The Risk of Cryptosporidiosis from Drinking Water

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Cryptosporidium spp. are intracellular protozoan parasites that are common in many animals including mammals, marsupials, reptiles, birds, and fish. The environmentally resistant thick-walled oocyst stage of the organism's life cycle is excreted in the feces of infected animals and can contaminate sources of drinking water. Although the disease is usually self-limiting in otherwise healthy humans, persistent infection can contribute to mortality in individuals with weakened immune systems. There have been many outbreaks of cryptosporidiosis associated with either drinking water or recreational water (Fayer et al., 1997; Fayer et al., 2000); the largest waterborne outbreak on record occurred in 1993 in Milwaukee with estimates of the affected population ranging from 15,000 to 400,000 individuals and up to 100 deaths (Hunter and Syed, 2001; MacKenzie et al., 1994). The continued detection of *Cryptosporidium* oocysts in source water and treated drinking water ensures that the organism remains a significant concern for the water industry and mandated monitoring under the Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR; USEPA, 2006) will determine whether water utilities need to install additional treatment based on the level of *Cryptosporidium* in their source water.

The genus *Cryptosporidium* contains at least 16 recognized species that infect a variety of vertebrates. The organisms are coccidian parasites placed within the Phylum Apicomplexa. (Fayer et al., 2008) Although *C. parvum* and *C. hominis* are the species most often isolated from humans, other species have also been detected in immune-compromised individuals. These include *C. canis*, *C. felis*, *C. meleagridis*, and *C. muris* (Fayer et al., 2001; Gatei et al., 2002; Morgan et al., 2000; Morgan-Ryan et al., 2002; Pedraza-Diaz et al., 2001; Pieniazek et al., 1999; Xiao et al., 2001). However, most cases of human cryptosporidiosis are attributed to *C. parvum* and *C. hominis*. Infections in humans may be asymptomatic but more frequently result in a variety of self-limiting

acute enteric symptoms characterized by profuse diarrhea, and infection of severely immune-compromised patients can contribute to mortality.

Reports on the occurrence of *Cryptosporidium* spp. oocysts in untreated surface waters vary widely. Studies conducted in the years immediately following the Milwaukee outbreak demonstrated that the average proportion of river, lake, and well water samples that were contaminated with oocysts ranged from 9 to 100% (Rose et al., 1997). A large survey of North America spanning 1988–1993 reported that 60.2% of samples (N = 347) were positive for *Cryptosporidium* oocysts (LeChevallier and Norton, 1995). A similar study in Canada demonstrated lower levels of contamination with oocysts detected in 6.1%, 4.5%, and 3.5% of raw sewage, raw water, and treated drinking water, respectively (Wallis et al., 1996). Additional studies have reported the occurrence of oocysts in 6% of stream samples in Wisconsin (Archer et al., 1995), 63% of river samples in Pennsylvania (States et al., 1997), and 13% of surface waters in New Zealand (Ionas et al., 1998). A large watershed survey conducted by the Metropolitan Water District of Southern California (MWD) detected oocysts in 11% of samples (N = 189) and 24% of first flush samples (N = 34) with extrapolated oocyst concentrations up to 417/L following storm events (Ferguson et al., 1998). The Information Collection Rule (ICR) survey of 5,838 untreated source waters throughout the U.S. reported an average occurrence of 6.8% with a mean concentration of 0.067 oocysts/L (Messner and Wolpert, 2003).

Sixty seven percent and 33% of waterborne outbreaks were caused by *C. hominis* and *C. parvum*, respectively (N = 22; McLauchlin et al., 2000; Sulaiman et al., 1998). Ninety three percent (N = 29) of storm water samples analyzed by a PCR-RFLP targeting the SSU rRNA gene, were positive for *Cryptosporidium* spp. (Xiao et al., 2000). None of the 12 detected genotypes matched those typically found in human, farm animal, or domestic animal samples. However, four were identical or closely related to *C. baileyi*, and *Cryptosporidium* genotypes from opossums and snakes indicating that wildlife was the primary source of oocyst contamination of surface water during storms. The same method was also used to analyze untreated surface water and wastewater samples. *Cryptosporidium* was detected in 45.5% of surface water samples (N = 55) and 24.5% of raw wastewater samples (N = 49; Xiao et al., 2001). The predominant genotypes in surface water matched the profiles of *C. parvum* and *C. hominis* while *C. andersoni* was most commonly detected in wastewater.

While oocysts are resistant to chlorine disinfection at the concentrations typically applied during drinking water treatment (2 – 6 mg/L), correctly operating treatment plants that utilize filtration usually remove oocysts from source water with high efficiency. However, oocysts have been detected in 3.8 – 40% of treated drinking water samples at concentrations up to 48 oocysts/100 L (Rose et al., 1997). A survey of treatment plants in Wisconsin detected oocysts in 4.2% (N = 72) of finished water samples (Archer et al., 1995). Fifteen years after the Milwaukee outbreak, *Cryptosporidium* contamination of drinking water continues to represent a public health threat for the water industry. However, the magnitude of the threat is uncertain.

Following the development of cell culture-based methods for assessing *Cryptosporidium* infectivity and the demonstration that cell culture is equivalent to animal models for measuring infectivity (Rochelle et al., 1997, 2001; Slifko et al., 1997, 2002; Di Giovanni et al., 1999), various cell culture methods have been used to detect infectious *Cryptosporidium* in water (Table 1).

Table 1. Prevalence of infectious *Cryptosporidium* spp. in various types of water

Type of water	Number of samples	Positive	Reference
Finished drinking water	1,690	1.4%	Aboytes et al., 2004
Filter backwash water	121	7.4%	Di Giovanni et al., 1999
Source water	560	3.9%	LeChevallier et al., 2003
Source water	122	4.9%	Di Giovanni et al., 1999
Disinfected reclaimed effluent	15	40%	Gennaccaro et al., 2003
Raw wastewater	18	33%	Gennaccaro et al., 2003

There are currently two species of *Cryptosporidium* that cause the majority of human infections, *C. parvum* and *C. hominis*. However, the source of contamination of environmental waters is often livestock or feral animals that can shed species of oocysts that are not infectious to humans and so represent minimal public health risk. The condition of the oocysts is also very important in determining the risk of infection. Oocysts are exposed to many conditions in the environment that can reduce their infectivity before entering a water treatment plant. The length of time post-shedding from the carriage animal, water temperature, and the amount of ultraviolet (UV) exposure from sunlight can reduce oocyst infectivity. Although oocysts are considered environmentally resistant, they exhibit considerable loss of infectivity as environmental temperature increases (Figure 1). Above 10°C oocysts lose infectivity at a rate of 0.004-log × temperature (°C) per day. In addition, surface waters are exposed to natural UV irradiation in sunlight which may damage oocyst DNA thereby inhibiting DNA replication and reducing infectivity. Once oocysts enter a drinking water treatment plant, they are exposed to additional conditions that can reduce their ability to cause infection in humans.

The risk of infection due to *Cryptosporidium* in drinking water depends on a combination of factors, many of which are poorly understood. These include the concentration of oocysts in source water, survival of oocysts in the environment, efficacy of treatment, virulence and dose response of the pathogen, species or strain of the pathogen, susceptibility to infection of individual water consumers, and the volume of water consumed.

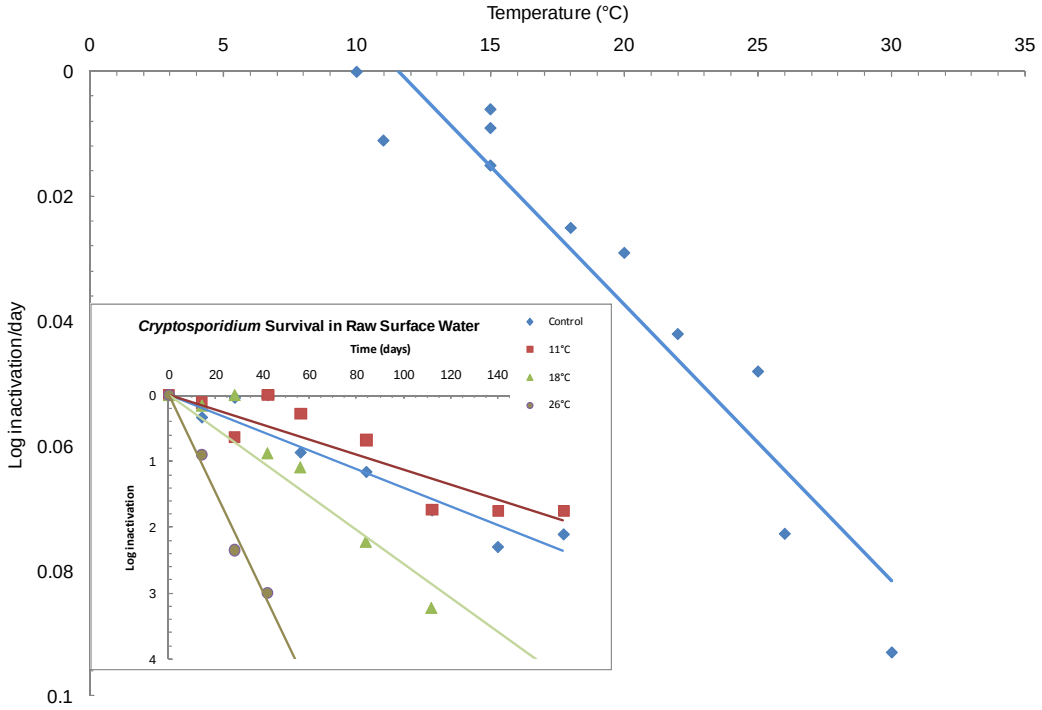

Figure 1. Reduction of *Cryptosporidium parvum* infectivity in response to increasing environmental temperature as measured by HCT-8 cell culture combined with RT-PCR quantification of infection (Rochelle et al., 2002).

According to the only study on the occurrence of infectious *Cryptosporidium* oocysts in conventionally filtered drinking water in the U.S., 27% of surface water treatment plants (N = 82) released infectious oocysts in their finished water at least once and overall, 1.4% of treated drinking water samples (N = 1,690) contained infectious oocysts (Aboytes et al., 2004). Using the calculation below, this occurrence data translates to an annual risk of cryptosporidiosis of 52 infections per 10,000 people (U.S. national risk = 1.6 million cases per year), which is much higher than the annual risk of infection goal set by the U.S. Environmental Protection Agency (USEPA).

$$Annual\ Risk = 1-(1-Daily\ Risk)^{350}$$

$$Daily\ Risk = water\ consumption \times concentration \times infection\ index$$

Where:
Water consumption = 1.2 L/day
Concentration in finished water = (number positive samples/total number samples) × (1/recovery efficiency) = 4.4 × 10^{-4} oocysts/L
Infection index = 0.028 for an unknown strain (according to Messner et al,. 2001)

Reduction of sporadic cryptosporidiosis cases following installation of additional treatment demonstrated that drinking cold, unboiled tap water was a leading independent risk factor for infection (Goh et al., 2005). However, since many oocysts in surface waters belong to species other than *C. hominis* and *C. parvum*, the public health benefits of the risk assessment framework underlying the LT2ESWTR, based solely on FITC-positive oocysts with no speciation or genotyping may be questioned. In implementing the Surface Water Treatment Rule in 1989, the USEPA determined that an acceptable annual risk of infection (the chance of one person being infected during one year) of 1/10,000 should be the goal of water treatment plants. In calculating this number, the recovery efficiency of the method, the concentration of the oocysts in water, and the infection index of the organism (the ability of the oocyst to cause an infection if ingested) must be considered. A frequent assumption for these calculations is that the average person ingests 1.2 L of unboiled tap water per day but changing consumer habits and the increasing popularity of bottled water add unknown variability to these assumptions. Estimates for daily risk of *Cryptosporidium* infection are typically in the range 1.5×10^{-5} – 3.8×10^{-4}. However, most of these estimates result in annual disease burdens that are orders of magnitude higher than the reported incidence of cryptosporidiosis cases from all sources in the U.S. In 2007, the Centers for Disease Control reported 11,170 cases of cryptosporidiosis from all sources nationwide with an annual average of 4,261 cases for the 10 years covering 1997 – 2007. The average annual incidence in the U.K. was 5.9 – 11.6/100,000 for a similar period. Even if only 1 in 100 cases are reported, the annual incidence from all sources is still far below most estimates of the risk from drinking water. Clearly, better estimates are needed to more accurately assess the threat to public health posed by *Cryptosporidium* in drinking water.

The current methods of *Cryptosporidium* detection in untreated surface water (Method 1622 and 1623; USEPA, 2005) use an antibody based detection method to identify oocysts. This method only provides presence/absence detection of oocysts. The absence of sporozoites within the oocyst (determined by DAPI staining and/or DIC microscopy) suggests that the oocyst is not infectious but the presence of sporozoites does not mean that the oocyst is infectious to humans. An intact oocyst may not be *C. parvum* or *C. hominis* or the oocyst may be sufficiently damaged that it will not cause infection in humans. The detection of non-infectious oocysts or oocysts belonging to a species that is not infectious for humans could cause unwarranted concern for a contaminant that may not be a significant public health risk.

In an ongoing study, treated water from conventional surface water filtration plants across a broad geographic area was sampled multiple times for the presence of infectious oocysts. Large volume samples (up to 1,000 L) were analyzed using a modification of USEPA method 1623 followed by in-vitro cell culture. A comparison of the three most commonly used cell culture-based infectivity methods for *Cryptosporidium* determined that the HCT-8 cell culture followed by immunofluorescence microscopy was the most appropriate method for the study (Figure 2; Johnson et al., 2007). Desirable characteristics of an infectivity method for finished water include: distinguishing infectious from non-infectious oocysts; eliminating or minimizing false positives and false negatives; robust enough to support infection despite environmental contaminants

that are isolated along with the oocysts; and allow for molecular analysis of positive samples to determine the species or genotypes responsible for infection.

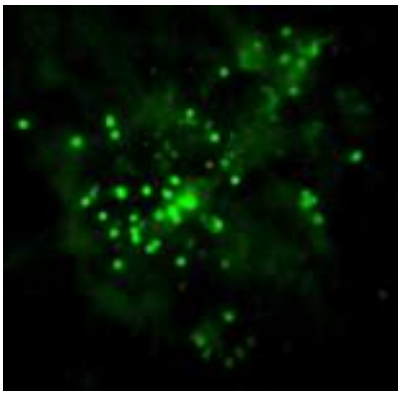

Figure 2. Infectious cluster of *Cryptosporidium parvum* in HCT-8 cells detected by immunofluorescence microscopy.

The study is ongoing but 201,000 L of water have been analyzed so far, with no positives yet detected. Positive controls and routine matrix spikes indicate that the method is working, so the lack of positives is not due to false-negative results. The eventual goal is to analyze 280,000 L. Assuming a single infectious cluster arises from one oocyst, if a single sample is positive, the annual risk will be calculated as 0.05 – 1.3 infections per 10,000 individuals, depending on the values for water consumption and risk of infection from a single oocyst selected for model input. Table 2 indicates the number of positive samples that will be needed for the risk calculation to exceed 1 in 10,000, based on a total volume of 280,000 L, exposure to drinking water for 365 days, and various values for the volume of unboiled drinking water consumed and the *Cryptosporidium* infection index.

The results of this study will be used to assess the risk of infection from *Cryptosporidium* in conventionally filtered drinking water.

Table 2. Risk of waterborne cryptosporidiosis

Water consumption (L/day)	Recovery efficiency (%)[a]	Infection index[b]	No. of positives to exceed 1/10,000 risk
0.5	71	0.028	4
0.5	71	0.0053	21
0.5	35	0.028	2
0.5	35	0.0053	11
1.2	71	0.028	2
1.2	71	0.0053	9
1.2	35	0.028	1
1.2	35	0.0053	5

[a] Average oocyst recovery efficiency using the modified version of USEPA Method 1623 was 71%.
[b] Infection index for an unknown strain in a population = 0.028; Infection index for Iowa isolate = 0.0053 (Messner et al., 2001).

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References

Aboytes, A., G. D. Di Giovanni, F. A. Abrams, C. Rheinecker, W. McElroy, N. Shaw and M. W. LeChevallier. 2004. Detection of infectious *Cryptosporidium* in filtered drinking water. *Jour. Amer. Wat. Works Assoc*., 96(9):88-98.

Archer, J. R., J. R. Ball, J. H. Standridge, S. R. Greb, P. W. Rasmussen, J. P. Masterson, and L. Boushon. 1995. *Cryptosporidium* spp. oocyst and *Giardia* spp. cyst occurrence, concentrations and distribution in Wisconsin waters. Wisconsin Department of Natural Resources, Publication No. WR420-95.

Di Giovanni, G. D., F. H. Hashemi, N. J. Shaw, F. A. Abrams, M. W. LeChevallier, and M. Abbaszadegan. 1999. Detection of infectious *Cryptosporidium parvum* oocysts in surface and filter backwash water samples by immunomagnetic separation and integrated cell culture PCR. *Appl. Environ. Microbiol*., 65:3427-3432.

Fayer, R., C. A. Speer, and J. P. Dubey. 1997. The General Biology of *Cryptosporidium*. In *Cryptosporidium and Cryptosporidiosis*, pp.1-41. Ed: R. Fayer. CRC Press, Boca Raton, Florida.

Fayer, R., U. Morgan, and S. J. Upton. 2000. Epidemiology of *Cryptosporidium*: transmission, detection, and identification. *Int. Jour. Parasitol*., 30:1305-1322.

Fayer, R., J. M. Trout, L. Xiao, U. M. Morgan, A. A. Lal, and J. P. Dubey. 2001. *Cryptosporidium canis* n. sp. from domestic dogs. *Jour. Parasitol*., 87: 1415-1422.

Fayer, R., 2008. General Biology, 1-42. *In* R. Fayer and L. Xiao (eds.), *Cryptosporidium and Cryptosporidiosis*, second edition. CRC Press, Boca Raton.

Ferguson, D. M., R. De Leon, W. D. Taylor, and M. H. Stewart. 1998. Surface water monitoring in two watersheds to identify sources of *Cryptosporidium* spp. and *Giardia* spp. *Proc. Source Water Assessment and Protection 98*. Dallas. pp. 149-156.

Gatei, W., R. W. Ashford, N. J. Beeching, S. K. Kamwati, J. Greensill, and C. A. Hart. 2002. *Cryptosporidium muris* infection in an HIV-infected adult, Kenya. *Emerg. Infect. Dis*., 8:204-206.

Gennaccaro, A. L., M. R. McLaughlin, W. Wuintero-Betancourt, D. E. Huffman, and J. B. Rose. 2003. Infectious *Cryptosporidium parvum* oocysts in final reclaimed effluent. *Appl. Environ. Microbiol*., 69:4983-4984.

Goh S., M. Reacher, D. P. Casemore, N. Q. Verlander, A. Charlett, R. M. Chalmers, M. Knowles, A. Pennington, J. Williams, K Osborne, S. Richards. 2005. Sporadic cryptosporidiosis decline after membrane filtration of public water supplies, England, 1996-2002. *Emerg Infect Dis*., 11(2):251-9.

Hunter, P. R. and Q. Syed. 2001. Community surveys of self-reported diarrhea can dramatically overestimate the size of outbreaks of waterborne cryptosporidiosis. *Wat. Sci. Technol*., 43:27-30.

Ionas, G., J. J. Learmouth, E. A. Keys, and T. J. Brown. 1998. Distribution of *Giardia* and *Cryptosporidium* in natural water systems in New Zealand- A nationwide survey. *Wat. Sci. Technol*., 38:57-60.

Johnson, A. M., G. D. Di Giovanni, P. A. Rochelle. 2007. Detection of infectious *Cryptosporidium* in treated drinking water using multiple cell culture assays and genotyping. AWWA Water Quality Technology Conference, 2007.

LeChevallier, M. W., and W. D. Norton. 1995. *Giardia* and *Cryptosporidium* in raw and finished water. *Jour. AWWA*, 87:54-68.

LeChevallier, M. W., G. D. Di Giovanni, J. L. Clancy, Z. Bukhari, S. Bukhari, J. S. Rosen, J. Sobrinho, and M. M. Frey. 2003. Comparison of Method 1623 and cell culture-PCR for detection of *Cryptosporidium* spp. in source waters. *Appl. Environ. Microbiol*., 69:971-979.

MacKenzie, W. R., N. J. Hoxie, M. E. Proctor, M. S. Gradus, K. A. Blair, D. E. Peterson, J. J. Kazmierczak, D. G. Addiss, K. R. Fox, J. B. Rose, and J. P. Davis. 1994. A massive outbreak in Milwaukee of *Cryptosporidium* infection transmitted through the public water supply. *New Engl. Jour. Med*., 331:161-167.

McLauchlin, J., C. Amar, S. Pedraza-Diaz, and G. L. Nichols. 2000. Molecular epidemiological analysis of *Cryptosporidium* spp. in the United Kingdom: Results of genotyping *Cryptosporidium* spp. in 1,705 fecal samples from humans and 105 fecal samples from livestock animals. *Jour. Clin. Microbiol*., 38:3984-3990.

Messner, M. J., C. L. Chappell, and P. C. Okhuysen. 2001. Risk assessment for *Cryptosporidium*: a hierarchical Bayesian analysis of human response data. *Water Res.*, 35(16):3934-3940.

Messner, M. J., and R. L. Wolpert. 2003. *Cryptosporidium* and *Giardia* occurrence in ICR drinking water sources: Statistical analysis of ICR data. In *Information*

Collection Rule Data Analysis, pp. 463-481. Eds. M. J. McGuire, J. L. McLain, and A. Obolensky. Awwa Research Foundation and the American Water Works Association, Denver, CO.

Morgan, U., R. Weber, L. Xiao, I. Sulaiman, R. C. A. Thompson, W. Ndiritu, A. Lal, A. Moore, and P. Deplazes. 2000. Molecular characterization of *Cryptosporidium* isolates obtained from human immunodeficiency virus-infected individuals living in Switzerland, Kenya, and the United States. *Jour. Clin. Microbiol*., 38:1180-1183.

Morgan-Ryan, U., A. Fall, L. A. Ward, N. Hijjawi, I. Sulaiman, R. Fayer, R. C. Thompson, M. Olson, A. Lal, and L. Xiao. 2002. *Cryptosporidium hominis* n. sp. (Apicomplexa: Cryptosporidiidae) from *Homo sapiens*. *Jour. Eukaryot. Microbiol*., 49:433-440.

Pedraza-Diaz, S., C. Amar, A. M. Iversen, P. J. Stanley, and J. McLauchlin. 2001. Unusual *Cryptosporidium* species recovered from human faeces: first description of *Cryptosporidium felis* and *Cryptosporidium* 'dog type' from patients in England. *Jour. Med. Microbiol*., 50:293-296.

Pieniazek, N. J., F. J. Bornay-Llinares, S. B. Slemenda, A. J. da Silva, I. N. Moura, M. J. Arrowood, O. Ditrich, and D. G. Addiss. 1999. New *Cryptosporidium* genotypes in HIV-infected persons. *Emerg. Infect. Dis*., 5:444-449.

Rochelle, P. A., D. M. Ferguson, T. J. Handojo, R. De Leon, M. H. Stewart, and R. L. Wolfe. 1997. An assay combining cell culture with reverse transcriptase-PCR to detect and determine infectivity of waterborne *Cryptosporidium parvum*. *Appl. Environ. Microbiol*., 63:2029-2037.

Rochelle, P. A., D. M. Ferguson, A. M. Johnson, and R. De Leon. 2001. Quantitation of *Cryptosporidium parvum* infection in cell culture using a colorimetric in situ hybridization assay. *Jour. Eukaryot. Microbiol*. 48:565-574.

Rochelle, P. A., Marshall, M. M., Mead, J. R., Johnson, A. M., Korich, D. G., Rosen, J. S., and De Leon, R. 2002. Comparison of in vitro cell culture and a mouse assay for measuring infectivity of *Cryptosporidium parvum*. *Appl. Environ. Microbiol*., 68:3809-3817.

Rose, J. B., J. T. Lisle, and M. LeChevallier. 1997. Waterborne cryptosporidiosis: Incidence, outbreaks, and treatment strategies, p.93-109. *In* R. Fayer (ed.), *Cryptosporidium* and Cryptosporidiosis. CRC Press, Boca Raton.

Slifko, T. R., D. Friedman, J. B. Rose, and W. Jakubowski. 1997. An in vitro method for detecting infectious *Cryptosporidium* oocysts with cell culture. *Appl. Environ. Microbiol*., 63:3669-3675.

Slifko, T. T., Huffman, D. E., Dussert, B., Owens, J. H., Jakubowski, W., Haas, C. N., and Rose, J. B. 2002. Comparison of tissue culture and animal models for assessment of *Cryptosporidium parvum* infection. *Exp. Parasitol*. 101:97-106.

States, S., K. Stadterman, L. Ammon, P. Vogel, J. Baldizar, D. Wright, L. Conley, and J. Sykora. 1997. Protozoa in river water: sources, occurrence, and treatment. *Jour. AWWA*, 89:74-83.

Sulaiman, I. M., L. Xiao, C. Yang, L. Escalante, A. Moore, C. B. Beard, M. J. Arrowood, and A. A. Lal. 1998. Differentiating human from animal isolates of *Cryptosporidium parvum*. *Emerg. Infect. Dis*., 4:681-685.

U.S. Environmental Protection Agency (USEPA). 2005. Method 1623: *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA. EPA 815-R-05-002, Office of Research and Development, Government Printing Office, Washington, DC.

U.S. Environmental Protection Agency (USEPA). 2006. National Primary Drinking Water Regulations: Long Term 2 Enhanced Surface Water Treatment Rule; Final Rule. Federal Register, 40 CFR Parts 9, 141 and 142, Vol. 71, No. 3:654-786.

Wallis, P. M., S. L. Erlandsen, J. L. Isaac-Renton, M. E. Olson, W. J. Robertson, and H. van Keulen. 1996. Prevalence of *Giardia* cysts and *Cryptosporidium* oocysts and characterization of *Giardia* spp. cysts isolated from drinking water in Canada. *Appl. Environ. Microbiol*., 62:2789-2797.

Xiao, L., K. Alderisio, J. Limor, M. Royer, and A. A. Lal. 2000. Identification of species and sources of *Cryptosporidium* oocysts in storm waters with a small-subunit rRNA-based diagnostic and genotyping tool. *Appl. Environ. Microbiol*., 66:5492-5498.

Xiao, L., C. Bern, J. Limor, I. Sulaiman, J. Roberts, W. Checkley, I. Cabrera, R.H. Gilman, and A.A. Lal. 2001. Identification of 5 types of *Cryptosporidium* parasites in children from Lima, Peru. *Jour. Infect. Dis*., 185:270-271.