SEASONAL VARIATION AND POTENTIAL SOURCES OF *CRYPTOSPORIDIUM* CONTAMINATION IN SURFACE WATERS OF CHAO PHRAYA RIVER AND BANG PU NATURE RESERVE PIER, THAILAND

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Abstract. Using molecular techniques, a longitudinal study was conducted with the aims at identifying the seasonal difference of *Cryptosporidium* contamination in surface water as well as analyzing the potential sources based on species information. One hundred forty-four water samples were collected, 72 samples from the Chao Phraya River, Thailand, collected in the summer, rainy and cool seasons and 72 samples from sea water at Bang Pu Nature Reserve pier, collected before, during and after the presence of migratory seagulls. Total prevalence of *Cryptosporidium* contamination in river and sea water locations was 11% and 6%, respectively. The highest prevalence was observed at the end of rainy season continuing into the cool season in river water (29%) and in sea water (12%). During the rainy season, prevalence of *Cryptosporidium* was 4% in river and sea water samples, but none in summer season. All positive samples from the river was *C. parvum*, while *C. meleagridis* (1), and *C. serpentis* (1) were obtained from sea water. To the best of our knowledge, this is the first genetic study in Thailand of *Cryptosporidium* spp contamination in river and sea water locations and the first report of *C. serpentis*, suggesting that humans, household pets, farm animals, wildlife and migratory birds may be the potential sources of the parasites. The findings are of use for implementing preventive measures to reduce the transmission of cryptosporidiosis to both humans and animals.

Keywords: *Cryptosporidium* species, river and sea waters, seasonal variation, potential sources, Thailand

INTRODUCTION

Cryptosporidium spp contamination in water source is a major concern. Due to its relative low infective dose (30 oocysts) and the robustness of oocysts in difficult environments, this parasite has become an important waterborne diarrheal causative agent (DuPont *et al*, 1995; Carey *et al*, 2004). Cryptosporidiosis has an impact on both public health and economy of 106 countries (Xiao and Fayer, 2008). The Milwaukee outbreak in 1993, for example, infected 403,000 individuals with more than 100 deaths, costing 92.6 million US

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dollars (Mac Kenzie *et al*, 1994; Corso *et al*, 2003). Water contamination was incriminated as the major route of transmission. Since the various causes cryptosporidiosis have been reported worldwide, resulting in health authorities, especially in the US and European countries, paying high attention on *Cryptosporidium* contamination in water.

The concentration of *Cryptosporidium* in water depends on such factors as seasonal variations affected by runoff water and difference of water temperature, source distribution and species of parasite, and type of water usage (*viz*. household, agriculture or waste). Thus an understanding of the nature of water source of *Cryptosporidium* contamination as well as the original source is very useful for appropriate water treatment and efficacious management.

The Chao Phraya River flows from the northern part of Thailand, through the capital city, Bangkok in the central region, before emptying into the Gulf of Thailand at Samut Prakan Province. The river is the source of water supply for approximately 12 million people in Bangkok and the surrounding provinces. At the mouth of Chao Phraya River, Bang Pu in Samut Prakan Province is where >10,000 seagulls migrate every year from China and remain during the cool season. There are three seasons in Thailand: summer season from March to May with an average temperature of 33-36ºC, rainy season from June to October with average temperature of 28-32ºC, and cool season from November to February with average temperature of 24-28ºC.

At present, there are 20 species of *Cryptosporidium* reported among humans, domestic animals and wild vertebrates (Plutzer and Karanis, 2009; Cabada and White, 2010) but only 7 species have been identified as being zoonotic, namely, *C. parvum, C. hominis, C. meleagridis*, *C. canis*, *C. felis, C. suis* and *C. muris* (Xiao and Frayer, 2008), of which the former three are of major concern with *C. parvum* and *C. hominis* being responsible for >90% of human cryptosporidiosis (Xiao and Ryan, 2004). Although 7 species of *Cryptosporidium* (*C. parvum, C. hominis, C. meleagridis, C. canis, C. felis*, *C. muris* and *C. parvum* bovine genotype 5) have been reported in HIV patients in Bangkok (Gatei *et al*, 2002; Tiangtip and Jongwutiwes, 2002; Saksirisampant *et al*, 2009), epidemiological studies concerning sources of such protozoa, transmission vehicle or zoonotic potential are limited in Thailand. Hitherto, there is no study concerning *Cryptosporidium* spp contamination in river and sea waters especially during the three seasons. Therefore, we conducted a longitudinal study with the aims at identifying seasonal variations of *Cryptosporidium* contamination in two settings of surface water, along the Chao Phraya River and sea, as well as analyzing the potential source of the contamination using species or genotype identification.

MATERIALS AND METHODS

Study sites

The study sites were 3 districts located at the north, central and south of Chao Phraya River, namely, Bang Sue with population of 139,750, Samphan Thawong with 28,762 and Rat Burana with 89,610 (wikipedia.org/wiki/List_of_Districts_of_Bangkok). Households occupying by humans and domestic pets as well as farm animal husbandry and wildlife along the river are potential sources of contaminants. In addition, the Bang Pu Nature Reserve pier, located about 12 km east of the town center of Samut Prakan Province, where more than 10,000 seagulls migrate annually from China after visiting Myanmar or Cambodia during the cool season was chosen.

Water samples

At the study sites, 20 liters of water samples were collected in plastic containers at 3 districts located along the Chao Phraya River during the 3 seasons: from November 2010 to February 2011 (cool season), March 2011 to May 2011 (summer) and from June 2011 to October 2011 (rainy season). Twenty liters of samples were collected at Bang Pu Nature Reserve pier during: 1) presence of migratory seagulls (January to March 2011), 2) absence of the birds (May to July 2011) and 3) prior to presence of migratory seagulls (August to October 2011). Temperature, turbidity and pH of each water sample were immediately determinded and recorded, and then all water samples were transported to the Department of Protozoology, Faculty of Tropical Medicine, Mahidol University, Bagkok, Thailand for further laboratory processing.

Isolation of *Cryptosporidium* from water samples

Twenty liters of each water sample were filtered through cellulose acetate membrane, 1.2 m pore size (PALL Life Science, Ann Arbor, MI). The plastic container was washed with 1 liter of washing buffer solution pH 7.2 (Graczyk *et al*, 1997) and filtered through the same filter membrane. The filter membrane was washed with 10 ml of washing buffer and mixed with sucrose solution to generate a final solution with specific gravity of 1.3 (Jenkins *et al*, 2010). The mixture was then left to stand for 10 minutes before centrifugation at 1,000*g* for 10 minutes at 8ºC. *Cryptosporidium* oocysts, if any, will float to the top of the solution and 15 ml of the upper layer were added to 30 ml of distilled water to make the specific gravity of solution of 1.0, which then was centrifuged at 2,700*g* for 15 minutes at room temperature. The sedimented samples were subjected for DNA amplification.

Amplification of *Cryptosporidium* DNA by nested PCR

The sedimented sample was suspended in 100 l of phosphate-buffered saline buffer. DNA was extracted using QIAmp DNA mini kit (Qiagen, Hilden, Germany) according to the manufacturer's protocols. The suspension was subjected to lysis by 5 cycles of freezing at -83ºC for 10 minutes and thawing at 56ºC for 5 minutes. The extracted DNA was stored at -20ºC until used. Nested PCR employed specific primers targeting 18S rRNA gene was carried out (Xiao *et al*, 1999, 2004b). In brief, 4 volumes (0.5 l to 3 l) of DNA solution were made up to 100 l reaction volume containing 200 M each dNTP, 200 nM each primary primer, 6 mM $MgCl_2$, 400 ng/ l non-acetylated BSA, 2.5 U *Taq* polymerase, and 1X PCR buffer. Primary PCR conditions consisted of a total of 35 cycles of 94ºC for 45 seconds, 55ºC for 45 seconds, and 72ºC for 60 seconds. The initial hot start was at 94ºC for 3 minutes and final step at 72ºC for 7 minutes. For the second PCR, BSA was not added, and double concentrations of each second primers (400 nM) were added, and 2 l of the primary amplicon template were used. Thermocycling conditions were identical to those of the primary PCR except that the annealing temperature was increased to 58ºC. Amplicons were visualized on 1.5% agarose gel with ethidium bromide under UV light. The primary amplicon was about 1,500 bp and second round amplicon was about 823 bp. These primers are specific for genus *Cryptosporidium* allowing detection of all species of *Cryptosporidium*.

Sequencing and genotyping

The nested PCR amplicons were purified by PureLink Quick Gel Extraction kit (Invitrogen, Carlsbad, CA) and sequenced using the second primer pair (Pacific Science, Bangkok, Thailand). The sequences were compared with published sequences of *Cryptosporidium* species and genotypes deposited at the NCBI (http://www.ncbi.nlm.nih.gov/BLAST/). The reference accession number for *C. parvum* were HQ651731.1, AB513881.1, *C. meleagridis* AJ493205.1 and *C. serpentis* AF093502.1.

RESULTS

Altogether 144 samples, 72 from the Chao Phraya River and 72 from Bang Pu Nature Reserve pier, were collected. The river and sea water samples were similar in terms of pH and temperature, but the mean turbidity was 3 times higher in sea water samples than in river water samples with the highest mean turbidity of the former of 188.6 (34.1-403.0) NTU during the period following the presence of seagulls and 79.8 (44.2-153.5) NTU in the latter in the summer season (Table1).

Total prevalence of *Cryptosporidium* contamination was 11% (8/72) in the river water samples and 6% (4/72) in the sea samples. The highest contamination (29%) of *Cryptosporidium* spp found in the Chao Phraya River samples was during the cool season and 12.5% in sea water during the presence of seagulls (January to March which was also the cool season) (Table 1). Low *Cryptosporidium* contamination (4%) was found during the rainy season in river water and in sea water before the presence of seagulls, but none was found in the summer and after the birds had gone, respectively. The highest prevalence of *Cryptosporidium* spp contamination was when the average temperature was 28.3ºC.

The distribution of *Cryptosporidium* spp along the Chao Phraya River indicated that the highest prevalence (17%) was at the northern part of Bangkok (Bang Sue District) where there is a moderate population density (Table 2). Water samples from both Samphanthawong District, with the highest population density, at the central and Rat Burana District, with the lowest population density, in the southern part of Bangkok were contaminated with *Cryptosporidium* spp at 8.3%. From PCR sequencing, all 8 positive samples from the Chao Phraya River were identified as *C. parvum*, whereas of the 4 positive samples from sea water, were 2 *C. parvum* and 1 each of *C. meleagridis* and *C. serpentis*.

DISCUSSION

In the present study, the overall prevalence of *Cryptosporidium* spp contamination in the Chao Phraya River was twice that in sea water at Bang Pu, Samut Prakarn Province which was in agreement with a previous study in the southwest costal of Thailand (12% of water samples were contaminated with *Cryptosporidium*) (Srisuphanunt *et al*, 2010). In another study in Thailand using 1,000 liters of underground water, a combination of immunomagnetic separation (IMS) and fluorescence assay revealed a higher *Cryptosporidium* contamination (35%) (Sutthikornchai *et al*, 2005). Temperate zone countries showed high contamination levels of up to 93% (Yang *et al*, 2008; Xiao *et al*, 2011; Budu-Amoaka *et al*, 2012a,b). These different results might not only reflect actual difference in *Cryptosporidium* contamination levels from various loca-

Table 1
Seasonal prevalence of *Cryptosporidium* spp contamination and physical properties of Chao Phraya River and sea water of Thailand during 2010 - 2011.

Sample source	Season	Turbidity (NTU) Mean (min-max)	Temperature (°C) (mean ± SD)	pH (mean ± SD)	No. of positive samples/total (%)	Identified species (No. of samples)
The Chao Phraya River	Summer[a]	79.8 (44.2 - 153.5)	31.1 ± 0.4	7.04 ± 0.02	0/24	-
	Rainy[b]	66.8 (26.0 - 192.0)	30.5 ± 0.4	7.23 ± 0.01	1/24 (4%)	(99% identity with HQ651731.1) *C. parvum* (1)
	Cool[c]	9.4 (3.3 - 76.8)	28.3 ± 1.2	6.61 ± 0.25	7/24 (29%)	(99% identity with AB513881.1) *C. parvum* (7)
Total					8/72 (11%)	
	Presence of seagulls					
Sea water	Before[d]	188.6 (34.1 - 403.0)	31.7 ± 0.6	7.52 ± 0.01	1/24 (4%)	(85% identity with AJ493205.1) *C. meleagridis* (1)
	During[e]	81.0 (11.4 - 270.0)	30.1 ± 2.3	6.48 ± 0.39	3/24 (12%)	(99% identity with JN247404.1) *C. parvum* (2) (99% identity with AF093502.1) *C. serpentis* (1)
	After[f]	247.3 (44.3 - 529.0)	31.2 ± 0.7	6.39 ± 0.04	0/24	-
Total *Cryptosporidium* contamination in sea water					4/72 (6%)	

[a]March - May, [b]June - October, [c]November - February, [d]August - October, [e]January - March, [f]May - July

Table 2
Prevalence of *C. parvum* contamination in Chao Phraya River in different seasons and locations.

District of Bangkok	Population density[a] (persons/km^2)	No. of positive (%)			No. of positive samples/total
		Summer (March-May)	Rainy season (June-Oct)	Cool season (Nov-Feb)	
Bang Sue (North)	13,040	-	1/8 (12.5%)	3/8 (37%)	4/24 (17%)
Samphan Thawong (Central)	21,993	-	-	2/8 (25%)	2/24 (8%)
Rat Burana (South)	5,928	-	-	2/8 (25%)	2/24 (8%)

[a]Jitramontree, 2008

tions, but also the difference in techniques used. We used a 20 liters water sample, which is the requirement of surface water sample volume recommended by the USEPA method 1623 (Yang *et al*, 2008). The sucrose floatation technique used was as effective as the IMS method (Koompapong *et al*, 2009).

The highest water turbidity was observed in the summer season in both river and sea water samples, and was higher than in other studies (Yang *et al*, 2008; Srisuphanunt *et al*, 2010). Particles tubidity in water may affect purification processes. Because the normal specific gravity of *Cryptosporidium* oocyst is 1.009-1.08, adhesion of oocysts to particles may increase the specific gravity of the oocysts resulting in less floatation (Dumetre *et al*, 2011), which will decrease the recovery rate. However, the sucrose solution used in this study has a specific gravity of 1.3, which is high enough to float oocysts with attached particles. Temperature and pH of water samples may have little effects on the recovery rate as temperature and pH of the water samples in this study differed from other studies (Fayer, 1994; Li *et al*, 2010).

Studies have indicated that the highest contamination of *Cryptosporidium* is in the rainy season, since the runoff water carries the parasite into surface water (Kaucner *et al*, 2005; Cizek *et al*, 2008; Miller *et al*, 2008). However, large amounts of runoff water can also dilute oocyst concentration. In Thailand, the rainy season in 2011 caused severe flooding in the north and central regions of Thailand as well as in Bangkok and including the Chao Phraya River. This could account for the 4.2% *Cryptosporidium* spp concentration in this season. Thus by the end of the rainy season and continuing into the cool season, the highest contamination level (29%) was found.

The highest contamination level of *Cryptosporidium* (12.5%) in sea water samples corresponded with the presence of migratory seagulls. The absence of *Cryptosporidium* in sea water after the departure of the birds might be attributed to high turbidity of the water causing low recovery rate.

The upper stream of the Chao Phraya River in Bangkok had twice the contamination prevalence than in the middle and lower stream. The upper stream receives the water from many tributaries from the northern part of Thailand, which run through animal farms, communities, and forests thus facilitating *Cryptosporidium* oocyst contamination. In the central region the highest population density of Bangkok (~22,000 persons/km^2 in Samphanthawong District) should have contributed to *Cryptosporidium* spp contamination of the Chao Phraya River more than other parts of Bangkok. However, contamination in this area and in the lower part was lower than in the upper part of Bangkok. This might be due to the waste water management being better in this area than in other parts of Bangkok.

All positive water samples in Chao Phraya River were contaminated with *C. parvum*, the zoonotic and the most common causative agent of cryptosporidiosis (Xiao *et al*, 2004a). Reports from Thailand have revealed that patients and cattle are infected with *C. parvum* (Gatei *et al*, 2002; Jittapalapong *et al*, 2006; Nuchjangreed *et al*, 2008; Saksirisampant *et al*, 2009; Inpankaew *et al*, 2010). Thus domestic animals, wildlife, farm animals and humans are potential sources of *C. parvum* in the river.

Mammalian animals and humans can be the sources of *C. parvum* in sea water. Avian species are potential sources of *C. meleagridis*. *C. serpentis* is recognized as being snake and lizard parasite (Xiao *et al*, 2004a). To the best of our knowledge, this is the first report of *C. serpentis* in Thailand sea water.

In summary, this is the first study of the seasonal variation of *Cryptosporidium* contamination and species in Chao Phraya River and marine water of Thailand. The cool season (from November to February) favored contamination than other seasons. Three species, *C. parvum*, *C. meleagridis* and *C. serpentis* were identified in marine water, but only *C. parvum* in river water. Further studies are needed to identify the original hosts of *Cryptosporidium* spp present in the water sources. The results of this study indicate that Bangkok water authorities should be aware of potential cryptosporidiosis outbreak among the 12 million citizens by paying attention to water treatment processes. Appropriate preventive measures utilizing the epidemiological findings of the present study will be beneficial in reducing the transmission of human and animal cryptosporidiosis.

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REFERENCES

Budu-Amoako E, Greenwood SJ, Dixon BR, *et al*. Occurrence of *Cryptosporidium* and *Giardia* on beef farms and water sources within the vicinity of the farms on Prince Edward Island, Canada. *Vet Parasitol* 2012a; 184: 1-9.

Budu-Amoako E, Greenwood SJ, Dixon BR, *et al*. Occurrence of *Giardia* and *Cryptosporidium* in pigs on Prince Edward Island, Canada. *Vet Parasitol* 2012b; 184: 18-24.

Cabada MM, White AC Jr. *Treatment* of cryptosporidiosis: do we know what we think we know? *Curr Opin Infect Dis* 2010; 23: 494-9.

Carey CM, Lee H, Trevors JT. Biology, persistence and detection of *Cryptosporidium parvum* and *Cryptosporidium hominis* oocyst. *Water Res* 2004; 38: 818-62.

Cizek AR, Characklis GW, Krometis LA, *et al*. Comparing the partitioning behavior of *Giardia* and *Cryptosporidium* with that of indicator organisms in stormwater runoff. *Water Res* 2008; 42: 4421-38.

Corso PS, Kramer MH, Blair KA, Addiss DG, Davis JP, Haddix AC. Cost of illness in the 1993 waterborne *Cryptosporidium* outbreak, Milwaukee, Wisconsin. *Emerg Infect Dis* 2003; 9: 426-31.

Dumetre A, Aubert D, Puech PH, Hohweyer J, Azas N, Villena I. Interaction forces drive the environmental transmission of pathogenic protozoa. *Appl Environ Microbiol* 2012; 78: 905-12.

DuPont HL, Chappell CL, Sterling CR, Okhuysen PC, Rose JB, Jakubowski W. The infectivity of *Cryptosporidium parvum* in healthy volunteers. *N Engl J Med* 1995; 332: 855-9.

Fayer R. Effect of high temperature on infectivity of *Cryptosporidium parvum* oocysts in water. *Appl Environ Microbiol* 1994; 60: 2732-5.

Gatei W, Suputtamongkol Y, Waywa D, *et al*. Zoonotic species of *Cryptosporidium* are as prevalent as the anthroponotic in HIV-infected patients in Thailand. *Ann Trop Med Parasitol* 2002; 96: 797-802.

Graczyk TK, Cranfield MR, Fayer R. Recovery of waterborne oocysts of *Cryptosporidium* from water samples by the membrane-filter dissolution method. *Parasitol Res* 1997; 83: 121-5.

Inpankaew T, Jiyipong T, Pinyopanuwat N, Chimnoi W, Thompson RC, Jittapalapong S. Prevalence and genotyping of *Cryptosporidium* spp. from dairy cow fecal samples in western Thailand. *Southeast Asian J Trop Med Public Health* 2010; 41: 770-5.

Jenkins MB, Liotta JL, Lucio-Forster A, Bowman DD. Concentrations, viability, and distribution of *Cryptosporidium* genotypes in lagoons of swine facilities in the Southern Piedmont and in coastal plain watersheds of Georgia. *Appl Environ Microbiol* 2010; 76: 5757-63.

Jitramontree P. Contamination of *Giardia* and *Cryptosporidium* in the Chao Phraya River. Bangkok, Mahidol University, 2008. Thesis. 231 pp.

Jittapalapong S, Pinyopanuwat N, Chimnoi W, Siripanth C, Stich RW. Prevalence of *Cryptosporidium* among dairy cows in Thailand. *Ann N Y Acad Sci* 2006; 1081: 328-35.

Kaucner C, Davies CM, Ferguson CM, Ashbolt NJ. Evidence for the existence of *Cryptosporidium* oocysts as single entities in surface runoff. *Water Sci Technol* 2005; 52: 199-204.

Koompapong K, Sutthikornchai C, Sukthana Y. *Cryptosporidium* oocyst detection in water samples: floatation technique enhanced with immunofluorescence is as effective as immunomagnetic separation method. *Korean J Parasitol* 2009; 47: 353-7.

Li X, Atwill ER, Dunbar LA, Tate KW. Effect of daily temperature fluctuation during the cool season on the infectivity of *Cryptosporidium parvum*. *Appl Environ Microbiol* 2010; 76: 989-93.

Mac Kenzie WR, Hoxie NJ, Proctor ME, *et al*. A massive outbreak in Milwaukee of cryptosporidium infection transmitted through the public water supply. *N Engl J Med* 1994; 331: 161-7.

Miller WA, Lewis DJ, Pereira MD, *et al*. Farm factors associated with reducing *Cryptosporidium* loading in storm runoff from dairies. *J Environ Qual* 2008; 37: 1875-82.

Nuchjangreed C, Boonrod K, Ongerth J, Karanis P. Prevalence and molecular characterization of human and bovine *Cryptosporidium* isolates in Thailand. *Parasitol Res* 2008; 103: 1347-53.

Plutzer J, Karanis P. Genetic polymorphism in *Cryptosporidium* species: an update. *Vet Parasitol* 2009; 165: 187-99.

Saksirisampant W, Prownebon J, Saksirisampant P, Mungthin M, Siripatanapipong S, Leelayoova S. Intestinal parasitic infections: prevalences in HIV/AIDS patients in a Thai AIDS-care centre. *Ann Trop Med Parasitol* 2009; 103: 573-81.

Srisuphanunt M, Karanis P, Charoenca N, Boonkhao N, Ongerth JE. *Cryptosporidium* and *Giardia* detection in environmental waters of southwest coastal areas of Thailand. *Parasitol Res* 2010; 106: 1299-306.

Sutthikornchai C, Jantanavivat C, Thongrungkiat S, Harnroongroj T, Sukthana Y. Protozoal contamination of water used in Thai frozen food industry. *Southeast Asian J Trop Med Public Health* 2005; 36 (suppl 4): 41-5.

Tiangtip R, Jongwutiwes S. Molecular analysis of *Cryptosporidium* species isolated from HIV-infected patients in Thailand. *Trop Med Int Health* 2002; 7: 357-64.

Xiao L, Escalante L, Yang C, *et al*. Phylogenetic analysis of *Cryptosporidium* parasites based on the small-subunit rRNA gene locus. *Appl Environ Microbiol* 1999; 65: 1578-83.

Xiao L, Fayer R, Ryan U, Upton SJ. *Cryptosporidium* taxonomy: recent advances and implications for public health. *Clin Microbiol Rev* 2004a; 17: 72-97.

Xiao L, Feng Y. Zoonotic cryptosporidiosis. *FEMS Immunol Med Microbiol* 2008; 52: 309-23.

Xiao L, Lal AA, Jiang J. Detection and differentiation of *Cryptosporidium* oocysts in water by PCR-RFLP. *Methods Mol Biol* 2004b; 268: 163-76.

Xiao L, Ryan UM. Cryptosporidiosis: an update in molecular epidemiology. *Curr Opin Infect Dis* 2004; 17: 483-90.

Xiao S, An W, Chen Z, Zhang D, Yu J, Yang M. Occurrences and genotypes of *Cryptosporidium* oocysts in river network of southern-eastern China. *Parasitol Res* 2012; 110: 1701-9.

Xiao L, Fayer R. Molecular characterisation of species and genotypes of *Cryptosporidium* and *Giardia* and assessment of zoonotic transmission. *Int J Parasitol* 2008; 38: 1239-55.

Yang W, Chen P, Villegas EN, *et al*. *Cryptosporidium* source tracking in the Potomac River watershed. *Appl Environ Microbiol* 2008; 74: 6495-504.