PREVALENCE 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*,

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1981b) 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).

Cryptosporidium is a coccidian protozoan pathogen that can cause life-threatening diarrhea in an immuno-compromised host. Following the first report of cryptosporidiosis in Philippine children made by Cross *et al* in 1985, local studies of *Cryptosporidium* as an etiologic agent for diarrhea have focused on its prevalence in children (Capeding and Saniel, 1990; Laxer *et al*, 1990; Jueco *et al*, 1991; Carlos *et al*, 1992; Paje-Villar *et al*, 1993, 1994; Menorca *et al*, 1994). A recent study by Rivera *et al* (2005) detected *Cryptosporidium* antibodies among Filipino cancer patients.

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

in distribution. All tests were two-tailed and considered significant at $p<0.05$.

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%).

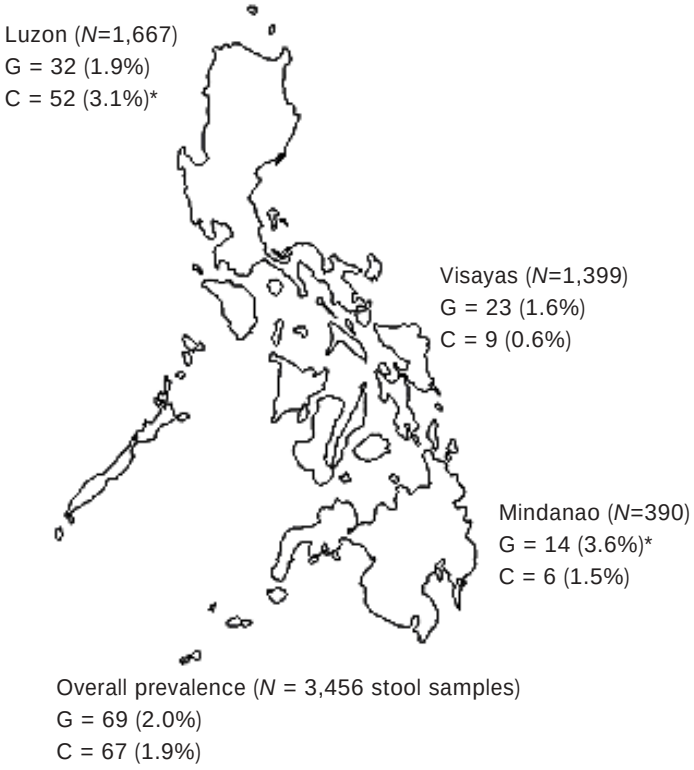

Fig 1–Overall prevalence of *Giardia* (G) and *Cryptosporidium* (C) in the Philippines, and their distribution in the 3 major islands. (* indicates the prevalence was significantly higher.)

Table 1
Summary of data of stool samples collected and microscopy.

Demographic profile	No. of stool samples	Prevalence Frequency (percentage)		
		Both protozoa[e]	*Giardia*	*Cryptosporidium*
Geographical location				
Luzon	1,667	83 (5.0)	32 (1.9)	52 (3.1)
Visayas	1,399	31 (2.2)	23 (1.6)	9 (0.6)
Mindanao	390	19 (4.9)	14 (3.6)	6 (1.5)
Overall (Philippines)	3,456	133 (3.8)[a]	69 (2.0)	67 (1.9)
Age group				
Pediatric (0-18 years old)	2,160	104 (4.8)	43 (2.0)	63 (2.9)
Adult (>18 years old)	1,245	26 (2.1)	24 (1.9)	3 (0.2)
Total	3,405[b]	130 (3.8)[c]	67 (2.0)	66 (1.9)
Sex				
Male	1,934	79 (4.1)	42 (2.2)	39 (2.0)
Female	1,520	54 (3.5)	27 (1.8)	28 (1.8)
Total	3,454[d]	133 (3.8)[a]	69 (2.0)	67 (1.9)

[a]Three patients had co-infection with *Giardia* and *Cryptosporidium*
[b]Fifty-one patients with unknown ages were excluded from analysis.
[c]Three patients of unknown ages were excluded from the analysis; 3 patients had co-infection with *Giardia* and *Cryptosporidium*.
[d]Two patients of unknown sex were excluded from analysis.
[e]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

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

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

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

Sex	*Giardia lamblia*		*Cryptosporidium* spp	
	Pediatric (*N*=2,160) Freq (%)	Adult (*N*=1,245) Freq (%)	Pediatric (*N*=2,160) Freq (%)	Adult (*N*=1,245) Freq (%)
Male	23 (1.1)	19 (1.5)	36 (1.7)	2 (0.2)
Female	20 (0.9)[a]	5 (0.4)	27 (1.2)[a]	1 (0.1)
Total	43 (2.0)	24 (1.9)[b]	63 (2.9)	3 (0.2)

[a]Significantly different (pediatric *vs* adult)
[b]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

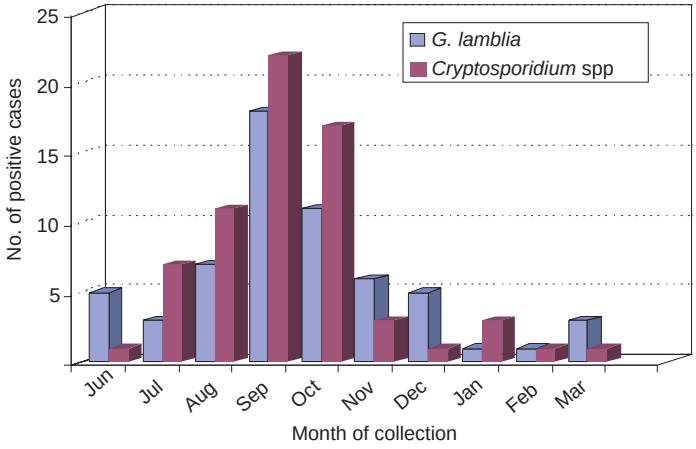

Fig 2–Seasonal variation in the prevalence rates of *G. lamblia* and *Cryptosporidium* spp among diarrhea patients in the Philippines. Data shown were for stools collected from June 2004 to March 2005.

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 reviews 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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