Chlorine Disinfection of Recreational Water for *Cryptosporidium parvum*

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We examined the effects of chlorine on oocyst viability, under the conditions of controlled pH and elevated calcium concentrations required for most community swimming pools. We found that fecal material may alter the Ct values (chlorine concentration in mg/L, multiplied by time in minutes) needed to disinfect swimming pools or other recreational water for *Cryptosporidium parvum*.

The small size of the *Cryptosporidium parvum* oocyst (4-6 µm) and its resistance to many chemical disinfectants (e.g., chlorine) pose a challenge for standard filtration and disinfection procedures (1). Moreover, the low dose required for infection and the prolonged excretion of high numbers of oocysts make *C. parvum* ideal for waterborne transmission. Chlorinated recreational water facilities, such as public swimming pools and water parks frequently used by large numbers of diapered children, have been implicated in numerous outbreaks of cryptosporidiosis during the last decade (Table 1).

Previous studies of chlorine inactivation of oocysts have used oxidant demand-free water and glassware or chlorine demand-free reactors (1,24-31); none were performed in simulated recreational water (i.e., pH balanced, $CaCl_2$ added for hardness, organic material added). Therefore, Ct values (chlorine concentration in mg/L multiplied by time in minutes) calculated under oxidant demand-free laboratory conditions for disinfection of microorganisms such as *Cryptosporidium* may not be directly applicable to recreational water environments where additional organic material, such as urine, feces, hair, sweat, sloughed cells, and lotion, is present, pH is controlled, and calcium concentration is elevated. We report that under recreational water conditions fecal material alone has a large negative effect on chlorine inactivation of *C. parvum* oocysts, and therefore on pool water quality and the potential for disease transmission.

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Study Design

Oocysts of the AUCP-1 isolate were extracted from the feces of experimentally infected calves and cleaned of fecal debris with cesium chloride (32). The short exposure to cesium chloride followed by thorough rinsing with deionized water has no deleterious effects on the oocyst wall or oocyst survival. Oocysts cleaned by this method appear free from all organic fecal debris and other microorganisms and thus are potentially more susceptible to disinfectants than are oocysts surrounded by debris. Oocysts were stored at 4°C until use and were less than 1 month old when used.

Two experiments were conducted to determine how long oocysts would remain infectious when exposed to two concentrations of chlorine at two temperatures. Stock solutions of 2.0 ppm and 10.0 ppm HOCl in demineralized water (resistance measured 18 mega-ohm) were prepared with commercial laundry bleach (CLOROX). Chlorine concentrations were monitored with a digital chlorine colorimeter kit (LaMotte model no. DC 1100, Chestertown, MD). In experiment 1, one centrifuge tube (15 ml polypropylene, screw-top Falcon, Becton Dickinson, Franklin Lakes, NJ) was prepared containing 1 x 10^6 oocysts for each of the 28 temperature and time combinations. Tubes were centrifuged at 1,500 g for 15 minutes; supernatants were decanted, and oocyst pellets were resuspended in 12 ml of stock chlorine

Table 1. Outbreaks of cryptosporidiosis in recreational water facilities

Facility	Location	Disinfectant	No. of cases estimated/ confirmed)	Date (year)	Ref.
Pool	Doncaster, UK	Chlorine	[a]/79	1988	2
Pool	Los Angeles County	Chlorine	44/5	1988	3
Pool	British Columbia	Chlorine	66/23	1990	4
Pool	Gloucestershire, UK	Ozone/chlorine	[a]/13	1992	5
Water slide	Idaho	Chlorine	500/[a]	1992	6
Pool (wave)	Oregon	Chlorine	[a]/52	1992	7
Pool (motel)	Wisconsin	Chlorine	51/22	1993	8,9
Pool (motel)	Wisconsin	Chlorine	64/[b]	1993	9
Pool	Wisconsin	Chlorine	5/[b]	1993	9
Pool	Wisconsin	Chlorine	54/[b]	1993	9
Pool (motel)	Missouri	Chlorine	101/26	1994	10
Lake	New Jersey	None	2,070/46	1994	11
Pool	Sutherland, New South Wales	Chlorine	[a]/70	1994	12
Pool	Kansas	[a]	101/26	1995	13
Water park	Georgia	Chlorine	2,470/62	1995	13
Water park	Nebraska	[a]	[a]/14	1995	13
Pool	Florida	[a]	22/16	1996	14[c]
Water park	California	Chlorine	3,000/29	1996	13,15[c]
Pool	Andover, UK	Chlorine	8/[a]	1996	16
Lake	Indiana	None	3/[a]	1996	13
River	NW England & Wales	None	27/7	1997	17[c]
Pool	SW England & Wales	Ozone & chlorine	[a]/9	1997	17[c]
Fountain	Minnesota	Sand filter	369/73	1997	18
Three pools	Canberra, Australia	[a]	[a]/210	1998	19[c],20[c]
Pool	Oregon	[a]	51/8	1998	21[c]
Pools	Queensland	[a]	129/[a]	1997	21[c]
Pools	New South Wales	[a]	370/[a]	1998	22[c]
Pools	Hutt Valley, New Zealand	[a]	[a]/171	1998	23[c]

[a]No data available.
[b]Reference did not identify cases as estimated or confirmed.
[c]Reference is not peer reviewed and may not reflect a rigorous investigation of the outbreak.

solution. Tubes were then placed in controlled temperature water circulators (model 9101; Polyscience, Inc., Niles, IL) and incubated at 20°C or 30°C. For all mice to be the same age at the time of infection, tubes were prepared on 7 successive days and placed in the water circulators so that all incubations ended and all mice were inoculated on the same day (Table 2).

Because of the small volume in each tube, it was not possible to monitor the chlorine concentration daily. Therefore, readings were taken initially and after incubation, immediately before the mice were inoculated. Upon removal from the water circulators, all tubes were centrifuged at 1,500 g for 15 minutes, supernatant was aspirated, and pelleted oocysts were resuspended in 1 ml of demineralized water. Oocysts in each tube were then administered orally to four neonatal BALB/c mice by gastric intubation. Each mouse received 150,000 oocysts. Oocysts remaining in each tube were counted with a hemacytometer to verify dosage levels. Individual oocysts were counted in the clumps of 2 to 4 oocysts observed on days 5 through 7. Mice were euthanized by CO_2 overexposure 96 hours after intubation. To assess infectivity, hematoxylin- and eosin-stained histologic sections of ileum from each mouse were examined by brightfield microscopy for developmental stages of *C. parvum* (24).

Because the results from experiment 1 indicated a need to examine shorter times of oocyst exposure to chlorine, a second experiment was conducted in which oocysts were tested for viability after exposure to chlorinated water for 6, 12, 24, 48, and 72 hours (Table 2). To simulate actual pool conditions (33), chlorinated water was balanced between pH 7.2 and 7.8, and $CaCl_2$ was added to a concentration of 200 ppm to 400 ppm.

To simulate a swimming pool fecal incident and thereby test the effectiveness of chlorine on

Table 2. Contact times and infectivity for purified *Crytosporidium parvum* oocysts subjected to chlorination (Experiments 1 and 2)[a]

			2 ppm			10 ppm	
		Ct[b]	Infectivity		Ct[b]	Infectivity	
Expt.	Days (min.)	value	20ºC	30ºC	value	20ºC	30ºC
2	(360)	720	**4/4**	**4/4**	3,600	**4/4**	0/4
2	(720)	1,440	**4/4**	**3/4**	7,200	0/4	0/4
1	1 (1440)	2,880	**4/4**[c]	0/4	14,400	0/4	0/4
2	1 (1440)	2,880	**4/4**	0/4	14,400	0/4	0/4
1	2 (2880)	5,760	0/4	0/4	28,800	0/4	0/4
2	2 (2880)	5,760	0/4	0/4	28,800	0/4	0/4
1	3 (4320)	8,640	**4/4**	0/4	43,200	0/4	0/4
2	3 (4320)	8,640	0/4	0/4	43,200	0/4	**3/4**
1	4 (5760)	11,520	0/4	0/4	57,600	0/4	0/4
1	5 (7200)	14,400	0/4	0/4	72,000	0/4	0/4
1	6 (8640)	17,280	0/4	0/4	86,400	0/4	0/4
1	7 (10,080)	20,160	0/4	0/4	100,800	0/4	0/4

[a]Treatments in which mice were found infected are shown in bold.
[b]Hypothetical Ct value calculated by assuming constant chlorine concentration.
[c]Fraction represents number of mice showing developmental stages of *C. parvum* in the intestinal epithelium over the total number of mice inoculated, e.g., 4/4 indicates 4 mice were found infected out of 4 mice inoculated.

oocysts in the presence of organic material, three aquariums were each filled with 30 L of tap water balanced to meet standard pool regulations (33). The pH was maintained by adding NaOH or HCl, and the calcium level was maintained by adding $CaCl_2$. In one aquarium, sufficient chlorine was added to achieve and maintain 2.0 ppm, a normal pool concentration. In another aquarium used to represent a response to water contamination, chlorine was maintained at 10 ppm. In the third aquarium, calcium and pH were held at standard pool conditions, but no chlorine was added. The aquariums were maintained at room temperature and were covered with a glass plate to prevent evaporation. Chlorine, pH, and calcium values were monitored 4 times a day between 8:00 a.m. and 4:30 p.m. and adjusted to target levels when necessary.

Calculations for simulating a pool fecal accident in an aquarium were based on a 700,000-L swimming pool and an infected person excreting approximately 500 g of fecal material into the water (490,000 mg feces per 700,000 L pool = 0.7 mg/L; 0.7 mg/L x 30/L aquarium = 20 mg feces). The estimated ratio of fecal mass to water volume necessarily correlates with large contamination to maintain a ratio based on the small size of the aquarium and the need for a sufficient quantity of feces and oocysts for testing. The number of oocysts added was based on laboratory experience for recovery of oocysts from numerous 5-g fecal samples of bovine feces.

To simulate a loose fecal mass but not fully dispersed feces, dialysis tubing (SPECTRUM Medical Industries, Inc., Los Angeles, CA) with a molecular weight cutoff of 6,000 to 8,000 was used to contain the oocysts and fecal mixture. Fecal material came from a calf that tested negative for *C. parvum*. Feces were mixed with water to form a diarrhea-like consistency. To ensure the recovery of sufficient oocysts for later bioassay in mice, 2 x 10^6 oocysts in 20 mg of the fecal slurry were introduced into the dialysis tubing and then filled with water from the appropriate aquarium. Time points of 0, 6, 12, 24, and 48 hours of exposure were tested. Therefore, additional dialysis tubing containing oocysts and fecal material was added to each aquarium at specified intervals, and all were removed at the end of the incubation time. Oocysts were aspirated from the dialysis tubing, they were concentrated by centrifugation (1,500 g, 15 minutes), and 150,000 were intubated into each of 3 to 5 neonatal BALB/c mice as before. Necropsy of mice and assessment for infectivity were performed as in the previous experiment.

Findings

In the first experiment, oocysts maintained at 20°C in 2 ppm chlorine for 1 and 3 days were infectious for mice. Oocysts maintained at higher temperatures or chlorine concentrations were not infectious for mice (Table 2).

In the second experiment, oocysts maintained at 20°C in 2 ppm chlorine remained infectious after exposure of 6 to 24 hours (Table

2). At 20°C and 10 ppm chlorine, oocysts exposed for 6 hours infected mice, whereas those exposed longer did not. At 30°C and 2 ppm chlorine, oocysts exposed for 6 hours infected all mice, those exposed for 12 hours infected 3 of 4 mice, and those exposed longer were not infectious. At 30°C and 10 ppm chlorine, oocysts held for 6, 12, 24, and 48 hours did not initiate infection; however, those held for 72 hours were infectious for 3 of 4 mice.

In the third experiment, tissues from all mice inoculated with the oocysts exposed to all incubation time points at 0, 2, or 10 ppm chlorine were found to contain developmental stages of the parasite in the intestinal epithelium. These findings indicated that oocysts in the presence of fecal material remained infectious even after exposure to 10 ppm chlorine for 48 hours.

Conclusions

Swimming is the second most popular recreational activity in the United States, with more than 350 million persons participating each year (34). The emergence of *C. parvum* as a major cause of recreational waterborne disease has prompted public health workers to reevaluate existing recommendations and regulations for water quality and use. Frequent fecal contamination of recreational water and the high level of *C. parvum* oocyst resistance to chlorine, the low oocyst dose required for infection, and high numbers of bathers make it imperative that we understand how oocyst inactivation is affected by recreational water conditions, including fecal contamination.

In our first experiment, to become noninfectious, purified *C. parvum* oocysts in chlorine demand-free deionized water required exposure to chlorine at a Ct value higher than 8,640. This value is relatively close to that obtained for disinfection under similar chlorine demand-free conditions (Ct = 7,200-9,600) (28). Purified *C. parvum* oocysts in chlorine demand-free water balanced to meet swimming pool standards (experiment 2) required even less time to be rendered noninfectious, i.e., exposure to 2 ppm chlorine for 2 days at 20°C or 1 day at 30°C. Incubation in 10 ppm chlorine rendered oocysts noninfectious in 6 hours or less at both temperatures, respectively.

The findings that oocysts in experiment 1 were infectious when exposed to 2 ppm at 20°C for 1 and 3 days but not for 2 days and that oocysts in experiment 2 infected 3 of 4 mice after exposure to 10 ppm chlorine at 30°C for 72 hours but not for shorter periods underscores the difficulty of performing these experiments. Such findings may be explained by the stickiness of the oocyst surface, which leads to clumping that can result in nonuniform sampling or possibly protection from inactivation. However, these outlying datapoints in experiments 1 and 2 are inconsistent with total oocyst inactivation observed in the shorter incubation times under the same conditions. It is unclear whether a few oocysts survived the exposure period or whether the infections were experimental artifacts. Environmental contamination of the mouse colony used for the bioassays is unlikely because no mice from the negative control litter used for this study or from >1,000 previous negative control mice used in this laboratory had developed a *C. parvum* infection. These data suggest that disinfection of *C. parvum*, in the absence of feces or other organic contaminants, may be less difficult than thought, particularly at the higher temperatures found in chlorinated recreational venues.

In contrast to experiment 2, in which fecal matter was absent, oocysts in experiment 3 were incubated under identical water and chlorine conditions but in the presence of feces (i.e., a simulated fecal accident) and remained infectious at all time points through 48 hours. Because this simulated accident was contained in a dialysis bag rather than being dispersed, it may not represent the best model for a dispersed diarrheal accident. Containment of oocysts with the organic material may actually have afforded some protection from inactivation. This highlights our incomplete understanding of *C. parvum* inactivation and the detrimental effect that organic or fecal contamination can play in recreational water.

Although the fecal accident simulated here could be considered major, the decrease in effective chlorine action is probably a conservative measure for recreational water since the simulation did not include additional biologic contaminants found in recreational water (i.e., sweat, hair, skin cells, lotion, urine, and algae). Because oocysts attach readily to biologic particles (35), such particles may provide a protective surrounding. The retarded inactivation of an already chlorine-resistant organism suggests that the current recommendation (36) for responding to fecal accidents (20 mg chlorine/ L for 9 hours to achieve a Ct value of 10,800) needs to be tested under appropriate conditions of water quality (33) in the presence of fecal and organic contaminants (both as tested here or dispersed in a pool) and revised as necessary.

Routine use of recreational venues by diapered children from day-care facilities, who have an elevated prevalence of *C. parvum* infection, increases the potential for waterborne disease transmission. Prevention plans that combine engineering changes (improved filtration and turnover rates, separate plumbing and filtration for high-risk "kiddie" pools), pool policy modifications (fecal accident response policies, test efficacy of barrier garments such as swim diapers), and patron and staff education should reduce the risk for waterborne disease transmission in public recreational water venues. Education efforts should stress current knowledge about waterborne disease transmission and suggest simple prevention measures such as refraining from pool use during a current or recent diarrheal episode, not swallowing recreational water, using proper diaper changing and handwashing practices, instituting frequent timed bathroom breaks for younger children, and promoting a shower before pool use to remove fecal residue.

Ms. Carpenter is a graduate student at Virginia Commonwealth University's Center for Environmental Studies. She is studying amoebae in Asian freshwater clams as biologic indicators of runoff from combined sewer outfalls into the James River. Ms. Carpenter's interests include the epidemiology of foodborne and waterborne parasitic protozoa.

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