Long-term study of *Cryptosporidium* and *Giardia* occurrence and quantitative microbial risk assessment in surface waters of Arizona in the USA

Hodon Ryu and Morteza Abbaszadegan

ABSTRACT

A four-year systematic study of microbial water quality in the surface waters of central Arizona was performed. The objectives of this study were to investigate the occurrence of microbial pathogens and indicators in the waters. A total of 192 water samples from five sites were analyzed for *Cryptosporidium*, *Giardia*, and various microbial and physical indicators. Ten percent (16/162) of the samples collected using EnviroChek filters were positive for *Cryptosporidium* oocysts, whereas no oocysts ($<$ 1 in 5 L) were detected in the grab samples (0/30). *Giardia* cysts were detected in 10% (3/30) of the grab samples and in 27% (44/162) samples collected using EnviroChek filters. Mean concentrations of oocysts in the source waters at the treatment plants were lower than the Bin 1 category of the USEPA Long Term 2 Enhanced Surface Water Treatment Rule; therefore no additional treatment is required by the plants. The annual risks of infection from *Cryptosporidium* met the annual acceptable risk of 10^{-4} at all sampling sites, whereas the risks of *Giardia* infection at the Verde River and the Salt River were 5.70E-04 and 2.66E-04, respectively.

Key words | *Cryptosporidium*, *Giardia*, microbial indicators, risk assessment

Hodon Ryu
Morteza Abbaszadegan (corresponding author)
Arizona State University,
National Science Foundation Water
 Quality Center,
Department of Civil and Environmental
 Engineering,
ECG 252, Tempe,
AZ 85287-5306,
USA
Tel.: +1 480 965 3868
Fax: +1 480 965 0557
E-mail: *abbaszadegan@asu.edu*

INTRODUCTION

The pathogenic protozoan parasites, *Cryptosporidium* and *Giardia*, are significant microbial contaminants in the U.S. (Craun 1990; Fayer *et al*. 1997) and have been found in surface waters and filtered drinking water supplies (LeChevallier *et al*. 1991*a*, *b*, 2003; LeChevallier & Norton 1995; Aboytes *et al*. 2004). *Cryptosporidium* and *Giardia* produce environmentally resistant oocysts and cysts, respectively, which allow the parasites to survive in water for extended periods. Many waterborne outbreaks of cryptosporidiosis and giardiasis have been reported in North America (Mackenzie *et al*. 1994; Kramer *et al*. 1996). Breakdowns or overloading of public water utilities with microbial pathogens have occasionally resulted in community outbreaks of gastroenteritis.

The microbial content of drinking water is regulated through the Total Coliform Rule and the Surface Water Treatment Rule (SWTR) in the U.S. According to the SWTR, a minimum treatment level of 99.9% removal (3-log reduction) of *Giardia* and 99.99% removal (4-log reduction) of enteric viruses is required (USEPA 1989). *Cryptosporidium* was listed as a potential contaminant of concern under the Safe Drinking Water Act in 1987 and then was included in an Interim Enhanced Surface Water Treatment Rule (IESWTR) in 1998, requiring the removal of 99.9% (3-log reduction) of oocysts (USEPA 1998). However, there are still no regulations in the U.S. which specifically address *Cryptosporidium* in potable supplies. Recently, the Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR) was promulgated by U.S. Environmental Protection Agency (EPA), which requires drinking water source monitoring for *Cryptosporidium* oocysts (USEPA 2006).

The greater Phoenix metropolitan area in central Arizona is one of the fastest growing urban areas in the United States (U.S.), and drinking water demands in this area have been increased significantly. Considering the diversity of pollutant sources in surface waters, it is prudent to undertake a wide range of analyses to establish the overall safety and quality of the water. Moreover, various water quality factors should be taken fully into account before deciding which technologies to employ in the treatment of source water. However, few studies on microbial surveillance in surface waters have been performed, and no characterization of protozoan parasites has been reported in this study area. The objectives of this study were to investigate the long-term occurrence of *Cryptosporidium* and *Giardia* in source waters of central Arizona, to perform risk assessment analyses for these pathogens to determine the public health significance associated with the sources of drinking water, and to analyze the correlation between the pathogens, indicator microorganisms, and physicochemical parameters.

MATERIALS AND METHODS

Sampling sites

The drinking water consumed in this area originates from the Verde River, the Salt River, and Central Arizona Project (CAP) canal water and groundwater. The three surface waters have different types of watershed areas. For example, cattle and horse ranching are the major activities in the watershed area of the Verde River which may be a probable source of microbial contamination, and Salt River water flowing from the eastern mountains travels through recreational areas where it may be exposed to high levels of anthropogenic contamination. The CAP canal starts from the Parker Dam in the middle of the Arizona-California state border. It delivers Colorado River water to central Arizona via an open canal (540 km long). The extensive watershed areas drain into the three surface water sources, thereby increasing the possibility of microbial contamination from wildlife and agricultural, rural, and urban runoff.

Three surface waters and two participating drinking water treatment plants (WTPs) were selected as the sampling sites (Figure 1). The surface waters are blended, and then are delivered to WTPs in the area via the metropolitan canal system. Briefly, the Granite Reef Diversion Dam, which is located downstream of the confluence of the Verde River and the Salt River, diverts water from the rivers into two major canals, the Arizona Canal and the South Canal. CAP water is also added to the canals below the Granite Reef Diversion Dam. Additionally, groundwater is added to canal water from several groundwater wells along the metropolitan canals.
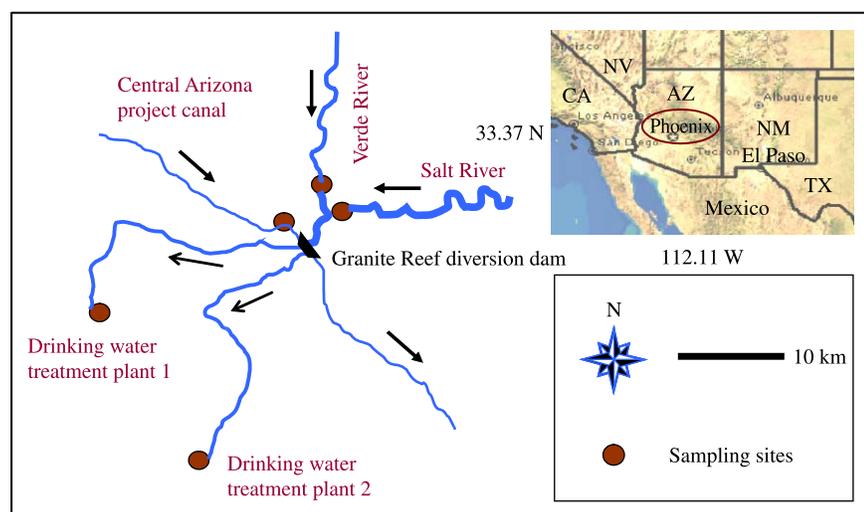

Figure 1 | Sampling sites in central Arizona.

Sample collection and processing

A total of 192 samples were collected at five designated sites on a monthly basis for four years (July 2002 through May 2006). The two sampling strategies used for *Cryptosporidium* oocysts and *Giardia* cysts included grab sampling (10 litres) or filtration (40–100 litres). The grab sampling technique was used for the first seven months, but the filtration technique was then adopted to increase sample volume. The grab samples were collected in a 10-litre plastic container and concentrated using centrifugation. The sample collection by filtration was performed using an Envirochek-HV sampling capsule (Gelman Sciences, Ann Arbor, MI) at flow rates of no more than 2 litres per min. Samples were delivered at 4°C to the Environmental Microbiology Laboratory at Arizona State University, Tempe, AZ and processed within 48 h of sample collection. For bacteria and coliphage analyses, grab samples were collected in sterilized 250-ml polypropylene bottles (Nalge Nunc International, Rochester, NY) and shipped in the same container along with the parasite samples. The samples for bacteria and coliphages were analyzed within 12 h and 36 h of sample collection, respectively. Samples for chemical parameters were collected in 500-ml amber glass bottles with Teflon liners (VWR, Plainfield, NJ). Turbidity, pH, temperature, and specific conductivity were measured during sample collection in the field.

Recovery efficiency of oocysts and cysts from seeded water samples

Cryptosporidium parvum oocysts (Iowa isolate) were obtained from the Sterling Parasitology Laboratory, the University of Arizona, Tucson, AZ. The oocysts were stored in antibiotic solution (100 μg/ml penicillin and 100 μg/ml gentamicin) containing 0.01% Tween 20. *Giardia lamblia* cysts (H3 isolate) were obtained from Waterborne (New Orleans, LA) as purified suspensions in phosphate-buffered saline (PBS) with penicillin, streptomycin, genta-micin, and amphotericin B. The number of oocysts and cysts in the stock suspensions were confirmed by direct count using a hemacytometer. Procedural recovery efficiency evaluations were performed, and mean percent recoveries and relative standard deviations (RSD) were calculated (USEPA 2001*a*). Briefly, 10 litres of water from each selected

source were seeded with 100–200 oocysts and cysts. The seeded water samples were filtered through Envirochek-HV capsules. Oocysts and cysts were purified using an immuno-magnetic separation (IMS) technique (Dynabeads GC-Combo; Dynal A.S., Oslo, Norway), labeled using immunofluorescence assay (IFA) (Hydroflour Combo; Strategic Diagnostics Inc., Newark, DE) and vital dye assay (4′,6′-diamidino-2-phenylindole [DAPI]), and enumerated by epifluorescence microscopy (BX-60, Olympus Optical Co., Ltd., Tokyo, Japan).

Recovery of oocysts and cysts from surface water samples

Samples were analyzed for *Cryptosporidium* oocysts and *Giardia* cysts using IMS followed by IFA as described in the USEPA Method 1623 (USEPA 2001*a*). An integrated cell culture-polymerase chain reaction assay (ICC-PCR) was used to detect infectious oocysts as described by Di Giovanni *et al*. (1999). Briefly, the filter was eluted and the concentrate was purified by IMS. Purified samples (110 μl) were divided into 60-μl and 50-μl aliquots, which were assayed by IFA microscopy and ICC-PCR, respectively.

Determination of average concentration of oocysts and cysts in the samples

Methods proposed to determine reliable average concentration include: 1) effective volume (EV)-weighted average, 2) arithmetic average, and 3) the no-zeros approach (treating zeros as ones) (Haas *et al*. 1996; Parkhurst & Stern 1998). Effective volume (EV) represents total accumulative sample volume assayed for each site over the period of study. This strategy was suggested by Parkhurst & Stern (1998) to obtain relatively unbiased estimates of mean concentrations of microbes in average volume. In the present study, the EV-weighted average method was used to calculate the concentrations of oocysts and cysts in surface water as follows,

$$\text{Average} = \frac{N}{EV}$$

Where, N is the total number of oocysts and cysts observed, and EV is the total effective volume of water assayed.

Indicator microorganisms

Ten-ml water samples were assayed for coliphages using the double agar layer technique (Adams 1959). *Escherichia coli* F_{amp} (ATCC 700891) and *E. coli* CN-13 (ATCC 700609) were used to detect male specific coliphages and somatic coliphages, respectively (USEPA 2001b). Five-ml samples were added to molten top agar with the appropriate host and plated on bottom agar plates in duplicate. The plates were incubated at 37°C overnight, and plaques were counted after 12 h. Positive and negative controls were included in each set of assays and for each coliphage group.

Total coliform and fecal coliform analyses were performed using the membrane filtration technique on mEndo LES agar (Difco, Sparks, MD) and mFC agar (EM Science, Gibbstown, NJ), respectively (APHA 1995). Samples in triplicate were filtered using 47-mm diameter cellulose acetate membranes with 0.45-μm pore size (Pall Gelman Laboratory, Ann Arbor, MI). The colony forming units for total coliforms and fecal coliforms were enumerated after overnight incubation at 35°C and 44.5°C, respectively. HPC bacterial analysis was performed using the spread plate technique on the standard minimal medium, R2A (Difco, Sparks, MD). Samples were spread uniformly on the agar with a glass spreader, and colonies were enumerated after five to seven days incubation at 27°C (APHA 1995).

Quantitative microbial risk assessment

The exponential dose-response model developed by Haas (1983) was chosen to determine the probability of infection from ingestion of various numbers of *Cryptosporidium* and *Giardia*. The exponential model is:

$$P_d = 1 - \exp\left(-\frac{N}{K}\right)$$

where P_d is the probability of infection resulting from daily ingestion of the number of pathogens (N). K is the average number of organisms which must be ingested to initiate an infection. The best-fit K values for *Cryptosporidium* for unknown strains and *Giardia* are 35.7 (80% confidence limits, 15.2–200.0) and 50.5 (95% confidence limits, 27.9–102.1), respectively (Rose *et al.* 1991; Messner *et al.* 2001). N is the number of *Cryptosporidium* and *Giardia* ingested daily by a person through potable water.

Exposure assessment was performed to estimate the average exposure (N) using the following Equation (Ryu *et al.* 2005):

$$N = C \times R^{-1} \times I \times 10^{-LR} \times V$$

where C is the concentration of *Cryptosporidium* and *Giardia* ((oo)cysts/ L), R is the recovery efficiency of the detection method, I is the fraction of detected pathogens capable of infection, LR is the removal efficiency of the pathogens during treatment processes, and V is a daily consumption of unboiled water for an average consumer. The parameters and assumptions for the calculation are presented in Table 1.

Estimates of daily risk may be extrapolated to the risk of infection over extended periods of time using the following Equation (Haas 1983):

$$P_t = 1 - (1 - P_d)^t$$

where P_t and P_d are the probability of infection after t (350) days and one day of exposure, respectively.

Assuming that the risks of infection from both parasites are independent, combined risks (P_{comb}) can be estimated using the following Equation (Teunis *et al.* 1997):

$$P_{comb} = 1 - (1 - P_c)(1 - P_g) = P_c + P_g - (P_c \times P_g)$$

where P_c and P_g are the risks of infection from *Cryptosporidium* and *Giardia*, respectively.

Statistical analysis

SPSS version 11.0 (SPSS Inc., Chicago, IL) was used for statistical analysis. Both the Kolmogorov-Smirnov and Shapiro-Wilk tests were performed for tests of normality, and data transformation was performed to convert a non-normal to normal distribution. The arithmetic means for each sampling season and each sampling site were compared using Analysis of Variance (ANOVA), and a Levene's test was performed to determine equality of variances prior to ANOVA. The analyses were performed at a significance level (α) of 0.05. If significant differences were observed, Duncan's post hoc test was performed to determine which values differed from all other values. For data which was not normally distributed, microbial

Table 1 │ Parameters and assumptions for estimating the risks of infection for *Cryptosporidium* and *Giardia* using an exponential model

Parameters for the calculation	$N = C \times R^{-1} \times I \times 10^{-LR} \times V$
	N: daily ingestion of the number of *Cryptosporidium* and *Giardia*
	C: mean concentration of *Cryptosporidium* and *Giardia* (Table 4)
	R: 0.81 and 0.31 for *Cryptosporidium* and *Giardia*, respectively
	I: 0.41 and 0.22 for *Cryptosporidium* and *Giardia*, respectively
	LR: 3.0 log − units reduction
	V: 2 litres
	$P_d = 1 - \exp(-N/K)$, $P_t = 1 - (1 - P_d)^t$
	P_t and P_d: probability of infection after t (350) days and one day of exposure
	K: average number of organisms which must be ingested to initiate an infection (35.7 for *Cryptosporidium* and 50.5 for *Giardia*)
Daily and annual risks	$C = 0.003 \ oocyst/1 \ L \ for \ the \ Verde \ River \ (Cryptosporidium)$
	$N \ (oocysts/day) \ = [0.003 \ \times \ 0.81^{-1} \times 0.41 \times 10^{-3} \times 2] = 3.04 \times 10^{-6}$
	$P_d \ = 1 - \exp(-3.04 \times 10^{-6}/35.7) = 8.51 \times 10^{-8}$
	$P_t \ = 1 - (1 - 8.51 \times 10^{-8})^{350} = 2.98 \times 10^{-5}$
	$C = 0.058 \ cyst/1 \ L \ for \ the \ Verde \ River \ (Giardia)$
	$N \ (cysts/day) = [0.058 \times 0.31^{-1} \times 0.22 \times 10^{-3} \times 2] = 8.23 \times 10^{-5}$
	$P_d \ = 1 - \exp(-8.23 \times 10^{-5}/50.5) = 1.63 \times 10^{-6}$
	$P_t \ = 1 - (1 - 1.63 \times 10^{-6})^{350} = 5.70 \times 10^{-4}$

prevalence was compared using the nonparametric Kruskal-Wallis test. In this study, means and standard deviations were calculated for HPC bacteria, total coliforms, fecal coliforms, and physicochemical parameters, and prevalence was calculated for *Cryptosporidium* spp., *Giardia* spp., male specific coliphages, and somatic coliphages. A nonparametric Spearman rank order correlation coefficient with a two-tailed P value was calculated for cross-correlations between indicator microorganisms, physicochemical parameters, and presence or absence of *Cryptosporidium* spp. and *Giardia* spp. P values of <0.05 were considered significant.

RESULTS AND DISCUSSION

Cryptosporidium spp. and Giardia spp

Mean recoveries and RSD of *Cryptosporidium* oocysts and *Giardia* cysts from four different types of seeded water samples were measured. The percent recovery efficiencies of oocysts and cysts from seeded central Arizona surface water averaged 81 ± 15 [mean ± RSD] and 31 ± 43, respectively. Although *Cryptosporidium* recovery efficiencies tended to be higher in less turbid water, there was no significant difference at an α level of 0.05 in the recovery of *Giardia* (Table 2). A total of 192 surface water samples were analyzed for *Cryptosporidium* oocysts and *Giardia* cysts. Ten percent (16/162) of the filtered samples were positive for *Cryptosporidium* oocysts using IMS-IFA technique, whereas no oocysts (<1 in 5 L) were detected in any of the grab samples (0/30) by either IFA or ICC-PCR assay. *Giardia* cysts were detected in 10% (3/30) of the grab samples and 27% (44/162) of the filtered samples. Filtration technique allowed higher sample volume and significantly increased ($P < 0.01$) the detection of oocysts and cysts in the study area.

Mean concentrations of *Cryptosporidium* spp. and *Giardia* spp. for five designated sampling sites ranged between 0.2 and 0.5 oocysts/100 litres and between 0.1

Table 2 | Mean percent recoveries for (oo)cysts and relative standard deviations (RSD) from seeded water samples

	Surface water			Reagent water (*n* = 3)
	Low turbidity[a] (*n* = 7)	Medium turbidity[b] (*n* = 14)	High turbidity[c] (*n* = 3)	
Cryptosporidium[d]	81 ± 15	37 ± 29	29 ± 26	83 ± 12
Giardia	31 ± 43	45 ± 28	ND[e]	56 ± 45

[a]Phoenix surface waters such as the Verde River, the Salt River, and Central Arizona Project canal water from the Colorado River were <5 NTU. The *Cryptosporidium* recovery efficiencies of low turbidity water and reagent water were not statistically different at the 95% confidence level ($P = 0.879$).
[b]Water turbidity ranged between 5 and 10 NTU. The samples were provided by USEPA on a quarterly basis (January of 2004 to June of 2005) to assess laboratory's performance for protozoan analysis.
[c]Rio Grande River water (>90 NTU) (Ryu *et al*. 2005).
[d]The results of oocyst recovery using IFA and ICC-PCR were comparable.
[e]Not determined.
Note: The percent values in the table are expressed as the mean ± RSD.

and 5.8 cysts/100 litres, respectively. *Giardia* spp. was detected in central Arizona surface water more often and at higher concentrations than *Cryptosporidium* spp. According to the final LT2ESWTR in the U.S., drinking water plants which use surface water as a drinking water source will be classified in one of the 4 bins (Table 3) based on mean concentrations of *Cryptosporidium* oocysts in raw water (USEPA 2006). The two participating drinking water plants for this study would be classified in Bin 1 (Table 4), which requires no additional treatment under LT2ESWTR. The fraction of viable oocysts and cysts determined by vital dye assay were 41% and 22% in central Arizona surface water, respectively, and these fractions were used for exposure assessment while performing quantitative microbial risk assessment. These

Table 3 | LT2ESWTR bin classification for conventional drinking water treatment plants

Bin number	*Cryptosporidium* concentraion (oocysts/L)	Additional treatment in conjunction with current IESWTR requirements[a]
1	< 0.075	No action
2	≧ 0.075 and < 1.0	1.0-log treatment
3	≧ 1.0 and < 3.0	2.0-log treatment
4	≧ 3.0	3.0-log treatment

[a]the removal of 99.9% (3-log reduction) of *Cryptosporidium* oocysts.

results are consistent with previous reports. LeChevallier *et al*. (1991a) reported 38% and 13% of viable oocysts and cysts in surface water, respectively. In another study, out of 18 IFA positive samples in surface water, 15 samples (87%) were found positive using the ICC-PCR technique (LeChevallier *et al*. 2003).

Risk assessment

To evaluate microbial water quality, the concentrations of both *Cryptosporidium* and *Giardia* need to be considered for microbial risk assessment. An annual acceptable microbial risk of infection of 1.00E-04 (10^{-4}) from waterborne exposure through potable water was applied for performing risk characterizations (Regli *et al*. 1991). The risks of infection from *Giardia* were approximately one order of magnitude higher than those from *Cryptosporidium*. The annual risks of infection from *Cryptosporidium* meet the annual acceptable risk of 10^{-4} at all sampling sites, falling within the range of 4.96E-05 to 1.98E-05, whereas the risks of infection from *Giardia* at the Verde River and the Salt River were 5.70E-04 and 2.66E-04, respectively (Table 4). Since the risks of infection from *Giardia* were much higher than those from *Cryptosporidium*, the combined risks from both parasites are almost identical to that of *Giardia*.

The probability of infection may have been overestimated in this study since all species of *Cryptosporidium* and *Giardia* were considered for risk calculation, not just those which infect humans. It is unlikely that all *Cryptosporidium* and *Giardia* detected in water samples are human pathogenic-species. Two species of *Cryptosporidium* (*hominis* and *parvum*) and *Giardia lamblia* are responsible for most human infections. However, USEPA Method 1623, which was used for this study, does not differentiate among species of *Cryptosporidium* or *Giardia*. In addition, a daily consumption of unboiled water for an average consumer could contribute to risk overestimations or underestimations. In the previous reports, various water consumption amounts were used for calculating microbial risk via drinking water: 100 – 500 ml (Teunis *et al*. 1997), 47 – 322 ml (Gale 2001), a median consumption of 0.96 litres (Roseberry & Burmaster 1992), 1.2 litres (LeChevallier *et al*. 2003), and 2 litres (Regli *et al*. 1991). In this study, for the

Table 4 | Estimated daily and annual risks of infection for *Cryptosporidium* oocysts and *Giardia* cysts

Sampling sites	Mean concentrations		Oocysts		Cysts		Oocysts + Cysts	
	Oocysts/L	Cysts/L	Daily	Annual	Daily	Annual	Daily	Annual
Verde River	0.003	0.058	8.51E-08	2.98E-05	1.63E-06	5.70E-04	1.72E-06	6.00E-04
Salt River	0.002	0.027	5.67E-08	1.98E-05	7.59E-07	2.66E-04	8.16E-07	2.85E-04
CAP water	0.004	0.001	1.13E-07	3.97E-05	2.81E-08	9.84E-06	1.42E-07	4.95E-05
WTP 1	N/A	0.005	N/A	N/A	1.41E-07	4.92E-05	1.41E-07	4.92E-05
WTP 2	0.005	0.003	1.42E-07	4.96E-05	8.43E-08	2.95E-05	2.26E-07	7.91E-05

N/A: Not available due to non-positive samples.

worst-case scenarios (i.e., overestimations), a daily consumption of 2-L was assumed. On the other hand, the best-fit K values used for the dose-response model must be considered when the dose-response relations are used to estimate the susceptibility of the entire population. The primary source of dose-response data has been human volunteer feeding studies. However, volunteers for such studies have been almost exclusively limited to healthy adult males. Therefore, the probability of infection may have been underestimated in our study.

Seasonality of parasites and indicator parameters

Sampling data was divided into four seasons, and the average values of various parameters for each group were compared using statistical analyses. The results are presented in Table 5. Generally, *Cryptosporidium* oocysts are detected in greater numbers in surface waters in most areas of North America during March-June, when spring rains increase run-off, and consequently rain-washed oocysts of *Cryptosporidium* are responsible for large outbreaks in the U.S. (Smith & Rose 1998). However, no significant seasonal variation in the prevalence of parasites was observed in the study area ($P > 0.1$). Relatively high numbers of total coliforms and fecal coliforms were detected during the summer (May–July) and monsoon (August–October) seasons ($P = 0.05$). In the Salt River, relatively high numbers of fecal coliforms were detected during spring (data not shown). These results indicate that human recreational activities and run-off due to rainfall during the monsoon season may be major factors contributing to microbial contamination in the surface

waters of central Arizona. Turbidity and indicator microorganisms, excluding somatic coliphages, were at their highest levels between February and April of 2005 in comparison to the previous two years. This is possibly due to record precipitation in central Arizona during early 2005.

Geographical (watershed) variation of parasites and indicator parameters

The average values of various parameters for five sampling sites were compared using statistical analyses. The results are presented in Table 6. *Giardia* cysts were found in greater prevalence in the Verde River and the Salt River, whereas no significant geographical variation in the prevalence of *Cryptosporidium* oocysts was observed. The results are comparable to risk estimates. Of the three surface waters, the highest numbers of indicator microorganisms were detected in the Verde River, followed by the Salt River and CAP canal water. In addition, higher turbidity and a lower level of specific conductivity were detected in the Verde River, whereas the highest level of specific conductivity and relatively low turbidity were observed in the Salt River ($P < 0.001$). As mentioned in the section on sampling sites, there were different watershed types in this study area. Relatively dense vegetation along the Salt River contributes to significantly reduced run-off, whereas the Verde River has less vegetation, resulting in high turbidity. CAP water turbidity was relatively stable (data not shown), and CAP water had the lowest level of bacteria ($P < 0.001$). Colorado River water is delivered via a well-protected CAP canal, and the water passes through a desert area over a long distance

Table 5 | Seasonal variation of pathogens and indicator parameters in surface waters of central Arizona

Sampling months	No. of samples	Temp (°C)[c,e]	Log10[a] Turbidity (NTU)[d,e]	HPC bacteria (cfu/100 ml)[f]	Total coliforms (cfu/100 ml)[e]	Fecal coliforms (cfu/100 ml)[e]	Male specific coliphages[g]	% of positive samples[b] Somatic coliphages[h]	Crypto-sporidium spp.[i]	Giardia spp.[j]
May–July	51	24.3 (4.1)[k]	0.62 (0.57)	6.64 (0.85)	2.12 (0.61)	1.50 (0.48)	25.0 (76)	15.0	7.8	33.3
August–October	55	24.5 (3.7)[k]	0.52 (0.48)	6.86 (0.65)	2.10 (0.57)	1.44 (0.61)	4.5 (62)	27.3	10.9	20.2
November–January	45	14.5 (3.2)[k]	0.68 (0.56)	6.60 (0.93)	1.57 (0.73)[k]	1.00 (0.59)[k]	21.2 (74)	33.3	6.7	28.9
February–April	30	19.5 (3.9)	0.75 (0.38)	6.45 (1.02)	1.74 (0.51)[k]	1.03 (0.55)[k]	26.7 (78)	26.7	6.7	16.7
February–April (2005)	11	16.5 (3.5)	1.52 (0.58)[k]	7.30 (0.69)[k]	2.63 (1.28)[k]	2.06 (0.78)[k]	36.4 (85)	18.2	9.1	18.0
Total	192	20.9 (5.7)	0.69 (0.58)	6.72 (0.84)	1.99 (0.74)	1.39 (0.64)	18.9	24.5	8.3	24.5

[a]Mean (standard deviation). Logarithmic data transformation was performed to convert a non-normal distribution into a normal one, prior to ANOVA.
[b]Prevalences were compared using the nonparametric Kruskal-Wallis test.
[c]Mean (standard deviation).
[d]Mean (standard deviation). NTU, nephelometric turbidity units.
[e–f]The P values for the differences between values are 0.053[e] and <0.001[f], as determined by ANOVA.
[g–j]Percent of positive samples (mean rank). The P values for the differences between values are 0.045[g], 0.437[h], 0.943[i], and 0.419[j], as determined by the Kruskal-Wallis test.
[k]Value is significantly different at the 0.05 (95%) level from the other values, as determined by Duncan's post hoc test.

Table 6 | Watershed (sites) variation of pathogens and indicator parameters in surface waters of central Arizona

Sampling sites	No. of samples	Conductivity (µS/cm)[c,e]	Log10 (cfu/100 ml)[a] Turbidity (NTU)[d,e]	HPC bacteria (cfu/100 ml)[e]	Total coliforms (cfu/100 ml)[e]	Fecal coliforms (cfu/100 ml)[f]	Male specific coliphages[g]	% of positive samples[b] Somatic coliphages[h]	Crypto-sporidium spp.[i]	Giardia spp.[j]
Verde River	42	416 (146)[k]	1.02 (0.57)[k]	7.15 (0.72)[k]	2.09 (0.69)	1.65 (0.56)[k]	18.8	46.9 (88.5)	9.4	53.0 (90.5)
Salt River	43	1279 (560)[k]	0.58 (0.56)	7.17 (0.69)[k]	2.40 (0.75)[k]	1.62 (0.64)[k]	25.0	31.3 (76.6)	6.1	42.0 (82.8)
CAP water	36	778 (243)	0.46 (0.25)	6.12 (0.63)[k]	1.53 (0.66)[k]	1.24 (0.43)	8.0	4.0 (57.3)	12.0	12.0 (60.8)
WTP 1	36	926 (264)	0.86 (0.50)[k]	6.63 (0.74)	1.99 (0.67)	1.08 (0.61)	18.5	18.5 (67.9)	3.7	12.0 (60.8)
WTP 2	35	1018 (492)	0.42 (0.67)	6.36 (0.88)[k]	1.83 (0.66)	1.15 (0.74)	22.2	14.8 (64.7)	11.0	7.7 (58.0)
Total	192	887 (488)	0.69 (0.58)	6.72 (0.84)	1.99 (0.74)	1.39 (0.64)	18.9	24.5	8.3	24.5

[a]Mean (standard deviation). Logarithmic data transformation was performed to convert a non-normal distribution into a normal one, prior to ANOVA.
[b]Prevalences were compared using the nonparametric Kruskal-Wallis test.
[c]Mean (standard deviation).
[d]Mean (standard deviation). NTU, nephelometric turbidity units.
[e–f]The P values for the differences between values are <0.001[e] and 0.001[f], as determined by ANOVA.
[g–j]Percent of positive samples (mean rank). The P values for the differences between values are 0.673[g], 0.002[h], 0.800[i], and <0.001[j] as determined by the Kruskal-Wallis test.
[k]Value is significantly different at the 0.05 (95%) level from the other values, as determined by Duncan's post hoc test.

Table 7 | Indicator parameters and proportions of samples positive for coliphages and parasites at various levels of fecal coliforms

Fecal coliforms (cfu/100 ml)[f]	No. of samples	Log10[a]			% of positive samples[b]			
		Turbidity (NTU)[c]	HPC bacteria (cfu/100 ml)[d]	Total coliforms (cfu/100 ml)[c]	Male specific coliphages[e]	Somatic coliphages[f]	Crypto-sporidium spp.[g]	Giardia spp.[h]
<1	18	0.25 (0.50)[i]	6.24 (0.69)[i]	1.15 (0.62)[i]	26.7	6.7	6.7	13.3 (59.2)
1–10	47	0.68 (0.45)	6.52 (0.72)[i]	1.72 (0.67)	11.1	29.6	14.8	7.4 (55.1)
11–100	104	0.77 (0.58)	6.91 (0.70)	2.07 (0.49)	13.3	25.3	6.7	36.0 (74.8)
>101	23	0.98 (0.70)[i]	7.30 (0.55)[i]	2.69 (0.93)[i]	23.8	28.6	9.5	33.3 (76.3)

[a]Mean (standard deviation). Logarithmic data transformation was performed to convert a non-normal distribution into a normal one, prior to ANOVA.
[b]Prevalences were compared using the nonparametric Kruskal-Wallis test.
[c–d]The P values for the differences between values are <0.001[c] and 0.003[d], as determined by ANOVA.
[e–h]Percent of positive samples (mean rank). The P values for the differences between values are 0.438[e], 0.335[f], 0.627[g], and 0.014[h], as determined by the Kruskal-Wallis test.
[i]Value is significantly different at the 0.05 (95%) level from the other values, as determined by Duncan's post hoc test.

(540 km). Therefore, low levels of microbial influx to the canal and natural die-off of microorganisms may account for relatively low levels of microorganisms.

In the intake raw water samples from WTP2, relatively low numbers of bacteria were detected. It may be ascribed to the natural die-off and dilution effect due to the addition of groundwater into the canals. However, increases in the number of fecal coliforms were observed several times in the samples from WTP1 (data not shown). Also, sporadic increases in the concentrations of male specific coliphages were detected in intake raw water from WTP2 (data not shown), suggesting a need for further microbial monitoring and microbial source tracking along the canals to elucidate anthropogenic impact.

Correlation between parasites, indicator microorganisms, and turbidity

The indicator microorganisms such as HPC bacteria, total coliforms, fecal coliforms, somatic coliphages, and turbidity showed moderate cross-correlations (correlation coefficients of 0.26 to 0.57); correlation coefficients of somatic coliphages with HPC bacteria and fecal coliforms were low but significant (0.19 and 0.17, respectively). However, male specific coliphages showed no significant correlation ($P > 0.05$) with any other parameters. The presence of *Giardia* spp. correlated significantly with the presence of *Cryptosporidium* spp., HPC bacteria, and fecal coliforms (correlation coefficients of 0.21, 0.26, and 0.27, respectively).

Table 8 | Indicator parameters and proportions of samples positive for coliphages and parasites at various levels of turbidity

Turbidity (NTU)	No. of samples	Log10[a]			% of positive samples[b]			
		HPC bacteria (cfu/100 ml)[c]	Total coliforms (cfu/100 ml)[d]	Fecal coliforms (cfu/100 ml)[e]	Male specific coliphages[f]	Somatic coliphages[g]	Crypto-sporidium spp.[h]	Giardia spp.[i]
0–2	36	6.24 (1.02)	1.94 (0.88)	1.54 (0.62)	21.2 (68.9)	15.2 (60.7)	6.1	18.2
2.1–10	104	6.67 (0.64)	1.92 (0.60)	1.19 (0.61)[j]	10.3 (61.3)	19.2 (63.8)	10.0	22.2
10.1–100	46	7.30 (0.60)[j]	2.24 (0.90)	1.73 (0.64)	28.6 (75.1)	42.9 (81.7)	3.1	27.2
>101	6	7.45 (0.95)[j]	1.98 (1.00)	1.64 (0.62)	33.3 (87.0)	16.7 (66.5)	0	0

[a]Mean (standard deviation). Logarithmic data transformation was performed to convert a non-normal distribution into a normal one, prior to ANOVA.
[b]Prevalences were compared using the nonparametric Kruskal-Wallis test.
[c–e]The P values for the differences between values are <0.001[c], 0.288[d], and 0.002[e], as determined by ANOVA.
[f–i]Percent of positive samples (mean rank). The P values for the differences between values are 0.039[f], 0.030[g], 0.137[h], and 0.443[i], as determined by the Kruskal-Wallis test.
[j]Value is significantly different at the 0.05 (95%) level from the other values, as determined by Duncan's post hoc test.

Sampling data were divided into four groups, based on the presence or absence of *Cryptosporidium* spp. and *Giardia* spp., to compare average concentrations of indicator microorganisms and turbidity in each group. The levels of HPC bacteria and fecal coliforms were greater in the presence of *Giardia* spp. ($P < 0.01$), whereas the other parameters were not different significantly in the presence or absence of *Cryptosporidium* spp. and *Giardia* spp. In addition, average concentrations of indicator parameters and prevalence of coliphages and parasites at four different levels of fecal coliforms or turbidity were compared (Tables 7 and 8). The levels of HPC bacteria, total coliforms, and turbidity were greater at a higher concentration of fecal coliforms ($P < 0.001$). The prevalence of coliphages and parasites did not vary significantly except for *Giardia* spp. at two concentrations of fecal coliforms (less or more than 10 cfu/100 ml, $P = 0.014$). These results were comparable to the correlation coefficients between parasites and indicator microorganisms as discussed previously. Although male specific coliphages and somatic coliphages were more prevalent at higher turbidity levels ($P < 0.05$), there was no correlation between turbidity levels and prevalence of *Cryptosporidium* spp. and *Giardia* spp. (Table 8). The results do not support a hypothesis that turbidity may be a good indicator of high parasite levels. One possible explanation for the low correlation between turbidity and parasites is that higher turbidity contributes to lower equivalent sample volumes, due to limitations of IMS in the USEPA 1623 Method used for this study. Consequently, the reduced sample volume may have diminished the probability of detecting the parasites.

CONCLUSIONS

This study was the first systematic study of microbial water quality and long-term monitoring of the protozoan parasites *Cryptosporidium* spp. and *Giardia* spp. in the surface waters of central Arizona. The annual risks of infection from *Cryptosporidium* met the annual acceptable risk of 10^{-4} at all sampling sites, whereas the risks of *Giardia* infection at the Verde River and the Salt River were 5.70E-04 and 2.66E-04, respectively. Mean concentrations of *Cryptosporidium* oocysts at the two participating drinking water

treatment plants were significantly lower than the Bin 1 category of the U.S. EPA LT2ESWTR; therefore no additional treatment is required. The data can be considered as grandfather data for source water monitoring under the LT2ESWTR. Greater numbers of coliform bacteria were detected during the summer and monsoon seasons, and the presence of fecal coliform bacteria correlated significantly with the presence of *Cryptosporidium* and *Giardia* ($P < 0.05$). These results indicate that human recreational activities and run-off due to rainfall may be major factors contributing to microbial contamination in the surface waters of central Arizona. In order to help manage non-point sources of pollution in watersheds in the study area, further study is needed to identify the type and sources of fecal contamination. This research endeavor facilitates the advancement of scientific knowledge which can serve as a platform to achieve the aspired environmental and water quality sustainability goals of the community.

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