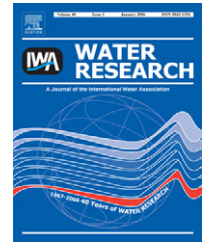


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Impact of bathers on levels of *Cryptosporidium parvum* oocysts and *Giardia lamblia* cysts in recreational beach waters

Deirdre Sunderland^a, Thaddeus K. Graczyk^{a,b,c,*}, Leena Tamang^a, Patrick N. Breyse^a

^aDepartment of Environmental Health Sciences, Division of Environmental Health Engineering, Johns Hopkins Bloomberg School of Public Health, 615 N. Wolfe Street, Baltimore, MD 21205, USA

^bJohns Hopkins Water and Health Center, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 21205, USA

^cDepartment of Molecular Microbiology and Immunology, Johns Hopkins Bloomberg School of Public Health, 615 N. Wolfe Street, Baltimore, MD 21205, USA

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ABSTRACT

Recreational beach water samples collected on weekends and weekdays during 11 consecutive summer weeks were tested for potentially viable *Cryptosporidium parvum* oocysts and *Giardia lamblia* cysts using the multiplexed fluorescence *in situ* hybridization (FISH) method. The levels of oocysts and cysts on weekends were significantly higher than on the weekdays ($P < 0.01$). Concentrations of oocysts in weekend samples ($n = 27$) ranged from 2 to 42 oocysts/L (mean: 13.7 oocysts/L), and cyst concentration ranged from 0 to 33 cysts/L (mean: 9.1 cysts/L). For the samples collected on weekdays ($n = 33$), the highest oocyst concentration was 7 oocysts/L (mean: 1.5 oocysts/L), and the highest cyst concentration was 4 cysts/L (mean: 0.6 cysts/L). The values of water turbidity were significantly higher on weekends than on weekdays, and were correlated with the number of bathers and concentration of *C. parvum* oocysts and *G. lamblia* cysts ($P < 0.04$). The study demonstrated positive relationships between number of bathers and levels of waterborne *C. parvum* oocysts and *G. lamblia* cysts in recreational beach water. It is essential to test recreational waters for *Cryptosporidium* and *Giardia* when numbers of bathers are greatest, or limit the number of bathers in a recreational beach area.

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1. Introduction

According to the US Environmental Protection Agency (US EPA), approximately 30% of the US population visit coastal areas each year for entertainment, fitness, and sport (US EPA, 2006). The National Survey on Recreation and the Environment found that 42% of respondents (i.e., approximately 89 million individuals) reported swimming in recreational

waters annually (Wade et al., 2006). However, along with these benefits comes a hidden risk to public health; contained in these waters are a variety of viral, bacterial, and protozoan pathogens. *Cryptosporidium parvum* and *Giardia lamblia* are enteric protozoans that cause significant adverse health effects in humans (WHO, 2002; Craun et al., 2005). These parasites are widely distributed throughout the world, and their transmissive stages, i.e., oocysts and cysts, respectively,

*Corresponding author. Department of Environmental Health Sciences, Johns Hopkins Bloomberg School of Public Health, 615 N. Wolfe Street, Baltimore, MD 21205, USA. Tel.: +1 410 614 4984; fax: +1 410 955 0105.

E-mail addresses: dsunderland@jhsph.edu, dsunderland@hotmail.com (D. Sunderland), tgraczyk@jhsph.edu (T.K. Graczyk), ltamang1@jhsph.edu (L. Tamang), pbreyse@jhsph.edu (P.N. Breyse).
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are very small and resilient to environmental stressors (Karanis et al., 2002; WHO, 2002; Center for Disease Control and Prevention, 2006; Farthing, 2006) and standard water chlorination (WHO, 2002; Standish-Lee and Loboschefs, 2006). This allows them to remain in the environment for long periods of time, creating a serious potential for exposure (Karanis et al., 2002; WHO, 2002). Evidence has shown that these pathogens can remain viable for several months to a year (Tamburrini and Pozio, 1999). *C. parvum* oocysts are round, 4–6 µm in diameter, and *G. lamblia* cysts are oval, 8–12 µm long and 7–10 µm wide (WHO, 2002). Although *Cryptosporidium* was only discovered to be infectious to humans in 1976, *Giardia* has been observed in humans for hundreds of years (WHO, 2002; Farthing, 2006). The emergence of the HIV/AIDS epidemic in the mid-1980s drew public attention to these protozoa (WHO, 2002; Farthing, 2006). Due to the weakened immune system of HIV/AIDS patients, there was a subsequent increase in the infection rates of these pathogens, sparking greater investigation of their exposure and pathogenicity. *Giardia* and *Cryptosporidium* are similar in both their sources of contamination and clinical presentation. Although some evidence exists that inhalation of *Cryptosporidium* oocysts is possible (Tzipori and Ward, 2002), the most common route of transmission of these protozoa is through ingestion (Ignatavicius and Workman, 2002; Stuart et al., 2003). This involves the fecal–oral route such as person-to-person transmission (i.e., touching door handles, changing diapers and then touching the mouth), swallowing contaminated recreational or drinking water, and ingesting contaminated food (Lemos et al., 2005; Coupe et al., 2006; Graczyk et al., 2006). Abundant evidence exists showing an increased risk of morbidity and mortality from these pathogens associated with children, immunocompromised individuals (i.e., HIV/AIDS patients or persons undergoing treatment for cancer), and the elderly (Karanis et al., 2002; WHO, 2002; DiGiorgio et al., 2002; Farthing, 2006). The most common symptom is diarrhea, but symptoms can include stomach cramps/pain, nausea, vomiting, and fever. In susceptible populations, infections can lead to severe dehydration and death (Farthing, 2006). Children who experience chronic infections may suffer from stunted growth and development due to malabsorption depriving them of nutrients and fluids required for growth (Ignatavicius and Workman, 2002; Stuart et al., 2003).

Cryptosporidium and *Giardia* may enter surface waters from urban runoff, agricultural runoff, wastewater discharges, leaking septic systems, direct fecal waste from wildlife, and human fecal accidents or diapered children shedding protozoa while bathing (Anderson et al., 1998; US Geological Survey, 2006a,b). The small size and omnipresence of these pathogens has led to many public health outbreaks in both drinking and recreational waters (Craun et al., 2005; Coupe et al., 2006). In 1971, the Center for Disease Control (CDC) and US EPA began collecting information on waterborne outbreaks under a voluntary surveillance system, and since 1978, recreational waters were included in the surveillance. Each year the number of reported outbreaks in recreational waters continues to climb. In 2001–2002, there was a record-breaking 65 outbreaks, including over 2500 illnesses, 61 hospitalizations, and eight deaths (Yoder et al., 2004). Of the 61 non-

chemically related outbreaks, 18% were related to *Cryptosporidium*, 1.6% to *Giardia*, and 19.6% had unknown etiology (Yoder et al., 2004). Similar numbers of outbreaks and illnesses were seen in 2003–2004 (Dziuban et al., 2006). These numbers were dwarfed by a recent study by Given et al. (2006), which suggested that as many as 1.4 million excess cases of gastrointestinal illness occur annually in Los Angeles and Orange county beaches alone. This illustrates the under-reporting of this public health issue. In addition to the threat to public health, there is a risk to the economic revenue of coastal regions. Areas that depend on recreational waters for their employment and financial stability are at risk from the impact of waterborne pathogens. In 2004, the US Geological Survey (US GS) concluded that the economic impact of visitors to marine recreational beaches in Florida, Hawaii, and California alone was 150 billion US dollars (US Geological Survey, 2006a,b). Not only is there an indirect risk but also the cost of the treatment course of the illnesses is substantial. The estimated health care cost associated with gastrointestinal illness from recreational beaches for Los Angeles and Orange counties ranged from 21 to 414 million US dollars per year (Given et al., 2006).

The only federal regulation regarding pathogens in recreational water is the Beaches Environmental Assessment and Coastal Health (BEACH) Act of 2000. The act requires that states adopt 1986 US EPA standards, which recommend that *E. coli* and *Enterococci* be used as bacterial indicators of fecal contamination (Indest, 2003; Wade et al., 2006). There are no requirements for testing recreational waters for *Cryptosporidium* or *Giardia*, although it has been shown through outbreaks that these pathogens can be shed into recreational water (Yoder et al., 2004; Craun et al., 2005). Unlike swimming pools, recreational beach areas have underlying sediment that can contain up to 1000 times as many fecal bacterial indicators as overlying water (Indest, 2003). Disturbance of this sediment may lead to re-suspension of bacterial coliforms, contributing to their higher concentration in water (Indest, 2003; Brookes et al., 2004; US Geological Survey, 2006a,b).

The purpose of the present study was to determine if the number of bathers in a recreational beach area has an effect on the concentration of waterborne *C. parvum* oocysts and *G. lamblia* cysts in that area. To account for bathers who were in the water and contributed to the pathogen increase via sediment re-suspension and direct microbial load, approximately half of the samples were collected on weekends when numbers of bathers were highest, and the other half on weekdays when there were lower numbers of bathers in the water.

2. Materials and methods

Over the months of July, August, and September 2006, water samples were collected at the Hammerman Area of Gunpowder Falls State Park (76°22'W; 39°22'N). This recreational beach area on the Gunpowder River in Chase, MD, USA, offers swimming and water sports to the public. A total of 60 samples were collected (three samples per day) at three sites within a section of the bathing area. Of these, 33 were collected on weekdays

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