Disposable swim diaper retention of *Cryptosporidium*-sized particles on human subjects in a recreational water setting

James E. Amburgey and J. Brian Anderson

ABSTRACT

Cryptosporidium is a chlorine-resistant protozoan parasite responsible for the majority of waterborne disease outbreaks in recreational water venues in the USA. Swim diapers are commonly used by diaper-aged children participating in aquatic activities. This research was intended to evaluate disposable swim diapers for retaining 5-μm diameter polystyrene microspheres, which were used as non-infectious surrogates for *Cryptosporidium* oocysts. A hot tub recirculating water without a filter was used for this research. The microsphere concentration in the water was monitored at regular intervals following introduction of microspheres inside of a swim diaper while a human subject undertook normal swim/play activities. Microsphere concentrations in the bulk water showed that the majority (50–97%) of *Cryptosporidium*-sized particles were released from the swim diaper within 1 to 5 min regardless of the swim diaper type or configuration. After only 10 min of play, 77–100% of the microspheres had been released from all swim diapers tested. This research suggests that the swim diapers commonly used by diaper-aged children in swimming pools and other aquatic activities are of limited value in retaining *Cryptosporidium*-sized particles. Improved swim diaper solutions are necessary to efficiently retain pathogens and effectively safeguard public health in recreational water venues.

Key words | cryptosporidiosis, *Cryptosporidium*, diarrhea, recreational water illnesses, swim diapers, swimming pools

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INTRODUCTION

Cryptosporidium caused more than 82% of waterborne disease outbreaks in treated recreational water venues in the USA in 2005 and 2006 (Yoder *et al*. 2008). Surveillance for *Cryptosporidium* in the USA indicates that the reported incidence of infection has increased dramatically since 2004 (Yoder & Beach 2010). Both the total number of cases and the number of individual outbreaks of cryptosporidiosis have shown net upward trends since 2004 (Yoder *et al*. 2010). Yoder *et al.* (2010) showed a striking increase in the number of cases of cryptosporidiosis during the warmer months of the year from 2006 to 2008 when outdoor public pools are normally open in the USA. 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 showed that 13 (8.1%) were positive for *Giardia* or *Cryptosporidium* or both (Shields *et al*. 2008a). In a study involving 803 Oklahoma children, 58% of adolescents (ages 14 to 21) were seropositive for *C. parvum*, indicating 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

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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).

Chlorine is relatively slow and ineffective at inactivating *Cryptosporidium* oocysts, which is supported by surveillance data. The current product of free chlorine concentration and time (or Ct value) for a 3-log inactivation of fresh *Cryptosporidium* oocysts can be up to 15,300 mg/L min at pH 7.5 (Shields *et al*. 2008b). Therefore, a free chlorine concentration of 1 mg/L can take more than 10 days to inactivate 99.9% of *Cryptosporidium* oocysts. However, a large number of people may swim in the pool, be exposed, and potentially become infected during that 10-day period. It is important to recognize that a 3 log reduction of more than 100 million oocysts still leaves more than 100,000 viable oocysts in the water, and infected swimmers might return to the pool and further contaminate it. Sand filters could provide a secondary barrier to *Cryptosporidium* in pools, but sand filters (without a coagulant) typically only remove about 25% of oocysts per passage through the filter (Amburgey *et al*. 2007, 2008, 2009a,b). Based on the slow kinetics of chlorine inactivation of *Cryptosporidium*, the known inefficiency of sand filters to remove oocysts, and the recent increase in incidence of cryptosporidiosis in the USA, prevention of *Cryptosporidium* introduction appears critically important for effectively safeguarding public health.

Swim diapers are commonly used worldwide in swimming pools to contain solid fecal releases by young swimmers. Prior research has shown that three brands of swim diapers retained 98–99% of fine solids (i.e., a construction site soil sample) for 30 min of water immersion activity using a wooden block and two plastic bottles coated with neoprene to simulate a child (Maas *et al*. 2004). However, the performance of swim diapers in containing watery diarrheal releases common of *Cryptosporidium*-infected hosts is still in question. As *Cryptosporidium* is responsible for the majority of recreational water illness (RWI) outbreaks in the USA, it is critically important that we understand how this pathogen behaves in a recreational water setting. The primary objective of this research was to evaluate some common disposable swim diapers for retaining 5-μm diameter polystyrene microspheres of approximately the same size, shape, and density as *Cryptosporidium* oocysts. As chlorine and swimming pool sand filters are rather limited in their ability to inactivate or remove *Cryptosporidium*, knowing whether or not swim diapers are effective at preventing the introduction of *Cryptosporidium* into pools is instrumental in effectively protecting public health in recreational water environments.

METHODS

Equipment set-up

The original pump and filter were removed from a 757 L hot tub and replaced with an external centrifugal pump via a rectangular 51 mm diameter PVC pipe loop measuring 6.5 m in length as shown in Figure 1. The external pipe loop had a valved sample line, but it did not have a filter. The pump (Challenger 3 HP, Pentair Water, Samford, NC, USA) was used to recirculate water at 144 L/min, and the flow was measured with a digital paddle-wheel flow meter (SEM-40, FlowServe, Irving, TX, USA) and controlled with a PVC ball valve on the discharge side of the pump. The total hydraulic retention time of the hot tub, pump, and pipe loop was 5.6 min at a flow of 144 L/min.

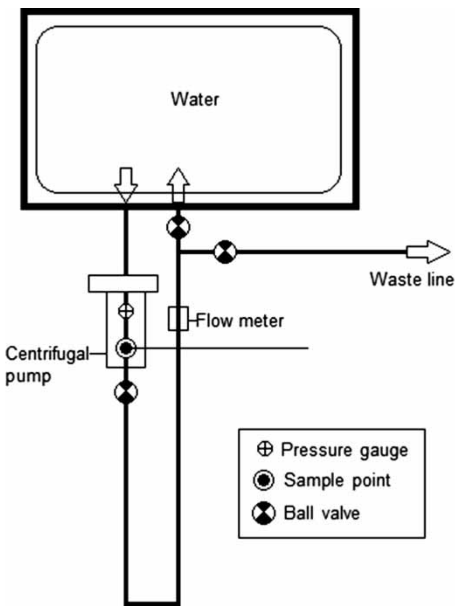

Figure 1 | Diagram of experimental set-up.

Simulated pool water

Simulated pool water was created for each experiment from 757 L of Charlotte, NC, USA tap water supplemented with sodium bicarbonate to an alkalinity of 150 mg/L as $CaCO_3$, with calcium chloride to a hardness of 250 mg/L as $CaCO_3$, with sodium hypochlorite to a free chlorine concentration of 2 mg/L, and with hydrochloric acid to a pH of 7.5. The water was held at temperature of approximately 35–38 °C during each experiment for bather comfort. The spa system was thoroughly cleaned between experiments with a minimum of three drain-and-fill rinses with recirculation at 227 L/min to minimize the potential for microspheres from a previous experiment to be present in a future experiment.

Simulated fecal releases

Approximately 10^7 4.87-μm diameter fluorescent green carboxylate-modified polystyrene microspheres (Fluoresbrite®, Polysciences, Inc, Warrington, PA, USA) were used in each of these experiments as a non-infective *Cryptosporidium* oocyst surrogate. The microspheres were introduced in 30 mL of ultrapure water directly into the swim diapers. The subject and swim diaper were both wet prior to the simulated watery diarrhea accident. The human subject(s) were allowed to play normally during experiments, but they were not allowed to leave the spa.

Products used in tests

The swim diapers (Huggies® Little Swimmers®, Kimberly-Clark, Neenah, WI, USA) were used according the weight guidelines on the packaging for each subject. The package also included a warning label that stated: 'To avoid transmission of diseases, do not allow children to enter the water if they are ill or experiencing diarrhea. Do not expect swim diapers to prevent the transmission of diseases'. Two types of swimming trunks were used in this study. The 'loose trunks' were made of 100% polyester and consisted of a thin outer shell with a coarse mesh inner lining commonly referred to as 'swimming trunks' in the USA. The swimming trunks were used as a control to establish a baseline for microsphere release rates when no 'diaper' was used. The snug trunks were actually advertised as 'swim diapers' and contained a fine weave liner with elastic around each opening sewn in underneath a pair of shorts (Speedo® Begin to Swim™ Boy's Swim Diaper, Speedo America, Los Angeles, CA, USA). Vinyl diaper covers (Gerber® Vinyl Pants, Gerber Childrenswear, Greenville, SC, USA) were also used in some experiments in combination with swim diapers or swimming trunks.

Sample collection and analysis

Nine sets of duplicate samples were collected from the recirculation line over the 40 min experimental period with five samples taken during the first 5 min. Duplicate 50 mL samples were collected in sterile 50 mL conical-bottomed plastic centrifuge tubes from a continuously flowing sample line. Sample volumes analyzed were adjusted to obtain between 10 and 150 microspheres per sample. Samples were filtered through 3-μm polycarbonate track-etched (PCTE) filters (GE/Osmonics, Minnetonka, MN, USA) in 25-mm glass microanalysis filter funnels (Millipore, Inc., Billerica, MA, USA) by a regulated three-place vacuum manifold. The filters were mounted on glass micro slides with one drop of polyvinyl alcohol-DABCO solution (Freer 1984) and a glass cover slip for enumeration under an epifluorescent microscope at ×100 total magnification.

Data adjustment

Any detection method losses will create variability within a dataset, but method losses were assumed universal. Hence, no corrections were made for the method losses. All data involving the percentages of microspheres released were based on the number of microspheres seeded. While this number (10^7) was held approximately constant, dispensing 22.8 μL of a 2.5% solids stock suspension led to some minor variability. By the conclusion of each 40 min experiment, an average of 91% of the target seed concentration was measured (with a range of 71–120%). To facilitate fair comparisons of the rates of microsphere release (particularly during the first few minutes of each experiment), all concentrations were adjusted (or normalized) based on the final sample's concentration being the 100% released value for that experiment. Overall, it makes little difference from a public health perspective whether 71 or 100% of a fecal accident is released when each can contain 100 million oocysts or more (Yoder & Beach 2010).

RESULTS AND DISCUSSION

The release of 5-μm microspheres (a *Cryptosporidium* surrogate) from swim diapers on human subjects is shown in Figure 2 along with the release of microspheres from standard lined swimming trunks (as a control). The swimming trunks seemed to be the worst case with 88% of the microspheres released within 1 min. However, the swim diapers tested in two sizes by human subjects performed only marginally better with releases of at least 50% of the microspheres released within 2 min of introduction. When dealing with 100 million or more parasites in a single accident, a 50% reduction is not going to eliminate the risk of waterborne disease outbreaks. Furthermore, even the 50% reduction was time-sensitive with at least 90% of microspheres released within 15 min.

Putting a vinyl diaper cover over a disposable swim diaper did slightly improve the performance as shown in Figure 3. The 50% release level was extended from 2 until

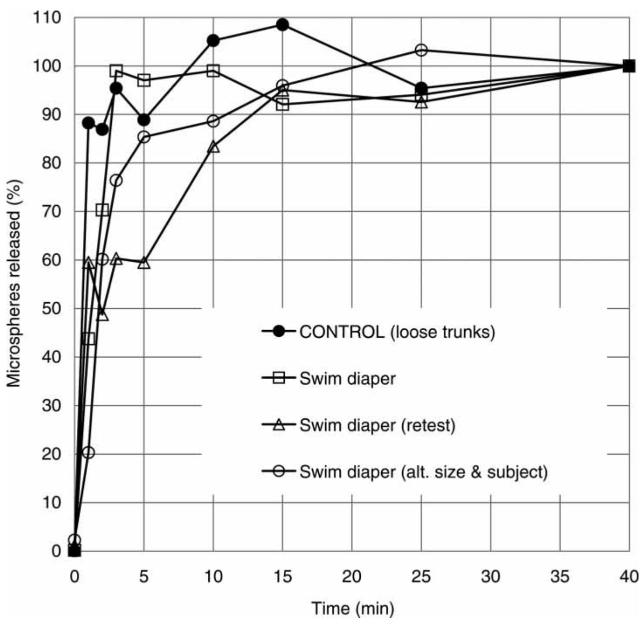

Figure 2 | Swimming trunks (control) and swim diaper release of microspheres versus time.

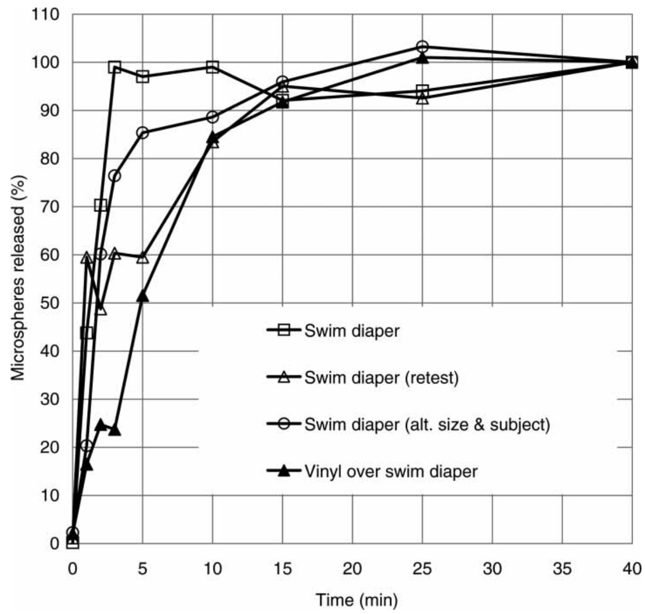

Figure 3 | Swim diaper with and without vinyl diaper cover release of microspheres versus time.

5 min, but the overall level of release was the same with and without the diaper cover after 15 min of activity. Based on the limited improvement with the diaper cover, alternate ways of using the vinyl diaper cover were explored such as putting the vinyl diaper cover under a pair of swimming trunks with the results shown in Figure 4. A vinyl diaper cover under either loose or snug swimming trunks slowed the release of microspheres versus the control, but the experiments with vinyl and swimming trunks did not perform better than the vinyl diaper cover over a swim diaper. In all experiments with vinyl diaper covers, at least 50% of the microspheres were still released within 5 min.

Figure 5 shows average release rates from all of the preceding experiments with the swim diaper trials and the vinyl diaper cover trials combined into groups. The use of swim diapers and/or vinyl diaper covers slowed the release of microspheres. However, none of the swim diaper solutions tested actually held the majority of *Cryptosporidium*-sized particles for more than 5 min. In all cases, at least 25% of the microspheres were detected in the bulk water within 2 min.

Swim diaper use offered only slightly better retention of 5-μm diameter surrogate particles used for *Cryptosporidium* oocysts than standard swimming trunks. Wearing a vinyl

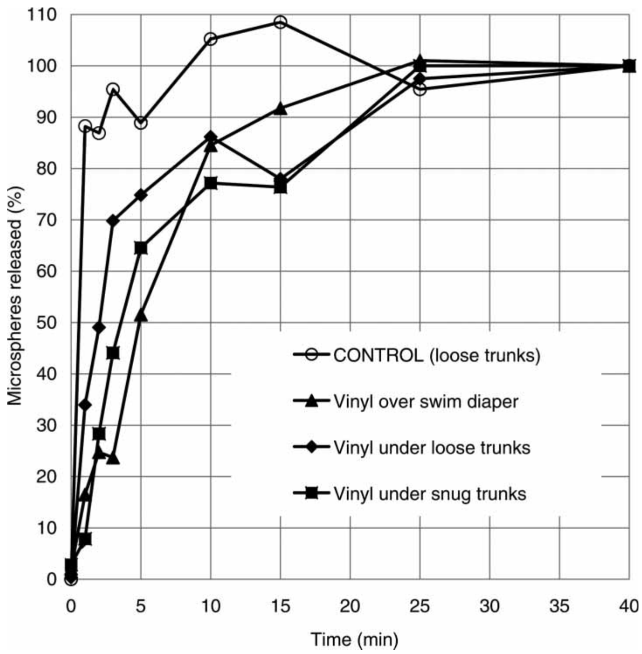

Figure 4 | Swimming trunks (control) and vinyl diaper cover release of microspheres versus time.

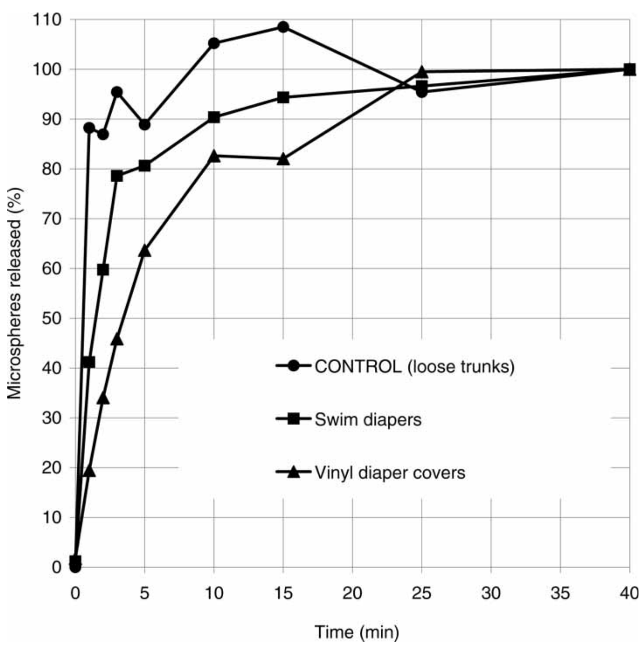

Figure 5 | Combined microsphere release data versus time.

diaper cover over a swim diaper showed another slight improvement in the retention of microspheres. Based on the improvement of vinyl diaper cover used over the swim diaper, vinyl diaper covers were tried underneath swimming trunks. While the vinyl diaper cover with trunks was better than trunks only, it was not as effective a vinyl diaper cover over a swim diaper. The vinyl diaper cover over the swim diaper was the most effective strategy tested, but the rapid release of particles associated with the simulated watery diarrhea accidents from all swim diaper experiments is cause for concern. Although previous research on swim diaper indicated excellent retention characteristics for solid materials, the current research shows that retention of pathogens in watery diarrhea will likely be much lower.

Cryptosporidium has been the leading cause of recreational water outbreaks in treated water venues for many years running. Each fecal release could contain in excess of 10^8 oocysts. Given the low infective dosages (i.e., 10–30 oocysts) and the chlorine-resistant nature of *Cryptosporidium* oocysts, the likelihood of future outbreaks with the best use of swim diapers and increased adult supervision is not likely to change significantly. A much more efficient swim diaper would be required to prevent the introduction of the majority of excreted oocysts. If the introduction of *Cryptosporidium* cannot be prevented with swim diapers, then the burden of protecting public health falls on the swimming pool treatment systems. As the pool water treatment approaches used in the past have not been entirely effective at preventing outbreaks, continuing with this approach is not likely to change anything. It is clear, however, that children or adults with diarrhea (or recently recovered from it) should not swim in public pools or other recreational water facilities.

CONCLUSIONS

Based on these experimental results, simulating fecal accidents by children with cryptosporidiosis, extremely close supervision would be required to achieve even marginal reductions in *Cryptosporidium* levels introduced in recreational water venues. Swim diapers offer only limited protection against the release of *Cryptosporidium* oocyst-sized particles into pool water with 25–70% release rates within 2 min of an accident for all swim diaper scenarios tested. Moreover, 50–97% of the particles were released within 5 min regardless of the preventive measures employed in this study. It appears that improved swim diaper solutions would be required to avoid the introduction of significant quantities of *Cryptosporidium* by diaper-aged hosts in recreational water venues. Swimming with or immediately following diarrhea should be strongly discouraged in the interests of public health.

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