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Cryptosporidium and *Giardia* Recoveries in Natural Waters by Using Environmental Protection Agency Method 1623

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Relatively few studies have examined recoveries from source waters by using Environmental Protection Agency method 1623 with organism spike doses that are environmentally realistic and at turbidity levels commonly found in surface waters. In this study, we evaluated the filtration capacities and recovery efficiencies of the Gelman Envirochek (standard filter) and the Gelman Envirochek high-volume (HV) sampling capsules under environmental conditions. We also examined the performance of method 1623 under ambient conditions with matrix spike experiments using 10 organisms/liter. Under turbid conditions, the HV capsule filtered approximately twice the volume filtered by the standard filter, but neither could filter 10 liters without clogging. In low-turbidity waters, oocyst, but not cyst, recoveries were significantly higher when the HV capsule was used. In turbid waters, organism recoveries were lower than those in nonturbid waters and were not significantly different for the different filters. When the HV capsule was used, *Cryptosporidium* recoveries ranged from 36 to 75%, and *Giardia* recoveries ranged from 0.5 to 53%. For both organisms, recoveries varied significantly by site. Turbidity could explain variation in *Giardia* recoveries ($r^2 = 0.80$) but not variation in *Cryptosporidium* recoveries ($r^2 = 0.16$). The inconsistent recoveries across sites suggested that the background matrix of the ambient water affected recovery by method 1623. A control sample collected at the height of the winter rainy season detected one organism, highlighting the difficulty of using this method to accurately measure pathogen abundance under natural conditions. Our findings support the use of the HV filter under field conditions but suggest that designing a cost-effective and statistically valid monitoring program to evaluate sources and loads of protozoan pathogens may be difficult.

Cryptosporidium and *Giardia* spp. are a group of protozoan parasites that can cause mild to severe diarrhea and potentially shorten the life spans of immunocompromised individuals. The importance of understanding occurrence and distribution of these parasites is magnified by their potential to infect a large population via drinking-water supplies.

The occurrence of numerous waterborne outbreaks with these parasites prompted the U.S. Environmental Protection Agency (EPA) to take a number of actions, including collection of data through the Information Collection Rule (ICR) and Information Collection Rule Supplemental Survey (ICRSS), as well as defining a protocol for developing pathogen total maximum daily loads (TMDL) (10). One step in establishing a TMDL is source assessment of the targeted pollutant, which involves determining the magnitude and location of the pollutant's origin.

The State of California's Department of Water Resources (DWR) initiated studies of pathogen source water contamination because the department operates and maintains the California State Water Project (SWP), which receives water from the Sacramento-San Joaquin Delta and its tributaries. Since SWP and Delta source waters originate over a large geographical area and winter turbidities can range from 2 to 350 nephelometric turbidity units (NTU), any method examining occurrence and concentration of *Cryptosporidium* and *Giardia* organisms must perform consistently over a wide range of environmental conditions. The current *Cryptosporidium* and *Giardia* detection method used in the United States, EPA method 1623 (9), is a considerable improvement over the previously used ICR methodology (1, 3, 5); however, relatively few studies have examined recoveries by this method at environmentally realistic concentrations of organisms (for example, 10 organisms/liter or lower) and at turbidity levels commonly found in the SWP or its major tributary rivers (3, 6).

The objectives of the study were twofold. First, we tested the relative capacities of the Gelman Envirochek standard and high-volume (HV) capsule filters. For an analyte that exhibits a nonhomogeneous distribution, sensitivity is linked to the volume of water filtered. Although marketed for filtration of large volumes of finished water, the HV filter could provide an advantage over the standard filter if it could filter larger volumes of turbid ambient waters without clogging. Second, we examined the recovery of *Cryptosporidium* and *Giardia* in ambient waters at environmentally low concentrations by using method 1623.

MATERIALS AND METHODS

Samples were collected at five points in the SWP and Delta system from December 1999 to May 2000. For the filtration capacity experiment, samples were analyzed at Barker Slough below Campbell Lake (Campbell Lake). For matrix spike experiments, samples were collected at the following locations: (i) immediately above Bethany Reservoir (Bethany), which is at the headwaters of the California Aqueduct; (ii) the Sacramento River at the town of Hood (Hood);

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TABLE 1. Comparison of volume capacities between the HV and standard Envirochek filters[a]

Sample site	Turbidity (NTU)	Vol filtered (liters) (mean ± SE)[b]	
		HV filter	Standard filter
Campbell Lake	88	3.2 ± 0.19 A	1.7 ± 0.32 B
Hood	99	7.0	4.0
Bethany	11	10	10
Barker Slough	36	10	NA[c]
Barker Slough	47	5.0	NA

[a] Three replicate samples were compared.
[b] Values followed by the same letter are not significantly different (*t* test, $P = 0.008$). Standard error could not be calculated for filter capacity comparisons in matrix spike experiments.
[c] NA, not analyzed.

(iii) the San Joaquin River near the town of Vernalis (Vernalis); and (iv) the forebay of the Barker Slough Pumping Plant (Barker Slough).

All filtration capacity samples were processed in the field by using the 1999 version of method 1623 (8). The component order in the filtration assembly followed EPA guidelines except that the flow controller was located after the pump. Three replicate filters per filter type (Gelman Envirochek and Envirochek HV sampling capsules) per source were used. Method recovery experiments using the 1999 version of method 1623 (8) were conducted at DWR's Municipal Water Quality Investigations field unit facility in West Sacramento, Calif. For each matrix spike experiment, approximately 132 liters of ambient water was collected at a site and transported on ice in 20-liter polyethylene carboys to the field unit facility. Subsamples were pooled into a clean, 208-liter polyethylene tank and allowed to reach ambient temperature. Water was thoroughly homogenized immediately before a 10-liter subsample was withdrawn for matrix spiking. Three replicate filters and one background control filter were used for each water and filter type. The background sample was filtered prior to the spiked triplicate samples. For spiked waters, the volume filtered was equal to the volume filtered through the appropriate background filter.

Both spiked and unspiked ambient samples were mixed for 10 min. If the ambient water was to be spiked with *Cryptosporidium* and *Giardia*, the spiking solution was vortexed for approximately 1 min and poured into the mixing 10-liter sample. Filter preparation was similar to that in the filtration capacity study except that Teflon-lined polyethylene tubing was used. During filtration, the 10-liter sample was mixed continuously.

Samples were sent on ice to Clancy Environmental Consultants, Inc. All laboratory analyses were completed within 72 h of sample spiking. Method 1623 standard procedures were followed with the exception that a packed pellet volume of up to 1 ml was subjected to a single immunomagnetic separation (IMS) step (2). In samples from the Sacramento River, the high turbidities produced a packed pellet volume that exceeded 1 ml. In this case, two subsamples, each equivalent to 1 ml of packed pellets, were subjected to individual IMS analyses. The manufacturer's instructions were followed for elution of the HV filters.

The Wisconsin State Laboratory of Hygiene provided *Cryptosporidium* and *Giardia* spiking suspensions. *Cryptosporidium parvum* spikes were prepared with the Iowa isolate, while spiking suspensions of *Giardia intestinalis* were prepared with the CH3 strain. Oocysts were obtained from the Sterling Parasitology Laboratory at the University of Arizona, Tucson. Cysts were obtained from Waterborne, Inc., New Orleans, La. *Cryptosporidium* oocysts were between 20 and 60 days old, and *Giardia* cysts were between 5 and 11 days old. Spike sample storage, preparation, enumeration, and quality assurance/quality control (QA/QC) were identical to those of EPA validation studies (11). For all experiments, spiking solutions contained an average of 100 (oo)cysts/10 liters. In all cases, the relative standard deviation of the spike mean was less than 3% ($n = 10$ or 12). Spiking solutions were used within 24 h after enumeration.

Percent recoveries were calculated by subtracting the number of organisms counted in the unspiked sample from the total number of organisms recovered from the spiked sample and then dividing this difference by the total number of oocysts spiked. Final values were multiplied by 100 to yield percent recoveries. Differences in filtration capacities and recoveries between sites were calculated by using either a *t* test or one-way analysis of variance (ANOVA). All parametric assumptions were checked prior to analysis.

RESULTS

At high turbidities (88 to 99 NTU), HV filters were typically able to filter more water than the standard filters, but at some sites neither could filter 10 liters (Table 1). At low turbidities (11 NTU), both filters were capable of filtering the full 10 liters.

Filtration capacity appeared to be affected by the nature of the source water. At Hood, with a turbidity of 99 NTU, the HV and standard filters were capable of filtering 7 and 4 liters, respectively. At Campbell Lake, with a turbidity of 88 NTU, filtration capacity was reduced by half (3.2 and 1.7 liters for the HV and standard filters, respectively). At the time of collection, the background water matrix of the Sacramento River water was dominated by sediment from recent rainfall activity, while turbidity at Barker Slough was dominated by algae. Capacity differences were also noted within the same water (for example, Barker Slough at 36 and 47 NTU).

In all waters tested, oocyst recovery with the HV filter was equivalent to or better than recovery with the standard filter (Table 2). In low-turbidity water (11 NTU), oocyst recoveries were significantly higher with the HV filter (arcsine transform, $P = 0.014$). No significant differences were observed between cyst recoveries (arcsine transform, $P = 0.18$). In a second recovery trial using high-turbidity waters (99 NTU), oocyst recoveries between the two filters were not significantly different (arcsine transform, $P = 0.77$). *Giardia* recoveries were <1%. Based on filter capacity and recovery comparisons, the remaining matrix spike recovery experiments were conducted with HV filters.

Cryptosporidium recoveries remained at or above 50% at all but the highest turbidity tested (Table 3). Oocyst recoveries between sites were significantly different (arcsine transform, *P*

TABLE 2. Comparison of average percent recoveries between the HV and standard Envirochek filters[a]

Organism	Site	Turbidity (NTU)	Spiking concn (organisms/liter)	% Recovery (mean ± SE)	
				HV filter	Standard filter
Cryptosporidium	Bethany	11	10.1	51 ± 0.02 A	43 ± 0.01 B
	Hood	99	9.4	36 ± 0.02	37 ± 0.05
Giardia	Bethany	11	10.3	53 ± 0.05	61 ± 0.06
	Hood	99	10.1	0.47 ± 0.00	0.83 ± 0.01

[a] Three replicate samples were compared. Data are untransformed values. All statistics were determined with arcsine-transformed data.
[b] Values followed by the same letter(s) are not significantly different (*t* test, arcsine-transformed data, $P = 0.014$).

TABLE 3. Mean oocyst and cyst recoveries by HV filter by site[a]

Organism	Site	Turbidity (NTU)	Spiking concn (organisms/liter)	% Recovery (mean ± SE)	CV[c] (%)
Cryptosporidium	Bethany	11	10.1	51 ± 0.02 AC	5.9
	Vernalis	20	9.7	75 ± 0.06 BCD	12.8
	Barker Slough	36	9.8	55 ± 0.12 ACD	38.5
	Hood	99	9.4	36 ± 0.02 A	11.8
Overall avg or CV				54	33.1
Giardia	Bethany	11	10.3	53 ± 0.06 C	20.2
	Vernalis	20	10.0	46 ± 0.03 C	10.9
	Barker Slough	36	10.3	2.6 ± 0.01 D	86.6
	Hood	99	10.1	0.5 ± 0.00 D	173.2
Overall avg or CV				25	100.8

[a] Three replicate samples were used. Data in table are untransformed values. All statistics were determined with arcsine-transformed data.
[b] Values followed by the same letter(s) are not significantly different ($P < 0.05$).
[c] CV, coefficient of variation.

= 0.02), with significant differences in recoveries occurring between the Vernalis and Hood sites (arcsine transform, $P < 0.05$). Variability between replicates at all sites was within the method's quality control criteria of 61% maximum relative percent difference; however, the coefficient of variation for samples collected at Barker Slough was higher than that of any other site tested. With one exception, oocysts were not detected in any of the ambient control samples. One oocyst was detected in storm water collected from the Hood site.

In contrast to *Cryptosporidium* recoveries, *Giardia* cyst recoveries declined steeply at turbidities above 20 NTU (Table 3). Statistically, there were no significant differences in recoveries at the two lowest turbidities; however, recoveries were significantly lower at turbidities beginning at 36 NTU (arcsine transform, $P = 0.001$) (Table 3). Coefficients of variation also illustrated the large variation associated with replicated sample recovery. No cysts were observed in ambient waters.

Filtration capacity experiments suggested that the matrix of the source water affected filtration capacity, but regression analysis of log-transformed data concerning turbidity versus oocyst recovery showed little predictive value ($r^2 = 0.16$) and the null hypothesis of zero slope could not be rejected ($P = 0.29$). For *Giardia*, turbidity was able to explain approximately 80% of the variability associated with recovery ($r^2 = 0.80$) while the slope of the line was significantly different from a slope of zero ($P = 0.001$).

DISCUSSION

Unlike the distribution of solutes in natural waters, (oo)cysts cannot be presumed to be homogeneously distributed throughout the water column. Therefore, the volume of water sampled becomes critical when trying to quantify concentration. In this study, as turbidity increased, neither the standard Envirochek nor the HV capsule filter could filter a full 10 liters without clogging. The HV filter did filter a larger volume of turbid water than the standard filter without compromising recovery efficiencies, suggesting that the HV filter may be a better choice for natural waters.

In this study, filter capacity was affected by relatively small changes in turbidity. Since turbidity instrumentation makes no distinction between the types or sizes of particles, sites with relatively minor differences in turbidity can produce relatively large changes in filtration capacity.

In the published literature, only the EPA's ICRSS has examined the method's performance over a wide range of turbidities while also using low organism spiking concentrations (3). Our study's overall recovery of 54% for oocyst matrix spike experiments was slightly higher than the 43% spiked recoveries obtained in the ICRSS, but our spiked cyst recoveries were considerably lower than the spiked source water recoveries in the ICRSS (25 versus 53%, respectively). One explanation for these divergent results may be the initial condition of the cysts used. In some cases, the quality of the *Giardia* cysts was poor; therefore, some cysts may have degraded during the sampling and handling process. In addition, the ICRSS study used cysts from the CDC:0284:1 strain obtained from EPA's Office of Research and Development. Our study used cysts from the CH3 strain.

Recoveries of 50% or less occurred in low- and high-turbidity waters, suggesting that the nature of the turbidity or the background matrix of the water was as important to recovery as was an absolute NTU value. In the case of oocyst recoveries, turbidity was unable to account for recovery differences between sites, while for cyst recoveries, turbidity could explain intersite differences. This dichotomy is similar to findings from the ICRSS (7), although that survey found only a weak correlation between turbidity and *Giardia* cyst recovery.

The ICRSS and our studies highlight the statistical difficulties of using method 1623 for either routine monitoring or TMDL studies. If a method is unaffected by the water source, then regardless of location, recoveries of organisms spiked at the same concentrations should not be statistically different. If a methodology behaves differently with different water matrices, then resulting distributions cannot be assumed to be similar across sample sites, rendering commonly used parametric and nonparametric statistical comparisons invalid. One solution may be to examine the overlap of 95% confidence intervals between sites (Christopher Frebis, EPA, personal communication).

Periods of rainfall, when the greatest mobilization of (oo)cysts could occur, corresponded to periods when filtration capacity and the method were most compromised. In this study, under high rainfall conditions, only 7 of the 132 liters originally sam-

pled from the river could be filtered through the HV filter. With a flow rate of 87,500 ft^3/s at the time, the volume filtered represented approximately 1.9×10^{-7} percent of the river volume. Recently, the EPA published proposed filtration guidelines that allow filtration of up to 50 liters of ambient water (4); however, as demonstrated in this study, filtering even 10 liters of turbid ambient water may be problematic. Increasing the sampling frequency can be critical to capturing a clumped distribution. It is likely that our one sample collected during a storm event was inadequate to characterize a clumped distribution, but the high cost of the method limited the number of samples collected.

Increasing sample size to offset method variability may be of little value with some waters. In an ongoing study at Barker Slough, laboratory recovery and precision of reagent-spiked water consistently fall within the EPA's QA/QC and coefficient of variation method guidelines, yet average recoveries of matrix spikes from Barker Slough are 15% with a coefficient of variation of 135% ($n = 9$). Monitoring and TMDL studies may face similar situations in their own watersheds, making it imperative to understand the nature of the confounding matrix on the method. Chelation between the slough's high levels of organic carbon (up to 38 mg/liter) and iron is one possible matrix effect interfering with the IMS portion of the method.

In conclusion, the data presented in this study suggest that the HV capsule filter may be a better choice for natural-water sampling programs, although the composition of the turbidity may still preclude filtration of 10 liters. Method 1623 is an improvement over the earlier ICR method, but our data suggest that matrix interference from the waters tested can compromise the method's accuracy and precision. Furthermore, increasing the number of replicates may be cost prohibitive and of little use. Statistical comparisons must also be approached with caution because of the problems matrix interference causes with the method. Regardless of the method used, the patchy distribution of the target organism must be accounted for to create a meaningful study design. This is a significant hurdle in its own right, and sampling designs to overcome this built-in environmental handicap need to be investigated fully.

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