Assessment of waterborne protozoan passage through conventional drinking water treatment process in Venezuela

Walter Q. Betancourt and Kristina D. Mena

ABSTRACT

Three drinking water treatment plants (DWTPs) differing in source water and treatment capacity were investigated for the potential passage of waterborne protozoan (oo)cysts through conventional processing. DWTP I (15,000 L/s), DWTP II (7,500 L/s) and DWTP III (4,300 L/s) provide drinking water for approximately 2.7 million inhabitants of the Metropolitan District of Caracas (Venezuela). The US Environmental Protection Agency Method 1623 for detection of *Cryptosporidium* and *Giardia* was used to analyze raw water and finished drinking water samples collected from the three plants. (Oo)cyst recovery efficiencies varied between 23 and 84%. The concentration of confirmed (oo)cysts detected in raw water samples ranged between 1 and 100 per 100 L. (Oo)cyst levels in finished water samples ranged from 2 to 25 per 100 L. These data indicated that the conventional treatment process to produce finished water at two filtration plants was not effective in preventing the passage of protozoan (oo)cysts. Monitoring strategies that include multiple microbial indicators and waterborne pathogens are strongly recommended for accurate source water characterization and for verification of the effectiveness of treatment process barriers to microbial breakthrough in the finished water.

Key words | *Cryptosporidium*, drinking water, *Giardia*, passage, treatment, Venezuela

Walter Q. Betancourt (corresponding author)
Venezuelan Institute for Scientific Research (IVIC),
Microbiology and Cell Biology Center (CMBC),
APDO 20632
Caracas 1020A,
Venezuela
E-mail: *wbetanco@ivic.gob.ve; walter.betancourt@fulbrightmail.org*

Walter Q. Betancourt
Kristina D. Mena
Epidemiology,
Human Genetics and Environmental Sciences,
School of Public Health,
The University of Texas Health Science Center at Houston,
1100 North Stanton,
Suite 110,
El Paso,
Texas 79902 USA

INTRODUCTION

It is well known that adequate and fully operational conventional drinking water treatment processes (coagulation, sedimentation, filtration, and disinfection) contribute to the reduction of microbial contaminants of public health concern in raw source water (LeChevalier & Au 2004). Treatment capacity and operation reliability (i.e., effective coagulation and filtration) are key components of the conventional treatment process that control the passage of waterborne protozoan (oo)cysts to the distribution system (Betancourt & Rose 2004; WHO 2008). These components, including the degree of deterioration in source water quality (i.e., levels of protozoan pathogens in raw source waters), may vary significantly between different locations and even within the same location (WHO 2008). Higher levels of pathogens in surface water supplies – including density and variety – are expected in areas where sewage treatment is marginal or nonexistent (Geldreich 1996).

Cryptosporidium and *Giardia* are two protozoan parasites of major concern for water utilities worldwide (Teunis & Havelaar 2002; Betancourt & Rose 2004; WHO 2008). The species *Cryptosporidium parvum* and *Giardia duodenalis* account for the majority of water-associated outbreaks occurring globally (Karanis *et al.* 2007). The high excretion levels of infectious (oo)cysts in human feces (10^8–10^9 (oo)cysts per gram of stool), the low infectious doses for cryptosporidiosis (9–1042 *C. parvum* oocysts, Okhuysen *et al.* 1999) and giardiasis (25–100 *G. duodenalis* cysts, Rendtorff 1954; Smith *et al.* 2006), and the relative resilience of (oo)cysts to conventional disinfection practices (i.e., water chlorination) highlight the need to establish appropriate monitoring strategies for public water systems

doi: 10.2166/wh.2012.118

in order to assess the safety of drinking water and the health risks to consumers.

Cryptosporidium and *Giardia* are part of the complex group of parasitic, bacterial, and viral diseases that impair the ability to achieve full potential and impair development and socio-economic improvements in the developing world (Savioli *et al.* 2006). The occurrence of these waterborne parasites in surface waters, including the reliability of treatment processes to assure optimal finished water quality (pathogen-free) in developing countries with upper-middle income economies, have not been fully investigated. Ninety percent of sewage systems in developing countries do not treat sewage before releasing it into the environment. Moreover, different types of malfunction and damage in the sewer systems frequently occur in these countries (Kavvas 2002), thereby creating serious environmental and water quality problems. Hence, studies that evaluate the potential risk of infection associated with waterborne pathogens in drinking water supplies in developing countries are necessary in order to develop and implement the appropriate measures to protect water resources and human health.

Venezuela is classified according to the World's Bank main criterion (i.e., gross national income, GNI) among nations with upper-middle income economies. Water supply and sanitation services in Venezuela are provided by HIDROVEN, the national agency that regulates and supervises the operations, development, and management of the water resource sector of the country (http://www.hidroven.gob.ve). HIDROVEN is a state-owned enterprise with ten affiliated regional water companies (Empresas Hidrológicas Regionales) that operate and maintain the public water supply system of the Capital District and major Venezuelan States. HIDROCAPITAL is the water company that operates the surface water treatment plants that supply water to the Metropolitan District of Caracas, which includes the capital city of Venezuela and four other municipalities of the states of Miranda and Vargas. The Metropolitan District water supply relies on a

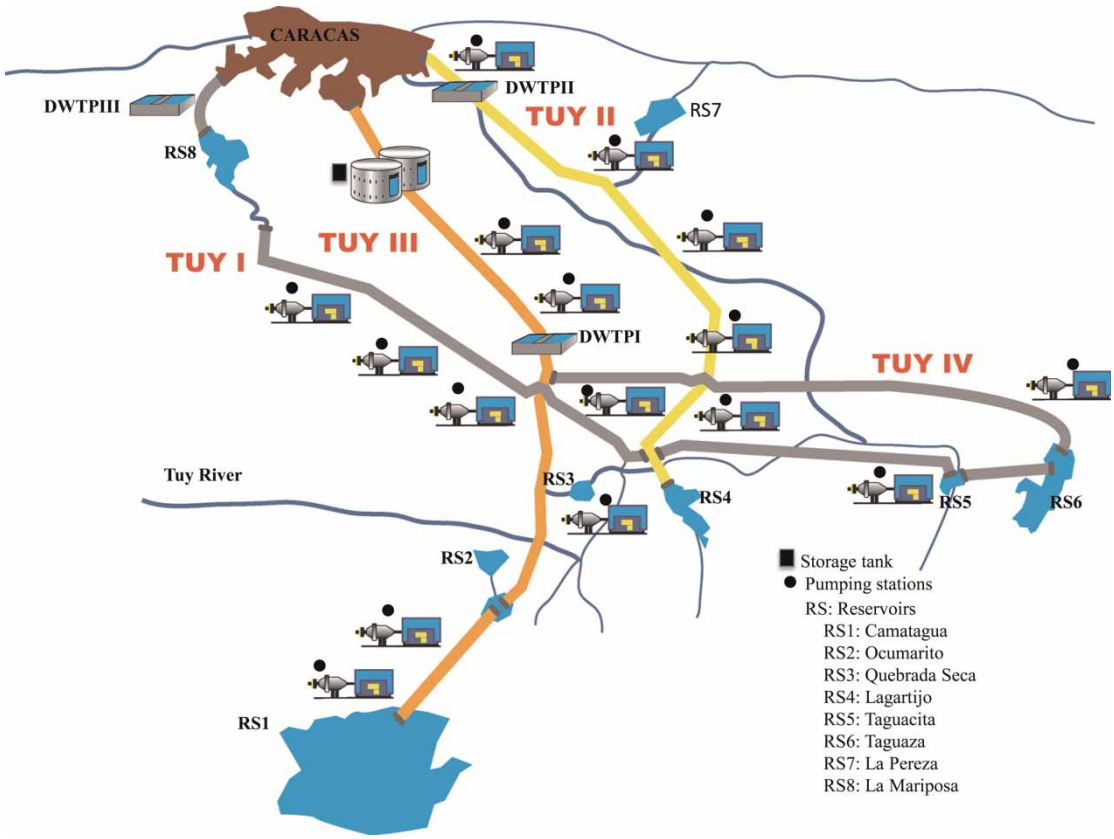

Figure 1 | Schematic of the drinking water supply system that serves the population of the Metropolitan District of Caracas, Venezuela.

distribution network composed of four production systems (Tuy Production Systems I, II, III and IV) that deliver source water for treatment from eight river reservoirs (Camatagua, Lagartijo, Ocumarito, Taguaza, La Mariposa, Taguacita, Quebrada Seca, La Pereza). These reservoirs draw surface water from four large rivers (Guarico, Tuy, Taguaza, Lagartijo) and provide water for irrigation, in addition to providing flood control and recreation benefits such as swimming, fishing, and boating. The distribution system serves approximately 2.7 million people and consists of numerous pumping stations connected by 3,000 km of a vast pipe network including a large storage tank to meet demand at satisfactory pressure (Figure 1).

The Drinking Water Guidelines of World Health Organization (WHO) recommends end product monitoring as an appropriate strategy to verify the effectiveness of the drinking water treatment barriers against microbial breakthrough in the finished water. This approach derives important management information that can be applied to determine any performance deficiency, including the assessment of any corrective procedure needed to ensure water safety and public health protection (WHO 2008). Monitoring of conventional drinking water treatment performance to determine pathogen passage and removal has not been considered previously by health authorities and service providers in Venezuela. In this study, end product monitoring of protozoan (oo)cysts was used to verify the effectiveness of conventional treatment barriers in preventing the passage of pathogenic parasites at three major water facilities that serve the Metropolitan District of Caracas in Venezuela. The monitoring strategy also involved the analysis of microbial parameters of water treatment performance (total coliforms) and water quality safety (*Escherichia coli*), along with chlorine residual measurements as essential parameters recommended for the minimum monitoring of community supplies (WHO 2008).

MATERIALS AND METHODS

Drinking water supplies

Three water treatment facilities in the Metropolitan District of Caracas were selected for this study on the basis of their source waters and their treatment capacities. Drinking water treatment plant I (DWTP I) treats raw water sources that come from Camatagua and Lagartijo reservoirs and from the Tuy River. Camatagua is the largest dammed river reservoir with a storage capacity of 1,573,000 million liters of surface water from Guarico River. This reservoir supplies the majority of drinking water for the city of Caracas and also supports multiple recreational and agricultural activities. Camatagua is a protected reservoir that unfortunately receives domestic wastes from unplanned settlements and its catchment area has been continuously affected by deforestation. Lagartijo reservoir has a storage capacity of 80,000 million liters of source water from Lagartijo River. This reservoir is used for drinking water supply and recreation. It is affected by high water level fluctuations due to high water demand which requires pumping of treated water (sedimentation and chlorination processes) from the Tuy River to the reservoir's raw water intake. The maximum capacity of DWTP I is 15,000 L/s and the average monthly production is 27,232,100 m^3.

DWTP II (La Guairita) processes raw water sources from three local reservoirs (Lagartijo, Taguacita, and Taguaza) and imported water from Tuy River plus surface water from Quebrada Seca and La Pereza reservoirs. Taguaza reservoir stores 184,000 million liters of source water from Taguaza River. This reservoir is located in a protected area with no human activities in its catchment basin. Taguacita is a compensatory reservoir with a maximum capacity of 120,000 million liters. This reservoir captures surface water from Taguacita River and is also located in a protected area. Quebrada Seca is a non-protected reservoir that stores 7,000 million liters of source water from the Tuy River. Its catchment basin is highly impacted by industrial and domestic wastewater pollution. La Pereza is a compensatory reservoir that stores 8,000 million liters of source water from the Tuy River. The reservoir is used for drinking and recreational purposes. It is surrounded by high eroded hills with pig breeding farms and livestock operations (bovine and equine) located in the reservoir basin area. The maximum capacity of DWTP II is 7,500 L/s and the average monthly production is 17,071,392 m^3.

DWTP III (La Mariposa) draws its raw water from La Mariposa reservoir. This reservoir stores 8,000 million liters of source water from the Tuy River. Its catchment area is

highly impacted by slum dwellers that lack sewerage systems. The reservoir supplies water to western Caracas and also supports multiple recreational activities. La Mariposa reservoir experiences high and frequent level fluctuations due to a high water demand that is satisfied with water pumped from Camatagua and Lagartijo reservoirs. The maximum production capacity of DWTP III is 4,300 L/s with an average monthly production of 10,979,178.58 m^3.

Water treatment at all facilities consists of coagulation and flocculation using alum and polymers, dual media filtration (anthracite coal and sand with a gravel support) and chlorine disinfection.

Water sample collection and processing

Finished water sampling was conducted at each plant at the point of entry into the distribution system. Samples were collected during five separate sampling events (between November 2009 and May 2010) and analyzed for *Cryptosporidium* and *Giardia* following standard procedures described in Method 1623 with slight modifications (USEPA 1999). Briefly, water samples were filtered through the Envirochek HV capsules (Pall Corporation) connected directly to a tap with flow rates maintained at 2–3.5 L m^{-1}. The volumes of sample filtered were 20 and 100 L for raw water and finished water, respectively. After filtration, the water content remaining in the capsule (~150 mL) was poured into a sterile plastic bottle. The capsule was filled with eluting solution (200 mL) and transported to the laboratory for the elution of (oo)cysts. All fractions, including the volumes of the first and second elution steps, were combined (~450 mL) and concentrated through centrifugation (2,500 × *g*, 15 min) into a final volume of approximately 5 mL (finished water) and 15 mL (raw water). Although the focus of the research was end-monitoring of protozoan (oo)cysts to verify the effectiveness of the existing conventional water treatment process in preventing microbial pathogen breakthrough in the finished water, the raw water intakes of the treatment plants were sampled the same day and analyzed for *Cryptosporidium* and *Giardia* in order to estimate levels of (oo)cysts entering the drinking water plants.

Cryptosporidium and *Giardia* (oo)cysts were purified by immunomagnetic separation using the Dynal GC-Combo kit (Prod. No. 730.02, DYNAL A.S., Oslo, Norway) which included two dissociation steps with 100 μL of 0.1 N hydrochloric acid. Immunomagnetic separation concentrates were fixed with absolute methanol and stained with fluorescein isothiocyanate-conjugated anti-*Cryptosporidium* and anti-*Giardia* monoclonal antibodies (EasyStain™, BTF A bioMérieux Company, Sydney, Australia) following standard procedures. Confirmation of (oo)cysts was carried out by staining with the fluorochrome 4′,6-diamidino-2-phenylindole (DAPI) along with Nomarski differential interference contrast microscopy to look at the internal morphology of (oo)cysts.

Recovery efficiency experiments were carried out as described in the April 1999 version of USEPA method 1623 to demonstrate acceptable method performance and included: (i) initial precision and recovery tests, (ii) matrix spikes, and (iii) method blanks. Distilled water was used as the reagent water sample for blanks and initial precision and recovery tests. The matrix spikes were carried out with three 100 L finished water and three 20 L raw water samples collected from each plant. ColorSeed™ (BTF A bioMérieux Company, Sydney, Australia) was used as internal calibration standard to determine the percentage recovery of (oo)cysts achieved in these samples. Naturally occurring (oo)cysts were easily identified and adjusted to the corresponding recovery efficiency in order to determine the number of (oo)cysts present in finished and raw water samples.

Microbial and physicochemical water quality data

The analysis of total coliforms and *E. coli* was conducted in accordance with the membrane filtration procedure described in the 20th edition of *Standard Methods for the Examination of Water and Wastewater* (1998) using m-ColiBlue 24 broth (Hach Company), which detects both bacterial indicators in 24 h with no confirmatory step required. Turbidity levels expressed as nephelometric turbidity units (NTU) were measured with a HACH Model 2100 A turbidimeter. The pH of water was measured with a pH meter (HANNA Instruments Model HI 98129) and spectrophotometric determinations for water color were performed with a HACH DR/890 colorimeter. Disinfectant residual was determined with the diethyl paraphenylene diamine

indicator test. These parameters were provided by each water utility. Conventional PCR amplification of the 16S rDNA of Bacteroidales using primers developed by Dick & Field (2004) was included to test an alternative assay for detection of general fecal pollution in raw and finished water samples.

PCR was carried out in a volume of 25 μL reaction mixture containing 2 μL of sample DNA. Each 25 μL PCR reaction consisted of 0.2 μm of each primer, 1× PCR buffer containing 2.0 mM $MgCl_2$ (TaKaRa BIO Inc., Otsu, Shiga, Japan) 0.2 mM of each deoxynucleoside triphosphate and 0.025 U Ex Taq DNA polymerase (TaKaRa BIO Inc.). The amplification conditions included a 94 °C denaturation step for 3 min, followed by 35 cycles of 94 °C 1 min, 60 °C for 45 sec, 72 °C for 45 sec, and a final extension step at 72 °C for 7 min in a MJ Mini BioRad thermal cycler (Hercules, CA, USA).

Statistics

The data derived from the recovery efficiency experiments were analyzed using descriptive statistics (i.e., mean and standard deviation). The Pearson correlation coefficient, *r*, was applied to determine the extent of association between pairs of variables using SYSTAX Version 13 statistical package.

RESULTS

The results of the initial precision and recovery tests demonstrated acceptable method performance based on the mean *Cryptosporidium* and *Giardia* (oo)cysts recoveries using method 1623 with the Envirochek HV filter. Mean percentage recoveries and relative standard deviation (RSD, mean/SD × 100) for *Cryptosporidium* and *Giardia* ($n = 5$) were 57% (RSD, 33%) and 45% (RSD, 39%), respectively. Following these assays, end-product monitoring of protozoan (oo)cysts was conducted during five separate sampling events at the three conventional drinking water treatment facilities that serve the Metropolitan District of Caracas.

Table 1 describes the levels of (oo)cysts detected in samples of raw and finished water, including the results of the recovery efficiency experiments with estimations of the adjusted number of (oo)cysts based on the recovery assay data obtained from three matrix spikes. The recovery rates for *Cryptosporidium* oocysts in raw water samples ranged between 25 and 55% (33 ± 27 [mean ± RSD]) while recovery rates for *Giardia* cysts ranged from 23 to 66% (33 ± 37 [mean ± RSD]). The level of naturally occurring *Cryptosporidium* and *Giardia* (oo)cysts detected in DWTP I was lower than the level of (oo)cysts detected in raw water samples from DWTP II and DWTP III. Overall, the number of *Cryptosporidium* and *Giardia* (oo)cysts detected at these sites ranged from 1 to 100 per 100 L.

The level of (oo)cysts detected in finished water produced by each plant varied among the three plants surveyed. The percentage recoveries for *Cryptosporidium* in these samples ranged from 56 to 84% (65 ± 14 [mean ± RSD]) while the percentage recoveries for *Giardia* cysts ranged from 25 to 67% (46 ± 30 [mean ± RSD]). *Cryptosporidium* was less frequently detected in finished water samples and the levels of oocysts detected were lower than the levels of *Giardia* cysts. *Cryptosporidium* oocysts were only detected in finished water produced by DWTP III, while *Giardia* cysts were detected in finished water produced by DWTP II and DWTP III. The numbers of oocysts detected in finished water samples from these plants ranged from 2 to 7 oocysts/100 L, while the number of *Giardia* cysts ranged from 5 to 25 per 100 L.

Table 2 summarizes the microbial and physicochemical water quality data obtained for raw and finished water samples collected from each treatment plant. *E. coli* was never detected in finished drinking water samples therefore all water utilities were in compliance with WHO and Venezuelan guidelines (<1 *E. coli* per 100 mL of drinking water and 0 fecal coliform/100 mL, respectively). Similarly, all the values for water turbidity, color, and chlorine residual in finished water were either below or according to the maximum acceptable value indicated by the guidelines (≤5 NTU, <15 true color units, <3 mg/L, respectively). The National Primary Drinking Water Standards are established in the Norms of the Sanitary Quality of Water by the Ministry of Health and Social Assistance under the Official Gazette of the Republic of Venezuela (No. 36.395, February 1998).

Table 1 | *Cryptosporidium* and *Giardia* (oo)cyst levels in three water treatment plants serving the Metropolitan District of Caracas, Venezuela

		***Cryptosporidium* oocysts/100 L**[a]		***Giardia* cysts/100 L**[@]	
Water facility	**Sample**	**Raw**	**Finished**	**Raw**	**Finished**
DWTP I	1	<2 (25%, NA[b])	<0.5 (72%, NA)	<2 (32%, NA)	<0.5 (27%, NA)
	2	1 (32%, 3)	<0.5 (56%, NA)	<1 (27%, NA)	<0.5 (48%, NA)
	3	<1	<0.2	<1	<0.2
	4	2	<0.2	1	<0.2
	5	1 (55%, 2)	<0.2 (62%, NA)	<1 (33%, NA)	<0.2 (57%, NA)
DWTP II	1	<50 (27%, NA)	<6.4 (67%, NA)	<50 (35%, NA)	6.4 (54%, 12)
	2	100 (41%, 244)	<8 (59%, NA)	50 (23%, 217)	<8 (67%, NA)
	3	50	<8	100	25
	4	25	<1	25	<1
	5	25 (35%, 71)	<5 (84%, NA)	50 (25%, 200)	<5 (25%, NA)
DWTP III	1	<15 (32%, NA)	<4 (72%, NA)	<15 (29%, NA)	<4 (35%, NA)
	2	<10 (25%, NA)	7 (59%, 12)	10 (26%, 38)	7 (58%, 12)
	3	<25	<3	25	<3
	4	50	5	50	5
	5	25 (30%, 83)	2 (56%, 4)	25 (66%, 38)	5 (41%, 12)

[a]The first number in parenthesis corresponds to percentage of recovery efficiency obtained with ColorSeed spikes followed, where applicable, by adjusted levels of (oo)cysts based on the corresponding recovery rates for those samples.
[b]NA, not applicable.

The molecular amplification of 16S rDNA of fecal *Bacteroidales* was used as an alternative assay to detect general fecal pollution based on the distinct advantages over the standard microbial water quality indicator assays (i.e., total coliforms and *E. coli*) as previously described by Dick & Field (2004). The standard bacterial indicators are associated with feces of warm-blooded animals; however they have also been found in freshwater environments associated with soil and vegetation (Geldreich 1996; Fujioka *et al.* 1999; Olapade *et al.* 2006; Whitman *et al.* 2006). The fecal marker is associated with anaerobic bacteria of the order *Bacteroidales* which are restricted to warm-blooded animals making up a significant portion of the fecal flora (Fiksdal *et al.* 1985; Bernhard & Field 2000), which is even greater than current fecal indicator bacteria such as coliforms and enterococci (Savage 2001).

The detection of both 16S rDNA of *Bacteroidales* and *E. coli* in raw water intake samples indicated the presence of fecal contamination in source waters. The fecal marker was never detected in effluent waters despite the detection of low levels of total coliforms (0.3 CFU/100 mL) in one sample from DWTP I and DWTP III along with the detection of protozoan (oo)cysts in finished waters from DWTP III. The presence of coliforms in treated water has been associated with microbiological water quality deterioration (McFeters *et al.* 1986). Hence coliforms are considered especially useful in operational monitoring since they may indicate treatment ineffectiveness, loss of disinfectant, or breakthrough (Geldreich 1996; Rompre *et al.* 2002; WHO 2008). The sporadic detection of low densities of coliform bacteria in effluent waters in this study may well indicate breaks in the conventional treatment barriers that lead to microbial breakthroughs, however, careful attention must be given to the analysis and results as discussed below.

The multiple tube fermentation technique is commonly used by water utilities in Venezuela to assess the bacteriological quality of raw and finished water based on total coliform counts. Nevertheless, inconsistencies in

Table 2 | Microbial and physicochemical water quality data obtained from raw and finished drinking water samples at each plant

		Physicochemical water quality data				Microbial water quality data[a]			
Water facility	**Sample no.**	**pH**	**Turbidity (NTU)**	**Color**	**Chlorine (mg/L)**	**Total coliforms**	***E. coli***	***Bacteroides* 16S rDNA**	**(Oo)cysts**
DWTP I									
Raw	1	7.5	1	15		77	1	+	−
	2	7.5	1.8	15		80	3	+	+
	3	7.3	1.6	15		55	2	+	−
	4	7.5	2.4	20		120	2	+	−
	5	7.6	1.4	20		89	4	+	+
Finished	1	7.5	1.3	10	2	<0.1	<0.1	−	−
	2	7.4	1.6	10	1.8	<0.1	<0.1	−	−
	3	7.2	1.2	10	1.7	<0.1	<0.1	−	−
	4	7.2	2.7	10	2	<0.1	<0.1	−	−
	5	7.4	1	10	1.7	0.3	<0.1	−	−
DWTP II									
Raw	1	6.9	5.2	28		55	2	+	−
	2	6.8	48.1	100		90	3	+	+
	3	6.7	40	50		100	1	+	+
	4	6.7	179	100		137	2	+	+
	5	7	70	100		200	1	+	+
Finished	1	6.5	0.33	10	1.68	<0.1	<0.1	−	+
	2	6.6	1.06	8	1.83	<0.1	<0.1	−	−
	3	6.6	2.0	10	1.60	<0.1	<0.1	−	+
	4	6.7	3.44	10	1.53	<0.1	<0.1	−	−
	5	6.6	2	10	1.7	<0.1	<0.1	−	−
DWTP III									
Raw	1	7.12	9.8	20		50	5	+	−
	2	6.98	48.2	100		55	7	+	+
	3	7.4	20	20		100	3	+	−
	4	7.18	73	20		80	4	+	+
	5	7.3	7	20		98	2	+	+
Finished	1	7.12	1.3	5	1.4	<0.1	<0.1	−	−
	2	6.64	1.6	5	1.08	<0.1	<0.1	−	+
	3	7.02	1.2	5	1.75	<0.1	<0.1	−	−
	4	6.84	2.7	5	2.8	0.3	<0.1	−	+
	5	6.92	0.5	5	2.5	0.1	<0.1	−	+

[a]The volumes of water examined for bacterial indicators, including the general fecal pollution marker, were 100–500 mL for raw water and 1,000 mL for finished water.

methodological and quality control procedures were observed during the study and therefore comparisons could not be made for indicator bacterial counts obtained with the two methods. Total coliform counts in samples of raw and finished water provided by water utilities were always reported as <2 MPN (most probable number) per 100 mL, which differed significantly from total coliform counts obtained with m-ColiBlue 24 broth in this study (Table 2).

The sensitivity of the molecular assay for detection of the fecal marker was not investigated, neither was the applicability of the quantitative molecular amplification method (qPCR) which is more sensitive and faster than the standard PCR method (Dick & Field 2004).

The correlation among water quality parameters (microbial and physicochemical) and (oo)cyst (levels and occurrence) in water samples was determined using the Pearson coefficient correlation, *r*. A strong association between *Cryptosporidium* and *Giardia* (oo)cysts was found ($r = 0.71$) in water samples collected from raw water taps thereby indicating the co-occurrence of both fecal parasites at these sites. In raw water samples, moderate associations were found between *Cryptosporidium* oocysts and water turbidity ($r = 0.48$), between *Giardia* cysts and total coliform counts ($r = 0.41$), and between *Cryptosporidium* and *Giardia* (oo)cysts and water color ($r = 0.47$). There were weak associations between total coliform counts and the occurrence of *Cryptosporidium* oocysts ($r = 0.22$), between oocyst occurrence and *E. coli* counts ($r = -0.14$), and between *Giardia* cysts and *E. coli* counts ($r = 0.3$). No associations were found between microbial water quality data and physicochemical parameters in finished water samples. Protozoan (oo)cysts were found in effluent waters that were negative for indicator bacteria and with turbidity levels between 0.3 and 2.7 NTU. These results indicate that drinking water supplies in compliance with national drinking water standards still do not achieve efficient removal of protozoan (oo)cysts.

DISCUSSION

There is very limited research on protozoan parasites in drinking water supplies in South America. Few studies have been conducted following standard procedures with inclusion of recovery efficiency tests, which facilitates further comparison with protozoan data reported in this study and studies conducted elsewhere. In addition, very few studies in South America have evaluated the occurrence of (oo)cysts in both raw and finished water samples. In urban areas of Brazil, *Cryptosporidium* and *Giardia* were analyzed in water samples from watershed catchments and treated water sources (Razzolini *et al.* 2010). Both protozoa were found in raw and treated water with more frequent detection of *Giardia* than *Cryptosporidium*. Maximum levels of 340 cysts and 10 oocysts/100 L were found in raw water while 6 cysts and 1 oocyst/100 L were detected in finished water. No associations were determined between microbial parameters and physicochemical data. In another study, the Atibaia River source for the Surface Water Treatment Plant of Campinas city, São Paulo, Brazil was monitored for *Giardia* and *Cryptosporidium*. Both protozoa were found at concentrations ranging from 250 to 12,000 cysts/100 L and from 1,500 to 6,000 oocysts/100 L. The high concentration of (oo)cysts was associated with frequent discharges of untreated sewage to Atibaia River (Neto *et al.* 2010). Source waters in Santa Fe Province, Argentina contained higher levels of *Cryptosporidium* (530 oocysts/100 L) than *Giardia* (65 cysts/100 L). Although statistically significant relationships were found between (oo)cysts and water turbidity as well as between (oo)cysts and bacterial indicators, the relationships varied by sampling place, source, and level of water contamination (Abramovich *et al.* 2001). Previous research in a drinking water facility servicing around 1,500,000 people of the second largest city in western Venezuela (Maracaibo, Zulia State) found *Cryptosporidium* oocysts at a higher frequency than *Giardia*, including oocyst concentrations in raw (211 oocysts/100 L vs. 2.38 cysts/100 L) and finished water samples (33.5 oocysts/100 L vs. 4.4 cysts/100 L). No correlations were found between the concentration of bacterial indicators and either protozoa (Quintero-Betancourt & Botero 2000).

The results obtained from this research demonstrated that the passage of (oo)cysts from raw water to finished water occurred at two DWTPs, DWTP II and DWTP III. These results may illustrate how the level of deterioration in source water quality (levels of (oo)cysts) influences the removal efficiency of (oo)cysts through conventional

treatment processes. (Oo)cyst removals could not be efficiently accomplished by those water facilities that depended on drinking water sources highly impacted with domestic, agricultural, and industrial wastewater discharges. Moreover, deficiencies in operational conditions might have also contributed with the passage of (oo)cysts through the conventional treatment process applied at the different water facilities. DWTP I processes raw water that originates from relatively protected surface source waters. The levels of protozoan (oo)cysts found in raw waters were the lowest among the three plants (1–2 (oo)cysts/100 L). In addition, (oo)cysts were never detected in treated water produced at this water facility. DWTP II and DWTP III receive a mixture of waters that originate from catchment areas and reservoirs directly impacted with polluted discharges. The levels of (oo)cysts found in raw waters varied between 25 and 100 per 100 L while the levels of (oo)cysts found in finished waters varied between 2 and 25 per 100 L (Table 1). *Cryptosporidium* and *Giardia* were found at relatively similar concentrations and frequencies in both drinking water supplies; although relatively better removal of *Cryptosporidium* oocysts was accomplished by DWTP II than by DWTP III.

Surface water resources in Venezuela are protected by environmental laws and water quality regulations; however, they are not enforced and compliance with pollution control laws and water quality regulations demands more effective government participation. In addition, partnerships and collaboration among multiple stakeholders (water suppliers, water management agencies, industries, research institutions, and the general population) are strongly required to preserve the nation's water resources. The current Norms for Classification and Control of Surface Water Quality and Effluent Disposal are established under the Organic Law of the Environment (Official Gazette No. 5.021, December 18, 1995). All surface waters in the country are classified to protect different uses or special characteristics of a water body. The microbiological water quality guideline for all waters used for water supply purposes or intended for future water supply is based on total coliform counts (geometric mean of $<$1,000 MPN/100 mL or $\leq$5,000 MPN/100 mL for any individual sample). Water sources for drinking water supply are classified as Type I Waters with three subtypes (IA, IB, and IC). Waters of Subtype IB correspond to surface waters that meet the total coliform counts specified above; these waters require conventional processing for drinking water supply purposes. Based on the results of the coliform tests in this study, source waters used to supply the Metropolitan District of Caracas are classified as Waters of Subtype B.

Monitoring for protozoan parasites in surface water sources is not established under current regulations. As previously discussed, multiple sources of pollution including livestock operations and wastewater discharges from unplanned settlements located within the water catchment areas are most likely responsible for the frequent occurrence of protozoan (oo)cysts in waters for supply in Venezuela. The relative contribution of each source to surface source water quality impairment in Venezuela needs to be continuously monitored in order to verify the overall quality of drinking water sources.

Since treatment barrier instability plays an important role on pathogen passage and occurrence in drinking water supplies worldwide (Geldreich 1996; LeChevalier & Au 2004), plant operators must continuously ensure proper functioning of key processes that control the passage of protozoan (oo)cysts to effluent water. Owing to the resistance of *Cryptosporidium* oocysts to inactivation by chlorine disinfection, optimal coagulation/flocculation, and filtration are required in order to achieve efficient (oo)cyst removals (Dugan *et al.* 2001; States *et al.* 2002; Emelko 2003; Betancourt & Rose 2004; Gitis *et al.* 2005). However, due to increasing water demand the three water facilities that serve the City of Caracas implement operational practices for filters that may adversely affect the quality of the effluent waters. For instance, during the filter ripening process the filters are brought into operation immediately after backwashing, this operational practice increases the potential for microbial breakthrough due to poor filter performance as indicated by LeChevalier & Au (2004). Previous studies also indicated that filters left out of service a few hours produced better effluent water sooner than filters washed and placed into service immediately (Pizzi 1996). Therefore, the application of adequate operational practices combined with the promotion of source water protection programs will ensure the effectiveness of current conventional treatment processes in preventing (oo)cysts and other microbial contaminants from entering the finished waters, thus

ensuring the continuous provision of safe drinking water to consumers.

In the USA, the Long Term Secondary Enhanced Surface Water Treatment Rule (LT2ESWTR) establishes key provisions for reduction of levels of *Cryptosporidium* in finished drinking water (USEPA 2007). According to this rule, large systems (servicing >10,000 people) that use surface water or ground water under the direct influence of surface water (GWUDI) as a source are required to conduct source water monitoring for *Cryptosporidium*, *E. coli*, and/or turbidity. Systems must comply with additional *Cryptosporidium* treatment requirements, by implementing one or more treatment processes or control strategies (i.e., pre-filtration, treatment, additional filtration, and inactivation). Surface water systems with levels of *Cryptosporidium* >0.075 oocysts/L (7.5 oocysts/100 L) are required to upgrade to 3-log (99.9%) removal or inactivation using additional treatment. Similarly, those systems with *Cryptosporidium* levels above 1 oocyst/100 L (100 oocysts/100 L) or higher (>3 oocysts/L or 300 oocysts/100 L) must achieve at least 1.0-log of additional treatment. Removal and inactivation processes must be accomplished using either one or a combination of different treatments (e.g., bag filters, bank filtration, cartridge filters, chlorine dioxide, membranes, ozone, or UV). The treatment technique (TT) requirements of the LT2ESWTR are expected to increase the level of protection from exposure to *Giardia* as well (USEPA 2007).

The *Cryptosporidium* and *Giardia* (oo)cyst levels detected in the raw and finished water samples for DWTP II and DWTP III were translated to public health risks (Tables 3 and 4). DWTP I had non-detects for all finished water samples, therefore estimate risk reductions were not calculated for this plant. Daily infection risks were estimated for both *Cryptosporidium* and *Giardia* using the exponential model:

$$P_i = 1 - \exp(-N/k),$$

where P_i = the probability of infection, N = the number of infectious protozoa in the exposure, and 1/k refers to the fraction of *Cryptosporidium* oocysts and *Giardia* cysts that survive and are capable of initiating an infection (0.0042 and 0.0199, respectively). This model assumes a random distribution of parasites in the water and a 2 L daily exposure.

Overall, daily infection risk estimates for raw water were approximately the same for both DWTP II and DWTP III (Tables 3 and 4). For finished water, daily infection risks ranged from 8/100,000 for *Cryptosporidium* to almost 1/100 for *Giardia*, both at DWTP II. When addressing water treatment and infection risk reduction, in most cases a one-log reduction in predicted number of infections was achieved at both plants. DWTPII actually achieved an overall higher infection risk reduction associated with both *Cryptosporidium* and *Giardia* (oo)cysts than DWTP III.

Table 3 | Daily human health risk estimates associated with exposure to waterborne *Cryptosporidium* and *Giardia* – DWTP II

Organism	Sample	Raw	Finished	Log reduction
Cryptosporidium	1	1.54×10^{-2}[a]	8.02×10^{-4}[a]	2
	2	2.03×10^{-2}	1.14×10^{-3}[a]	1
	3	4.19×10^{-3}	6.72×10^{-4}	1
	4	2.10×10^{-3}	8.40×10^{-5}	2
	5	5.98×10^{-3}	5.00×10^{-4}[a]	1
Giardia	1	5.53×10^{-2}[a]	4.71×10^{-3}	1
	2	8.30×10^{-2}	4.74×10^{-3}[a]	1
	3	5.77×10^{-2}	9.90×10^{-3}	1
	4	9.90×10^{-3}	3.98×10^{-4}	1
	5	7.65×10^{-2}	7.93×10^{-3}[a]	1

[a]Based on detection limit.

Table 4 | Daily human health risk estimates associated with exposure to waterborne *Cryptosporidium* and *Giardia* – DWTP III

Organism	Sample	Raw	Finished	Log reduction
Cryptosporidium	1	3.93×10^{-3}[a]	4.67×10^{-4}[a]	1
	2	3.36×10^{-3}[a]	9.97×10^{-4}	1
	3	2.10×10^{-3}	2.52×10^{-4}	1
	4	4.19×10^{-3}	4.20×10^{-4}	1
	5	6.98×10^{-3}	3.00×10^{-4}	1
Giardia	1	2.04×10^{-2}[a]	4.54×10^{-3}[a]	1
	2	1.52×10^{-2}	4.79×10^{-3}	1
	3	9.90×10^{-3}	1.19×10^{-3}	0
	4	1.97×10^{-2}	1.99×10^{-3}	1
	5	1.50×10^{-2}	4.84×10^{-3}	1

[a]Based on detection limit.

Specifically, higher infection risk reductions were observed at DWTP II for *Cryptosporidium*. Raw water (oo)cyst levels were similar for both DWTP II and DWTP III. When comparing risks associated with *Cryptosporidium* with those estimated for *Giardia*, *Giardia* was associated with higher predicted daily infection risks for both raw water and finished water at both plants. If assuming yearly exposures in the analyses above, almost all of the infection risk estimates closely approach 1.

The infection risks estimated in this study for enteric protozoa in raw and finished waters can inform performance targets (i.e., pathogen removal targets) to determine if the conventional treatment processes can achieve the microbial reduction necessary to ensure the safety of drinking water and, thus, a specified level of tolerable risk (10^{-6} DALY (disability-adjusted life years) per person per year). *Cryptosporidium* is used as a reference pathogen that, if controlled, would ensure control of other pathogens of concern like *Giardia*. For drinking water consumption of 2 L per day and a raw water concentration of 1 oocyst/L the performance target for *Cryptosporidium* corresponds to 5.2 log units (WHO 2008). The concentrations of (oo)cysts found in this study and the corresponding log reductions between 1 and 2 log units for *Cryptosporidium* and *Giardia* (Tables 3 and 4) would indicate that DWTP II and DWTP III do not meet the performance targets for waterborne protozoan (oo)cysts.

The Norms of the Sanitary Quality of Water in Venezuela are currently under revision to include *Cryptosporidium* monitoring of source waters and finished drinking waters. This research may provide water utilities with relevant information on appropriate methods for monitoring *Cryptosporidium* and *Giardia* in drinking water supplies. It is also the first study in Venezuela that uses quantitative microbial risk assessment to better understand the health risks associated with exposure to enteric protozoa in drinking water. Venezuela urgently needs appropriate water quality studies addressed to the identification of major sources of fecal pollution and their relative contributions to pollution loads deteriorating drinking surface water supplies. These research measures would be intended to adequately inform government regulators of the problems associated with multiple sources of water pollution in the watersheds, and consequently to demonstrate the need for implementing more effective enforcement mechanisms to prevent and control surface water pollution.

The suitability of current and newer culture-based methods for quantification of indicator bacteria in drinking water supplies has played an important role towards water compliance monitoring. Nevertheless, numerous studies have demonstrated significant differences in the performance of methods used for detection of total coliforms and *E. coli* in water samples (Rompre *et al.* 2002; Macy *et al.* 2005). Additionally, these microbial parameters have been demonstrated to be inadequate, as in this study, for assessing

and controlling exposure to waterborne protozoan pathogens in drinking water (Rose *et al.* 1996; Barrell *et al.* 2000). Therefore, the application of monitoring strategies that include multiple microbial indicators and waterborne pathogens is strongly recommended for accurate source water characterization and for verification of the effectiveness of treatment process barriers to microbial breakthrough in the finished water.

CONCLUSIONS

This study demonstrated that the conventional treatment process to produce finished water at two filtration plants was not effective in preventing the passage of protozoan (oo)cysts. *Cryptosporidium* and *Giardia* (oo)cysts were found in raw source waters and effluent waters of two major treatment facilities that provide water for consumption to more than two million inhabitants of the metropolitan city of Caracas. Full conventional treatment was not able to remove (oo)cysts effectively from drinking water supplies that depended on source waters highly impacted by illegal dumping and pollution discharges. In addition, the modification of plant operational practices to meet growing drinking water demands might be contributing to the passage of (oo)cysts to the finished waters. It is therefore fundamental to prevent waterborne (oo)cysts and other microbial contaminants from entering source waters and to maintain optimal treatment operations in order to ensure a safe and dependable drinking water supply.

The data of the present investigation were based on microscopic counts following procedures included in US EPA Method 1623; further genotyping of the protozoan parasites and infectivity assays are strongly necessary for accurate estimations of public health risks. The analysis of additional waterborne pathogens (e.g., viruses) in source waters may allow derivation of location-specific data required for identifying pathogen removal targets and appropriate control measures necessary to improve the water treatment process. Furthermore, the analysis of multiple microbial indicators (e.g., coliforms and *E. coli*, enterococci, *Clostridium perfringens*, coliphages, and host-specific fecal microbial markers), and waterborne pathogens is highly recommended in order to assess the performance of current and alternative surrogate indicators of pathogen removal at full-scale conventional water treatment level. The development of reliable databases on occurrence and exposure to waterborne pathogens is warranted to safeguard public health.

ACKNOWLEDGEMENTS

The authors thank HIDROCAPITAL and personnel from each water facility for logistical support. Funding for this project was provided under the Organic Law of Science and Technology (LOCTI-990) and by the Venezuelan Institute for Scientific Research (IVIC-883). The schematic of the drinking water supply system created by the IVIC Department of Design is greatly appreciated.

REFERENCES

Abramovich, B. L., Gilli, M. I., Haye, M. A., Carrera, E., Lurá, M. C., Nepote, A., Gómez, P. A., Vaira, S. & Contini, L. 2001 *Cryptosporidium* and *Giardia* in surface water. *Rev. Argent. Microbiol.* **33**, 167–176.

Barrell, R., Hunter, P. R. & Nichols, G. 2000 Microbiological standards for water and their relationship to health risk. *Commun. Dis. Public Health* **3**, 8–13.

Bernhard, A. E. & Field, K. G. 2000 Identification of nonpoint sources of fecal pollution in coastal waters by using host-specific 16S ribosomal DNA genetic markers from fecal anaerobes. *Appl. Environ. Microbiol.* **66**, 1587–1594.

Betancourt, W. Q. & Rose, J. B. 2004 Drinking water treatment processes for removal of *Cryptosporidium* and *Giardia*. *Vet. Parasitol.* **126**, 219–234.

Dick, L. K. & Field, K. G. 2004 Rapid estimation of numbers of fecal *Bacteroidetes* by use of a quantitative PCR assay for 16S rRNA genes. *Appl. Environ. Microbiol.* **70**, 5695–5697.

Dugan, N. R., Fox, K. R., Owens, J. H. & Miltner, R. J. 2001 Controlling *Cryptosporidium* oocysts using conventional filtration. *J. Am. Water Works Assoc.* **93**, 64–77.

Emelko, M. R. 2003 Removal of viable and inactivated *Cryptosporidium* by dual- and tri-media filtration. *Water Res.* **37**, 2998–3008.

Fiksdal, L., Make, J. S., LaCroix, S. J. & Staley, J. T. 1985 Survival and detection of Bacteroides spp., prospective indicator bacteria. *Appl. Environ. Microbiol.* **49**, 148–150.

Fujioka, R., Dian-Denton, C. & Castro, J. 1999 Soil: the environmental source of *Escherichia coli* and enterococci in Guam's streams. *J. Appl. Microbiol.* **85**, 83S–89S.

Geldreich, E. E. 1996 Waterborne pathogen invasions: a case for water quality protection in distribution. In: *Microbial Quality of Water Supply in Distribution Systems* (E. E. Geldreich ed.). CRC Press, New York, pp. 359–410.

Gitis, V., Freger, V., Haugth, R. C. & Clark, R. M. 2005 Removal of *Cryptosporidium parvum* oocysts by rapid sand filtration with ballasted flocculation-filtration and intermediate downwashes. *Acta Hydrochim. Hydrobiol.* **33**, 355–364.

Karanis, P., Kourenti, C. & Smith, H. 2007 Waterborne transmission of protozoan parasites: A worldwide review of outbreaks and lessons learnt. *J. Water Health* **5**, 1–38.

Kavvas, M. 2002 An appraisal of sewer system problems in developing countries. *Water Policy* **4**, 323–331.

LeChevalier, M. W. & Au, K. 2004 *Water Treatment and Pathogen Control: Process Efficiency in Achieving Safe Drinking Water*. World Health Organization. IWA Publishing, London, UK, pp. 1–110.

Macy, J. T., Dunne, E. F., Angoran-Benie, Y. H., Kamelan-Tano, Y., Kouadio, L., Djai, K. A. & Luby, S. P. 2005 Comparison of two methods for evaluating the quality of stored drinking water in Abidjan, Côte d'Ivoire, and review of other comparisons in the literature. *J. Water Health* **3**, 221–228.

McFeters, G. A., Kippin, J. S. & LeChevalier, M. W. 1986 Injured coliforms in drinking water. *Appl. Environ. Microbiol.* **51**, 1–5.

Neto, R. C., dos Santos, L. U., Sato, M. I. & Franco, R. M. 2010 *Cryptosporidium* spp. and *Giardia* spp. in surface water supply of Campinas, southeast Brazil. *Water Sci. Technol.* **62**, 217–222.

Okhuysen, P. C., Chappell, C. L., Crabb, J. H., Sterling, C. R. & DuPont, H. L. 1999 Virulence of three distinct *Cryptosporidium parvum* isolates for healthy adults. *J. Infect. Dis.* **180**, 1275–1281.

Olapade, O. A., Depas, M. M., Jensen, E. T. & McLellan, S. L. 2006 Microbial communities and fecal indicator bacteria associated with *Cladophora* mats on beach sites along Michigan shores. *Appl. Environ. Microbiol.* **72**, 1932–1938.

Pizzi, N. 1996 Optimizing your plant's filter performance. *OpFlow* **22** (5), 1–5.

Quintero-Betancourt, W. & Botero, L. 2000 Descriptive study on the presence of protozoan cysts and bacterial indicators in a drinking water treatment plant in Maracaibo, Venezuela. *Int. J. Environ. Health Res.* **10**, 51–61.

Razzolini, M. T., da Silva, S. & Bastos, V. K. 2010 Detection of *Giardia* and *Cryptosporidium*/oocysts in watersheds and drinking water sources in Brazil urban areas. *J. Water Health* **8**, 399–404.

Rendtorff, R. C. 1954 The experimental transmission of human intestinal protozoan parasites. 1. *Giardia lamblia* cysts given in capsules. *Am. J. Hyg.* **59**, 209–220.

Rompre, A., Pierre-Servais, J. B., de Roubin, M. R. & Laurent, P. 2002 Detection and enumeration of coliforms in drinking water: current methods and emerging approaches. *J. Microbiol. Methods* **49**, 31–54.

Rose, J. B., Lisle, J. T. & Haas, C. N. 1996 The role of pathogen monitoring in microbial risk assessment. In: *Modeling Disease Transmission and its Prevention by Disinfection* (C. J. Hurst, ed.). Cambridge University Press, New York, USA, pp. 1–408.

Savage, D. C. 2001 Microbial biota of the human intestine: a tribute to some pioneering scientists. *Curr. Iss. Intest. Microbiol.* **2**, 1–15.

Savioli, L. H., Smith, H. V. & Thompson, A. 2006 *Giardia* and *Cryptosporidium* join the 'Neglected Diseases Initiative'. *Trends Parasitol.* **22**, 203–208.

Smith, H. V., Cacciò, S. M., Tait, A., McLauchlin, J. & Thompson, R. C. 2006 Tools for investigating the environmental transmission of *Cryptosporidium* and *Giardia* infections in humans. *Trends Parasitol.* **22**, 160–167.

Standard Methods for the Examination of Water and Wastewater 1998 20th edition. American Public Health Association/ American Water Works Association/Water Environment Federation, Washington, DC.

States, S. M., Tomko, R., Scheuring, M. & Casson, L. 2002 Enhanced coagulation and removal of *Cryptosporidium*. *J. Am. Water Works Assoc.* **94**, 67–77.

Teunis, P. F. M. & Havelaar, A. H. 2002 Risk assessment for protozoan parasites. *Int. J. Biodeter. Biodegrad.* **50**, 185–193.

USEPA 1999 *Method 1623: Cryptosporidium and Giardia in Water by Filtration/IMS/FA. EPA-821-R-99-006*. US Environmental Protection Agency, Washington, DC, USA.

USEPA 2007 *The Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR) Implementation Guidance. EPA 816-R-07-006*. US Environmental Protection Agency, Washington, DC, USA.

Whitman, R. L., Nevers, M. B. & Byappanahalli, M. N. 2006 Examination of the watershed-wide distribution of *Escherichia coli* along southern Lake Michigan: an integrated approach. *Appl. Environ. Microbiol.* **72**, 7301–7310.

WHO 2008 *Guidelines for Drinking-Water Quality*. 3rd edition. World Health Organization, Geneva.

First received 19 July 2011; accepted in revised form 5 March 2012. Available online 12 May 2012