PRESENTATION RATES OF GIARDIA AND CRYPTOSPORIDIUM AMONG DIARRHEIC PATIENTS IN THE PHILIPPINES

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Abstract. The prevalence of Giardia and Cryptosporidium among 3,456 diarrheic patients corrected from May 2004 to May 2005 in the Philippines was determined. Of 133 (3.8%) positive samples, 69 (2.0%) were positive for Giardia and 67 (1.9%) for Cryptosporidium. Three samples had co-infection with Giardia and Cryptosporidium. Luzon had the highest positive samples (5.0%) followed by Mindanao (4.9%), then Visayas (2.2%). Giardia was most prevalent in Mindanao (3.6%) while Cryptosporidium was most prevalent in Luzon (3.1%). The prevalence of Giardia (2.0%) among pediatric patients (0-18 years) did not significantly differ from that (1.9%) among adults (>18 years old). However, for Cryptosporidium, the prevalence (2.9%) among pediatric patients was significantly higher compared to that (0.2%) among adult patients. In the pediatric population, the highest percentage of patients with Giardia was the 5-9 year old age group, while that of Cryptosporidium was in the 0-4 year old group. The prevalence of Giardia, but not Cryptosporidium, was significantly higher in male than female adults. Seasonality had a distinct peak in September with Cryptosporidium more prevalent in the rainy (2.6%) than dry season (0.9%).

INTRODUCTION

Diarrhea is considered a major cause of morbidity, especially in developing countries. In the Philippines, it was the leading cause of morbidity for the years 2001 and 2003, and the second in 2002 (National Statistics Office, 2006). Common causes of diarrhea are infections due to viruses, bacteria, helminthes and protozoa. These causative agents are either food-borne or water-borne.

Among enteric protozoa, Giardia lamblia (syn. G. intestinalis or G. duodenalis) and Cryptosporidium spp are the most commonly reported causes of water-borne diarrhea outbreaks.

G. lamblia is especially prevalent in children in developing countries (Bryan et al, 1994), and is the most commonly diagnosed flagellate in international travel (Marshall et al, 1997). So far, all outbreaks of giardiasis have been associated with water-borne transmission.

G. lamblia was first reported in the Philippines in 1977 (Cross et al, 1977) and since then has been identified as a common intestinal parasite. Studies done in Luzon (Carney et al, 1981a; Adkins et al, 1987; Auer, 1990; Paje-Villar et al, 1993; Lee et al, 2000), in various localities in the Visayas (Cross et al, 1977; Carney et al, 1980; Salas, 1997), and in the southern islands of Mindanao (Carney et al,
indicate wide distribution of Giardia in the Philippines. It has also been reported among children living in various residential institutions (Bustos et al, 1991; Baldo et al, 2004), and among measles patients with diarrhea (Carlos et al, 1992).


Stool examinations in the Philippines typically included the identification of the common etiologic agents of diarrheas such as rotavirus and bacteria (Escherichia coli, Shigella, Campylobacter, Salmonella, and Vibrio cholerae). In major tertiary hospitals in the Philippines, routine stool examinations may include G. lamblia, which can be readily identified by microscopy. However, identification of Cryptosporidium is not routinely done, unless specifically requested by a physician.

The present study is the most recent nationwide survey of Giardia and Cryptosporidium and provides basic information on the prevalence of these enteric protozoa in the Philippines.

MATERIALS AND METHODS

Samples collection and examination

Stool samples were collected from patients who consulted for diarrhea in collaborating hospitals and health centers from May 2004 to May 2005 in the three main islands of the Philippines. There were 31 collaborators from Luzon, 39 from the Visayas, and 9 from Mindanao. Patients or relatives were asked to fill out an information sheet that provided the demographic data for this work.

Single fecal samples were collected from each patient and 1 ml of each sample was placed in a 15 ml polypropylene tube containing 9 ml of 10% formalin. The fixed fecal samples were stored at 4ºC until transported to the Research and Biotechnology Division of St Luke's Medical Center, Quezon City, Philippines, for concentration and microscopic examination. All formalin-fixed stool specimens were concentrated using the formalin-ethyl acetate method.

To detect Cryptosporidium and Giardia, 5 µl from each stool concentrate and 5 µl of detecting antibodies from the MeriFluor® Cryptosporidium-Giardia direct fluorescence detection kit (Meridian Diagnostics, Cincinnati, Ohio) were mixed on a slide. Each slide was scanned under a 20x objective (Zeiss Axiolab microscope). Giardia and Cryptosporidium showed apple-green fluorescence with a blue excitation filter of 450 nm (09B, Zeiss). Giardia cysts are oval, measuring approximately 11-14 µm, while Cryptosporidium oocysts are round and smaller (4-6 µm). Cyst/oocyst morphology, was carried out under a 100x objective using a phase contrast microscope.

Ethical clearance

This project was given ethical clearance by the St Luke's Institutional Ethics Review Board, and informed consent was obtained from patients or their relatives.

Statistical analysis

Data from the information sheets were encoded in Microsoft Excel. Data processing and analysis were performed using SPSS ver 14 software. Descriptive statistics, such as means and proportions, were used to describe the patients’ socio-demographic characteristics. The chi-square or Fisher's exact and t-test statistics were used to test for differences.
RESULTS

A total of 3,456 stool samples were collected for this study. Samples came from patients who consulted due to diarrhea in various hospitals and health centers. Collection was done over a 13-month period from May 2004 to May 2005 in all 3 major groups of islands in the Philippines (Fig 1, Table 1). The highest number (1,667 or 48.2%) of samples came from Luzon, the largest group of islands in the northern Philippines. The Visayas were next with 1,399 samples (40.5%) and Mindanao in the south had 390 samples (11.3%).

The summary of data from sample collection is given in Table 1. Patients were from <1 to 95 years old with 2,160 (63.4%) samples from pediatric (0-18 years old) patients and 1,245 (36.6%) from adult (>18 years old) patients. The ratio of male to female patients was 1.3:1.

Of the 3,456 stool samples examined, 133 stools (3.8%) were positive for Giardia lamblia and/or Cryptosporidium spp (Fig 1 and Table 1). Three samples showed co-infection with Giardia and Cryptosporidium. Thus, the total number of isolated protozoa was 136. There was no significant difference (p=0.862) in the frequency distribution between G. lamblia (69; 2.0%) and Cryptosporidium spp (67; 1.9%).

Fig 1—Overall prevalence of Giardia (G) and Cryptosporidium (C) in the Philippines, and their distribution in the 3 major islands. (* indicates the prevalence was significantly higher.)
Table 1
Summary of data of stool samples collected and microscopy.

<table>
<thead>
<tr>
<th>Demographic profile</th>
<th>No. of stool samples</th>
<th>Prevalence Frequency (percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Both protozoae</td>
</tr>
<tr>
<td>Geographical location</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Luzon</td>
<td>1,667</td>
<td>83 (5.0)</td>
</tr>
<tr>
<td>Visayas</td>
<td>1,399</td>
<td>31 (2.2)</td>
</tr>
<tr>
<td>Mindanao</td>
<td>390</td>
<td>19 (4.9)</td>
</tr>
<tr>
<td>Overall (Philippines)</td>
<td>3,456</td>
<td>133 (3.8)a</td>
</tr>
<tr>
<td>Age group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pediatric (0-18 years old)</td>
<td>2,160</td>
<td>104 (4.8)</td>
</tr>
<tr>
<td>Adult (&gt;18 years old)</td>
<td>1,245</td>
<td>26 (2.1)</td>
</tr>
<tr>
<td>Total</td>
<td>3,405b</td>
<td>130 (3.8)f</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1,934</td>
<td>79 (4.1)</td>
</tr>
<tr>
<td>Female</td>
<td>1,520</td>
<td>54 (3.5)</td>
</tr>
<tr>
<td>Total</td>
<td>3,454d</td>
<td>133 (3.8)e</td>
</tr>
</tbody>
</table>

*Three patients had co-infection with Giardia and Cryptosporidium
*Fifty-one patients with unknown ages were excluded from analysis.
*Three patients of unknown ages were excluded from the analysis; 3 patients had co-infection with Giardia and Cryptosporidium.
*Two patients of unknown sex were excluded from analysis.
*Refers to Giardia and/or Cryptosporidium.

The positivity rates for Giardia and/or Cryptosporidium were 5.0% from Luzon, 4.9% from Mindanao, and 2.2% from the Visayas (Fig 1 and Table 1). Among the 3 islands, only the Visayas had a significant difference (p<0.001) in proportion of positive samples from Luzon and Mindanao. There was no difference (p=0.930) in the distribution of positive samples between Mindanao and Luzon.

With respect to the individual protozoa, G. lamblia was most prevalent in Mindanao (p=0.050) while Cryptosporidium spp was the most prevalent in Luzon (p<0.001).

In Luzon, the prevalence of Cryptosporidium spp (3.1%) was significantly higher than that of G. lamblia (1.9%). However, in the Visayas, it was significantly lower (0.6% for Cryptosporidium spp vs 1.6% for G. lamblia). There was not sufficient evidence to conclude that the prevalence of Cryptosporidium spp (1.5%) in Mindanao was statistically different from that of G. lamblia (3.6%).

The overall positivity rate in the Philippines for Giardia and Cryptosporidium among pediatric patients was 4.8% and in adults it was 2.1%. (Table 1). Among pediatric patients, the prevalence of G. lamblia (2.0%) was significantly lower (p=0.049) than that of Cryptosporidium spp (2.9%). On the other hand, the prevalence of Cryptosporidium spp (0.2%) was significantly lower than that of G. lamblia (1.9%) among adults (p<0.001).

The prevalences of Cryptosporidium spp were statistically different between pediatric and adult patients (2.9% vs 0.2% respectively; p<0.001) but not for G. lamblia (2.0% vs 1.9% respectively; p=0.899).

Based on age-specific distribution, the
Prevalence Rates of Giardia and Cryptosporidium

Table 2
Age-specific distribution of patients with Giardia and Cryptosporidium Infections.

<table>
<thead>
<tr>
<th>Age group (Years)</th>
<th>Total no. of samples</th>
<th>No. (%) with Giardia</th>
<th>No. (%) with Cryptosporidium</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-4</td>
<td>1,605</td>
<td>21 (1.3%)</td>
<td>58 (3.6%)</td>
</tr>
<tr>
<td>5-9</td>
<td>255</td>
<td>17 (6.7%)</td>
<td>2 (0.8%)</td>
</tr>
<tr>
<td>10-18</td>
<td>300</td>
<td>5 (1.7%)</td>
<td>3 (1.0%)</td>
</tr>
<tr>
<td>19-29</td>
<td>412</td>
<td>12 (2.9%)</td>
<td>2 (0.5%)</td>
</tr>
<tr>
<td>30-39</td>
<td>244</td>
<td>4 (1.6%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>40-49</td>
<td>195</td>
<td>6 (3.1%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>50-59</td>
<td>168</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>≥60</td>
<td>226</td>
<td>2 (0.9%)</td>
<td>1 (0.4%)</td>
</tr>
<tr>
<td>Total</td>
<td>3,405</td>
<td>67 (2.0%)</td>
<td>66 (1.9%)</td>
</tr>
</tbody>
</table>

Table 3
Prevalence rates for G. lamblia and Cryptosporidium spp among pediatric and adult patients by sex.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Giardia lamblia</th>
<th>Cryptosporidium spp</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pediatric (N=2,160)</td>
<td>Adult (N=1,245)</td>
</tr>
<tr>
<td></td>
<td>Freq (%)</td>
<td>Freq (%)</td>
</tr>
<tr>
<td>Male</td>
<td>23 (1.1)</td>
<td>19 (1.5)</td>
</tr>
<tr>
<td>Female</td>
<td>20 (0.9)</td>
<td>5 (0.4)</td>
</tr>
<tr>
<td>Total</td>
<td>43 (2.0)</td>
<td>24 (1.9)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Significantly different (pediatric vs adult)
<sup>b</sup>Significantly different (male vs female)

5-9 year old age group and the 0-4 year old age group had the highest percentage of patients with Giardia and Cryptosporidium infection, respectively (Table 2). In our collection, Giardia was seen to have a wider age distribution than Cryptosporidium.

Table 3 compares the prevalence rates of G. lamblia and Cryptosporidium spp among pediatric and adult patients by sex. Among males, there were no significant differences (p>0.05) between pediatric and adult patients. On the other hand, the prevalence rates for the two protozoa among females were significantly higher (p<0.001) in pediatric patients than in adults. For pediatric patients, there were no significant differences (p>0.05) between males and females. However, among adults, the prevalence of G. lamblia, but not Cryptosporidium spp, was significantly different (p=0.004) between males and females.

The seasonality of the occurrence of G. lamblia and Cryptosporidium spp among diarrhea patients in the Philippines revealed an increasing trend during the rainy season (May-October), with a distinct peak in September (Fig 2). This coincided with an increasing number of stool samples collected. There seemed to be a higher (p=0.002) prevalence of
Cryptosporidium, but not of Giardia, during the rainy than the dry season (2.6% vs 0.9%). However, the positivity rates between the two protozoa were not significantly different (p>0.050) for the two seasons.

**DISCUSSION**

Over a period of more than 20 years, numerous studies conducted regarding the prevalence of intestinal parasites have documented the ubiquity of *G. lamblia* and *Cryptosporidium* spp in the Philippines. Carney et al (1980, 1981a,b) conducted surveys of intestinal parasites and found *G. lamblia* to be present in all the areas studied in Luzon, the Visayas and Mindanao. In a review of studies done over a period of 17 years, Cross and Basaca-Sevilla (1984) documented the prevalence of intestinal parasitic infections, including *G. lamblia*, in all the major islands of the Philippines.

In the present study, 3,456 stool samples came from all the major islands in the Philippines. There was no significant difference between the overall prevalences of *G. lamblia* (2.0%) and *Cryptosporidium* spp (1.9%) in the population of patients with diarrhea included in the study.

In the Philippines, a wide range of prevalences have been reported, depending on the study population. Studies done in various residential institutions tended to have higher prevalence rates for Giardia both in children and adults: 17.6% in a mass survey of inmates (Bustos et al, 1991), 11.6% in children of residential institutions in Metro Manila (Baldo et al, 2004), and 9.7% in a mental institution (Rivera et al, 2006).

Field surveys have had varying results. In a study done in 1973, Cross et al (1977) reported a prevalence of 3% for *G. lamblia* from stool samples collected in Samar Province in the Visayas. The infection rate in Bohol, another province in the Visayas, was reported by Carney et al (1980) as 6%. Rivera et al (1998) obtained a significantly low rate of 0.26% in the northern Philippines.

At an outpatient clinic at Clark Air Force Base Hospital in Luzon, Giardia lamblia was found in 2% of American military personnel with diarrhea (Echeverria et al, 1979). Hospital-based surveys gave surprisingly low rates of 0.6% in a 2-year survey of etiologic agents of diarrheal disease at San Lazaro Hospital, Manila (Adkins et al, 1987), and 0.4% at a university hospital (Paje-Villar et al, 1993).

In children, the prevalence of Giardia infection has been studied under various conditions and in different environments. Among the urban poor in Metro Manila, Auer (1990) found a 20% prevalence of *G. lamblia* in children age 8 months to 15 years, and Lee et al (2000) found a 7.8% prevalence rate in children and adolescents in a rural community in southern Luzon. Carlos et al (1992) studied enteropathogens among measles patients...
with diarrhea in urban Filipino children. They reported that among measles patients with diarrhea age 0-9 year, the prevalence of Giardia was 5.7%. In measles patients without diarrhea age 0-10.5 years, the prevalence was 3.1%. Carney et al (1980) reported an infection rate of 11% among children age 0-9 years, while Cross et al (1985) reported a frequency of 4% in children 1-9 years old.

In the present study, the highest frequency of Giardia infection was 6.7%, seen in children age 5-9 years old. In children 0-9 years old, the frequency was 2.0%. This value is much lower than that previously reported.

The prevalence (1.9%) for Cryptosporidium in our nationwide survey was slightly higher than that obtained by Jueco et al (1991) who reported a prevalence of 1.8% in patients of all ages, but lower than the 2.6% rate seen by Cross et al (1985) among patients age 1 month to 75 years. While our study was a nationwide survey, the latter studies were done on a limited hospital-based population in Metro Manila.

Reports on cryptosporidiosis among diarrhea patients in the Philippines have been mainly regarding its prevalence in children: 2.8% in children 0-5 years old (Jueco et al, 1991), 2.9% in children 6-20 months old (Cross et al, 1985), 2.5% in children 0-2 years old (Kainama, 1989), 4% in children 7-19 months old (Capeding and Saniel, 1990), 8.5% in children 7-24 months old (Laxer et al, 1990), 6.3-7.1% in children 6-27 months old (Carlos et al, 1992), and 2.54% in children less than 12 years old (Paje-Villar et al, 1994).

Our findings show a prevalence of 3.6% in children age 0-4 years, which is similar to the prevalences of those previously reported. Our results show that Cryptosporidium occurs more frequently in children than in adults in the Philippines, consistent with reports from developing countries (Thamlitkul et al, 1987). This trend is expected, since children, especially those below 5 years old, are particularly vulnerable due to the high prevalence of malnutrition and poor immunity that leads to persistent diarrhea. Hunter and Nichols (2002) in a review of the literature found that cryptosporidiosis is more common and more severe in malnourished than in well-nourished children. In the Philippines, studies by Paje-Villar et al (1993) and Menorca et al (1994) underscore the role of immune status and malnutrition in cryptosporidiosis among children.

Malnutrition continues to persist in the Philippines despite improvements in primary healthcare. A nationwide survey conducted by Cerdeña et al in 2001 of 12,425 Filipino children age 0-10 years indicated that about 6 out of every 100 pre-schoolchildren suffered from acute malnutrition, and 31 out of every 100 children were underweight.

Unlike previous studies in the Philippines, the present work compared the two enteric protozoa G. lamblia and Cryptosporidium spp with respect to geographical, age and sex distribution. G. lamblia was the most prevalent in Mindanao and Cryptosporidium was the most prevalent in Luzon. Among pediatric patients, the prevalence of G. lamblia was significantly lower than the prevalence of Cryptosporidium spp. However, in adults the prevalence of Cryptosporidium spp was significantly lower than G. lamblia.

Our study found a significant difference between pediatric patients and adults in regards to the prevalence of Cryptosporidium spp, but no difference in the prevalence of G. lamblia. In the pediatric group, Cryptosporidium had the highest prevalence among the 0-4 year olds and G. lamblia was the most prevalent among 5-9 year olds. In the Philippines, Cross et al (1977) and Baldo et al (2004) obtained similar results, showing a tendency for Giardia to decrease with age.

Differences in sex distribution for G. lamblia and Cryptosporidium had no signifi-
cant impact due to inconsistencies in various reports. In our study, the prevalence rates of both G. lamblia and Cryptosporidium spp in pediatric patients were not significantly different between males and females. However, Salas (1997) showed G. lamblia infection to be higher in males than in females among children 0 to 10 years of age. Among adults, only G. lamblia had a significantly higher prevalence in males than females.

Our study showed that Giardia and Cryptosporidium infections in the Philippines were correlated with the rainy season, which in turn was correlated with the higher incidence of diarrhea during the same period. In their 2-year study, Adkins et al (1987) showed the number of patients with diarrhea in Manila increased with the onset of the monsoon rains and peaked during the months of maximum rainfall. Similarly, Capeding and Saniel (1990) associated cryptosporidiosis in acute diarrhea in children, with episodes predominating during the rainy months of June to September. Salas (1997), studying G. lamblia infections in Cebu, located in the Visayas, found infections to be low during the summer and high during the rainy months.

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