

## Removal of *Cryptosporidium* and polystyrene microspheres from swimming pool water with sand, cartridge, and precoat filters

James E. Amburgey, Kimberly J. Walsh, Roy R. Fielding and Michael J. Arrowood

### ABSTRACT

*Cryptosporidium* has caused the majority of waterborne disease outbreaks in treated recreational water venues in the USA for many years running. This research project evaluated some common US swimming pool filters for removing *Cryptosporidium* oocysts, 5- $\mu$ m diameter polystyrene microspheres, and 1- $\mu$ m diameter polystyrene microspheres. A 946 L hot tub with interchangeable sand, cartridge, and precoat filters was used at room temperature for this research. Simulated pool water for each experiment was created from Charlotte, NC (USA) tap water supplemented with alkalinity, hardness, chlorine, and a mixture of artificial sweat and urine. Precoat (i.e., diatomaceous earth and perlite) filters demonstrated pathogen removal efficiencies of 2.3 to 4.4 log (or 99.4–99.996%). However, sand and cartridge filters had average *Cryptosporidium* removals of 0.19 log (36%) or less. The combined low filter removal efficiencies of sand and cartridge filters along with the chlorine-resistant properties of *Cryptosporidium* oocysts could indicate a regulatory gap warranting further attention and having significant implications on the protection of public health in recreational water facilities. The 5- $\mu$ m microspheres were a good surrogate for *Cryptosporidium* oocysts in this study and hold promise for use in future research projects, field trials, and/or product testing on swimming pool filters.

**Key words** | *Cryptosporidium*, diatomaceous earth, granular media filtration, precoat filtration, recreational water, swimming pools

### INTRODUCTION

*Cryptosporidium* is a human protozoan parasite transmitted by the fecal-oral route while enclosed in a protective outer shell called an oocyst. The oocyst makes it highly-resistant to chlorine disinfection with Ct values on the order of 15,300 mg/L min (Shields *et al.* 2008b). *Cryptosporidium* caused more than 80% of reported waterborne disease outbreaks in treated recreational water venues (e.g., swimming pools) in the USA from 2005 to 2006 (Yoder *et al.* 2008). Cryptosporidiosis lasts an average of 12 days (with rare instances lasting as long as 4 weeks) in immunocompetent individuals with symptoms that can include: watery diarrhea, nausea, vomiting, fever, and abdominal

cramping (Daniel 1996; Hoxie *et al.* 1997; Yoder & Beach 2010). Surveillance of cryptosporidiosis in the USA indicates that the reported incidence of infection has increased dramatically since 2004 (Yoder & Beach 2010). Both the number of reported cases and the number of individual outbreaks have shown overall upward trends since 2004 (Yoder *et al.* 2010). While it is difficult and expensive to assess the prevalence of protozoan parasites in public pools during normal non-outbreak conditions, a study of 160 filter backwash water samples from Atlanta, GA, USA showed that 13 (8.1%) were positive for the presence of *Giardia* or *Cryptosporidium* or both although the viability of the

James E. Amburgey (corresponding author)  
Roy R. Fielding  
University of North Carolina at Charlotte,  
9201 University City Blvd.,  
Charlotte,  
NC 28223-0001,  
USA  
E-mail: jeamburg@uncc.edu

Kimberly J. Walsh  
EP Minerals,  
9785 Gateway Dr,  
Reno, NV,  
USA

Michael J. Arrowood  
Centers for Disease Control and Prevention,  
1600 Clifton Rd.,  
Atlanta, GA,  
USA

parasites was not reported (Shields *et al.* 2008a). In a study of 803 Oklahoma children, 58% of adolescents (ages 14 to 21) were seropositive for *C. parvum*, which indicates prior infection by the pathogen (Ford 1999). The true burden of cryptosporidiosis is not known with certainty, but recent estimates have ranged from 300,000 to 748,000 cases annually in the USA (Yoder & Beach 2007; Beach 2011). Multiple sources have indicated that weaker subpopulations (e.g., very young children, elderly people, pregnant women, and the immunocompromised) could die from cryptosporidiosis (Daniel 1996; Hoxie *et al.* 1997; Ford 1999). A quantitative risk assessment model of *Cryptosporidium* in swimming pools recently confirmed there is a 'significant public health risk' (Pintar *et al.* 2010). As in the drinking water industry, the burden for safety often falls primarily on physical removal (i.e., filtration).

### Accidental fecal release scenario

It is challenging to define a 'typical' accidental fecal release of a person with cryptosporidiosis in a swimming pool primarily because pools vary so widely in volume and in the number of swimmers. If the number of swimmers with cryptosporidiosis in a given pool increases proportional to the number of swimmers in the pool, then the volume of the pool becomes less critical. So, for a typical hotel or apartment complex with a pool volume of 100,000 L and 100 visitors per day on average, let us assume only one of the swimmers has cryptosporidiosis. As many cases of cryptosporidiosis are asymptomatic and a person can still excrete oocysts for up to 50 days after cessation of diarrhea (Yoder & Beach 2010), it might be reasonable to assume at least one person in the pool is introducing fresh oocysts as they swim. A single bowel movement from an infected person can contain  $10^8$ – $10^9$  oocysts (Yoder & Beach 2010), so  $10^8$  oocysts will be used here for a conservative estimate. If we assume the oocysts are quickly and uniformly distributed throughout the entire volume of pool water by the activity of the swimmers (as opposed to having localized regions of higher and lower concentrations), then the initial concentration would be 1,000 oocysts/L (or 1 oocyst/mL). The average adult swallows 16 mL of water each time they swim while younger swimmers swallow an average of 37 mL (Dufour *et al.* 2006). In this scenario, the average

swimmer would swallow 16 or 37 oocysts depending on the age of the swimmer. *Cryptosporidium* has a low infectious dose, and as few as 10–30 oocysts can cause infection in healthy persons (Yoder & Beach 2010). So, the average swimmer would ingest enough oocysts to potentially become infected for as long as it takes the pool's treatment system to lower the concentration of infective oocysts to a safe level. This scenario probably borders on best-case conditions since many pools (e.g., plunge pools, kiddie pools) are smaller and multiple swimmers could be introducing oocysts at the same time. This scenario also ignores the potential for accumulation of oocysts over multiple days.

### Pool water treatment overview

Swimming pools are generally complex systems where a body of water experiences heterogeneous mixing and variable inputs of multiple contaminants. The filtration and disinfection of the water in a pool typically occurs as the water is recirculated through a treatment area at regular intervals that could be as high as 4–8 h on average (via a system of interconnected inlets and outlets spaced around the pool by the designer). A target pH (e.g., 7.2 to 7.6) and free chlorine level (e.g., 2 to 4 mg/L) are typically maintained in pool water (often by an automatic controller) to achieve some level of residual disinfection between treatment cycles. Unfortunately, the residual disinfectant is quite slow at inactivating chlorine-resistant pathogens like *Cryptosporidium*. Automatic control systems for filters exist, but most swimming pool filters are still operated manually. The overall quality and safety of the pool water depends on many variables (e.g., bather inputs, mixing dynamics, length of treatment cycle, disinfection process type(s), filtration effectiveness, as well as the training/performance of the pool operator), but the current study focused solely on the effectiveness of the filtration process for removal of particles of two specific sizes.

### Filtration practices

Relatively little is known about the capabilities of common US swimming pool filters to remove waterborne pathogens. Research has shown that 'high-rate' (i.e., 25–49 m/h filter loading rate) swimming pool sand filters can only

consistently deliver 0.11 to 0.28 log (22 to 48%) removal of *Cryptosporidium* oocysts and/or a microsphere surrogate without coagulation (Amburgey et al. 2007, 2008, 2009a, b; Croll et al. 2007). These levels of removal appear inadequate to prevent outbreaks of cryptosporidiosis, which is supported by surveillance data on outbreaks investigated each year in the US. However, drinking water treatment research has shown that granular media filters can remove in excess of 6 log (99.9999%) of *Cryptosporidium* oocysts (States et al. 2002). Although sand filters are similar for swimming pool and drinking water treatment facilities, there are two important differences between drinking water treatment and pool water treatment practices. First, drinking water treatment facilities perform chemical coagulation prior to filtration, which greatly enhances the ability of the filters to remove the pathogens by reducing the natural electrostatic repulsion that exists between negatively charged pathogens and negatively charged filter media in water (Hendricks 2006). Second, the filter loading rates (flow per unit of surface area of filter) are typically at least 4 to 5 times lower in US drinking water filters than in pools (e.g., 5 to 10 m/h for drinking water versus 37 to 49 m/h in pools).

Diatomaceous earth (DE) is also used in drinking water treatment. However, the two important differences between sand filters used for drinking water and pool water treatment (stated previously) do not apply to DE filtration. The filter loading rates used for DE filters in both industries are similar, and neither industry necessarily practices coagulation as pretreatment for DE filters (Logsdon, 2008; NSPF, 2009). Unlike sand filters, DE filters rely dominantly on the size exclusion principle to prevent pathogens from passing through the tiny pores in the DE media. This is why sand filter media depths are a minimum of 0.25 m (and commonly called 'depth filters') while DE media depths are only 1.6–3.2 mm because they are 'surface filters'. Drinking water research has shown *Cryptosporidium* removal in excess of 6 log for DE filters (Ongerth & Hutton 1997; Ongerth & Hutton 2001). Nevertheless, two important differences still exist between the DE filtration practices used in the drinking water and pool water industries. The grades of DE used in drinking water treatment are of a finer grade, which is typically measured as 'permeability'. Typical permeability values of the DE media used for swimming pools is 4–5  $\mu\text{m}^2$ , but the permeability of the DE media used in drinking

water treatment is typically less than 1  $\mu\text{m}^2$  (Hendricks 2006). The finer-grained precoat media has proportionally smaller pores between the grains and removes smaller particles more effectively. Secondly, the amount of DE media loaded into each filter per unit surface area of the media support material is typically two-fold greater (at 1 kg/m<sup>2</sup>) in the drinking water industry (Logsdon 2006). More media per unit surface area creates a thicker layer of DE 'cake' that particles must pass through to avoid removal.

Pool filters are commonly designed to keep swimming pools looking clear and pleasing to the eye (i.e., turbidity removal), which is not the same as for effective pathogen removal. The US swimming pool industry has traditionally relied on disinfectants, such as free chlorine, to control the spread of waterborne diseases. However, chlorine is inefficient at inactivating *Cryptosporidium*, so the burden of microbial safety falls on filtration. The drinking water industry also relied heavily on chlorine until chlorine-resistant pathogens forced changes in the 1980s (for *Giardia*) and the 1990s and beyond (for *Cryptosporidium*). These chlorine-resistant pathogens forced the drinking water industry to put considerable regulatory emphasis on filtration optimization to achieve physical removal of these pathogens. US drinking water regulations have become increasingly stringent on pathogen removal in recent years in order to safeguard public health. The swimming pool industry could be forced to take the same approach. However, the average level of training and human resources devoted to swimming pool operations tends to be considerably less than for drinking water operations, which necessitates simple yet effective filtration options. Constantly optimizing coagulation and closely monitoring filtered water turbidity might not be the most practical approach for the majority of US swimming pools.

### Research objectives

The primary objective of this research was to evaluate some common swimming pool filtration technologies for removing *Cryptosporidium* oocysts, 5- $\mu\text{m}$  polystyrene microspheres, and 1- $\mu\text{m}$  polystyrene microspheres from a simulated pool water. The filter types chosen were sand, cartridge, and precoat. Standard commercially available filters and media were used in these experiments. Knowing the key differences between drinking water and pool water

practices for both sand and precoat filters, a limited number of additional experiments were conducted in an effort to achieve better pathogen removal performance with the sand and precoat filters.

## METHODS

### Experimental setup (model pool)

A 946 L commercial hot tub was used at room temperature (20 °C) without aeration to serve as the pool (or water tank) for this research. The original pump and filter were removed and replaced by a commercially available (to the pool industry) 2.2 kW centrifugal pump and a set of three interchangeable filters (i.e., sand, cartridge, and precoat) connected via a 51 mm diameter PVC pipe loop measuring approximately 6.5 m in length. A schematic of the experimental setup is shown in Figure 1, and Table 1 contains detailed information about the materials used in this research. The flow was measured with a digital paddle-wheel flow meter and controlled with a 51 mm diameter PVC ball valve.

### Filter descriptions

The sand filter contained approximately 35 cm of sand (with an effective size of 0.49 mm and uniformity

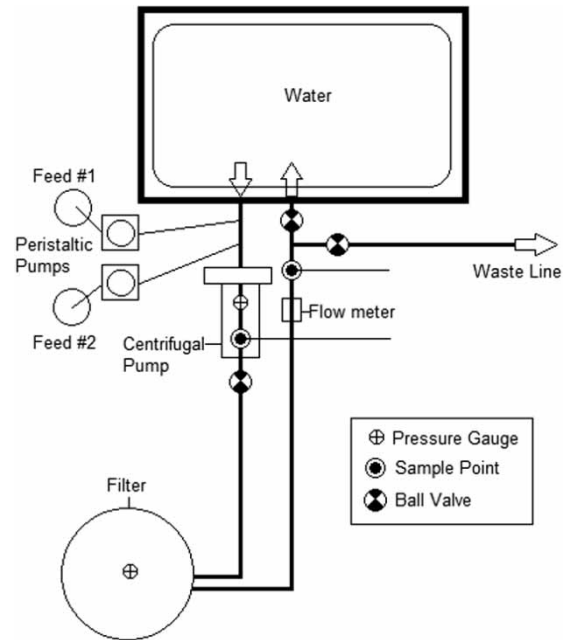


Figure 1 | Diagram of experimental setup.

Table 1 | Materials and equipment details

Item	Model	Manufacturer	Address
Sand filter	Triton <sup>®</sup> II TR 40	Pentair Water	Samford, NC
Precoat filter	FNS Plus 24	Pentair Water	Samford, NC
Cartridge filter	Clean & Clear Plus 240	Pentair Water	Samford, NC
Centrifugal pump	Challenger <sup>®</sup> 3 HP	Pentair Water	Samford, NC
Standard DE	Celatom <sup>®</sup> Standard	EP Minerals	Reno, NV
Fine DE	Celatom <sup>®</sup> Premium	EP Minerals	Reno, NV
Fine perlite	Proprietary product	EP Minerals	Reno, NV
Coagulant	Super Blue <sup>®</sup>	Arch Chemicals Inc.	Norwalk, CT
Flow meter	SEM-40	FlowServe	Irving, TX
Peristaltic pumps	505 Di	Watson Marlow	Wilmington, MA
Magnetic stirrer	Cimarec <sup>®</sup>	Thermo Fisher	Waltham, MA
Microspheres	Fluoresbrite <sup>®</sup> YG	Polysciences, Inc.	Warrington, PA
Fluorescent stain	Zymed <sup>®</sup> IgGAM (H + L)	Invitrogen Corp.	Carlsbad, CA
PCTE filters	K30CP02500	GE Osmonics	Minnetonka, MN
PCTE filters	K06CP02500	GE Osmonics	Minnetonka, MN
Filter funnels	xx10 025 00	Millipore, Inc.	Billerica, MA
Microscope	Standard 25	Carl Zeiss	Oberkochen, Germany

coefficient of 1.5), but only about 25 cm of the sand was necessarily used for filtration as the remainder was below the surface of the laterals. A cross-sectional drawing of the 0.18 m<sup>2</sup> sand filter appears in Figure 2. The surface areas of the precoat and cartridge filters were 2.2 and 22.3 m<sup>2</sup>, respectively. The three types of precoat media used in this study were standard pool grade DE with a permeability of 4.4 μm<sup>2</sup>, fine DE with a permeability of 1.2 μm<sup>2</sup>, and fine perlite with a permeability of 1.5 μm<sup>2</sup>. The manufacturer-supplied filter cartridges were used in the cartridge filter, but no pore size rating was given in the product literature. All filters were from the same manufacturer and similar in size, shape, and construction materials despite the very different filtration technologies and surface areas being utilized.

### Pathogen and microsphere seeding

Inline feed of the oocyst/microsphere suspensions was made possible by a digital peristaltic pump feeding directly into the PVC pipe just upstream of the centrifugal pump. The oocyst/microsphere suspensions were made in a 1-L glass Erlenmeyer flask of simulated pool water and stirred continuously with a magnetic stirrer and Teflon<sup>®</sup>-coated stir bar prior to and during the experiments. The inline feed system allowed for feeding the oocysts/microspheres into the system without stopping and restarting the precoat filters during each experiment, which had been observed to hinder the filter performance in pre-trial runs.

### Filter operation

The sand and cartridge filters were operated differently because the stop/start process was not expected to alter

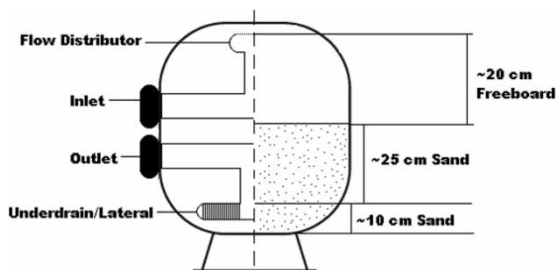


Figure 2 | Cut-away view of sand filter.

their performance once the flow stabilized. For the sand and cartridge filter experiments, the flow of water was stopped just prior to beginning the oocyst/microsphere seeding, and valves were repositioned to redirect the filtered water to the drain piping thereby preventing recirculation. Preliminary experiments (data not shown) revealed that the low removals associated with the sand and cartridge filters allowed rapid accumulation of particles in the spa water with recirculation, which caused the influent number of oocysts/microspheres to increase rapidly and potentially alter the accuracy of calculations as well as the filter performance itself. Sand filters were backwashed with simulated pool water prior to experiments to prevent the pore water from changing chemically during the experiments while the other two types of filters were simply drained completely and filled with simulated pool water at the beginning of the experiment.

All filters were pressure filters (as opposed to gravity or vacuum filters). The filter loading rates were chosen at the maximum rated value for each filter in order to assess the worst-case scenarios for each filter in terms of *Cryptosporidium* removal. The spa system was capable of pumping water at up to 227 L/min. The precoat and cartridge filters were operated at 227 L/min for filter loading rates of 6.1 and 0.61 m/h, respectively. Water was pumped through the sand filter at 144 L/min for a filter loading rate of 49 m/h. All filters were designed and approved by the National Sanitation Foundation (Ann Arbor, MI, USA) to operate at the prescribed flow rates according to their product labels. The DE or perlite media was added to the precoat filter (in slurry form) in the amount of 0.49 kg/m<sup>2</sup> of filter surface area. However, one exception was that the amount of fine DE was increased to 1.5 kg/m<sup>2</sup> in the second experiment only.

### Coagulant application

The coagulant, when used, was poured directly into the spa following an initial dilution in 10 L of simulated pool water and allowed to recirculate and mix for a period of time prior to seeding oocysts and/or microspheres. The minimum recommended dosage on the bottle (1.6 mg/L as product) was used without any optimization or zeta potential measurements. This product was chosen because of its history and prevalence in the US swimming pool market. Currently, coagulant use is not required with any type of filter. Use is

at the discretion of the pool operator, which often means it is not used at all by a given facility. When a coagulant is used, it is often in response to a real or perceived water quality problem. Coagulant usage guidelines are not well-established, and the directions on the bottle tend to be vague about the manner of application and frequency of use. While the ingredients and formulation information are proprietary, the primary active ingredient is a cationic polymer.

### Simulated pool water

Simulated pool water was created for each experiment from 946 L of Charlotte, NC (USA) tap water supplemented with sodium bicarbonate to an alkalinity of 150 mg/L as CaCO<sub>3</sub>, with calcium chloride to a hardness of 250 mg/L as CaCO<sub>3</sub>, with sodium hypochlorite to a free chlorine concentration of 2 mg/L, with hydrochloric acid to a pH of 7.5, and with a mixture of artificial sweat and urine described in Table 2 to a final total organic carbon (TOC) concentration of approximately 20 mg/L as C. This TOC concentration was chosen to match the campus pool at UNC Charlotte, which is near the upper end of the 3.0 to 23.6 mg/L average TOC concentration range observed in a survey of 23 indoor pools in the Southeastern USA (Kanan 2010).

### Experimental procedure and sampling

Two experiments were performed for each set of conditions. For each experiment, duplicate samples were collected from

**Table 2** | Artificial sweat and urine formula

Component	Concentration (mg/L)	Concentration (mM)
Acetic acid	12.6	0.21
Ammonium chloride	48.4	0.90
Creatinine	1.8	0.016
Lactic acid	32.8	0.36
Potassium chloride	4.0	0.053
Sodium chloride	59.6	1.02
Sodium phosphate (monobasic)	4.2	0.030
Sodium sulfite	2.0	0.016
Urea	18.7	0.31

the filter influent and effluent pipes. Sampling began approximately 1 min after the start of an experiment. The hydraulic detention time of the pipe loop from the point of microsphere injection through the pump and sand filter to filter effluent sample point was calculated to be 35 s at a flow rate of 144 L/min. So, roughly two hydraulic detention times passed prior to the collection of the first sample and four detention times between replicate sets of samples. A delay of one hydraulic detention time was used between collection of the influent and effluent paired samples in an attempt to sample the same water volume before and after filtration. The hydraulic detention time of the system including the hot tub was only 7.2 min, so the duration of the microsphere seeding experiments was limited to less than 6 min each for the sand filter configuration. The cartridge and precoat filter setups used a higher flow rate and proportionally shorter hydraulic retention times (25 s) with entire experiments lasting no more than 4 min each. So, it was not possible to look at performance changes or headloss development rates versus time of filtration as part of this study.

### Microspheres and *Cryptosporidium*

Fluorescent-green carboxylate-modified polystyrene microspheres (4.869 µm, std. dev. 0.246 µm) were fed simultaneously with *Cryptosporidium* oocysts, but the smaller diameter microspheres (0.951 µm, std. dev. 0.010 µm) were used in separate experiments. A 1 L microsphere suspension was fed inline during the experiment to achieve the target filter influent concentration of approximately 15–150 oocysts/microspheres per mL of water. Influent samples of 50 mL were collected in sterile 50 mL conical-bottomed plastic centrifuge tubes, and the volume of the effluent samples varied from 50 mL to 1 L with the larger samples collected in glass media bottles. A maximum of approximately 10<sup>8</sup> heat-inactivated (55 °C for 30 min) *Cryptosporidium parvum* oocysts (Iowa isolate; purified according to Arrowood & Donaldson (1996)) and/or YG fluorescent carboxylate-modified polystyrene microspheres were used in each experiment. The microspheres also served as an internal control for oocyst staining procedure since the microspheres do not have to be stained to be seen under an epifluorescence microscope. Further, microspheres are not susceptible to excystation (breaking open)

during experiments, and they are much easier to identify under an epifluorescence microscope. The *Cryptosporidium* oocysts were stored in water at 4 °C with antibiotics prior to use and obtained from Dr Michael Arrowood's laboratory at the Centers for Disease Control and Prevention in Atlanta, GA, USA.

### Sample analysis

Samples were filtered through 3- $\mu$ m polycarbonate track-etched (PCTE) filters in 25-mm glass microanalysis filter funnels by a regulated 3-place vacuum manifold. Sample volumes analyzed were adjusted to obtain between 10 and 150 oocysts and/or microspheres per sample. The staining method used for *Cryptosporidium* involved placing 600  $\mu$ L of *Cryptosporidium*-specific monoclonal antibody (obtained from Dr. Arrowood's lab) on top of the filter for 30 min at room temperature, rinsing twice with 750  $\mu$ L of 1 $\times$  PBS (pH 7.4), applying 500  $\mu$ L of a 1:150 dilution of a fluorescein isothiocyanate (FITC)-labeled goat anti-mouse antibody in the dark at room temperature for 30 min, and rinsing twice more with 1 $\times$  PBS. The filters were mounted on glass micro slides with one drop of polyvinyl alcohol-DABCO solution (Freer 1984) and a 25-mm square glass cover slip for enumeration under epifluorescence microscope at 100 $\times$  (for microspheres) or 250 $\times$  (for oocysts) total magnification. Separate counts were performed for microspheres and oocysts whenever they were used together. The raw counts per mL from the filter influent and effluent sample pairs were used to calculate the percent and log removals. The fluorescent filter set used in the microscope had a 450–490-nm excitation wavelength range, a 510-nm dichroic filter, and a 520-nm emission filter. The PCTE filters with 3- $\mu$ m pores were used for the 5- $\mu$ m microspheres and oocysts, which were assayed simultaneously. A 0.65- $\mu$ m pore size PCTE filter was used for the experiments using only the 1- $\mu$ m microspheres. Samples were stored at 4 °C prior to analysis.

### Quality control measures

The spa system was thoroughly cleaned between experiments with a minimum of three drain-and-fill rinses with recirculation at 227 L/min, and samples were collected prior to

seeding in each experiment to measure any potential carryover between experiments. A control experiment was used to determine whether or not the oocysts and microsphere were destroyed by the pump and/or lost due to surface attachment within the system. All statistical calculations were performed on the percent removal data (not the log-manipulated values) with commercially available software (Microsoft Office Excel 2007; Microsoft, Redmond, WA, USA).

## RESULTS AND DISCUSSION

Particle challenge tests were performed for three categories of filters (i.e., sand, cartridge, and precoat). Subcategories included sand filtration with and without coagulant as well as precoat filtration with three individual filter media products. For each of the preceding filtration scenarios, particle removal was determined separately for *Cryptosporidium* oocysts, 5- $\mu$ m microspheres, and 1- $\mu$ m microspheres. Data from individual experiments are summarized in Table 3.

### Sand and cartridge filter removals of *Cryptosporidium*

Percent *Cryptosporidium* removals and log *Cryptosporidium* removals under varying conditions are shown in Figures 3 and 4, respectively. *Cryptosporidium* removals averaged 0.16 log (31%) for the sand filter operated at 49 m/h and 0.19 log (36%) for the cartridge filter operated at 0.61 m/h. Sand filter removals nearly doubled to a mean of 0.41 log (61%) with the addition of a coagulant to the system. The coagulant dose was not optimized, which might explain the relatively low removals in comparison with drinking water research results or the precoat media used in this study. Charge neutralization is a process that requires the number of positive charges (from the coagulant) to balance the negative surface charges on the particles. The improvement with coagulant addition indicates promise, but further trials with alternate coagulant dosages were beyond the scope of this project. Furthermore, the coagulant was added and recirculated for a minimum of 4 h prior to adding the oocysts. This practice seemed consistent with what might actually occur before a fecal accident in a swimming pool intermittently employing a coagulant, but this method of coagulation is

**Table 3** | Removal data for individual experiments for each type of filter and particle

		<i>Cryptosporidium</i>		5- $\mu$ m Microspheres		1- $\mu$ m Microspheres	
		% removal	Log removal	% removal	Log removal	% removal	Log removal
Sand	Exp. 1-1	31	0.16	26	0.13	39	0.22
	Exp. 1-2	24	0.12	32	0.17	6	0.03
	Exp. 2-1	25	0.13	32	0.16	23	0.11
	Exp. 2-2	45	0.26	37	0.20	25	0.12
	<b>Mean</b>	<b>31</b>	<b>0.16</b>	<b>32</b>	<b>0.17</b>	<b>23</b>	<b>0.11</b>
	<b>Std. Dev.</b>	<b>9</b>	<b>-</b>	<b>5</b>	<b>-</b>	<b>14</b>	<b>-</b>
Cartridge	Exp. 1-1	48	0.28	43	0.24	10	0.05
	Exp. 1-2	32	0.17	33	0.18	0	0.00
	Exp. 2-1	27	0.13	21	0.10	33	0.17
	Exp. 2-2	38	0.21	31	0.16	16	0.08
	<b>Mean</b>	<b>36</b>	<b>0.19</b>	<b>32</b>	<b>0.17</b>	<b>15</b>	<b>0.07</b>
	<b>Std. Dev.</b>	<b>9</b>	<b>-</b>	<b>9</b>	<b>-</b>	<b>14</b>	<b>-</b>
DE (standard)	Exp. 1-1	99.6	2.43	99.4	2.22	56	0.36
	Exp. 1-2	99.4	2.26	99.4	2.25	49	0.29
	Exp. 2-1	99.5	2.31	99.4	2.25	73	0.57
	Exp. 2-2	99.6	2.40	99.5	2.29	61	0.41
	<b>Mean</b>	<b>99.5</b>	<b>2.35</b>	<b>99.4</b>	<b>2.25</b>	<b>60</b>	<b>0.40</b>
	<b>Std. Dev.</b>	<b>0.1</b>	<b>-</b>	<b>0.04</b>	<b>-</b>	<b>10</b>	<b>-</b>
Perlite (fine)	Exp. 1-1	99.93	3.18	99.95	3.33	54	0.34
	Exp. 1-2	99.95	3.29	99.93	3.15	50	0.30
	Exp. 2-1	99.95	3.31	99.91	3.06	65	0.46
	Exp. 2-2	99.93	3.15	99.94	3.19	62	0.42
	<b>Mean</b>	<b>99.94</b>	<b>3.23</b>	<b>99.93</b>	<b>3.17</b>	<b>58</b>	<b>0.37</b>
	<b>Std. Dev.</b>	<b>0.01</b>	<b>-</b>	<b>0.02</b>	<b>-</b>	<b>7</b>	<b>-</b>
Sand + Coagulant	Exp. 1-1	52	0.32	41	0.23	63	0.44
	Exp. 1-2	43	0.24	48	0.28	68	0.49
	Exp. 2-1	76	0.63	82	0.73	63	0.44
	Exp. 2-2	73	0.57	78	0.66	56	0.35
	<b>Mean</b>	<b>61</b>	<b>0.41</b>	<b>62</b>	<b>0.42</b>	<b>63</b>	<b>0.43</b>
	<b>Std. Dev.</b>	<b>16</b>	<b>-</b>	<b>21</b>	<b>-</b>	<b>5</b>	<b>-</b>
DE (fine)	Exp. 1-1	99.991	4.07	99.994	4.21	75	0.61
	Exp. 1-2	99.994	4.24	99.995	4.30	79	0.67
	Exp. 2-1 <sup>a</sup>	99.999	4.98	99.999	5.16	96	1.36
	Exp. 2-2 <sup>a</sup>	99.996	4.45	99.997	4.59	95	1.30
	<b>Mean</b>	<b>99.995</b>	<b>4.33</b>	<b>99.996</b>	<b>4.44</b>	<b>86</b>	<b>0.86</b>
	<b>Std. Dev.</b>	<b>0.00</b>	<b>-</b>	<b>0.00</b>	<b>-</b>	<b>11</b>	<b>-</b>
Control (no filter)	Exp. 1-1	-4	-0.02	12	0.05	11	0.05
	Exp. 1-2	2	0.01	-17	-0.07	9	0.04
	<b>Mean</b>	<b>-1</b>	<b>0.00</b>	<b>-3</b>	<b>-0.01</b>	<b>10</b>	<b>0.05</b>
	<b>Std. Dev.</b>	<b>5</b>	<b>-</b>	<b>21</b>	<b>-</b>	<b>1</b>	<b>-</b>

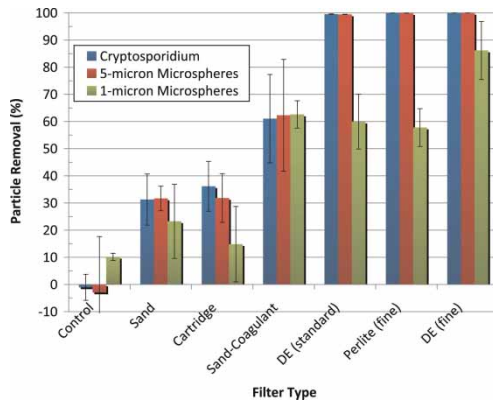
<sup>a</sup>Indicates precoat media was loaded at 1.5 Kg/m<sup>2</sup>.

significantly different than that practiced in drinking water production. In drinking water treatment, adding a continuous feed of coagulant immediately following oocysts/microspheres (just prior to filtration) is expected, but alternate coagulant feeding scenarios were not explored in the present study. These two preceding coagulant addition scenarios could be termed 'passive' and 'active' treatment, respectively.

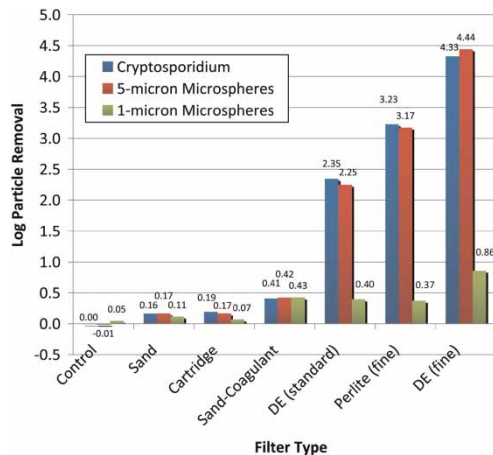
### Precoat filter removals of *Cryptosporidium*

Precoat filter removals of *Cryptosporidium* oocysts were significantly higher than for the other two types of filters as shown in Figure 4. The standard grade of DE used in swimming pools removed 2.3 log (99.6%) of oocysts. Two finer-grained (lower permeability) types of precoat media each





**Figure 3** | Percentage particle removal for each filtration scenario. Error bars extend one standard deviation in each direction.



**Figure 4** | Log particle removal for each filtration scenario.

produced higher removals of oocysts than the standard DE media. Perlite and a finer grade of DE removed oocysts with an average removal efficiency of 3.2 and 4.3 log, respectively. The filtration rate remained constant at 6.1 m/h throughout all of the precoat filtration experiments, but the amount of precoat media increased in one experiment. The mass of standard DE and perlite was identical in all experiments (i.e., 1.1 kg or 0.5 kg/m<sup>2</sup>), but the lower density of perlite produced a precoat layer of approximately double the thickness of the DE precoat layer. Half of the data points with fine grade of DE were also produced at 0.5 kg/m<sup>2</sup>, but the other half were produced with 1.5 kg/m<sup>2</sup> of DE. The thickness of the precoat layer did appear to make some difference in the *Cryptosporidium* removal as shown in Figure 5, but the number of replicate samples ( $n = 2$ ) was insufficient for statistical analysis. The

dominant factor impacting *Cryptosporidium* removal was likely the permeabilities of the precoat media, which were 4.4, 1.5, and 1.2  $\mu\text{m}^2$  for the standard DE, perlite, and fine DE medias, respectively. A series of heteroscedastic, 2-tailed Student's *t*-tests showed that the differences in oocyst removals between each of the media/filter types were statically significant ( $\alpha = 0.05$ ) with the exception of the sand filter versus the cartridge filter. For the statistical analyses, data from all four of the fine DE experiments were grouped together.

### Five-micron microsphere (*Cryptosporidium* surrogate) removals

The microspheres were used as a potential non-infectious surrogate for *Cryptosporidium* oocysts as they have nearly identical size, shape, and density. If the microspheres behave like *Cryptosporidium* oocysts in pools, then they could be used to safely evaluate any full-scale pool filtration system under normal operating conditions without posing any risk to bathers. The percent removals and log removals of the 5- $\mu\text{m}$  microspheres are also shown in Figures 3 and 4, respectively. The *Cryptosporidium* removal results in Figures 3 and 4 are very similar to the microsphere removals. A series of heteroscedastic, 2-tailed Student's *t*-tests did not indicate any statistically significant differences ( $\alpha = 0.05$ ) between the oocyst and 5- $\mu\text{m}$  microsphere removals for any of the experiments with identical treatment. So, the microspheres appeared to be a good surrogate for *Cryptosporidium* oocysts under all test conditions in these filters. Polystyrene microspheres have also been found to be a good surrogate for oocysts in multiple drinking water research studies (Dai & Hozalski 2003; Emelko et al. 2003; Amburgey et al. 2005; Brown & Emelko 2009).

### One-micron microsphere removals

The percent removals and log removals of the 1- $\mu\text{m}$  microspheres are shown in Figures 3 and 4, respectively. The 1- $\mu\text{m}$  microspheres were used in this study to evaluate how each type of filtration performed at removing particles even smaller than *Cryptosporidium* oocysts. These experiments sought to test the perceived limits of each filtration scenario. Mean sand and cartridge filter removals dropped

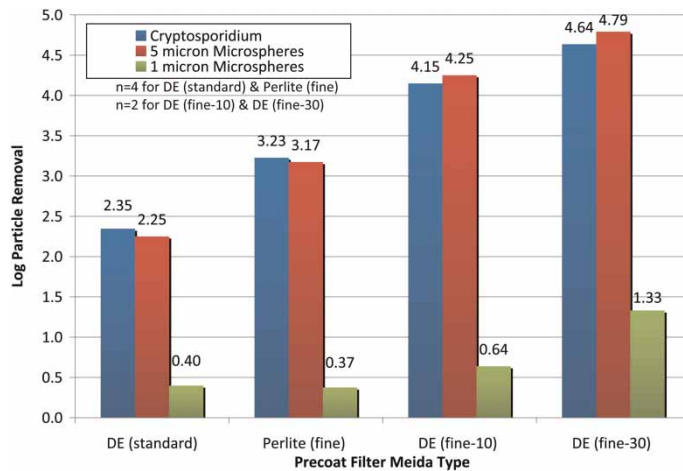


Figure 5 | Log particle removal for each precoat media type.

below 0.12 log (25%) and approached the control (no filter) removal rate of 0.05 log (10%).

Mean removals tended to be lower across each filtration condition with the exception of the sand filter-coagulant combination where the mean removal remained almost constant. The sand filter with coagulant actually showed slightly higher average removals than the standard DE or perlite for the 1- $\mu$ m microspheres. There was one unplanned difference in the experimental procedure between the oocyst/5- $\mu$ m microsphere experiments and the 1- $\mu$ m microsphere experiments using the sand filter with coagulant that could have contributed to relative increase in removal efficiency between sand filter with coagulant versus the other filtration scenarios for the 1- $\mu$ m removal experiments. The coagulant was added and recirculated for approximately 30 min prior to the 1- $\mu$ m removal experiments instead of at least 4 h as was done in the oocyst/5- $\mu$ m microsphere experiments. The shorter interval between coagulant addition and the beginning of the seeding experiment could have increased the removal efficiency of the coagulant/sand filter system, which could indicate that this was a time-dependent treatment process for this system. It could be important that the ratio of surface area of the sand grains to the volume of water in this research setup was much larger than in a typical swimming pool, which could have exerted an increased coagulant demand on the system due to the natural negative surface charge of sand grains in water. The preceding design issue, if significant, calls the real-world applicability of the coagulation experiments into question, but not the nature of the coagulation results, which was

a general improvement over no coagulant. However, the impact of this system's surface area ratios would only be expected to influence the coagulation trials since none of the other trials were adding chemicals that would potentially alter the charge of any surface (i.e., the other experiments were purely physical removal without a chemical component).

As shown in Figure 5, the precoat filters removals were considerably lower at 0.37 to 1.3 log for the 1- $\mu$ m microspheres than for the larger 5- $\mu$ m microspheres and oocysts (2.2 to 4.8 log). Coagulant use may hold promise for increasing the filtration removals of all three types of filters though demonstrated herein only for the sand filter. Hendricks (2006) stated that coagulant use improved the removal of smaller particles in DE filters, but there was a corresponding increase in the rate of headloss accumulation. The removal of the 1- $\mu$ m microspheres was highest for the fine DE media (with a mean of 86% or 0.85 log for two loading levels used for the fine DE) as shown in Figure 3 and 4. Only with the finest grade of DE at triple the standard loading rate, were any of the filters tested capable of removing greater than 1 log (90%) of the 1- $\mu$ m microspheres. Removals of 1- $\mu$ m microspheres were 0.61–0.67 log (75–79%) and 1.3–1.4 log (95–96%) for the standard and tripled media loading rates for fine DE, respectively.

## CONCLUSIONS

The results show that swimming pool sand filters currently thought to be used by the majority of the pools in the USA

(as well as cartridge filters) have limited effectiveness for pathogen removal with overall removals averaging less than 0.19 log (36%) for *Cryptosporidium* oocysts and both sizes of microspheres under the conditions studied. Based on this finding, it seems logical that the pool industry would want to support new research to identify techniques for improving sand filter performance to better safeguard the health of bathers. Coagulation prior to sand filtration is one technique that warrants further study since it is required prior to sand filtration in drinking water treatment operations throughout the world as well as in the majority of European public swimming pool facilities. While there are no obvious barriers to coagulation prior to sand filtration, there is a significant lack of comprehensive data regarding the current filter design and operating practices in the USA. The size and depth of filter media, filter loading rates, and backwashing practices are known to have significant impacts on filter performance, but accurate values for these parameters are not currently available to regulators, researchers, pool designers, or even filter manufacturers. A survey of US aquatic facilities could establish a baseline of current practices and help identify (through future research) the best strategies for improving filter performance and protecting public health.

Diatomaceous earth and perlite produced higher levels of pathogen removal with mean removals ranging from 2.3 log (99.4%) to 4.4 log (99.996%) with the higher pathogen removals obtained in experiments with finer grades of DE and perlite. The performance of the finer grades of precoat media was further enhanced by increasing the thickness of the media layer in the filter. The 1- $\mu$ m microsphere removals by the precoat filters were considerably lower than for the 5- $\mu$ m microsphere removals with removals averaging 0.37 to 0.85 log. The impact of stopping and restarting a precoat filter without changing the media and pathogen distribution within the filter media is a topic that appears to warrant further investigation. The effects of integrity and cleanliness of precoat media support materials over time on pathogen removal is still unknown. The use of precoat media on top of the sand in a sand filter might be a way to quickly improve the performance of existing sand filters, but this approach would require long-term full-scale studies at multiple locations to gain wide-spread acceptance.

The 5- $\mu$ m microspheres appeared to be an adequate surrogate for *Cryptosporidium* in terms of filter removals for all

three types of filters and under all tested conditions in simulated swimming pool water. This finding could be important for future research studies, new product testing protocols, as well as for full-scale performance evaluations of swimming pool filters. While the risk factors associated with *Cryptosporidium* outbreaks extend beyond swimming pool filtration removal efficiency, there does appear to be significant room for improvement in this area.

Based on the low average removal efficiency  $\leq 0.19$  log (36%) average removal efficiency of sand and cartridge filters for *Cryptosporidium*, the prevalence of these filters in existing recreational water facilities, and the highly chlorine-resistant properties of *Cryptosporidium* oocysts; further safeguarding public health in recreational water facilities in the USA could be quite challenging. The epidemiological data (both endemic and outbreak) suggest that this problem is not going away on its own. The leaders in the pool industry should take an active role in fully assessing this problem, identifying potential solutions, and making the critical decisions required to resolve this problem.

---

## ACKNOWLEDGEMENTS

EP Minerals generously provided funding for this research as well as the precoat filter media. Pentair Water sized and donated the centrifugal pump, three pool filters, and the PVC valves used to direct water into and out of the filters.

---

## DISCLAIMER

The findings and conclusions in this paper are those of the authors and do not necessarily represent those of the Centers for Disease Control and Prevention.

---

## REFERENCES

- Amburgey, J. E., Amirtharajah, A., York, M. T., Brouckaert, B. M., Spivey, N. C. & Arrowood, M. J. 2005 Comparison of conventional and biological filter performance for *Cryptosporidium* and microsphere removal. *Journal of the American Water Works Association* **97** (12), 77–91.
- Amburgey, J. E., Fielding, R. R. & Arrowood, M. J. 2007 Removing *Cryptosporidium* oocysts from swimming pools with sand filters. In: *Proceedings National Swimming Pool Foundation*

- (NSPF) 2007 World Aquatic Health™ Conference (WAHC). Cincinnati, OH, USA.
- Amburgey, J. E., Fielding, R. R. & Arrowood, M. J. 2008 *Cryptosporidium* oocysts properties & control with swim diapers and filters. In: *Proceedings 2008 World Aquatic Health™ Conference*, Colorado Springs, CO, USA.
- Amburgey, J. E., Fielding, R. R. & Arrowood, M. J. 2009a Filtration removals and swim diaper retention of *Cryptosporidium* in swimming pools. In: *Proceedings 2009 Swimming Pool and Spa International Conference*, London, UK. (CD-ROM).
- Amburgey, J. E., Fielding, R. R. & Arrowood, M. J. 2009b Latest developments in crypto removal by swimming pool filters. In: *Proceedings 2009 World Aquatic Health™ Conference*, Atlanta, GA, USA.
- Arrowood, M. J. & Donaldson, K. 1996 Improved purification methods for calf-derived *Cryptosporidium parvum* oocysts using discontinuous sucrose and cesium chloride gradients. *Journal of Eukaryotic Microbiology* **43** (5), S89.
- Beach, M. J. 2011 Infectious diseases, cryptosporidiosis, and recreational water use in the united states: current trends and possible long-term solutions. In: *Invited Lecture at the 4th International Conference Swimming Pool and Spa, Porto, Portugal in March, 2011*.
- Brown, T. J. & Emelko, M. B. 2009 Chitosan and metal salt coagulant impacts on *Cryptosporidium* and microsphere removal by filtration. *Water Research* **43**, 331–338.
- Croll, B. T., Hayes, C. R. & Moss, S. 2007 Simulated *Cryptosporidium* removal under swimming pool filtration conditions. *Water and Environment Journal* **21**, 149–156.
- Dai, X. & Hozalski, R. M. 2003 Evaluation of microspheres as surrogates for *Cryptosporidium parvum* oocysts in filtration experiments. *Environmental Science and Technology* **37**, 1037–1042.
- Daniel, P. A. 1996 *Cryptosporidium*: a risk assessment. *Water Supply* **14**, 387–401.
- Dufour, A. P., Evans, O., Behymer, T. D. & Cantu, R. 2006 Water ingestion during swimming activities in a pool: a pilot study. *Journal of Water and Health* **4**, 425–430.
- Emelko, M. B., Huck, P. M. & Douglas, I. P. 2003 *Cryptosporidium* and microsphere removal during late in-cycle filtration. *Journal of the American Water Works Association* **95** (5), 173–182.
- Ford, T. E. 1999 Microbiological safety of drinking water: united states and global perspectives. *Environmental Health Perspectives* **107** (Supplement 1), 191–206.
- Freer, S. M. 1984 A permanent wet-mount for fluorescent microscopy of surface stained lymphoid cells. *Journal of Immunological Methods* **66**, 187.
- Hendricks, D. W. 2006 *Water Treatment Unit Processes: Physical and Chemical*. CRC Press (Taylor & Francis Group) Boca Raton, FL.
- Hoxie, N. J., Davis, J. P., Vergeront, J. M., Nashold, R. D. & Blair, K. A. 1997 Cryptosporidiosis-associated mortality following a massive outbreak in Milwaukee, Wisconsin. *American Journal of Public Health* **87**, 2032–2035.
- Kanan, A. A. 2010 *Occurrence and Formation of Disinfection By-Products in Indoor Swimming Pools Water*. PhD Dissertation, Clemson University, Clemson, SC. UMI Number: 3402530.
- Logsdon, G. S. 2008 *Water Filtration Practices: Including Slow Sand Filters and Precoat Filtration*. American Water Works Association, Denver, CO.
- National Swimming Pool Foundation (NSPF) 2009 *Pool and Spa Operator Handbook*. NSPF, Colorado Springs, CO. p. 139.
- Ongerth, E. & Hutton, P.E. 1997 DE filtration to remove *Cryptosporidium*. *Journal of the American Water Works Association* **89** (12), 39–46.
- Ongerth, E. & Hutton, P. E. 2001 Testing of diatomaceous earth filtration for removal of *Cryptosporidium* oocysts. *Journal of the American Water Works Association* **93** (12), 54–63.
- Pintar, K. D. M., Fazil, A., Pollari, F., Charron, D. F., Waltner-Toews, D. & McEwen, S. A. 2010 A risk assessment model to evaluate the role of fecal contamination in recreational water on the incidence of cryptosporidiosis at the community level in ontario. *Risk Analysis* **30**, 49–64.
- Shields, J. M., Gleim, E. R. & Beach, M. J. 2008a Prevalence of *Cryptosporidium* spp. and *Giardia* intestinalis in swimming pools, Atlanta, Georgia. *Emerging Infectious Diseases* **14**, 948–950.
- Shields, J. M., Hill, V. R., Arrowood, M. J. & Beach, M. J. 2008b Inactivation of *Cryptosporidium parvum* under chlorinated recreational water conditions. *Journal of Water and Health* **6**, 513–520.
- States, S., Tomko, R., Scheuring, M. & Casson, L. 2002 Enhanced coagulation and removal of *Cryptosporidium*. *Journal of the American Water Works Association* **94** (11), 67–77.
- Yoder, J. S. & Beach, M. J. 2007 Cryptosporidiosis surveillance—United States, 2003–2005. *MMWR* **56** (No. SS-7), 1–10. <http://www.cdc.gov/mmwr/PDF/ss/ss5607.pdf>.
- Yoder, J. S. & Beach, M. J. 2010 *Cryptosporidium* surveillance and risk factors in the United States. *Experimental Parasitology* **124**, 31–39.
- Yoder, J. S., Hlavsa, M. C., Craun, G. F., Hill, V. R., Roberts, V., Yu, P. A., Hicks, L. A., Alexander, N. T., Calderon, R. L., Roy, S. L. & Beach, M. J. 2008 Surveillance for waterborne diseases and outbreaks associated with recreational water use and other aquatic facility-associated health events – United States, 2005–2006. *MMWR* **57** (No. SS-9), 1–38. <http://www.cdc.gov/mmwr/PDF/ss/ss5709.pdf>.
- Yoder, J. S., Herral, C. & Beach, M. J. 2010 Cryptosporidiosis surveillance – United States, 2006–2008. *MMWR* **59** (No. SS-6), 1–14. <http://www.cdc.gov/mmwr/pdf/ss/ss5906.pdf>.

First received 2 May 2011; accepted in revised form 19 September 2011. Available online 14 October 2011