EPIDEMIOLOGY OF GIARDIASIS AND CRYPTOSPORIDIOSIS IN JAMAICA

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Abstract. We report the findings of a cross-sectional epidemiologic study of Giardia lamblia and Cryptosporidium infections in Jamaica. Three hundred twenty-eight stool samples from patients less than one to 81 years of age were examined using formalin-ether concentration for G. lamblia, Ziehl-Neelsen staining for Cryptosporidium, and the Prospect® rapid enzyme immunoassay (EIA; Alexon, Sunnyvale, CA) for parasite diagnosis. The Prospect® Giardia rapid assay detected 17 cases of G. lamblia infection compared with six by formalin-ether concentration. However, the Prospect® Cryptosporidium EIA did not increase the rate of detection of Cryptosporidium when compared with Ziehl-Neelsen staining. Cryptosporidium infections were most frequently diagnosed in children less than five years old and prevalence decreased with age. In contrast, the prevalence of giardiasis increased as children became older. There were no associations between the infections and stool consistency, clinical manifestations, or sex of the individuals. The contribution of the parasites to childhood morbidity will depend on accurate laboratory diagnosis.

Cryptosporidium infections (especially by C. parvum) are increasingly recognized as an emerging cause of diarrhea in humans worldwide. They are especially important in immunocompromised individuals and have been studied extensively in patients with acquired immunodeficiency syndrome (AIDS). Prevalence of infection with C. parvum among AIDS and immunocompromised patients in developing countries is thought to be between 8.7% and 48% with a mean of 24%. The prevalence of infections among these patients is somewhat lower in the developed world (14%, range = 6–70%). Similarly, there is a striking difference between the prevalence of C. parvum among immunocompetent persons in developed and developing countries. Whereas 2.2% of individuals with diarrhea in developed countries are expected to be infected with the parasite, the prevalence among immunocompetent individuals in developing countries was 6.1%.

Despite the focus of studies on immunocompromised patients, C. parvum infection is a major cause of self-limiting diarrhea in immunocompetent individuals including children and travelers. Close contact transmission of the highly infectious organism is an important route of transmission as shown by epidemics among children in day-care facilities and household clustering of infection.

The protozoan flagellate Giardia lamblia shares several epidemiologic characteristics with C. parvum. Both organisms are water-borne and this route has been the cause of epidemics of diarrhea in both adults and children. Furthermore, like C. parvum, G. lamblia is a cause of childhood diarrhea and may be transmitted by close-contact and is often associated with epidemics of diarrhea in day care facilities. The prevalence of both G. lamblia and C. parvum is generally higher among very young children and this may be related to more efficient fecal-oral transmission of the infective stages or enhanced susceptibility due to lack of immunity.

While the prevalence of cryptosporidiosis and giardiasis is well documented in patients and children with human immunodeficiency virus (HIV) and AIDS, data are not widely available on the cross-sectional distribution of these infections. Water-borne outbreaks of infections with the parasites have highlighted the fact that morbidity may occur outside of these focus groups in endemic areas.

Reliable epidemiologic data require the most accurate diagnosis of infections. Enzyme immunoassays (EIAs) are commercially available that provide rapid, sensitive, and specific diagnosis of infections with the G. lamblia and C. parvum. These assays are ideal for use in epidemiologic studies in which only a single stool sample is examined, due to low sensitivity of microscopy in these cases.

We report the results of a pilot study of the epidemiology of C. parvum and G. lamblia infections at The University Hospital of the West Indies, (UHWI) Jamaica using conventional microscopic methods and commercially available EIAs for parasite diagnosis.

The objectives of this study were to determine the cross-sectional distribution of giardiasis and cryptosporidiosis in a hospital population in Jamaica, specifically, to determine epidemiological characteristics of both infections in the same population. This data may be useful for determining screening criteria for the parasites in the Jamaican population. The study also evaluated the potential benefits of using EIA to diagnose G. lamblia and C. parvum on a single fecal sample submitted for microscopy.

MATERIALS AND METHODS

Stool samples from 328 individuals that were submitted to the Department of Microbiology at UHWI in Kingston to be analyzed for enteric bacteria or intestinal parasites were included in the study. Due to its location, the UHWI receives patients from both the city (Kingston) and surrounding rural communities. It is the major referral facility in Jamaica and patients are admitted from various locations on the island. Patients’ age, sex, clinical manifestations, and consistency of stool samples were recorded at the time of submission of the samples. Minimum inclusion criteria were age and sex of the patient and submission of a quantity of stool that was adequate for diagnosis using microscopy. The project was approved by the Ethics Committee of the University Hospital of the West Indies. Verbal informed consent was obtained from each study participant at the time of sample collection and after explanation of the study objectives.

All samples were processed using formalin-ether concentration for ova and parasites, which is the method used for routine fecal analysis in the hospital laboratory. Fecal sam-
samples are often submitted in 10% formalin only and this made them inappropriate for permanent staining using trichrome stain. Consequently, only wet mount examination was performed to detect G. lamblia cysts microscopically. All samples were analyzed for C. parvum by modified Ziehl-Neelsen staining.\textsuperscript{31}

Unconcentrated stool samples were analyzed for antigens of G. lamblia and Cryptosporidium using commercially available EIA kits (Prospect\textsuperscript{R} Giardia Rapid Assay and Prospect\textsuperscript{R} Cryptosporidium Rapid Assay; Alexon, Inc., Sunnyvale, CA). The assays were conducted following the instructions of the manufacturer and were read visually to observe a color change indicative of the presence of parasite antigens.

### RESULTS

Three hundred twenty eight stool samples were examined in the study. Of these, 197 were from female and 131 were from male patients. The age of the patients ranged from less than one to 81 years with a median age of 19 years.

Formalin-ether concentration detected six (1.8\%) cases of G. lamblia infection on a single stool sample. However, the Prospect\textsuperscript{R} Giardia Rapid Assay detected these six plus an additional 11 cases of G. lamblia infection (17 of 328 or 5.2\%, Table 1). In contrast, the results from detection of C. parvum using modified Ziehl-Neelsen staining were similar to those using the Prospect\textsuperscript{R} Cryptosporidium Rapid Assay. Of 14 (4.3\%) samples positive for C. parvum by Ziehl-Neelsen staining, 13 (4.0\%) were positive by EIA (Table 2). No mixed infections with G. lamblia and C. parvum were detected in the study.

Based on the EIA, 11 (85\%) of 13 cases of C. parvum infections were detected in the stools of children nine years old or less, whereas 13 (76\%) of the 17 cases of G. lamblia were diagnosed in children between 0 and 12 years old. Of particular note was the high frequency of infection in children two years old and less. The prevalence of C. parvum infection in this group was 11.5\% (9 of 78) and that of G. lamblia was 5.1\% (4 of 78) (Figure 1). In the 0–12-year-old age group, the prevalence of infection with C. parvum decreased with age, whereas the prevalence of G. lamblia infections increased with age and was highest in the 7–8-year old age class (Figure 1). Infections with C. parvum were also diagnosed in three males who were 25, 29, and 34 years old, and were associated with HIV infection. Three cases of G. lamblia infections were seen in individuals between 10 and 19 years of age and one case each in individuals between 20 and 30 and 40 and 50 years of age.

<table>
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<th>Positive</th>
<th>Negative</th>
<th>Total</th>
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<tbody>
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<tr>
<td>Positive</td>
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<td>11</td>
<td>17</td>
</tr>
<tr>
<td>Negative</td>
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<td>311</td>
<td>311</td>
</tr>
<tr>
<td>Total</td>
<td>6</td>
<td>322</td>
<td>328</td>
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*EIA = enzyme immunoassay.

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<tr>
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<tr>
<td>Total</td>
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<td>314</td>
<td>328</td>
</tr>
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</table>

*EIA = enzyme immunoassay.

Sex of participant was not significantly associated with infection with either of the parasites studied.

Data were recorded on the consistency of 322 of the 328 stool samples submitted to the laboratory for analysis. About half (179 or 55.6\%) were soft, 64 (19.9\%) were watery, and 79 (24.5\%) were formed. Of the 13 cases of C. parvum detected by EIA, nine (5\%, 9 of 179) were in soft stools, three (4.7\%, 3 of 64) were in watery stools, and one was detected in a formed stool. Similarly, 13 of 16 cases of G. lamblia were detected in soft stools (7.3\%, 13 of 179) and two (3.1\%, 2 of 64) and one case in watery (1.6\%) and formed (1.3\%) stools, respectively. There were no significant differences between the rates of detection of G. lamblia or C. parvum in soft versus watery stool samples ($P = 0.37$ and 1.00, respectively, by Fisher’s exact test: two-tailed).

The clinical manifestations reported among the study participants by the attending physicians included no symptoms (stool submitted as part of a routine medical examination), diarrhea (or other gastrointestinal illness including gastroenteritis and dysentery), ova and parasites, HIV/AIDS, and others (these included manifestations not associated with the parasite under study, and which had small sample sizes such as sickle cell disease, mesenteric adenitis, neuroblastoma, and liver abscesses). The frequencies of detection of G. lamblia and C. parvum with respect to clinical manifestations among patients in the study are shown in Table 3. Two (1.3\%) and seven (7.5\%) of the 140 samples submitted for diarrhea (or other gastrointestinal illnesses) were positive for C. parvum and G. lamblia infections, respectively. Similarly, three (4.7\%) and seven (10.9\%) of the 64 stool samples submitted for investigation of ova and parasites were positive for C. parvum and G. lamblia, respectively.

### DISCUSSION

The study examined the epidemiology of C. parvum and G. lamblia infections concurrently in a hospital population in Jamaica using microscopic methods and sensitive EIAs for parasite diagnosis. The Prospect\textsuperscript{R} Giardia Rapid Assay detected 4\% more G. lamblia infections than formalin-ether concentration, which is the method used routinely in the hospital laboratory. Trichrome staining was not used in the study and it is not known if this method would have increased the rate of detection of the parasite when compared with formalin-ether concentration. On the other hand, diagnosis of C. parvum using the EIA did not improve the rate of detection of the parasite in stool samples. In comparison with stool examination, the immunoassays were found to be rapid and specific and there was no need for extensive train-
FIGURE 1. Prevalence of Cryptosporidium and Giardia lamblia infections in Jamaican children.

Table 3
Association between clinical manifestation of participants and infection with Cryptosporidium and Giardia lamblia

<table>
<thead>
<tr>
<th>Clinical manifestation</th>
<th>Number of participants (%)</th>
<th>Number positive for Cryptosporidium by EIA (%)</th>
<th>Number positive for G. lamblia by EIA (%)</th>
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<tr>
<td>Routine medical</td>
<td>17 (5.2)</td>
<td>1 (5.8)</td>
<td>0 (0)</td>
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<tr>
<td>Diarrhea/GI symptoms</td>
<td>140 (24.8)</td>
<td>2 (1.3)</td>
<td>7 (7.5)</td>
</tr>
<tr>
<td>Ova and parasites</td>
<td>64 (19.9)</td>
<td>3 (4.7)</td>
<td>7 (10.9)</td>
</tr>
<tr>
<td>HIV or AIDS</td>
<td>4 (1.2)</td>
<td>3 (75)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Other§</td>
<td>89 (48.8)</td>
<td>4 (3.1)</td>
<td>3 (2.5)</td>
</tr>
<tr>
<td>Total</td>
<td>314</td>
<td>13 (4.1)</td>
<td>17 (5.4)</td>
</tr>
</tbody>
</table>

* EIA = enzyme immunoassay; HIV = human immunodeficiency virus; AIDS = acquired immunodeficiency syndrome.
† % calculated column wise.
‡ % calculated row wise.
§ Other includes manifestations not associated with Giardia and Cryptosporidium such as iron deficiency anemia, eosinophilia, and sepsis.

The results of the EIAs based on visual reading were unequivocal. Therefore, the assays are ideal for both clinical diagnosis and large-scale studies of the epidemiology of C. parvum and G. lamblia infections in developing countries, where enormous numbers of stool samples will be examined.

Based on the results of the EIA, the study revealed differences between the epidemiology of C. parvum and G. lamblia in the same population. Cryptosporidium parvum was diagnosed most frequently in children less than two years old and prevalence appeared to decrease with age. In contrast, the prevalence of giardiasis increased with the age of the children and was not as highly clustered in a particular age group. Children 2–10 years of age were most likely to be infected with C. parvum in Nepal.32 A study designed to determine guidelines for screening stool samples for Cryptosporidium in the United Kingdom recommended that the main selection criterion should be the age of the patient.33 The study suggested that all samples from patients up to and including adults 44 years old should be screened for C. parvum. The present study showed that in Jamaica, most infections with Cryptosporidium occurred in young children (less than nine years old). The three infections diagnosed by EIA outside of this age group were in males who were 25, 29 and 34 years old and were associated with HIV infection.

There were no mixed infections with the parasites and they were diagnosed at equivalent rates in males and females. Similarly, in a large study in Mexico City, the gender of the children studied did not influence the rate at which C. parvum infections were detected.17 In contrast, studies in Nigeria and Nepal showed that girls were more likely to be infected with the parasite than boys.32,34 Male sex was associated with a significantly higher relative risk for the first episode of symptomatic giardiasis in rural Egypt, although the rate of infection with the parasite was lower in boys than girls.35 Similarly, the prevalence of giardiasis was higher among boys than girls in a study in northern Jordan.36

Neither stool consistency or clinical diagnosis were accurate indicators of infection with G. lamblia or C. parvum when the EIA was used for diagnosis. These findings are similar to those recommended to laboratories for screening for Cryptosporidium in the United Kingdom.33 The parasite was detected significantly more frequently in watery stools...
than in formed stools in a study in Nigeria that used formalin-methylene blue staining for oocysts.34

The reasons for marked differences in the epidemiology of the infections studied in the same population are not known. The risk factors for acquiring G. lamblia and C. parvum in Jamaica are apparently dissimilar and this is reflected in their age-prevalence profiles. Alternately, age-related acquisition of immunity to infection or loss of infection may be different for the parasites and this may contribute to the observed patterns.15 Most infections with the parasites were in children, and clinical diagnosis was not an accurate indicator of infection. Factors such as malnutrition and bottle feeding as seen in Liberia, Costa Rica, and Mexico may be important in determining the epidemiology of the infections in Jamaica.3,17,37 The prevalence of G. lamblia infections increased with age and this may be reflective of the increasing exposure as children attend schools and day-care facilities. Perhaps bottle feeding or infection from untreated water may be more important for transmission of C. parvum in young children.

This pilot investigation was the first to examine the epidemiology of giardiasis and cryptosporidiosis in Jamaica. It established that Cryptosporidium and G. lamblia infection may be underdiagnosed infections in children. The prevalence of C. parvum was seen to decrease with age while the frequency of G. lamblia increased as children became older. There were no identifiable clinical manifestations associated with the infection, and assessment of their contribution to morbidity (especially in children) will require accurate laboratory diagnosis:

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REFERENCES