Environ. Sci. Technol. **2005**, *39,* 8600−8605

Microbial Characterization and Population Changes in Nonpotable Reclaimed Water Distribution Systems

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Changes in the microbial quality of nonpotable reclaimed water distribution systems in seven metropolitan areas of the southwestern United States were investigated by performing pathogen monitoring and bacterial growth. Water samples were collected from tertiary-treated effluents at point of entry and point of use in the distribution systems. The samples were analyzed for *Cryptosporidium* oocysts, *Giardia* cysts, enteroviruses, microbial indicators, and assimilable organic carbon (AOC). *Cryptosporidium* and *Giardia* were detected in 16% (12/77) and 43% (33/77) of nonpotable reclaimed water samples, whereas no infectious *Cryptosporidium parvum* were detected in any of the samples. No infectious enteroviruses were detected in any sample (0/27). At point of entry total coliform and fecal coliforms ranged from 0.7 to 280 and from 0 to 1.9 colony-forming units (cfu)/100 mL, respectively. Increases in the number of coliforms were observed as water passed through the reclaimed water distribution systems. However, no such increase in the number of coliphages was found. The chlorination practices at some utilities were not sufficient to inactivate coliforms and coliphages, but supplemental ultraviolet disinfection resulted in lower numbers of these microbial indicators. AOC levels decreased by 3-fold as water passed through the distribution systems, which inversely correlated with bacterial regrowth.

Introduction

Diminishing freshwater supplies and increasing municipal water demands in highly populated areas make water reuse a significant global concern. Historically, reclaimed water was used to irrigate crops (*1*). In the last 30 years, dramatic changes in the quantity and quality of reclaimed water produced by megacities have resulted in new application scenarios. The water reuse trend has shifted toward unconventional uses such as urban irrigation, toilet and urinal flushing, commercial and industrial uses, and indirect potable reuse (*2*). Southwestern states of the United States such as Arizona, California, Nevada, and Texas have extensive water reuse programs (*3*). However, concerns about the microbial content of reclaimed water limit its applications.

A wide variety of enteric microbial pathogens, including viruses, bacteria, and protozoan parasites, may be found in wastewater (*4*). Occurrence of microbial pathogens in reclaimed water and the related risks have been examined over the last several decades (*5*−*9*). Recently, reports of infectious *Cryptosporidium parvum* oocysts in reclaimed water plant effluents have been published (*10*, *11*). However, microbial water quality and growth in reclaimed water distribution systems remains to be considered. Reclaimed water, even tertiary-treated, usually contains higher amounts of organic matter than drinking water, indicating that consumption of chlorine in the reclaimed distribution systems could occur rapidly, resulting in conditions conducive for bacterial growth. Moreover, concentrations of assimilable organic carbon (AOC), which serves as nutrients for bacterial growth, are much higher in reclaimed water than in drinking water. On the basis of these two factors (*12*), it is expected that high nutrient levels in combination with low chlorine residuals initiate bacterial growth in reclaimed water distribution systems. Many studies on bacterial growth in drinking water distribution systems have been reported (*13*−*17*); however, few studies on microbial surveillance have been performed in nonpotable reclaimed water distribution systems.

No federal regulations for water reuse exist in the United States. Individual states are responsible for the development and implementation of water reuse criteria (*2*, *18*). Reclaimed water samples from seven metropolitan areas in the southwestern United States were analyzed for a variety of microorganisms and chemical parameters. This study characterizes water quality in reclaimed water distribution systems by monitoring pathogens and investigating microbial growth. The results of this study will aid in building a database of common microbes and their occurrence to be used in revising guidelines and disinfection practices for reclaimed water.

Experimental Section

Site Selection and Sampling Strategy. On the basis of utility size, treatment processes, and water reuse application, seven nonpotable reclaimed water distribution systems were selected (Table 1). In each distribution system two sampling sites (point of entry, POE, and point of use, POU) were selected for periodic monitoring for water quality indicators. Water samples from all the sampling sites were collected between June 2002 and June 2003. Samples for *Cryptosporidium* oocysts, *Giardia* cysts, male-specific coliphages, somatic coliphages, total coliforms, fecal coliforms, and HPC bacteria were assayed on a bimonthly basis, whereas samples for enteroviruses were collected and assayed on a quarterly basis only at POU in each distribution system. Chemical parameters such as AOC, dissolved organic carbon (DOC), and total chlorine residuals were also measured quarterly.

Sample Collection and Processing. The samples for protozoan parasites and enteroviruses were collected in a 100-L plastic container and dechlorinated by adding 500 mL of 10% sodium thiosulfate. The water samples for *Cryptosporidium* and *Giardia* were filtered through an Envirochek-HV sampling capsule (Gelman Sciences, Ann Arbor, MI) at flow rates of no more than 2 L/min, and the samples for viruses were filtered through a 1 MDS filter (CUNO Inc., Meriden, CT) at 20 L/min. The water samples were continuously mixed during filtration process. Sample volumes for protozoan parasites and enteroviruses ranged from 20 to 170 L and from 114 to 341 L, respectively. Sample volumes varied due to differences in water turbidity at each utility. Filter cartridges retained in filter housings were shipped overnight at 4 °C to the Environmental Microbiology Laboratory at Arizona State University, Tempe, AZ. Samples for *Cryptosporidium*, *Giardia*, and enteric viruses were

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10.1021/es050607l CCC: $30.25
Published on Web 10/13/2005

TABLE 1. Summary of Treatment Plant and Distribution System Characteristics

	infrastructure						
		distribution system					
utilities	avg water production (mgd)	total length (mi)	distance to sampling sites from plants (mi)	residence time (h)	tertiary treatment processes[a]	disinfectants	applications
CA1	15 (medium)	30	20	18−24	anthracite coal and sand	chlorine, chloramine	landscape and agricultural irrigation (vegetables, fruits), industrial uses, commercial toilet flushing
CA2	3.6 (small)	50	0[b]		anthracite coal and sand	chlorine	industrial, commercial, and landscape irrigation
CA3	30 (large)	40	10	12−15	anthracite coal	chlorine	irrigation, cooling towers, construction, dust suppression
TX1	31 (large)	64	25	22−30	sand	chlorine	commercial and industrial users, discharge into Medina and San Antonio Rivers
TX2	9.2 (medium)	31	7.6	5−12	sand	UV,[c] chlorine	industrial uses, golf courses, parks, landscape and agricultural irrigation (cotton, citrus fruit, vegetables)
NV	10 (medium)	17	6	6−10	automatic backwash filters	UV,[c] chlorine	golf courses, parks
AZ	2−4 (small)	12	3	4−8	automatic backwash filters	UV,[c] chlorine	golf courses

[a] Tertiary treatment processes include primary treatment (bar screens, grit chamber, primary clarification), secondary treatment (activated sludge aeration basin, secondary clarification), and filtration. [b] Only final effluent (POE) was collected. [c] UV dose of 100 mJ/cm^2.

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 parasites and virus samples. The samples for bacteria and coliphages were analyzed within 12 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). Total chlorine residuals, turbidity, pH, and temperature were measured during sample collection by utility personnel.

Recovery Efficiency of Oocysts and Cysts from Seeded Water Samples. *Cryptosporidium parvum* oocysts (Iowa isolate) were obtained from the Sterling Parasitology Laboratory, 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 antibiotic solution (PBS with penicillin, streptomycin, gentamicin, and amphotericin B). The number of oocysts and cysts in the stock suspensions were confirmed by direct count using a hemocytometer. Procedural recovery efficiency evaluations were performed with reclaimed water from site AZ, two different surface waters, and laboratory reagent-grade water. Mean percent recoveries and relative standard deviations (RSD) were then calculated (*19*). Briefly, 10 L water samples 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 by an immunomagnetic separation (IMS) technique (Dynabeads GC-Combo, Dynal A. S., Oslo, Norway) (*19*), labeled by an immunofluorescence assay (IFA) (Hydrofluor Combo, Strategic Diagnostics Inc., Newark, DE), and enumerated by epifluorescence microscopy (BX-60, Olympus Optical Co., Ltd., Tokyo, Japan).

Recovery of Oocysts and Cysts from Reclaimed Water Samples. Samples were analyzed for *Cryptosporidium* oocysts and *Giardia* cysts by IMS followed by IFA as described in U.S. Environmental Protection Agency (EPA) method 1623 (*19*). An integrated cell culture−polymerase chain reaction assay (ICC−PCR) was used to detect infectious oocysts as described by Di Giovanni et al. (*20*). Briefly, the filter was eluted and the concentrate was purified by IMS. Purified samples (110 μL) were divided into 60 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-zeroes approach (treating zeroes as ones) (*21*, *22*). Effective volume (EV) represents the total accumulative sample volume assayed for each site over the period of study. This strategy was suggested by Parkhurst and Stern (*22*) 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 reclaimed 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.

Additionally, confidence limits (CLs) for mean concentration of oocysts and cysts were determined to take into account the special characteristics of the data set such as unpredictability of the distribution of both parasites in the study areas (*22*, *23*).

Detection of Enteroviruses. The samples were eluted from 1 MDS filters and assayed for enteroviruses following a method described by Abbaszadegan et al. (*24*) with a minor modification. Briefly, the filters were eluted with a sterilized solution containing 1.5% beef extract (Difco, Sparks, MD) and 0.05 M glycine (Sigma Chemical Co., St. Louis, MO) (pH 9.4). Each eluent was concentrated by organic flocculation, and sample concentrates were extracted with Vertrel XF (Micro Care Marketing Services, New Britain, CT) to facilitate

TABLE 2. Mean Percent Recoveries for (oo)Cysts from Seeded Water Samples[a]

		surface water		
	reclaimed water ($n = 4$)	high turbidity[b] ($n = 3$)	low turbidity[c] ($n = 7$)	reagent water ($n = 3$)
Cryptosporidium	48 ± 53	29 ± 26	81 ± 15	83 ± 12
Giardia	26 ± 33	ND[d]	31 ± 43	56 ± 45

[a] Percent values in the table are expressed as mean ± RSD (relative standard deviation). [b] Rio Grande River water (>90 NTU). [c] Phoenix surface waters such as the Verde River, the Salt River, and Central Arizona Project canal water from the Colorado River (<5 NTU). *Cryptosporidium* recovery efficiencies of low turbidity water and reagent water were not statistically different at the 95% confidence level ($P = 0.879$). [d] Not determined.

dispersion of viral clumps. Each sample concentrate was assayed for enteroviruses by use of buffalo green monkey (BGM) kidney cells.

Indicator Microorganisms. Water samples (10 mL) were assayed for coliphages by the double agar layer technique (*25*). *Escherichia coli* F_{amp} (ATCC 700891) and *E. coli* CN-13 (ATCC 700609) were used to detect male-specific coliphages and somatic coliphages, respectively (*26*). Samples (5 mL) 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 by the membrane filtration technique on mEndo LES agar (Difco, Sparks, MD) and mFC agar (EM Science, Gibbstown, NJ), respectively (*27*). Samples in triplicate were filtered through 47-mm diameter cellulose acetate membranes with 0.45 μm pore size (Pall Gelman Laboratory, Ann Arbor, MI). The plates for total coliforms and fecal coliforms were counted after 24-h incubation at 35 and 44.5 °C, respectively. HPC bacterial analysis was performed by 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 counted after 5−7 days of incubation at 27 °C (*27*).

Chemical Parameters. All glassware for chemical analyses was thoroughly cleaned and rendered free of organic carbon by combustion at 550 °C for 4 h. Teflon-lined silicone septa were soaked in a 10% sodium persulfate solution for 1 h at 60 °C and rinsed with carbon-free deionized (DI) water three times. To alleviate dissolved carbon leaches, organic-carbon-free 25-mm glass fiber filters (type GF/F, Whatman Ltd., Maidstone, MI) were rinsed with 25 mL of carbon-free DI water. After dechlorination, 500 mL of water was filtered through an in-line filter system. Twelve 45-mL organic-carbon-free borosilicate glass vials with Teflon-lined silicone septa were filled to the shoulder of the vials (approximately 40 mL) with the filtered samples. Nine, one, and two vials were prepared for AOC measurement, growth control, and DOC analysis, respectively.

AOC was measured by Standard Methods procedure 9217 (*27*). Briefly, vegetative cells present in water samples were killed by pasteurization at 70 °C for 30 min. The samples were inoculated with *Pseudomonas fluorescens* strain P17 and *Spirillum* NOX. After 7, 8, and 9 days of incubation at 15 °C in the dark, P17 and NOX colonies were counted for each incubation day in R2A medium (Difco, Sparks, MD). Following the Standard Methods procedure (*27*), the average bacterial growths of P17 and NOX observed during the incubation days were converted into AOC units as micrograms of acetate carbon per liter and micrograms of oxalate carbon per liter, respectively.

Samples for DOC analysis were collected in two 45-mL vials, acidified with concentrated hydrochloric acid, and stored at 4 °C prior to analysis. DOC analysis was performed on a TOC analyzer (TOC-5050A, Shimadzu, Columbia, MD) with an autosampler (ASI-5000A, Shimadzu, Columbia, MD).

Statistical Analysis. All of the data were entered into a Microsoft Excel spreadsheet. SPSS version 11.0 (SPSS Inc., Chicago, IL) was used for statistical analysis. The mean values were compared by *t*-test, and a Leven's test was performed to determine equality of variances prior to the *t*-test; *p* values of <0.1 were considered significant.

Results and Discussion

Enteric Protozoan Parasites. 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 reclaimed water averaged 48% ± 53% [mean ± RSD] and 26% ± 33%, respectively, which are similar to another reported study (*11*). The highest mean recovery efficiency of oocysts and cysts was achieved in laboratory reagent-grade water followed by low-turbidity water, reclaimed water, and high-turbidity water (Table 2).

Over the period of study, frequent variation in the limit of detection of oocysts and cysts among samples was observed. This variation was caused by differences in the equivalent sample volumes processed by IMS. This variance is the direct implication of changes in sample turbidity, which makes it difficult to obtain an unbiased estimate of the average concentrations. To address this issue, EV-weighted mean concentrations were considered in this study. Table 3 presents descriptive statistics for oocyst and cyst concentrations in reclaimed water. *Cryptosporidium* oocysts and *Giardia* cysts were detected in 16% (12/77) and 43% (33/77) of reclaimed water samples, respectively, by IMS−IFA. IFA cannot be used to identify *C. parvum*. Therefore, the detected oocysts are referred to as *Cryptosporidium* spp. in this study.

The samples were also assayed for infectious *C. parvum* oocysts by ICC−PCR, but no infectious oocysts were detected in any sample. The highest concentration of *Cryptosporidium* was 43 oocysts in 271 L of total EV of the samples at site CA1, which translates into a mean concentration of 15.9 oocysts/100 L (Figure 1). The highest concentration of *Giardia* was 558 oocysts in 264 L of total EV of the samples at site CA2, which translates into a mean concentration of 211 cysts/100 L (Figure 1). In nonpotable reclaimed water, *Giardia* cysts were detected more often and at higher concentrations than *Cryptosporidium* oocysts.

Recently, Quintero-Betancourt et al. (*11*) studied tertiary-treated reclaimed water with ≥4 mg/L chlorine and reported that the levels of total oocysts and infectious *C. parvum* detected by IFA and focus detection method most probable number (FDM-MPN) assay in the effluents of reclaimed waters ranged from 2 to 319 oocysts/100 L and from 2 to 18 MPN/100 L, respectively. They also reported that a minimum of 5−10 oocysts is required for the detection of infectious *C. parvum* by an FDM-MPN assay, possibly due to the low ratio of infectious to total *Cryptosporidium* oocysts in the samples as well as effective disinfection practices. All the participating utilities in this study practiced chlorination, and only three utilities had supplemental UV disinfection. The chlorine residual ranged from 0.5 to 5.7 mg/L. Eighty-three percent

TABLE 3. Effective Volume Weighted Mean Concentrations of *Cryptosporidium* and *Giardia* in Reclaimed Water

		Cryptosporidium (oocysts/100 L)			*Giardia* (cysts/100 L)		
sampling sites	N	% ND samples[a]	mean	upper 95% CL[b]	% ND samples[a]	mean	upper 95% CL[b]
AZ	12	83	0.53	1.35	75	1.58	2.75
NV	12	100	0.00	0.71	42	31.2	36.0
CA1	12	58	15.9	21.4	33	36.5	44.5
CA2	6	67	0.76	2.73	0	211	229
CA3	11	73	0.46	1.35	91	0.46	1.35
TX1	12	100	0.00	0.66	42	6.64	9.16
TX2	12	100	0.00	0.38	92	2.29	3.46
total	77	84			57		

[a] Ratio of nondetected (ND) to total samples. [b] CL, confidence limit.

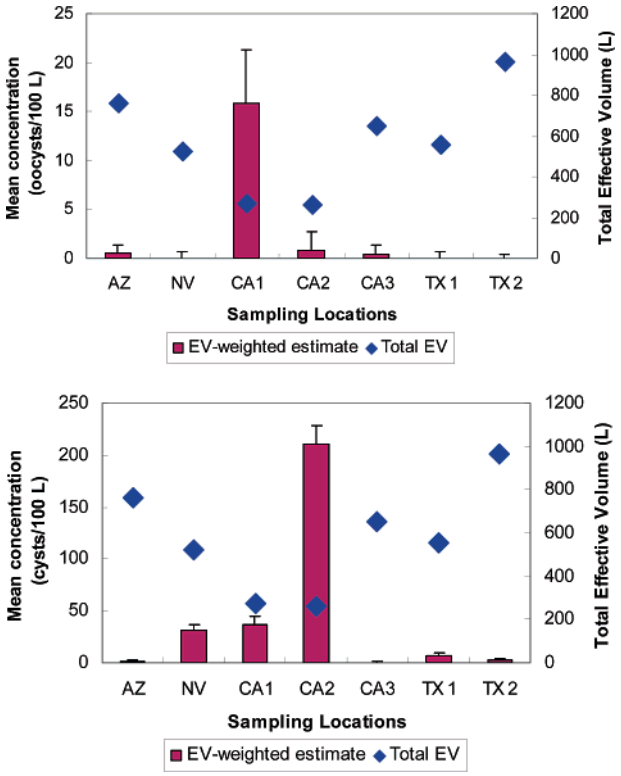

FIGURE 1. Geographical variation of mean concentrations of *Cryptosporidium* oocysts (top) and *Giardia* cysts (bottom) with upper 95% confidence limits and total effective sample volume.

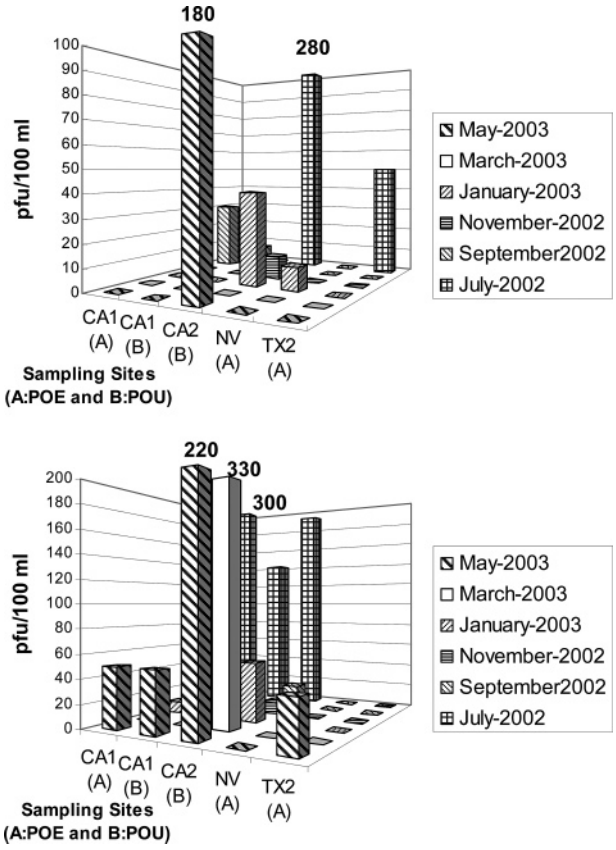

FIGURE 2. Male-specific coliphages (top) and somatic coliphages (bottom) in reclaimed water.

(10/12) of the positive samples for *Cryptosporidium* had less than 5 oocysts. It has been reported that probability of finding infectious *Cryptosporidium* is very slim in samples containing less than 5 oocysts (*11*), which may be the reason for nondetection of infectious oocysts in the samples.

In the present study oocysts and cysts were not detected in many samples, but a very high number of oocysts and cysts were found in a few samples. Normalization of oocyst and cyst occurrence data for sample volume revealed an inverse correlation between mean concentrations of oocysts and cysts and total EV of the samples (Figure 1). This suggests that enteric protozoan parasites are detected more often in turbid reclaimed water (*6*, *7*, *10*), suggesting a need for additional monitoring and correlation analysis.

In most instances (67%), *Cryptosporidium* oocysts were found in greater concentrations at point of use (POU) than at point of entry (POE). At sites CA1, AZ, and CA3, water carriage through the distribution systems resulted in an increase in oocyst concentrations on two, one, and one occasions, respectively. In contrast, water carriage through distribution systems resulted in a decrease in concentration of oocysts on one occasion at sites CA1 and CA3. No clear impact of water carriage on *Giardia* concentrations in reclaimed distribution systems was determined. In 41% of samples, higher concentrations of *Giardia* cysts were found at POU than at POE. The observed differences in the concentrations of oocysts and cysts can be attributed to either environmental factors (e.g., variance in occurrence, retention, and sporadic release of oocysts and cysts from biofilms or microbial degradation) or procedural factors (e.g., variance in detection sensitivity). The relative standard deviation (RSD) was calculated to discern the probable contribution of environmental and procedural factors. The variation in occurrence of oocysts and cysts was generally within the RSD range (Table 2). However, on three occasions the oocyst concentration increase was beyond the RSD range, which can be attributed mainly to environmental factors.

Viruses. No enteroviruses were detected in any sample (0/27) by a cell culture method. High water turbidity levels decreased the volumes of samples filtered. Equivalent sample volumes for cell culture assays ranged from 13 to 114 L, and those for 93% (25/27) of the samples were less than 75 L. In

TABLE 4. Changes of Bacterial Population and Other Factors between Points of Entry and Use in Distribution Systems[a]

utility	disinfection practices	AOC (μg/L)	AOC/DOC (%)	chlorine residual (mg/L)	HPC (cfu/mL)	total coliform[b,c] (cfu/100 mL)	fecal coliform[b] (cfu/100 mL)
CA1	chlorination	518 ± 158 to 105 ± 38 ($p = 0.040$)	5.9 ± 2.8 to 1.3 ± 0.5 ($p = 0.047$)	1.67 ± 0.58 to 0.01 ± 0.00 ($p = 0.038$)	$4.8 \times 10^3 \pm 2.8 \times 10^2$ to $1.5 \times 10^5 \pm 2.8 \times 10^4$ ($p = 0.017$)	280 ± 396 to 527 ± 740 [2/4]	0.1 ± 0.3 to 0.3 ± 0.7
CA3	chlorination	1226 ± 870 to 310 ± 171 ($p = 0.083$)	10.9 ± 6.8 to 5.5 ± 3.1 ($p \geq 0.1$)	5.73 ± 1.37 to 0.04 ± 0.05 ($p < 0.01$)	$5.4 \times 10 \pm 9.8 \times 10$ to $2.2 \times 10^3 \pm 1.9 \times 10^3$ ($p = 0.069$)	1.8 ± 3.5 to 1.8 ± 3.5 [0/4]	no positives
TX1	chlorination	594 ± 105 to 345 ± 84 ($p = 0.020$)	9.7 ± 2.3 to 5.8 ± 2.1 ($p = 0.069$)	0.55 ± 0.07 to 0.24 ± 0.22 ($p \geq 0.1$)	$1.5 \times 10^4 \pm 1.6 \times 10^4$ to $1.0 \times 10^5 \pm 6.3 \times 10^4$ ($p = 0.039$)	3.0 ± 4.2 to 76 ± 74 [2/4]	1.9 ± 2.1 to 6.6 ± 5.7
TX2	chlorination, UV inactivation	463 ± 42 to 848 ± 97 ($p = 0.036$)	6.7 ± 0.9 to 11 ± 1.6 ($p = 0.077$)	1.78 ± 0.58 to 2.84 ± 1.89 ($p \geq 0.1$)	$1.0 \times 10^3 \pm 1.7 \times 10^3$ to $1.8 \times 10 \pm 1.9 \times 10$ ($p \geq 0.1$)	no positives [0/4]	no positives
NV	chlorination, UV inactivation	523 ± 393 to 703 ± 490 ($p \geq 0.1$)	5.3 ± 3.8 to 6.6 ± 2.4 ($p \geq 0.1$)	0.68 ± 0.26 to 0.30 ± 0.01 ($p \geq 0.1$)	$1.0 \times 10^3 \pm 1.4 \times 10^3$ to $2.4 \times 10^4 \pm 3.5 \times 10^4$ ($p \geq 0.1$)	0.7 ± 0.6 to 9.0 ± 5.6 [3/4]	no positives
AZ	chlorination, UV inactivation	829 ± 615 to 508 ± 155 ($p \geq 0.1$)	10 ± 7.0 to 8.1 ± 5.1 ($p \geq 0.1$)	0.82 ± 0.26 to 0.27 ± 0.26 ($p = 0.061$)	$2.7 \times 10^4 \pm 1.3 \times 10^4$ to $1.6 \times 10^4 \pm 2.6 \times 10^4$ ($p \geq 0.1$)	1.0 ± 1.4 to 5.5 ± 6.4 [2/4]	no positives

[a] Values in the table are expressed as mean $\pm$ standard deviation; p values were obtained via t-test. [b] A t-test was not performed for total coliform bacteria because a Leven's test for equality of variances suggested equal variances for test variables could not be assumed. [c] The ratio of bacterial growth occasions to total cases is given in brackets.

addition, the limitations imposed by toxicity during cell culture assays resulted in low equivalent sample volumes for viral assays. Moreover, more than 40% of the samples were chlorinated and UV-disinfected, which may be a reason for nondetection of infectious enteroviuses.

Male-specific coliphages and somatic coliphages were detected in 10% (8/78) and 18% (14/78) of the water samples, respectively (Figure 2). No increase in the number of coliphages was observed during water passage through the distribution systems. Relatively high numbers of coliphages were detected in the samples from sites CA1 and CA2 during summer months (July 2002 and May 2003).

Bacterial Population Changes in the Distribution Systems. Total coliforms and fecal coliforms were detected in 42% (49 of 117) and 20% (23 of 118) of the water samples, respectively. The numbers of total coliforms and fecal coliforms ranged from 1 to 6.0×10^4 cfu/100 mL and from 1 to 1.1×10^2 cfu/100 mL, respectively. Total coliforms were detected in higher numbers and with greater incidence frequency in the samples from sites CA1 and TX1 where chlorination was the only disinfection practice (Table 4).

AOC serves as a nutrient source for bacterial growth in any body of water. The high levels of AOC in reclaimed water distribution systems makes them a favorable ecological niche for the growth of heterotrophic bacteria, and low or nonexisting chlorine residuals can stimulate their growth (*12*). Other factors that influence bacterial growth include temperature, residence time, and material and age of the distribution system. The significant variation in AOC concentrations in distribution systems is potentially due to the oxidation of organic matter by chlorine and the consumption of AOC by bacteria (*16*). Chlorine could react with organic matter rapidly, resulting in AOC concentrations that initially increase. When the oxidation is completed, bacteria could consume AOC while water travels through the distribution system. AOC is generally a very small portion of DOC in water (*28*). In the present study, the variation in the ratio of AOC to DOC between the two sampling points followed a pattern similar to the change in AOC concentrations (Table 4), indicating that DOC variation did not affect AOC levels.

Significant bacterial growth was observed in distribution systems at sites CA1, CA3, and TX1, where chlorination alone was used for disinfection. AOC concentrations and chlorine residuals decreased significantly at these sites as water passed through the distribution systems (Table 4). AOC consumption by bacteria seems to be a main factor for bacterial growth in these systems. Relatively high concentrations of HPC bacteria at site TX1 were observed, which can be attributed to low chlorine residuals. At utilities using a combination of chlorination and UV disinfection (TX2, NV, and AZ), both oxidation of organic matter by chlorine and bacterial consumption of AOC seem to control bacterial growth in the distribution systems (Table 4). At site TX2, the distribution system contained very high levels of chlorine residuals, which caused oxidation of organic matter and a subsequent increase in AOC. Low levels of bacteria at this site were possibly due to high levels of chlorine residuals. At site NV, low levels of chlorine residuals resulted in high concentrations of HPC bacteria, but AOC increase by oxidation of organic matter seemed to offset AOC consumption by bacteria. As water passed through the distribution system at site AZ, there was an increase in the concentration of total coliforms with no significant change in HPC bacteria. The factors responsible for this phenomenon were not clear. One possible explanation is that coliform bacteria are released from biofilms intermittently. Disinfection practices (UV irradiation and low chlorine) at sites NV and AZ seemed to have no significant effect on bacterial populations. High chlorination could be considered for achieving sufficient inactivation of microorganisms. However, high DOC levels in reclaimed water make it undesirable to use higher chlorine doses because the combination of DOC and chlorine results in greater formation of disinfection byproducts (DBPs). Therefore, additional DOC removal is recommended prior to chlorination. This scenario suggests a need for an ecological risk assessment of DBPs formed in reclaimed water distribution systems.

In summary, *Cryptosporidium* oocysts and *Giardia* cysts were detected in 16% (12/77) and 43% (33/77) of reclaimed water samples by IMS−IFA. Mean concentrations of oocysts and cysts seem to be related to the total effective volume of the samples investigated, suggesting that enteric protozoan parasites may be detected more often in turbid reclaimed water. Neither infectious *C. parvum* oocysts nor infectious enteroviruses were detected in any sample. An increase in the number of total coliforms was observed with water carriage through the reclaimed water distribution systems, whereas no such increase in the number of coliphages was noted. The chlorination practice used at some utilities was

not sufficient to inactivate coliphages and coliforms, while plants using a combination of chlorination and UV disinfection had fewer positive samples and lower levels of microorganisms. More than a 3-fold decrease in AOC with a significant decrease in chlorine residuals was observed during water carriage/transmission through the distribution systems, which may be due to bacterial growth and chlorine consumption by organic matter.

Acknowledgments

This research was supported by the American Water Works Association Research Foundation (Project 2703) and the National Science Foundation Water Quality Center at Arizona State University. We acknowledge the help of Mr. Ramesh Narasimhan and Dr. John Brereton at Narasimhan Consulting Services, Inc., for their assistance in coordinating with the participating utilities. We also thank the personnel at the utilities for collecting water samples.

Literature Cited

(1) World Health Organization. *Water Sanitation and Health (WSH)*; available from www.who.int/water_sanitation_health/wastewater/2003.

(2) *Guidelines for Water Reuse*; EPA/625/R-92/004; U.S. Environmental Protection Agency and U.S. Agency for International Development: Washington, DC, 2004.

(3) Department of the Interior/Bureau of Reclamation. *Water 2025: Preventing Conflict and Crisis in the West*; available from www.doi.gov/water2025/, 2003.

(4) Bitton, G. *Wastewater Microbiology*, 2nd ed.; Wiley−Liss: New York, 1999.

(5) Asano, T.; Leong, L. Y. C.; Rigby, M. G.; Sakaji, R. H. Evaluation of the California wastewater reclamation criteria using enteric virus monitoring data. *Water Sci. Technol.* **1992**, *26*, 1513−1524.

(6) Jolis, D.; Pitt, P.; Hirano, R. Risk assessment for *Cryptosporidium parvum* in reclaimed water. *Water Res.* **1999**, *33*, 3051−3055.

(7) Rose, J. B.; Dickson, L. J.; Farrah, S. R.; Carnahan, R. P. Removal of pathogenic and indicator microorganisms by full-scale water reclamation facility. *Water Res.* **1996**, *30*, 2785−2797.

(8) Tanaka, H.; Asano, T.; Schroeder, E. D.; Tchobanoglous, G. Estimating the safety of wastewater reclamation and reuse using enteric virus monitoring data. *Water Environ. Res.* **1998**, *70*, 39−51.

(9) Yanko, W. A. Analysis of 10 years of virus monitoring data from Los Angeles County treatment plants meeting California wastewater reclamation criteria. *Water Environ. Res.* **1993**, *65*, 221−226.

(10) Gennaccaro, A. L.; McLaughlin, M. R.; Quintero-Betancourt, W.; Huffman, D. E.; Rose, J. B. Infectious *Cryptosporidium parvum* oocysts in final reclaimed effluent. *Appl. Environ. Microbiol.* **2003**, *69*, 4983−4984.

(11) Quintero-Betancourt, W.; Gennaccaro, A. L.; Scott, T. M.; Rose, J. B. Assessment of methods for detection of infectious *Cryptosporidium* oocysts and *Giardia* cysts in reclaimed effluents. *Appl. Environ. Microbiol.* **2003**, *69*, 5380−5388.

(12) LeChevallier, M. W.; Welch, N. J.; Smith, D. B. Full-scale studies of factors related to coliform regrowth in drinking water. *Appl. Environ. Microbiol.* **1996**, *62*, 2201−2211.

(13) Abbaszadegan, M.; Ghatpande, P.; Brereton, J.; Alum, A.; Narasimhan, R. Laboratory testing protocol to identify critical factors in bacterial compliance monitoring. *Water Sci. Technol.* **2003**, *47*, 131−136.

(14) Carter, J. T.; Rice, E. W.; Buchberger, S. G.; Lee, Y. Relationship between levels of heterotrophic bacteria and water quality parameters in a drinking water distribution system. *Water Res.* **2000**, *34*, 1495−1502.

(15) Escobar, I.: Randall, A.; Taylor, J. Bacterial growth in distribution systems: Effect of assimilable organic carbon (AOC) and biodegradable dissolved organic carbon (BDOC). *Environ. Sci. Technol.* **2001**, *35*, 3442−3447.

(16) Liu, W.; Wu, H.; Wang, Z.; Ong, S. L.; Hu, J. Y.; Ng, W. J. Investigation of assimilable organic carbon and bacterial regrowth in drinking water distribution system. *Water Res.* **2002**, *36*, 891−898.

(17) Volk, C. J.; LeChevallier, M. W. Assessing biodegradable organic matter. *J. Am. Water Works Assoc.* **2000**, *92*, 64−76.

(18) *Code of regulations, Title 22, Division 4, Chapter 3 Water recycling criteria*; State of California: Sacramento, CA, 2000; Sections 60301 et Seq.

(19) *Method 1623: Cryptosporidium and Giardia in water by filtration/IMS/FA*; EPA/821/R-01/025; Office of Water, U.S. Environmental Protection Agency: Washington, DC, 2001.

(20) Di Giovanni, G. D.; Hashemi, F. H.; Shaw, N. J.; Abrahams, F. A.; LeChevallier, M. W.; Abbaszadegan, M. Detection of infectious *Cryptosporidium parvum* oocysts in surface and filter backwash water samples by IMS−CC−PCR. *Appl. Environ. Microbiol.* **1999**, *65*, 3427−3432.

(21) Haas, C. N.; Crockett, C. S.; Rose, J. B.; Gerba, C. P.; Fazil, A. M. Assessing the risk posed by oocysts in drinking water. *J. Am. Water Works Assoc.* **1996**, *88*, 131−136.

(22) Parkhurst, D. F.; Stern, D. A. Determining average concentrations of *Cryptosporidium* and other pathogens in water. *Environ. Sci. Technol.* **1998**, *32*, 3424−3429.

(23) Fisher, L. D.; van Belle, G. *Biostatistics: A methodology for the health sciences*; John Wiley & Sons: New York, 1993.

(24) Abbaszadegan, M.; Stewart, P.; LeChevallier, M. W. A strategy for detection of viruses in groundwater by PCR. *Appl. Environ. Microbiol.* **1999**, *65*, 444−449.

(25) Adams, M. *Bacteriophages*; Interscience Publishers: New York, 1959.

(26) *Method 1601: Male specific and somatic coliphages in water by two-step enrichment procedure*; EPA-821-R-01-030; Office of Water, U.S. Environmental Protection Agency: Washington, DC, 2001.

(27) American Public Health Association, American Water Works Association, Water Environmental Federation. In *Standard methods for the examination of water and wastewater*, 19th ed.; Eaton, A. D., Clesceri, L. S., Greenberg, A. E., Eds.; APHA: Washington, DC, 1995.

(28) Van der Kooij, D. Assimilable Organic Carbon (AOC) in Drinking Water. In *Drinking Water Microbiology*; McFeters, G. A., Ed.; Springer-Verlag: New York, 1990; pp 57−87.

Received for review March 29, 2005. Revised manuscript received September 2, 2005. Accepted September 9, 2005.

ES050607L