistent diarrhea and abdominal pain. The strong association of cryptosporidiosis with vomiting is unusual, especially when it is the sole symptom, and has been observed in other studies in Egypt and Tanzania. Together with persistent diarrhea, vomiting exacerbates the morbidity, especially dehydration, associated with cryptosporidiosis.

Our results indicate that anthropoontic transmission is the main mode of infection in Kenya. This indicates that direct or indirect human-to-human transmission is the main mode of spread rather than water or environmental sources contaminated with zoonotic cryptosporidium oocysts. This has important public health implications because it indicates that an improvement in personal hygiene could significantly reduce transmission of cryptosporidiosis in the region. However, the presence of zoonotic species including C. canis, C. felis, C. muris, and C. meleagridis indicates that animal reservoirs are still important. No attempt was made to investigate the HIV status of the children, and it is not clear if the immune status may have predisposed some of the children to zoonotic species of Cryptosporidium parasites. However, current molecular analyses of diverse isolates from both immunocompetent and immunocompromised persons indicate that the unusual zoonotic species are common in both populations. Only C. parvum, C. meleagridis, and C. muris among the zoonotic species had been identified in Kenya. Our study is the first to demonstrate that most species of Cryptosporidium that have been identified in humans, including C. canis and C. felis, also occur in humans in Kenya.

In conclusion, our study has shown that cryptosporidiosis is a common enteric parasite in Kenya. There is a need to include its detection in routine ova and parasite diagnosis using acid-fast staining. The seasonal occurrence and the dominant anthropoontic type of cryptosporidiosis indicate that there can be targeted public health education to limit its transmission. Recently, nitazoxanide drug has been introduced for treatment of cryptosporidiosis. However, it is still not readily available and control measures will largely depend on improved public health, including personal hygiene and sanitation.

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