J. Protozool. Res. 17, 32-38 (2007)

Survey of C*ryptosporidium* sp. infection in two impoverished communities in Metro Manila, Philippines

Claveria, F.G.*, Alonzo, C., Bacal, A.M. and Mayugba, Z.M.
Biology Department, College of Science, De La Salle University-Manila, Taft Avenue, Manila, Philippines
*Corresponding author: Claveria, F.G., E-mail: claveriaf@dlsu.edu.ph

ABSTRACT

Human excreta from households of squatter communities in Singalong and Leveriza, Metro Manila, Philippines were examined for *Cryptosporidium* sp. infection. Fecal concentration procedure and the modified specific Ziehl-Neelsen staining technique were used. Of the 54 positive cases, 30 and 24 cases were detected in solid and semisolid excreta, respectively, with the infection rate being significantly higher ($p= 0.008$) in Singalong. Oocysts were detected among 53.7% children (≤10 years old) compared to 46.3% in older age groups. The gender associated difference in infection rate in both study sites was insignificant. Oocysts density ranged from very few to moderate to numerous. While the results confirmed the susceptibility of both genders and of all age groups to *Cryptosporidium* sp., the difference in percent infection between children and older age groups could not be clearly established owing to the small sampled population. In the absence of information on the health status of the respondents upon fecal collection, no association can be made on the pathogenicity of the parasite. We therefore recommend educating the communities on the risks of them contacting waterborne infections through contaminated waters and providing them overall improvement in basic sanitary facilities.

Key words: *Cryptosporidium*; Ziehl – Neelsen acid fast; impoverished communities; fecalysis

INTRODUCTION

Genus *Cryptosporidium* is a protozoan apicomplexan parasite that comprises several species that reside in epithelial tissues of the respiratory and gastrointestinal tract of a wide range of vertebrate hosts, like humans, cattle, dogs, cats, rodents, birds, fishes, snakes and lizards (Xiao *et al*., 2004; Current and Reese, 1983). Cows serve as a major host of *Cryptosporidium* worldwide (Llinares *et al*., 1999; Scott *et al*., 1994; Fayer and Ungar, 1986; Anderson, 1981; Panciera *et al*., 1971), causing potentially high risk to the human population. *Cryptosporidium hominis, Cryptosporidium muris and Cryptosporidium parvum* have been documented in humans and cattle (Carreno *et al*., 2001).

The transmission of *Cryptosporidium* sp. to vertebrate hosts is mainly thru the ingestion of food and water contaminated with feces containing oocysts (King *et al*., 2005; Fayer and Ungar, 1986). River systems and water sources generally become contaminated with oocysts from land grazed by livestock and human excreta, as well as, by way of sewage overflow, waste discharge, direct animal fecal deposition in waterways and water run-off (Davies *et al*., 2004; Chauret *et al*., 2001; Ono *et al*., 2001; Rose *et al*., 1991; LeChevallier *et al*., 1991). Gastrointestinal illness is one important manifestation of cryptosporidiasis in both immunocompetent and immunodeficient people (Nime *et al*., 1976). Most patients with this infection experience mild diarrhea and children and acquired immunodeficiency syndrome (AIDS) patients are highly susceptibility (Pitlik *et al*., 1983).

In the Philippines, documented studies on cryptosporidiosis are scanty. Earlier reports were among diarrheic patients, including children living in Metro Manila and from nearby provinces in Northern Luzon, Leyte, Samar and Mindanao (Capeding and Saniel, 1990; Laxer *et al*., 1988; Cross *et al*., 1985). Salazar *et al*. (1986) documented eight cases among AIDS patients and mentally retarded and malnourished children and 8-12 months old babies with acute respiratory infection and diarrhea. Worldwide, water-borne transmission route of parasitic infections is considered a major public health problem particularly in poor and developing countries (Xiao *et al*., 2004), like the Philippines where potable water supply is inadequate. In this paper, we report our findings of *Cryptosporodium* sp. infection in two impoverished urban communities in Manila located alongside riverbanks and stagnant canals.

Materials and Methods

Study sites and collection of fecal samples

A total of 150 fecal samples obtained from infants, toddlers, adolescents, adults and old-aged people residing in two separate squatter areas in Singalong and Leveriza, Metro Manila (Fig. 1) were subjected to fecalysis. Each respondent was provided an envelope containing a vial with half-full 10% formalin and a popsicle stick to pick up a pea size excreta and placed into a vial. The fixed excreta were then brought to the Parasitology Laboratory at the STRC building, DLSU-Manila for processing.

Fecal concentration procedure

To obtain the desired sediments for oocysts detection, the fecal concentration procedure (World Health Organization, 1994) was used with slight modification as follows: To obtain a 10 ml fecal-formalin mixture, 3 grams of semisolid feces was mixed with 7 ml 10% formalin; while 2 grams solid feces was mixed with 8 ml 10% formalin. The fecal-formalin mixture was stirred and the suspension was filtered using a three-fold surgical gauze and poured into a 15 ml centrifuge tube. To the filtrate, 10% formalin was added to attain the 10 ml mark to which 3 ml of ether was added. The tube capped with a rubber stopper was vigorously shaken for 10 sec and centrifuged at 400-500xg (2,500 – 2,800 RPM) for 3 min. Post-centrifugation, the top three layers (ether, fatty debris and formalin) were discarded, retaining only the bottom sediment for the detection of *Cryptosporidium* oocysts.

Fecal sediment staining and oocysts density scoring

The Ziehl-Neelsen (ZN) acid-fast staining procedure specific for the detection and identification of *Cryptosporidium* oocysts was used (World Health Organization, 1994), with some modification. The standard ZN staining technique uses carbol-fuchsin, 1% HCl-ethanol solution, HCl-methanol solution and 0.25% malachite green. Since the fecal samples were earlier fixed in 10% formalin, the 1% HCl-methanol solution was discarded in the present study. Deep red to pink stained spherical or slightly ovoid oocysts containing black granules and measuring 4-6 μm which are characteristics of *C. parvum*, *C. meleagridis* and *C. hominis* that have been documented in humans (Xiao *et al*., 2004) were noted. Oocysts density was determined according to the oocysts scoring system of Dagnall Teaching Laboratory, Liverpool School Tropical Medicine (1998), as follows: rare (+) for ≤ 5 oocysts per slide; few to moderate (++) for 1-10 oocysts per field of view; and numerous (+++) for 11 or more oocysts per field of view. Per smear, 20 viewing fields were covered at 1,000x magnification.

Figure 1. Collection sites of human excreta in Metro Manila. A & B. Singalong. C & D. Leveriza.

Data Analysis

Prevalence of infection per study site and distribution of infection between different age groups and gender and oocysts density were noted and the difference analyzed using the Z-test at p=0.05.

RESULTS AND DISCUSSION

Examination of 150 fecal samples revealed an overall 36.0% prevalence, with the infection rate being significantly higher (p= 0.008) in the Singalong study site. Of the 54 positive cases, 30 and 24 cases were detected in solid and semisolid excreta, respectively. No watery stools were provided. Eighteen (33.0%) males and 36 (67.0%) females were infected (Table 1). Oocysts were detected among 53.7% children (≤10 years old) compared to 46.3% in older age groups. The difference in infection rate between sexes in both study sites is insignificant.

Table 1. Survey results of fecalysis in 150 respondents in Singalong and Leveriza for *Cryptosporidium* sp. infection.

Collection Site	No. fecal samples examined	No. fecal samples infected (%)		Total (%)
		♂	♀	
Singalong	62	12 (40.0)	18 (60.0)	30 (48.4)
Leveriza	88	6 (25.0)	18 (75.0)	24 (27.3)
Total (%)	150	18 (33.0)	36 (67.0)	54 (36.0)

In Singalong, 17 (56.7%) children (≤10 years old) were infected compared to only 13 (43.3%) among the older age groups (Table 2; Fig. 2). Owing to the small sample size per age group, the difference in infection rates between children and older age groups could not be statistically tested. In Leveriza, children and older age groups had similar 50% infection (Table 3; Fig. 2). Oocysts density ranged from rare (≤5 oocysts per smear) to numerous (≥11 oocysts per field of view) (Tables 2&3; Fig. 3). In the two study sites combined, 35.0%, 28.0% and 37.0% of the infected respondents manifested rarely, few to moderate, and numerous oocysts, respectively.

Results confirmed the susceptibility of both sexes and of all age groups to *Cryptosporidium* sp. However, the difference in percent infection between children and older age groups could not be clearly established owing to the small sample size for the different age groups. A follow-up study addressing this limitation in the present study is warranted.

Table 2. Age and sex distribution and oocysts density of *Cryptosporidium* sp. among respondents of Singalong. (N=62)

Age Range (years)	Sex		Total infected (%)	Oocysts Density		
	♂ No. examined (no. infected)	♀ No. examined (no. infected)		+	++	+++
≤1-5	11 (4)	8 (4)	8 (42.1)	2	2	4
6-10	7 (6)	5 (3)	9 (75.0)	2	2	5
11-15	1 (1)	1 (0)	1 (50.0)	0	0	1
16-20	1 (0)	3 (2)	2 (50.0)	1	0	1
21-25	0 (0)	4 (1)	1 (25.0)	0	0	1
26-30	1 (0)	4 (3)	3 (60.0)	1	1	1
31–35	2 (1)	1 (1)	2 (66.7)	1	1	0
36-40	1 (0)	2 (0)	0 (0.0)	0	0	0
41-62	3 (0)	7 (4)	4 (40.0)	1	3	0
Total	27 (12)	35 (18)	30 (48.4)	8	9	13

Legends: + ≤5 oocysts per smear (1,000x) ++ 1-10 oocysts per field of view (1,000x)
+++ ≥11 oocysts per field of view (1,000x)

While percent infection was significantly higher among respondents of Singalong relative to Leveriza, the two study sites are similar in terms of their level of sanitation, and having adjacent overcrowded households along the esteros (=stagnant canals). The respondents in the area have been honest to admit that they, most of the time, throw their wastes, including fecal excreta into the river tributaries and canals which exacerbates the problem of environmental sanitation. From personal interviews conducted during the survey, during high tide especially acute in the rainy season, the water from the canals and river tributaries flows out and into their houses, contaminating the water supplies. Such situation favors the transmission of pathogens like *Cryptosporidium* spp. (Davies *et al*., 2004).

Table 3. Age and sex distribution and oocysts density of *Cryptosporidium* sp. among respondents of Leveriza. (N = 88).

Age Range (years)	Sex		Total infected (%)	Oocyst Density		
	♂ No. examined (no. infected)	♀ No. examined (no. infected)		+	++	+++
≤1–5	16 (3)	9 (6)	9 (36.0)	4	2	3
6-10	4 (1)	4 (2)	3 (37.5)	1	1	1
11-15	2 (0)	3 (1)	1 (20.0)	1	0	0
16–20	3 (0)	12 (4)	4 (26.7)	3	1	0
21–25	5 (0)	7 (3)	3 (25.0)	1	0	2
26-30	0 (0)	6 (1)	1 (16.7)	1	0	0
31-35	2 (0)	2 (0)	0 (0.0)	0	0	0
36-40	1 (0)	0 (0)	0 (0.0)	0	0	0
41-45	0 (0)	1 (0)	0 (0.0)	0	0	0
46-58	4 (2)	7 (1)	3 (27.3)	0	2	1
Total	37 (6.0)	51 (18.0)	24 (27.3)	11	6	7

Legends: + ≤5 oocysts per smear (1,000x) ++ 1-10 oocysts per field of view (1,000x)
+++ ≥11 oocysts per field of view (1,000x)

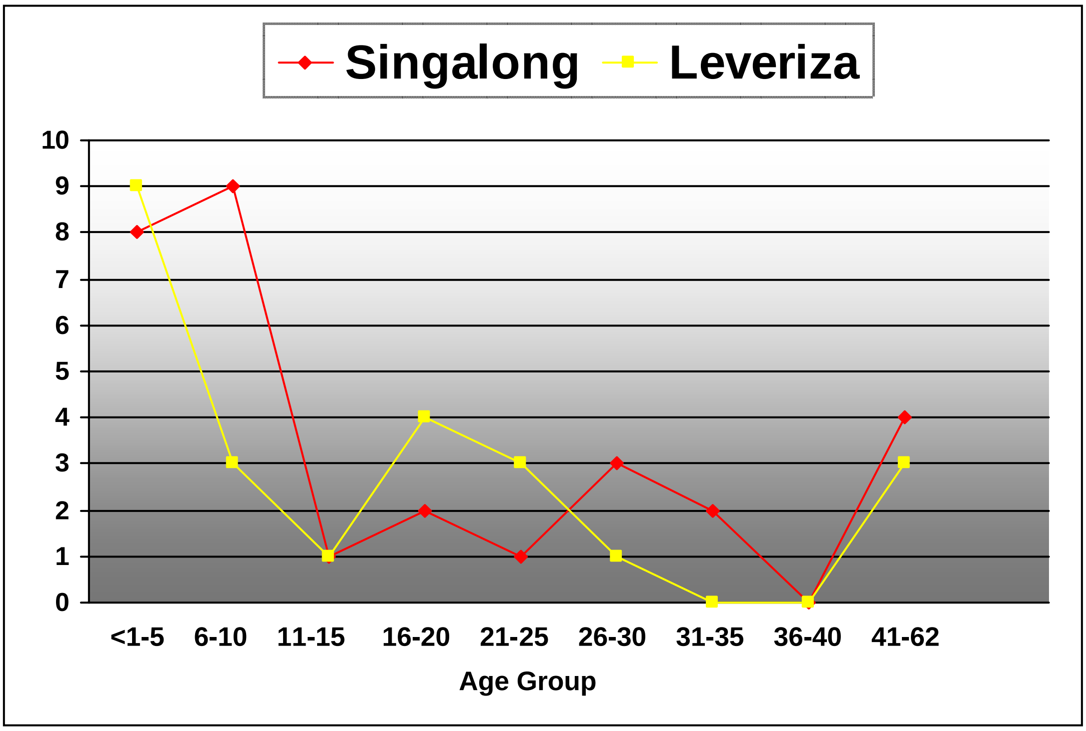

Figure 2. Distribution of infection in Singalong (N=30) and Leveriza (N=24) according to age groups. Note Higher infection among the 10 years old and younger respondents.

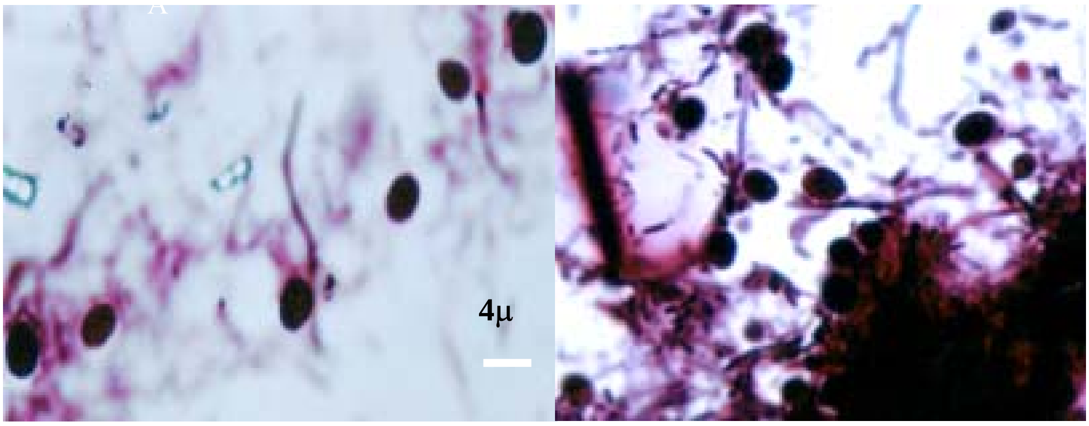

Figure 3. Oocysts showing black granules.

Interesting these are the conditions prevailing in the present study sites. Koch *et al*., (1985) considered person to person transmission as a common cause of human cryptosporidiosis. Present data on *Cryptosporidium* sp. infection in these two communities are considerably higher compared to those earlier reported in the country (Capeding and Saniel, 1990; Laxer *et al*., 1988; Salazar *et al*. 1986; Cross *et al*., 1985). In the absence of information on the health status of the respondents upon fecal collection, no association can be made on the pathogenicity of the parasite. Providing these communities avenues to understand and realize the risks of them contacting waterborne infections through contaminated waters and overall improvement in basic sanitary facilities are highly recommended.

REFERENCES

Anderson, B. 1981. Patterns of shedding of cryptosporidial oocysts in Idaho calves. J. Am. Vet. Med. Assoc. 178:982-984.

Capeding, M. and Saniel, M. 1990. Cryptosporidiosis in Filipino children: Association with acute diarrhea. Phil. J. Microbiol. Infect. Dis. 19:49-50.

Carreno, R., Pokorny, N., Lee, H., Trevors, J. and De Grandis, S. 2001. Phenotypic and genotypic characterization of *Cryptosporidium* species and isolates. J. Indian Microbiol. Biotech. 26:95-106.

Chauret, C., Radziminski, C., Lepuil, M., Creason, R. and Andrews, C. 2001. Chlorine dioxide inactivation of *Cryptosporidium parvum* oocysts and bacterial spore indicators. Appl. Environ. Microbiol. 67:2993-3001.

Clark, D. 1999. New insights into human cryptosporidiosis. Clin. Microbiol. Rev. 12:554-563.

Cross, J., Alcantara, A., Alquiza, L., Zaraspe, G. and Ranoa, C. 1985. Cryptosporidiosis in Philippine children. Southeast Asian J. Trop. Med. Publ. Hlth. 16:257-260.

Current, W., Reese, N., Ernst, J.V., Bailey, W.S., Heyman, M.B. and Weinstein, W.M. 1983. Human cryptosporidiosis in immunocompetent and immunodeficient persons: Studies of an outbreak and experimental transmission. New Engl. J. Med. 308:1252-1257.

Dagnall Teaching Laboratory, Liverpool School of Tropical Medicine. 1998. pp.106.

Davies, C., Ferguson, C., Kaucner, C., Krogh, M., Altavilla, N., Deere, D. and Ashbolt, N. 2004. Dispersion and transport of *Cryptosporidium* oocysts from fecal pats under simulated rainfall events. Appl. Environ. Microbiol. 70:1151-1159.

Fayer, R. and Ungar, B. 1986. *Cryptosporidium* spp. and cryptosporidiosis. Microbiol. Rev. 50:458-483.

King, B., Keegan, A., Monis, P. and Saint, C. 2005. Environmental temperature controls *Cryptosporidium* oocyst metabolic rate and associated retention of infectivity. Appl. Environ. Microbiol. 71:3848-3857.

Koch, K., Phillips, D., Aber, R. and Current, W. 1985. Cryptosporidiosis in hospital personnel. Evidence for person-to-person transmission. Annual. Internal. Med. 102:593-596.

Laxer, M., Alcantara, A., Javato-Laxer, M., Cui, M., Leano, R., Bautista, S., Ramilo, N. and Gavina, T. 1988. *Cryptosporidium* from Palawan, Republic of the Philippines. Phil. J. Microbiol. Infect. Dis. 17:1-3.

LeChevallier, M.W., Norton, W.D. and Lee, R.G. 1991. *Giardia* and *Cryptosporidium* spp. in filtered drinking water supplies. Appl. Environ. Microbiol. 57:2617–2621.

Bornay-Llinares, F.J., da Silva, A.J., Moura, I.N., Myjak, P., Pietkiewicz, H., Kruminis-Łozowska, W., Graczyk, T.K. and Pieniazek, N.J. 1999. Identification of Cryptosporidium felis in a cow by morphologic and molecular methods. Appl. Environ. Microbiol. 65:1455–1458.

Nime, F., Burek, J., Page, D., Holscher, M. and Yardley, J. 1976. Acute enterocolitis in a human being infected with the protozoan *Cryptosporidium*. Gastroenterology 70:592-598.

Ono, K., Tsuji, H., Rai, S., Yamamoto, A., Masuda, K., Endo, T., Hotta, H., Kawamura, T. and Uga, S. 2001. Contamination of river water by *Cryptosporidium parvum* oocysts in western Japan. Appl. Environ. Microbiol. 67:3832-3836.

Panciera, R., Thomassen, R. and Garner, F. 1971. Cryptosporidiosis in a calf. Vet. Pathol. 8:479-484.

Pitlik, S., Fainstein, V., Rios, A., Guarda, L., Mansell, P. and Hersh, E. 1983. Cryptosporidial cholecystitis. New Engl. J. Med. 308:967.

Rose, J.B., Gerba, C.P. and Jakubowski, W. 1991. Survey of potable water supplies for *Cryptosporidium* and *Giardia*. Envtl. Sci. Tech. 25:1393-1400.

Salazar, N., Gonzaga, N. and Saniel, M. 1986. Cryptosporidial diarrhea: Isolation and identification of causative organisms. Phil. J. Microbiol. Infect. Dis. 15:53-56.

Scott, C.A., Smith, H.V. and Gibbs, H.A. 1994. Excretion of *Cryptosporidium parvum* oocysts by a herd of beef suckler cows. Vet. Rec. 134: 172.

World Health Organization. 1994. Bench aids for the diagnosis of intestinal parasites. Plate 2.

Xiao, L., Fayer, R., Ryan, U. and Upton, S. 2004. *Cryptosporidium* Taxonomy: Recent advances and implications for public health. Clin. Microbiol. Rev. 17:72-97.