Construction of Laboratory Network on the Characterization of Enteric Protozoan Pathogens Prevalent in Asia and Pan-Pacific:

Philippine Report

2005 - 2008

Study Director: **Dr. Takuro Endo**

National Institute of Infectious Diseases

Tokyo, Japan

TABLE OF CONTENTS

INTRODUCTION

The Department of Health in the Philippines recognizes the importance of access to safe drinking water for the promotion and protection of public health. Thus, the Philippine National Standards for Drinking Water 2007 has set standards for drinking water under Administrative Order (AO) 2007-0012 dated March 9, 2007 (Appendix Ia). The objective of the AO is to protect public health, safety, and welfare by ensuring quality and safety of drinking water.

A high percentage of the population in the Philippines is exposed to health risks caused by unsafe drinking water supply. Philippine Health Statistics show that drinking water and other water-borne diseases still rank among the leading causes of morbidity in the country. In 2004 and 2005, acute watery diarrhea ranked 3rd among the 10 leading causes of morbidity in the Philippines. In 2006, it ranked 2nd (Appendix Ib).

Some of the factors that contribute to these risks include accessibility to safe water supply and existence of sanitary toilet. According to statistics from the National Epidemiology Center (NEC) of the Department of Health, the percentage of households with access to safe water supply ranges from 81.8-83% while those with sanitary toilet ranges from 69.4-75.4% for 2004 to 2006, respectively (Appendix Ic).

According to the 2004-2006 data from NEC, the incidence of acute watery diarrhea was highest in children aged 1-4 years, followed by those aged less than 1 year (Appendix Id).

In a study by Carlos and Saniel (1990) the most common causes of diarrhea in the Philippines are rotavirus and enterotoxigenic *E. coli*. In previous studies (Saniel *et al.* 1985, 1988), *Cryptosporidium* and *E. histolytica* were ranked as number 9 and 10 among the causes of diarrhea. Recent local studies on enteric protozoa include studies on *Iodamoeba butschlii*, *Endolimax nana*, *Entamoeba coli*, and *Giardia lamblia* by Lee *et al.* (2000); *G. lamblia*, *E. histolytica*, and *Blastocystis hominis* by Baldo *et al.* (2004); *E. histolytica/E. dispar* by Rivera *et al.* (2006); *Cryptosporidium* by Rivera *et al.* (2005); and *G. lamblia* by Yason and Rivera (2007).

The present study involved the collection of diarrheic stool samples and the identification of enteric protozoa (*Giardia* and *Cryptosporidium*). These 2 pathogenic protozoa are the most common causes of water-borne outbreaks around the world. *Giardia* is the most common cause of water-borne outbreaks in the United States (Herwaldt *et al.* 1992). Ingestion of unfiltered, inadequately chlorinated surface water or ground water is the most commonly documented causes of giardiasis. The Milwaukee outbreak in 1993 was traced to have been due to *Cryptosporidium* oocysts that contaminated the community water supply. This served as an eye-opener on the importance of surveillance and routine screening for *Cryptosporidium*.

METHODOLOGY

Collaborating hospitals

There were 78 collaborating hospitals/clinics that served as the collection sites for diarrheic stool samples used in this study: 31 from Luzon, 38 from Visayas, and 9 from Mindanao (Appendices IIa, b, c).

Patient information

A Patient Information Sheet (Appendix III) was used to gather relevant data from the patients. Gathered data included clinical symptoms, immunostatus, demography, and drinking water source. The data were encoded using Microsoft Access.

Stool samples

Collection. About 1 ml of stool sample from a diarrheic patient was added into a polypropylene tube containing 9 ml of 10% formalin. From May 2004 to June 2005, samples were collected in the 3 major islands of the Philippines (Luzon, Visayas, and Mindanao). From January 2006 to January 2008, collection was done in Visayas and Metro Manila only. About 1 to 3 ml of fresh stool sample was collected and sent to St. Luke's Medical Center.

Stool containers were labeled with patient's name and date of sample collection. Fresh stool samples from Metro Manila were prepared for microscopy upon arrival. Formalin-fixed and fresh samples from Visayas and Mindanao were stored at 4°C until sent for laboratory processing at the Research and Biotechnology Division of St. Luke's Medical Center in Metro Manila.

Processing. Formalin-fixed stool samples collected from 2004 to 2005 were concentrated using modified formalin-ethyl acetate method prior to microscopy. However, unfixed stool samples collected from 2006 to 2008 were directly used for microscopy. Purifications of microscopy-positive unfixed samples were done using sucrose gradient centrifugation and cesium chloride floatation prior to DNA extraction.

Modified formalin-ethyl acetate method. The tube containing 9 ml of 10% formalin and 1 ml stool sample was vortexed and filtered. The filtrate plus 2 ml ethyl acetate was mixed vigorously and centrifuged at 1,000 x g for 5 min at 4°C. The pellet plus 2 ml of 0.1% Tween 20 was centrifuged at 1,000 x g for 5 min at 4°C. The washed pellet was resuspend with 50 µl of PBS and used immediately for microscopy. The remaining suspension was stored at 4°C.

Sucrose gradient centrifugation and cesium chloride floatation. Fresh stool suspension (1 g of stool sample plus 9ml of 0.01% TritonX-100) was filtered. The filtrate was centrifuged at 1000 x g for 5 min at room temperature. The pellet was resuspended in 5 ml of 0.01% TritonX-100.

The resuspended pellet was underlain with 2 ml of 50% (w:v) sucrose in distilled water and centrifuged at 1,500 rpm for 5 min at room temperature. The interphase was mixed with distilled water to make 10 ml and centrifuged at 1,500 x g or 2,500 rpm for 5 min at room temperature. The pellet was resuspended in 0.01% Tween-80 to make 1 ml.

The resuspended pellet (350 µl) was laid over 700 µl of 20% (w:v) cesium chloride in distilled water and centrifuged at 12,000 rpm for 3 min at room temperature. The upper layer above the interphase or 250 µl if not visible was discarded. The interphase (650 µl) was transferred into a new tube containing 800 µl of distilled water and centrifuged at 12,000 rpm for 3 min at room temperature. The pellet was washed with 1 ml of sterile TE buffer and centrifuged at 12,000 rpm for 3 min at room temperature. The washed pellet was resuspended in 1 ml of sterile TE buffer and used immediately for DNA extraction. The remaining suspension was stored at –20°C.

Fluorescence microscopy using MeriFluor® Kit

Concentrated formalin-fixed or unconcentrated unfixed stool sample (5 µl) was dropped on a slide and stained with MeriFluor® kit. The slide was scanned under a fluorescence microscope with a blue excitation filter (450 nm, 09B, Zeiss) at x 400 magnification.

Photomicrographs of fluorescing *Giardia* cysts and *Cryptosporidium* oocysts were taken immediately for documentation.

Molecular Analyses

After microscopy, samples containing *Giardia* cyst or *Cryptosporidium* oocyst were stored at -18 °C until used for DNA extraction.

Extraction of Genomic DNA. DNA extraction and purification were done using QIAamp DNA stool minikit (Qiagen) following manufacturer's instruction. Another protocol used was glass beads method based on the procedures of McLauchlin *et al.* (2000). Then, genomic DNA of the parasites was extracted based on the procedures of Yagita and Izumiyama (2006, pers. com.). The latter is more successful in extracting *Cryptosporidium* DNA.

The DNA extraction protocol was performed as follows: approximately 200 µl of purified oocysts in sterile Tris-EDTA (TE) buffer was added to 100 mg of 0.5-mm glass beads plus 400 µl of 1% sodium dodecyl sulfate (SDS). The tube was vortexed for 2 min at maximum speed, boiled for 30 min, and incubated at 56°C overnight after adding 5 µl of proteinase K (10 mg/ml). DNA was isolated by adding 400 µl of buffered phenol and spinning at 12,000 rpm for 5 min at 27°C. DNA was precipitated by adding 40 µl of sterile 1 M sodium

chloride (NaCl) and 800 µl of absolute ethanol. The tube was centrifuged at 14,000 rpm for 5 min at 27°C. The supernatant was discarded while the pellet was washed with 400 µl of 70% ethanol and centrifuged at 14,000 rpm for 5 min at 27°C. The pellet was dried at room temperature. The DNA was eluted into 25 µl sterile TE buffer. DNA sample was either used directly for PCR amplification or stored at –20°C until used.

Nested PCR analysis for identification of *Giardia*. PCR analysis for *Giardia* was conducted by amplifying the glutamate dehydrogenase (gdh) gene fragment using the method described by Homan *et al.* (1998). The first PCR step was performed using 5 µl of *Giardia* genomic DNA in a 50-µl-reaction mixture containing 10x PCR buffer with 15 mM $MgCl_2$, 10 mM each of dNTPs, 20 µM of each primer, and 2.5 U of *Taq* polymerase. The primer pair (gdh1/4) used was 5'-ATC TTC GAG AGG ATG CTT GAG-3' and 5'-AGT ACG CGA CGC TGG GAT ACT- 3'. The cycling conditions were: an initial hot start of 94°C for 3 min; 45 cycles comprising of 94°C for 30 s, 55°C for 1 min, and 72°C for 1 min; and final extension of 72°C for 5 min.

For the second step, 1 µl of the first PCR product was re-amplified in a 50-µl-reaction mixture with the same contents used in the first PCR step. The primer pair (gdh 1seq/4seq) used was 5'-AGG ATG CTT GAG CCG GAG CG-3' and 5'-GGA TAC TTN TCC YTG ACC TC-3'. The cycling conditions were: an initial hot start of 94°C for 3 min; 30 cycles comprising of 94°C for 15 s, 58°C for 30 s, and 72°C for 1 min; and final extension of 72°C for 5 min.

PCR-RFLP analyses for identification of *Cryptosporidium*. DNA analyses were performed by PCR/-RFLP of polythreonine (poly-T), 18S SSU rRNA, and cpgp40/15 genes based on the procedures of Yagita *et al.* (2001), Xiao *et al.* (1999) and Izumiyama (2006, pers. com.) respectively.

PCR-RFLP of poly-T gene. Poly-T gene was amplified using 1 µl of *Cryptosporidium* genomic DNA in a 25-µl-reaction mixture containing 10x PCR buffer with 15 mM $MgCl_2$, 10 mM each of dNTPs, 20 µM of each primer, and 2.5 U of *Taq* polymerase. The primer pair used was (Cry 44) 5'-CTC TTA ATC CAA TCA TTA CAAC-3' and (Cry373) 5'-AGC AGC AAG ATA TGA TAC CG-3'. The following cycling conditions were used: an initial hot start of 94°C for 3 min; 40 cycles comprising of 94°C for 30 s, 53°C for 30 s, and 72°C for 30 s; and final extension of 72°C for 5 min.

RFLP analysis was performed by digesting the PCR product with *Rsa*I endonuclease under the conditions recommended by the supplier.

Nested PCR-RFLP of 18S SSU rRNA gene. The first PCR step was performed using 5 µl of *Cryptosporidium* genomic DNA in a 50-µl-reaction mixture containing 10x PCR buffer with 15 mM $MgCl_2$, 10 mM each of dNTPs, 20 µM of each primer, and 2.5 U of *Taq*

polymerase. The primer pair (CX0FR) used was 5'-TTCTAGAGCTAATACATGCG-3' and 5'-CCCATTTCCTTCGAAACAGGA-3'. A total of 40 cycles, each consisting of 94°C for 45 s, 55°C for 45 s, and 72°C for 1 min, was performed. An initial hot start at 94°C for 3 min and a final extension step at 72°C for 7 min were included.

For second PCR step, 1 μl of the first PCR product was re-amplified in a 50-μl-reaction mixture with the same contents used in the first PCR step. The primer pair (CX1FR) used was S5'-GGAAGGGTTGTATTTATTAGATAAAG-3' and 5'-AAGGAGTAAGGAACAACCTCCA-3'. The cycling conditions used were identical to the conditions used for the first step.

For restriction fragment analysis, 8 μl of the second PCR product was digested in a 10-μl reaction mixture containing 20 U of *Ssp*I (Invitrogen) for species diagnosis or 20 U of *Vsp*I (Promega) for distinguishing *C. parvum* from *C. hominis* and 1 μl of the appropriate restriction buffer at 37°C for 3 hours.

Nested PCR of cpgp40/15 gene. The first PCR step was conducted using 5 μl of *Cryptosporidium* genomic DNA in a 50-μl-reaction mixture containing 10x PCR buffer with 15 mM $MgCl_2$, 10 mM each of dNTPs, 20 μM of each primer, and 2.5 U of *Taq* polymerase. The primer pair used was (cpgpi0F/1R) 5'-ACT CTC CGT YAT AGT CTC CG-3' and 5'-AAA CGG AAG GAA CGA TGT ATC-3'. The following cycling conditions were used: an initial hot start of 94°C for 5 min; 45 cycles comprising of 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min; and final extension of 72°C for 10 min.

For second PCR step, 1 μl of the first PCR product was re-amplified in a 50-μl-reaction mixture with the same contents used in the first step. The primer pair used was (cpgpi1F/0R) 5'-TAG TCT CCG YTG TAT TCT CAG-3' and 5'-AAR GCA GAG GAA ACC AGC ATC-3'. The cycling conditions used were identical to the conditions used for the first step.

The primer pairs were designed by Dr. Shinji Izumiyama of the Department of Parasitology of the National Institute of Infectious Diseases, Tokyo, Japan.

All PCR and RFLP products were visualized after electrophoresis on 2% agarose gels.

RESULTS

Table 1 shows a summary of sample collection and prevalence of enteric protozoa, specifically *Giardia* and *Cryptosporidium*, for a 3-year period from 2005 to 2007. A total of 6302 stool samples were collected in the 3 major islands of the Philippines– Luzon, Visayas and Mindanao. Ninety-four (1.49%) out of the total sample collection were positive for *Giardia* while 92 (1.46%) were positive for *Cryptosporidium*.

In 2005, the total number of isolates was 136 but 3 of these samples had mixed infection of *Giardia* and *Cryptosporidium*. Thus, in terms of the number stool samples, only 133 were positive for this particular year.

Table 1. Summary of collection of diarrheic samples and prevalence of enteric protozoa in the Philippines, 2005-2007

Major islands	2005 (May 2004-June 2005)	2006 (Jan. 2006 – Jan. 2007)	2007 (Feb. 2007-Jan. 2008)	Consolidated (May 2004-Jan 2008)
Luzon	1667	1658	872	4197
Visayas	1399	295	21	1715
Mindanao*	390	0	0	390
TOTAL	**3456**	**1953**	**893**	**6302**
Prevalence of				
Giardia	69	14	11	94 **(1.49%)**
Cryptosporidium	67	21	4	92 **(1.46%)**

* Mindanao was not a collection site in 2006 and 2007.

Out of the total collection of 6302 stool samples for the 3-year period, 54.2% was from male and 45.8% from female patients (Table 2). The same trend is observed in the 3 major islands.

Table 2. Summary of distribution by sex of patients with diarrheic samples, 2005-2007

Major islands	2005 Number of		2006 Number of		2007 Number of		Consolidated Percentage of	
	Male	Female	Male	Female	Male	Female	Male	Female

Luzon	965	700	832	826	483	389	54.4	45.6
Visayas	753	646	150	145	14	7	53.5	46.5
Mindanao	216	174	---	---	---	---	55.4	44.6
TOTAL	**1934**	**1520**	**982**	**971**	**497**	**396**	**54.2**	**45.8**

In our patient population, the age ranged from 0-98 years in males, and 0-95 years in females (Table 3). As to age distribution, the % of pediatric patients (18 years and below) was 54.5%, while for adults it was 45.5%. This was also the trend in the 3 major islands. In Mindanao, the number of pediatric patients was almost twice that of adults.

Table 3. Summary of age classification of patients with diarrheic samples, 2005-2007

Age by Major islands	**2005** Number of		**2006** Number of		**2007** Number of		**Consolidated** Percentage of	
	Pedia-tric	Adult	Pedia-tric	Adult	Pedia-tric	Adult	Pedia-tric	Adult
Age range	0-95		0-98		0-86		0-98	
Luzon	1174	453	661	932	334	490	53.4	46.7
Visayas	737	651	191	103	18	3	55.2	44.9
Mindanao	249	141	---	---	---	---	63.3	36.8
TOTAL	**2160**	**1245**	**852**	**1035**	**352**	**493**	**54.5**	**45.5**

In Table 4, patients were divided into different age groups. Around 50% of the patients were less than 1 year to 14 years of age. This was higher than 37.3% for patients 15-49 years old. In the other extreme, there were only 3.6% of those above 65 years of age.

Table 4. Age group distribution of patients with diarrhea, 2005-2007

Age Group (years)	2005 Number (%)	2006 Number (%)	2007 Number (%)	Consolidated Number (%)
< 1	687 (20.2)	145 (7.7)	45 (5.3)	877 (14.3)
1 – 4	918 (27.0)	376 (19.9)	176 (20.8)	1470 (23.0)
5 – 14	429 (12.6)	284 (15.1)	109 (12.9)	822 (13.4)
15 – 49	977 (28.7)	851 (45.1)	460 (54.4)	2288 (37.3)
50 – 64	237 (7.0)	176 (9.3)	49 (5.8)	462 (7.5)
≥ 65	157 (4.6)	55 (2.9)	7 (0.8)	219 (3.6)
TOTAL	**3405 (100.0)**	**1887 (100.0)**	**846 (100.0)**	**6138 (100.0)**

In our sample population, the over-all prevalence of *Giardia* was 2.7% while for *Cryptosporidium* it was 2.5% (Tables 5 and 6). *Giardia* had the highest prevalence in Luzon (7.4%), followed by Mindanao (7.1%), and lowest in Visayas (3.4%). It was only in Luzon where the prevalence in adults was higher than in the pediatric age group. *Cryptosporidium* had a higher prevalence in children than in adults in all islands.

Table 5. Prevalence of *Giardia* among diarrheic patients in the Philippines, 2005-2007

Major islands	2005 Number of	2006 Number of	2007 Number of	Consolidated Percentage of
Luzon				
Pediatric	22	4	3	**2.3**
Adult	9	7	7	**5.1**
No information*	1	0	1	
Visayas				
Pediatric	10	2	0	**1.9**
Adult	12	1	0	**1.5**
No information*	1	0	0	
Mindanao				
Pediatric	9	--	--	**3.6**
Adult	5	--	--	**3.5**
TOTAL	**69**	**14**	**11**	**2.7**

* No information on age of 1 patient

Table 6. Prevalence of *Cryptosporidium* among diarrheic patients in the Philippines, 2005-2007

Major islands	2005 Number of	2006 Number of	2007 Number of	Consolidated Percentage of
Luzon				
Pediatric	50	12	4	**5.4**
Adult	1	6	0	**1.3**
No information	1	1	0	
Visayas				
Pediatric	7	2	0	**1.1**
Adult	2	0	0	**0.3**
Mindanao*				
Pediatric	6	--	--	**2.4**
Adult	0	--	--	**0**
TOTAL	**67**	**21**	**4**	**2.5**

* No collection in Mindanao for 2006 and 2007

The total collection of *Cryptosporidium* isolated for the 3-year period is 92. From this, only 48 have been identified up to the species level. *C. hominis* and *C. parvum* were the predominant species of *Cryptosporidium* in our collection (Table 7). Three samples were found to be co-infected with 2 species: 2 samples with both *C. hominis* and *C. parvum* and 1 sample with both *C. hominis* and *C. canis*. *C. canis* was found only in samples from the Visayas.

Table 7. Species of *Cryptosporidium* in the Philippines, 2005-2007

Species	2005 Number of	2006 Number of	2007 Number of	Consolidated Number of
Luzon				
C. hominis	10	3	1	**14**
C. parvum	9	16	0	**25**
Co-infection[a]	1	0	0	**1**
Visayas				
C. hominis	1	2	0	**3**
C. parvum	0	0	0	**0**
C. canis	1	0	0	**1**
Co-infection[b]	1	0	0	**1**
Mindanao[c]				
C. hominis	1	--	--	**1**
C. parvum	1	--	--	**1**
Co-infection[a]	1	--	--	**1**
TOTAL	**26**	**21**	**1**	**48**

[a] Co-infection of *C. hominis* and *C. parvum*

[b] Co-infection of *C. hominis* and *C. canis*

[c] No collection in Mindanao for 2006 and 2007

Risk factors for diarrhea that include source of drinking water and toilet sanitation were investigated (Tables 8 and 9). Only 17.1% used commercial bottled water while majority (45.7%) sourced their drinking water from the local water system distributed though pipes. Another 38% obtained their drinking water from underground sources. Other sources were untreated surface water (2.9%), and tanks from private distributors going around in trucks (0.5%). Toilet sanitation was based on the presence of sanitary toilet for excreta disposal. This was done only in 2006 and 2007. Almost 95% of those patients in this study who provided data used a sanitary toilet.

Table 8. Sources of drinking water of patients with diarrhea, 2005-2007

Water Sources	2005 Number (%)	2006 Number (%)	2007 Number (%)	Consolidated Number (%)
Pipe distribution	1459 (42.9)	278 (51.0)	153 (82.7)	1890 **(45.7)**

Underground water source	1317 (38.8)	221 (40.9)	31 (16.7)	1569 **(38.0)**
Bottled Water	619 (18.2)	79 (14.8)	8 (4.4)	706 **(17.1)**
Untreated surface water	88 (2.6)	31 (5.8)	1 (0.5)	120 **(2.9)**
Other sources	10 (0.3)	9 (1.7)	3 (1.6)	22 **(0.5)**

Table 9. Sanitary condition of households of patients with diarrhea, 2005-2007

Toilet type in household	**2005**	**2006** Number (%)	**2007** Number (%)	**Consolidated** Number (%)
Sanitary toilet	No information	506 (94.1)	176 (97.2)	682 **(94.9)**
No sanitary toilet	collected	32 (5.9)	5 (2.8)	37 **(5.1)**
TOTAL	No information collected	538 (100.0)	181 (100.0)	719 (100.0)

DISCUSSION

According to the 2007 ***Philippines Environment Monitor,*** the most significant environment-related health risks in the Philippines are air and water pollution, and the lack of good sanitation and hygiene practices. Environment–related diseases account for an estimated 22% of the reported disease cases and 6% of reported deaths, and cost PhP14.3 billion (approximately US$287 million) per year in lost income and medical expenses.

The report also states that water pollution and poor sanitation conditions, which cause diseases such as diarrhea, typhoid, cholera, and intestinal worms, are estimated to account for 17% of reported disease cases and 1.5% of the reported deaths in the Philippines.

This study focused on the detection of enteric protozoa (*Giardia* and *Cryptosporidium*), as these are the most common causative agents of water-borne disease outbreaks.

We collected 6302 diarrheic stool samples from all major islands in the Philippines from May 2004 to January 2008. There was a break in our collection period from July-December 2005. We completed a one year collection period from May 2004 to June 2005, and an interim analysis was done.

The 3-year collection included in this report included a wide range of ages from less than 0 to 98 years, from both genders, and from both rural and urban communities. The collection in Mindanao was not carried out in 2006 and 2007 due to the requirement of fresh stool specimens for the DNA extraction. This was not possible in view of transport limitations from the island to St. Luke's Medical Center in Quezon City.

In the present study, the presence of enteric protozoa across all ages regardless of gender, in both rural and urban areas in the Philippines, highlights the significance of safe drinking water supply and sanitation in public health programs.

In this report, the overall prevalence of *G. lamblia* is 1.49% and that of *Cryptosporidium* spp. is (1.46%). Our molecular studies show that the predominant species of *Cryptosporidium* in our collection are *C. hominis* and *C. parvum*. This may indicate that both anthroponotic and zoonotic transmission occur. Likewise, this underscores the importance not only of sanitation but also environmental quality monitoring in preventive measures.

Higher prevalence rates had been reported in the past, depending on the study population. Previous studies done on *Giardia* gave 17.6, (Bustos *et al.*, 1991), 11.6 (Baldo *et al.*, 2004), 9.3% (Rivera *et al.*, 2006) in various institutions. In children aged 8 months to 15 years, a 20% prevalence was found (Auer, 1990), while Lee *et al.*(2000) found a prevalence of 7.8% in children and adolescents.

On the other hand, 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% in a university hospital (Paje-Villar *et al.*, 1993).

The prevalence for *Cryptosporidium* of 1.46% in this study is lower than those obtained by Jueco *et al.* (1991) who reported a prevalence of 1.8% in patients of all ages and by Cross *et*

al. (1985) among patients aged 1 month to 75 years. While our study is 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 are mainly on its prevalence in children. This ranges from 2.5% [0-2 years old] (Kainama, 1989), 2.54% [less than 12 years old] (Paje-Villar *et al.*, 1994), 2.9% [6-20 months old] (16), 2.8 % [0-5 years old] (Jueco *et al.*1991), 4% [7-19 months old] (Capeding and Saniel, 1990), 7.1% [6-27 months old] (Carlos *et al.*, 1992), to 8.5% [7-24 months old] (Laxer *et al.*, 1990). In this study, the prevalence (2.92%) of *Cryptosporidiuim* in patients 0-18 years old, is within the range previously reported.

In the last few years, there has been some progress in addressing the need for access to safe drinking water and sanitary toilets. Private water concessionaires in Manila have provided water supply connections, especially to the urban poor. Investment in sanitary toilets and increases in household water supply connections have contributed to the reduction in many water-borne diseases such as diarrhea. There are now 43% fewer reported cases of diarrhea nationally compared to 10 years ago.

Results of the present study indicate a low incidence of the 2 enteric protozoa (*Giardia* and *Cryptosporidium*). While this low incidence might indicate a greater awareness of the importance of sanitation for ensuring safe drinking water, the infrastructure for health still needs to be improved. Improvement can be done by filling in the gaps in basic sanitation, expanding water resource development, and reducing the health risks of polluted surface and groundwater (2007 ***Philippines Environment Monitor***).

RECOMMENDATIONS

Our report seeks to increase awareness on the part of government agencies, health practitioners, civil society organizations, and the general public to help improve the health of Filipinos through a better environment. Good quality environment can bring about better sanitation and safe drinking water for the Filipino people. Our results indicate that there are still gaps in basic sanitation as well as in water resource development, thus there is a need

- to improve infrastructure for health
- to expand water resource development, and
- to reduce pollution of surface and ground water. through environmental regulation

REFERENCES

Auer C. (1990): Health status of children living in a squatter area of Manila, Philippines, with particular emphasis on intestinal parasitoses. Southeast Asian J. Trop. Med. Public Health, 21, 289-300.

Adkins, H. J., Escamilla, J., Santiago, L. T., Ranoa, C., Echeverria, P. and Cross, J. H. (1987): Two-year survey of etiologic agents of diarrheal disease at San Lazaro Hospital, Manila, Republic of the Philippines. J. Clin. Microbiol., 25, 1143-1147.

Baldo, E.T., Belizario, V.Y., De Leon, W.U., Kong, H.H. and Chung, D.I. (2004): Infection status of intestinal parasites in children living in residential institutions in Metro Manila, the Philippines. Korean J. Parasitol., 42, 67-70.

Bustos, M. D., Salazar, N., Espino, F. E. C., Montalban, C., Sabordo N. and Laurente, M. (1991): Ornidazole in the treatment of giardiasis in an institution for the mentally retarded. Phil. J. Microbiol. Infect. Dis., 20, 13-16

Capeding, M.R. and Saniel, M.C. (1990): Cryptosporidiosis in Filipino children: association with acute diarrhea. Philipp. J. Microbiol. Infect. Dis., 19, 49-50.

Carlos, C.C. and Saniel, M.C. (1990): Etiology and Epidemiology of Diarrhea. Philipp J Microbiol Infect Dis 19 (2): 51-53.

Carlos, C. C., Saniel, M. C., Lucero, G. M. and Evangelista, T. A. (1992): Enteropathogens among measles patients with diarrhea in urban Filipino children. Philipp. J. Microbiol. Infect. Dis., 21, 53-60.

Herwaldt, B.L., Craun, G.F., Stokes, S.L., and Juranek, D.J. (1992): Outbreaks of waterborne-disease in the United States: 1989-1990. J. Am. Water Works Assoc. 84:129-135.

Homan, W.L., Gilsing, M., Bentala, H., Limper, L., and van Knapen, F. (1998): Characterization of *Giardia duodenalis* by polymerase-chain-reaction fingerprinting. Parasitol Res, 84:707-714.

Jueco, N. L., Belizario, V. Y. Jr., de Leon, W. U., Bravo, L. C. and Gregorio, G. V. (1991): Cryptosporidiosis among selected patients in the Philippine General Hospital. Acta Medica Philippina, 27, 244-247.

Kainama, J. M. (1989): Prevalence of cryptosporidiosis among young children zero to two year old: a hospital-based study. Phil. Women's Univ. Forum, 4, 108.

Laxer, M. A., Alcantara, A. K., Javato-Laxer, M., Menorca, D. M, Fernando, M. T. and Ranoa, C. P. (1990): Immune response to cryptosporidiosis in Philippine children. Am. J. Trop. Med. Hyg., 42, 131-139.

Lee, K-J., Ahn, Y-K., and Yong, T-S. (2000): A small-scale survey of intestinal parasite infections among children and adolescents in Legaspi city, the Philippines. Korean J. Parasitol., 38, 183-185.

Mclauchlin, J., Amar, C., Pedraza-Dias, S., and Nichols, G.L. (2000): Molecular epidemiological analysis of *Cryptosporidium* spp. in the United Kingdom: results of genotyping *Cryptosporidium* spp. in 1705 fecal samples from humans and 105 fecal samples from livestock animals. J. Clin. Microbiol. 38(11):3984-3990.

Paje-Villar, E., Co, B., Carandang, E., Raymundo A., Lagamayo E., Hart, C. A., Lavadia E., and Rigor E. (1993): Diarrheas among Filipino infants and children: clinical and laboratory correlations. Philipp. J. Pediatr., 42, 1-22.

Rivera, W.L., Yason, J.AD.L. and Rivera, P.T. (2005): Serological detection of cryptosporidiosis among Filipino cancer patients. Parasitol. Res., 98, 75-76.

Rivera, W.L., Santos, S.R., and Kanbarra, H. (2006): Prevalence and genetic diversity of *Entamoeba histolytica* in an institution for the mentally retarded in the Philippines. Parasitol. Res. 98, 106-110.

Saniel, M.C., Morilles, R.R., Monzon, O.T. *et al.* (1988): The relative importance of various enteropathogens in the etiology of acute diarrhea: a hospital-based study in urban Philippines. In: SEAMIC Proceedings of the 14th SEAMIC workshop: gastro-intestinal infections in Southeast Asia (V). SEAMIC. Southeast Asian Medical Information Center/International Medical Foundation of Japan. 91-96.

Saniel, M.C., Sta. Maria, A., Sanvictories, E. *et al.* (1985): Prospective study of diarrhea in infants and young children or a peri-urban community; morbidity pattern and etiologies, In: Tzipori, S. ed. Infectious Diarrhea in the Young: Strategies for Control in Humans and Animals. The Nethernalnds: Elsevier. 113-116.

Xiao, L., Morgan, U.M., Limor, J., Escalante, A., Arrowood, M., Shulaw, W., Thompson, R.C., Fayer, R., and Lal, A.A. (1999): Genetic Diversity within *Cryptosporidium parvum* and related *Cryptosporidium* species. Appl Environ Micobiol. 65(8):3386-91.

Yagita, K., Izumiyama, S., Tachibana, H., Masuda, G., Iseki, M., Furuya, K., Kameoka, Y., Kuroki, T., Itagaki, T. and Endo, T. (2001): Molecular characterization of Cryptosporidium isolates obtained from human and bovine infections in Japan. Parasitol. Res. 87(11):950-5

Yason, J.A.D.L, and Rivera W.L. (2007): Genotyping of *Giardia duodenalis* isolates among residents of slum are in Manila, Philippines. Parasitol. Res. (2007) 101:681-687.

APPENDICES

Appendix Ia. Administrative Order 2007-0012.

Republic of the Philippines
Department of Health
OFFICE OF THE SECRETARY
San Lazaro Compound, Rizal Avenue, Sta. Cruz, Manila, Philippines 1003
Tel. No. (632) 743-8601 locals 1107, 1125; (632) 711-9502/03;
TeleFax: (632) 743-1829
Email Address: osec@doh.gov.ph Website: http://www.doh.gov.ph

09 March 2007

ADMINISTRATIVE ORDER
No. 2007- 0012

SUBJECT: Philippine National Standards for Drinking Water 2007

I. RATIONALE/INTRODUCTION

Access to safe drinking water is not only essential for the promotion and protection of public health but is a basic human right. Provision of safe water supply prevents the transmission of waterborne pathogens and reduces the exposure of individuals to chemical and physical hazards that could be ingested through contaminated drinking water. Diarrheas and other waterborne diseases still rank among the leading causes of illnesses in the country. It is apparent that continuous development or refinement of policies and programs geared towards minimizing the risk of contracting waterborne diseases should be supported to provide optimal health service for the population.

Setting standards for drinking water establishes threshold limits for different impurities found in drinking water. These limits are intended to minimize risk and therefore prevent deleterious health repercussions that result from lifelong exposure to these impurities through consumption of water. The Department of Health is mandated to formulate standards to this effect. Chapter II (Water Supply), Section 9 of the Code on Sanitation of the Philippines states that "Standards for drinking water and their microbiological and chemical examinations, together with the evaluation of results, shall conform to the criteria set by the National Drinking Water Standards."

The government recognizes recent quality-related developments in the water supply sector in the country and elsewhere such as the following:

1. New information on many chemicals. As an outcome of evolving agricultural, industrial and even domestic practices, new chemicals find their way into the environment and contaminate drinking water sources

2. Proliferation of water refilling stations as alternative (or main) sources of drinking water. The quality of "processed" water from these stations may require distinct standards compared to the water from large water systems.

Appendix Ib. Ten Leading Causes of Morbidity in the Philippines, 2004-2006.

Disease	Number of cases in 2004	Disease	Number of cases in 2005	Disease	Number of cases in 2006
ALRI* and pneumonia	776,562	ALRI* and pneumonia	690,566	ALRI* and pneumonia	670,231
Bronchitis/ bronchiolitis	719,982	Bronchitis/ bronchiolitis	616,041	**Acute watery diarrhea**	572,259
Acute watery diarrhea	577,118	**Acute watery diarrhea**	603,287	Bronchitis/ bronchiolitis	538.990
Influenza	379,910	Influenza	406,237	Hypertension	408,460
Hypertension	342,284	Hypertension	382,662	Influenza	339,881
TB respiratory	103,214	TB respiratory	114,360	TB respiratory	132,725
Chickenpox	46,779	Diseases of the heart	43,898	Diseases of the heart	38,482
Diseases of the heart	37,092	Malaria	36,090	Acute febrile illness	25,400
Malaria	19,894	Chickenpox	30,063	Malaria	22,284
Dengue fever	15,838	Dengue fever	20,107	Dengue fever	15,279

*Acute lower respiratory tract infection

Source: Annual Report, National Epidemiology Center, Department of Health