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RAPID COMMUNICATION

Bather density and levels of *Cryptosporidium*, *Giardia*, and pathogenic microsporidian spores in recreational bathing water

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Abstract The study demonstrated that the resuspension of bottom sediments caused by bathers and their direct microbial input resulted in elevated levels of *Cryptosporidium parvum* oocysts, *Giardia lamblia* cysts, and microsporidian spores, particularly *Enterocytozoon bieneusi*, in recreational beach water on days deemed acceptable for bathing by fecal bacterial standards.

Cryptosporidium parvum, *Giardia lamblia*, and human-virulent microsporidia (i.e., *Enterocytozoon bieneusi*, *Encephalitozoon intestinalis*, *Encephalitozoon hellem*, and *Encephalitozoon cuniculi*) are anthropozoonotic enteropathogens that inflict considerable morbidity on healthy people and can cause mortality (e.g., *Cryptosporidium* and microsporidia) in immunosuppressed individuals (Graczyk et al. 2007a). *Cryptosporidium* and *Giardia* are very frequently transmitted via water, which is also involved in the epidemiology of microsporidian spores (Graczyk et al. 2007a). These enteropathogens are category B biodefense agents on the National Institutes of Health list, and microsporidian spores are on contaminant candidate list of the U.S. Environmental Protection Agency (EPA 1998). The only federal regulation regarding pathogens in recreational waters is the Beaches Environmental Assessment and Coastal Health Act which requires the use of fecal bacterial indicators to assess water microbiological quality (Wade et al. 2006). As fecal coliforms in recreational bathing waters originate from bather-induced resuspension of bottom sediments (Hanes and Fosa 1970; Cheung et al. 1991; Steward et al. 2002) and from bathers themselves (Gerba 2000; Elmir et al. 2007), crowded beach waters usually have the highest fecal coliform levels (Kay et al. 1994; Elmir et al. 2007). Information on relationships between bather density and the levels of other human enteropathogens is scant; however, recent studies indicate that such relationships exist for waterborne protozoan pathogens as well (Graczyk et al. 2007b; Sunderland et al. 2007).

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Materials and methods

We selected a recreational beach that, during the summer season, attracts a large numbers of people for weekend recreation but very few during the weekdays; thus, the relationship between the bather density and the levels of *C. parvum*, *G. lamblia*, and human-virulent microsporidia could be studied. Water samples were collected during 11 consecutive summer weeks from the Chesapeake Bay recreational beach in Maryland, USA (76°22′W, 39°22′N).

Three, 4-l samples were collected during each of nine weekends and 11 weekdays, giving a total of 27-weekend and 33-weekday samples (Table 1). Water quality parameters were measured (Table 1). The numbers of bathers were counted and assigned a density score; score 5 (more than 50 bathers), score 4 (38–49), score 3 (25–37), score 2 (14–24), score 1 (2–13), and score 0 (0–1; Table 1). The 24-h rainfall data and tide levels corresponding to the water collection dates were obtained electronically (http://www.ncdc.noaa.gov and http://www.kayaktrips.net:81). The samples were filtered through a 1.2-μm pore size, 293-cm diameter cellulose acetate membrane (Millipore, Bedford, MA, USA), and the membranes were eluted with 50 ml of eluting fluid (Graczyk et al. 2006). The tubes with eluants were centrifuged (5,000×*g*; 5 min), the pellet transferred to a 15-ml plastic tube and processed by sugar-phenol flotation (Graczyk et al. 2007a). The top 1.5 ml was collected, placed in an Eppendorf tube, and the sugar was washed off by centrifugation two times (5,000×*g*; 5 min) using sterile phosphate-buffered saline (PBS; pH 7.4). The samples were coded, and the multiplexed fluorescence in situ hybridization (FISH) assays for *C. parvum* and *G. lamblia* and for *E. intestinalis*, *E. hellem*, *E. cuniculi*, and *E. bieneusi* were carried out (Graczyk et al. 2007a). Positive and negative controls were prepared as described previously (Graczyk et al. 2006). The pathogens were enumerated, and the samples were uncoded.

Results and discussion

The numbers of bathers on weekends were significantly greater than those on weekdays (Table 1). Water turbidity levels were also significantly higher than those on weekdays (Table 1). Overall, the water turbidity values were significantly correlated with the bather density (Spearman rank correlation: R=0.68, P<0.001); however, they were not related to the rainfall or tide levels (Table 1). The proportion of water samples containing human pathogens were significantly higher in weekend water collections than in weekday samples, and the concentrations of pathogens were significantly higher on weekends when compared to weekdays (Table 1). Overall, the concentration of waterborne pathogens was significantly correlated with the bather density score (Spearman rank correlation: R=0.53, P<0.01). Microsporidian spores were represented mostly by *E. bieneusi*; *E. intestinalis* spores were detected in a single weekend water sample.

In terms of microbiological water quality, bathers themselves are currently considered to be a non-point source of fecal coliform bacteria (Elmir et al. 2007). The present study provides evidence that both the resuspension of bottom sediments caused by bathers and their microbial load input into the water may be considered as non-point sources for contamination of recreational waters with *Cryptosporidium*, *Giardia*, and microsporidian spores virulent to humans. It was not determined whether sediment resuspension or bather microbial input played a more important role in water contamination; usually, both mechanisms are responsible for elevated fecal coliform counts in recreational bathing waters (Cheung et al. 1991; Gerba 2000; Steward et al. 2002; Elmir et al. 2007).

As all recreational water samples were collected on days acceptable for bathing by fecal bacterial indicator standards, the present study provides evidence that bathing in waters open to the public can result in exposure to human enteric pathogens. It also demonstrates that bacterial indicators, i.e., *Escherichia coli* and enterococci, are not reliable in predict-

Table 1 The results of testing (i.e., range and mean±SD) of recreational bathing water samples for potentially viable *Cryptosporidium parvum* oocysts, *Giardia lamblia* cysts, and human-virulent microsporidian spores, i.e., *Enterocytozoon bieneusi*, by the multiplexed FISH method and for water quality parameters

	Weekend samples n=27	Weekday samples n=33	P value
Number and (%) of oocyst-positive samples	13 (48)	2 (6)	0.02
Number and (%) of cyst-positive samples	10 (37)	2 (6)	0.02
Number and (%) of spore-positive samples	16 (59)	10 (30)	0.03
Oocyst concentration (oocyst per liter)	2–42 13.7±1.7	0–7 1.5±0.2	0.01
Cyst concentration (cysts per liter)	0–33 9.1±1.1	0–4 (0.6±0.1)	0.01
Spore concentration (spores per liter)	0–16 4.8±0.9	0–11 1.8±0.6	0.04
Bather density score	2–5 3.8±1.6	0–3 1.6±1.1	0.001
Water turbidity (NTU)	11–88 53.6±21.1	18–75 39.9±15.4	0.04
Rainfall (cm)	0–6.0 1.0±2.2	0–1.8 0.2±0.5	NS
Tide (m)	0.17–0.67 0.33±0.18	0.16–0.46 0.29±0.12	NS
Water salinity (ppt)	0.2–2.1 0.9±0.8	0.3–2.0 0.7±0.6	NS
Water temperature (°C)	26.5–32.4 29.6±2.0	22.9–33.0 30±3.0	NS
Dissolved O_2 (mg/l)	4.9–7.9 6.0±0.9	4.0–7.5 5.7±0.9	NS
Water conductivity (μS/m)	51–412 181±146	70–396 165±125	NS

ing the presence of other human waterborne pathogens (Elmir et al. 2007). The study supports the recommendation that people with gastroenteritis should be advised not to enter the water, as this can result in bather cross-infection (Cheung et al. 1991). The pathogens investigated in the present study have also been associated with a variety of wildlife (Graczyk et al. 2007a). Thus, their zoonotic origin is plausible, as wildlife was abundant in the surrounding park.

Although *E. bieneusi* spores were previously reported from recreational waters (Coupe et al. 2006), analysis of stools from HIV/AIDS patients with microsporidiosis has not suggested a link to recreational water (Conteas et al. 1998). However, as HIV/AIDS individuals are advised about opportunistic pathogens in untreated surface waters, they most likely avoid such exposure, which explains why this risk factor was not significant in the previous analysis (Conteas et al. 1998). Because microsporidia are emerging pathogens, the ID_{50} or minimal infectious dose is still unknown. However, the numbers of spores shed by an infected person are high. Asymptomatic children shed up to 1.5×10^5 per gram of *E. bieneusi* spores (Mungthin et al. 2005); in AIDS patients, concentration of spores varied from 4.5×10^5 to 4.4×10^8 per milliliter of diarrhetic feces, totaling 10^{11} spores in a 24-h period (Goodgame et al. 1999).

Means to decrease the negative impact of bathers on water quality include: (a) limiting bather numbers; (b) preventing diapered children from entering the water; (c) advising people with gastroenteritis to avoid bathing; and (d) recommending the use of showers before bathing. Whenever possible, recreational bathing areas should be located away from and upstream of point sources of contamination (Steward et al. 2002).

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