Cryptosporidium and Giardia in swimming pools in the Netherlands
F. M. Schets, G. B. Engels and E. G. Evers

ABSTRACT

The occurrence of Cryptosporidium and Giardia in indoor swimming pools in the Netherlands was studied at five locations. The backwash water from seven pool filters was analysed for the presence of Cryptosporidium oocysts and Giardia cysts for a period of 1 year. Of the 153 samples of filter backwash water analysed, 18 (11.8%) were found positive for either Cryptosporidium (4.6%), Giardia (5.9%) or both (1.3%). Oocysts and cysts were also detected in the water of one toddler pool and one learner pool. Although most of the (oo)cysts in the filter backwash water were dead, viable and potentially infectious oocysts were detected in the learner pool. On the basis of numbers of potentially infectious (oo)cysts detected in the learner pool, and assuming one visit to an infected pool per year, risk assessment indicated an estimated risk of infection with Cryptosporidium that exceeded the generally accepted risk of one infection per 10,000 persons per year. Guidelines for pool operators on how to manage faecal accidents and public information on the importance of hygiene in swimming pool complexes are recommended tools in controlling the risk of infection.

Key words | Cryptosporidium, Giardia, infection risk, swimming pool, water quality

INTRODUCTION

Cryptosporidium and Giardia are pathogenic protozoan parasites that cause gastro-enteritis in humans. Giardia infections may be asymptomatic and are usually self-limiting with clearance in 2–4 weeks, but chronic infections also occur. Individuals with a healthy immune response recover from Cryptosporidium infections in 1 or 2 weeks, but infections may be severe and life threatening in immunocompromised persons (Arrowood 1997). Many outbreaks of cryptosporidiosis and giardiasis due to consumption of contaminated drinking water or consumption or recreation in contaminated surface water have been reported throughout the years (Furtado et al. 1998; Fayer et al. 2000). Swimming pool related outbreaks of Cryptosporidium or Giardia infections have been frequently reported in the United States (Harter et al. 1984; Porter et al. 1988; MacKenzie et al. 1995), the United Kingdom (Joce et al. 1991; Hunt et al. 1994; Nichols 1999) and Australia (Hellard et al. 2000; Puech et al. 2001). In some cases Cryptosporidium oocysts could be detected in the pool water (Hunt et al. 1994; Lemmon et al. 1996) or the filter backwash water (McAnulty et al. 1994), which confirmed the source of the outbreak. Contamination by infected swimmers, malfunctioning of (part of) the water treatment system and minimal water treatment are reported as the cause of contaminated pool water (Harter et al. 1984; Porter et al. 1988; Joce et al. 1991).

To date no outbreaks of cryptosporidiosis or giardiasis have been reported in the Netherlands, although Cryptosporidium and Giardia do circulate in the Dutch population. From two studies on the incidence of gastro-enteritis in the Netherlands, a prevalence of 5% for Giardia and 2% for Cryptosporidium has been calculated (de Wit et al. 2003a, b). Infected persons may therefore contaminate the swimming pool water with these parasites if they use swimming pools especially while having diarrhoea. After shedding, infectious Cryptosporidium oocysts will probably be present in the pool water for some time, allowing faecal-oral person-to-person transmission.
Microbiologically safe swimming pool water is obtained by adding an instantly effective disinfectant, such as chlorine, to the water. To maintain good water quality, the pool water is continuously treated, and treatment processes, such as filtration, coagulation, flocculation, pH and temperature adjustment and disinfection, should be carefully balanced. When pool water treatment is performed such that process indicator values comply with legal standards, the applied chlorine concentrations of 0.5–1.5 mg l⁻¹ provide sufficient protection against bacterial or viral infections (Galbraith 1980). Protozoan organisms like Cryptosporidium and Giardia are less sensitive to chlorine than bacteria or viruses (Jarroll et al. 1981; Korich et al. 1990). Giardia cysts show some resistance to chlorine at water temperatures below 10°C, but at water temperatures commonly applied in swimming pools (≥20°C) and a chlorine concentration of 1.5 mg l⁻¹, 99% are inactivated within 10 min (CT₉₉ = 15; Jarroll et al. 1981). Chlorine concentrations used for swimming pool disinfection have no rapid inactivating effect on Cryptosporidium oocysts. CT₉₉ values reported for Cryptosporidium oocysts are high and range from 3,700 (Driedger et al. 2000) to 9,600 (Korich et al. 1990). Efficient elimination of infectious oocysts therefore relies mainly on coagulation and filtration.

Population studies showed that one of the risk factors for Giardia diarrhoea was swimming in the week before onset of the complaints. People who swam had a 16-fold (odds ratio 11.2, 95% confidence limits 2.0–63.6) higher risk of developing Giardia diarrhoea compared with people that did not swim (de Wit et al. 2001a, b). A case-control study, performed following the observation of an increased prevalence of Cryptosporidium in the stool samples of gastro-enteritis patients in a region in the western part of the Netherlands in late summer 1995, indicated an association between swimming in a swimming pool and the risk of infection with Cryptosporidium (van Asperen et al. 1996).

From the perspective of these indicated associations between swimming in a pool and the risk of infection with Cryptosporidium or Giardia, we studied the occurrence of these parasites in the backwash water of pool filters from indoor swimming pools in the Netherlands in non-outbreak situations for a period of 1 year. The pool water from four toddler pools and one learner pool was examined for several weeks immediately after or during peak hours. The obtained data were used to estimate the risk of infection with Cryptosporidium or Giardia by exposure to swimming pool water in the Netherlands.

**METHODS**

**Sampling of filter backwash and pool water**

From May 2000 to June 2001 the backwash water from seven swimming pool filters obtained from five pool complexes was sampled at biweekly intervals. The first 20–25 l of backwash water was sampled and collected in polypropylene vessels; chlorine was neutralized by adding sodium thiosulfate (30 g l⁻¹, 1 ml for each litre of sample). Samples were analysed within 24 h. Depending on the turbidity of the backwash water, which determined the amount of pellet after concentration, 1–25 l could be processed. From samples with low turbidity larger volumes could be processed.

Toddler pools, present in four of the five selected pool complexes, were sampled at weekly intervals from June to September 2001. Samples were taken immediately after swimming classes for parents and children less than 3 years of age. The pool water circulation was stopped while sampling to prevent possibly contaminated water from leaving the pool and to prevent clean water entering the pool. Envirochek HV (Pall Gelman, Ann Arbor, USA) filtration was used according to the manufacturers’ instructions: 200–400 l pool water was concentrated at a filtration speed of 2 l min⁻¹. Envirochek HV capsules were processed within 24 h and refrigerated (2–8°C) between sampling and processing. Pool water pH, turbidity and temperature were recorded. A sample for bacteriological analysis was taken according to ISO 5667, Part 2 and 3 (ISO 1985, 1991, respectively), transported to the laboratory on melting ice and examined within 24 h.

The pool water from a learner pool was sampled each week for a period of 6 weeks during parent-and-child swimming classes. About 1,000 l was filtered through Envirochek HV capsules at a filtration speed of
10 l min$^{-1}$ at the outlet side of the pool. Seeding experiments showed that the increased filtration speed (2 l min$^{-1}$ is recommended) had no negative effect on (oo)cyst recovery (data not shown). Filter operating pressure remained below the advised maximum of 2.1 bar. Pool water pH, turbidity and temperature were recorded and a sample for bacteriological analysis was taken and handled as described above.

**Sample processing, purification and (oo)cyst detection**

The pH of the backwash water samples was lowered to 2.5 by adding 1 N hydrochloric acid (HCl) prior to concentration. This partly dissolved the coagulant–particle complexes and resulted in reduced pellet volume after concentration, which enabled purification of a larger fraction or the total of the concentrated sample. Backwash water samples smaller than 5 l were concentrated by a two-step centrifugation method: 15 min of centrifugation at 1,050 × G was followed by partial aspiration of the supernatant (rest volume 0.50–100 ml) and resuspension of the pellet; next, the concentrate was centrifuged for 10 min at 1,080 × G, the supernatant was aspirated and the pellet volume was measured. Centrifuge brakes were not used to avoid whirling of the pellets. Samples larger than 5 l were concentrated by Envirochek HV filtration.

All filtered samples were eluted from Envirochek HV filters with 130 ml elution buffer (1 g Laureth-12, 10 ml 1 M Tris pH 7.4, 2 ml 0.5 M EDTA pH 8.0, 150 μl Antifoam A, 1 l distilled water) by agitation in a wrist-action laboratory shaker for 5 min at 600 rpm. The eluate was decanted into a 250 ml conical centrifuge tube and the elution procedure was repeated with another 130 ml elution buffer; this eluate was decanted into the same centrifuge tube. After centrifugation (10 min, 1,080 × G, without centrifuge brake) of the final eluate, aspiration of the supernatant and determining the pellet volume, the water concentrates were purified by immuno magnetic separation (IMS) using the Dynal GC-Combo system (Dynal, Oslo, Norway).

One ml of 10 × SL buffer A and 1 ml of 10 × SL buffer B (both supplied by Dynal) were added to a volume of 0.5 ml of the concentrated water sample. The final volume was adjusted to 10 ml with distilled water, followed by addition of 100 μl of resuspended anti- Cryptosporidium Dynabeads and 100 μl of resuspended anti- Giardia Dynabeads. The mixture was incubated on a rotating mixer (25 rpm) for 1 h at room temperature. The Dynabeads-(oo)cyst-complexes were collected by using a magnet (Dynal MPC-1); the supernatant was aspirated and the complexes were resuspended in 1 ml 1 × SL buffer A. The (oo)cyst-Dynabeads-complexes were captured with another magnet (Dynal MPC-M), the supernatant was carefully removed and, after vortexing for 10 s, the complexes were incubated for 10 min at room temperature in 50 μl 0.1 N HCl. The mixture was vortexed for another 10 s, after which the beads were retrieved with the MPC-M magnet. The purified (oo)cysts were brought on to Dynal Spot-on slides in 5 μl 1 N sodium hydroxide (NaOH), dried at 30–35°C and fixed by adding a drop of methanol.

Fixed slides were stained (30–45 min at 37°C) with fluorescein-isothiocyanate (FITC) labelled monoclonal antibodies directed to the Cryptosporidium oocyst and Giardia cyst wall (Cellabs Cryptosporidium/Giardia staining reagent, Brookvale, Australia) and propidium iodide (PI, 1 mg ml$^{-1}$ in phosphate buffered saline (PBS, 0.01 M, pH 7.2), 2 min at room temperature). Stained slides were rinsed with PBS once, dried and mounted. They were examined for the presence of presumptive Cryptosporidium oocysts and Giardia cysts by epifluorescence microscopy at magnification 250 × using a Zeiss Axioskop. Magnification 1,000 × and Nomarski differential interference contrast (DIC) microscopy were used for confirmation.

**Bacteriological analysis of pool water samples**

Pool water samples were analysed for the presence of total and faecal coliform bacteria on Laurylsulphate agar (Dutch Normalisation Institute 1981$^a$, b, respectively), while the direct plating method (Dutch Normalisation Institute 1990) was used for detection of Escherichia coli. Pseudomonas aeruginosa and (spores of) Clostridium perfringens were enumerated on modified Pseudomonas aeruginosa agar-B (mPA-B; Dutch Normalisation Institute 1987) and modified Clostridium perfringens agar
The total number of heterotrophic bacteria at 37°C was counted on plate count agar (PCA; Dutch Normalisation Institute 1979). Media for bacteriological analyses were obtained from Oxoid Limited, Basingstoke, UK, and prepared according to the manufacturers’ instructions, except for mCP and mPA-B, which were prepared from individual components according to Bisson and Cabelli (1979) and Dutka (1981), respectively.

Faecal accidents

Pool operators were asked to record their observations of faecal material in any of the pools during the second half-year of the study. They were also questioned on the action taken following a faecal accident. A form for completion was supplied.

Risk assessment

The infection risk for swimmers exposed to numbers of potentially infectious (oo)cysts as found in the examined learner pool, was estimated by using the exponential dose-response model (Haas 1983) for which: \( P_{\text{inf}} = 1 - e^{-\mu r} \) and \( \mu = C \times 1/R \times I \times V \). Dose-response parameter values \( r \) as published by Teunis et al. (1996) were used. One visit to a contaminated swimming pool per year was assumed.

RESULTS AND DISCUSSION

Filter backwash water

Of the 153 samples of filter backwash water analysed, 18 (11.8%) were found positive: seven samples (4.6%) contained Cryptosporidium oocysts, nine samples (5.9%) Giardia cysts and in two samples (1.3%) both Cryptosporidium and Giardia were detected. Numbers ranged from 0.11 to 17 oocysts and from 0.06 to 24 cysts per litre backwash water (Table 1). No abnormalities in pH and turbidity that might have been indications of the presence of (oo)cysts were observed in the positive samples. All pool filters were positive at least once, but none of the filters was persistently positive. Viability staining with PI, and DIC microscopy, showed that most of the oocysts (85.4%) and cysts (81.1%) isolated from the filter backwash water were dead or empty (oo)cyst shells. Stress during (oo)cyst stay in the filter bed, the filter backwash process and sample processing may have influenced the viability of these (oo)cysts negatively. The presence of Cryptosporidium oocysts and Giardia cysts in the filter backwash water, however, indicates the previous presence of those (oo)cysts in the swimming pool water. It is to be assumed that viable and infectious (oo)cysts were present in the pool water immediately after shedding by infected swimmers.

Toddler pools

A total of 13 samples of pool water from toddler pools were analysed. None contained total coliforms, faecal coliforms, E. coli, P. aeruginosa or (spores of) C. perfringens in 100 ml samples. In one sample (7.7%) Giardia cysts were detected (0.04 cysts l\(^{-1}\)); PI staining indicated that all cysts were dead. This sample exceeded the standard for heterotrophic plate count at 37°C (≤100 cfp ml\(^{-1}\)) by more than tenfold, indicating the poor hygienic quality of the pool water. The turbidity standard (≤0.5 NTU) was exceeded in three other samples taken from this pool, but no bacteria or protozoa were detected in those samples. The pool operator reported frequently observed problems with pool water quality, related to the small capacity of this pool (0.8 m\(^3\)) and the large number of toddlers using it. The average occupation of this pool during parent-and-child swimming classes was 25 toddlers and (at least one of) their parents. This pool was regularly emptied, cleaned and filled with clean water in an attempt to overcome water quality problems.

Learner pool

The learner pool was probably contaminated on or shortly before 24 September 2001. The sample taken on that day contained large numbers of Cryptosporidium oocysts and
Giardia cysts (Table 2). A minor reinfection on 1 October cannot be excluded, considering the equal number of Giardia cysts in the water on both dates. Samples that were taken on 8 October at different times during the day revealed that (oo)cysts were already present in the pool water before opening hours (about 12 h after closing the evening before). Neither during therapeutic swimming classes, nor during parent-and-child swimming classes, was an increase of the (oo)cyst concentration in the pool water observed. (Oo)cysts were still detectable 3 hours after the swimming classes were terminated. Sixty-one percent of the Cryptosporidium oocysts detected in the sample taken on 24 September were considered viable and potentially infectious (PI negative, with sporozoites). This declined to 2% in the sample that was taken on 8 October. Most of the Giardia cysts in the 24 September sample (90.1%) were dead (PI positive); Giardia cyst viability was not determined in later samples.

Table 1 | Cryptosporidium and/or Giardia positive filter backwash water samples. The third column displays the pool types corresponding to the pool filters: L, learner pool; R, recreational pool; S, slide pool; T, toddler pool; W, whirlpool

<table>
<thead>
<tr>
<th>Sample date</th>
<th>Filter code</th>
<th>Pool type</th>
<th>Volume examined (l)</th>
<th>Number</th>
<th>Concentration (in l⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cryptosporidium</td>
<td>Giardia</td>
<td>Cryptosporidium</td>
</tr>
<tr>
<td>220501</td>
<td>I</td>
<td>L,T,W</td>
<td>1.0</td>
<td>12</td>
<td>24</td>
</tr>
<tr>
<td>170700</td>
<td>II</td>
<td>R,S,T,W</td>
<td>0.5</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>240401</td>
<td></td>
<td></td>
<td>0.2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>091000</td>
<td>IIIa</td>
<td>R,S</td>
<td>4.0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>231000</td>
<td></td>
<td></td>
<td>4.0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>051200</td>
<td></td>
<td></td>
<td>4.0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>210501</td>
<td></td>
<td></td>
<td>2.0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>050601</td>
<td></td>
<td></td>
<td>2.0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>270201</td>
<td>IIIb</td>
<td>T,W</td>
<td>5.0</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>070800</td>
<td>IVa</td>
<td>S,T</td>
<td>0.5</td>
<td>17</td>
<td>0</td>
</tr>
<tr>
<td>050201</td>
<td></td>
<td></td>
<td>0.2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>030401</td>
<td></td>
<td></td>
<td>1.0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>010501</td>
<td></td>
<td></td>
<td>0.5</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>290500</td>
<td>IVb</td>
<td>L</td>
<td>2.0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>290501</td>
<td></td>
<td></td>
<td>17</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>050201</td>
<td>V</td>
<td>L</td>
<td>1.0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>030401</td>
<td></td>
<td></td>
<td>1.0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>010501</td>
<td></td>
<td></td>
<td>0.5</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>
Cleaning of the pool walls and ‘dead corners’ (which might have served as an (oo)cyst reservoir) with bristles, and backwashing of the pool filter daily, did not result in complete elimination of (oo)cysts, although concentrations declined gradually (Table 2). The pool operator reported large cracks in the filter bed on 12 October, which confirmed the supposed malfunctioning of the pool filter. The cracks allowed circulation of unfiltered pool water. A more stringent backwash procedure was applied, resulting in rearrangement of the filter material and consequent closing of the cracks. From 22 October onwards, Cryptosporidium and Giardia concentrations declined to below the detection limit.

The local public health service did not observe any clustered gastro-enteritis complaints with Cryptosporidium or Giardia in October 2001, when viable and potentially infectious (oo)cysts were detected in the learner pool. A cluster of Cryptosporidium or Giardia infections was not observed in November 2001 either. Individual cases associated with this contaminated pool may have gone unnoticed, because no attempts were made to trace and question individuals who visited their general practitioner with gastro-enteritis complaints. Small outbreaks may also go undetected because in the Netherlands clinical faecal samples are not routinely examined for Cryptosporidium and Giardia.

The pool water quality in the learner pool complied with bacteriological standards at the time of the incident, but the turbidity standard was exceeded on 24 September. An initial turbidity increase was observed on 1 October at the onset of the swimming class, but turbidity decreased to an acceptable level within 1 hour. In other samples that contained (oo)cysts, pool water turbidity complied with the standard; therefore this parameter is an unreliable indicator for the presence of protozoan (oo)cysts. Analyses of the pool water quality from all pools included in this study, by certified commercial laboratories, showed that 73% of the pool water samples (n = 128) fully complied with the legal standards during this study. In most samples that did not comply (21%), the concentration of free available chlorine exceeded the upper limit of 1.5 mg l⁻¹, indicating that pool water disinfection with regard to bacteria and viruses was sufficient. It is clear that compliance of pool water quality with legal standards does not guarantee the absence of pathogenic protozoa, although Giardia cysts will be rapidly inactivated at the temperatures and chlorine concentrations applied in swimming pools (Jarroll et al. 1981).

The learner pool incident illustrates the importance of a properly functioning coagulation and filtration process and stresses the need for guarding the efficiency of this process. Continuous monitoring of the turbidity of the pool filter filtrate and regular visual inspection of the condition of the filter bed may be useful tools that result in early notice of poor filtration.

### Table 2

<table>
<thead>
<tr>
<th>Sample date</th>
<th>Concentration (n l⁻¹)</th>
<th>pH</th>
<th>Turbidity (NTU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>240901</td>
<td>2.31</td>
<td>6.9</td>
<td>1.64</td>
</tr>
<tr>
<td>011001</td>
<td>0.23</td>
<td>7.4</td>
<td>0.74</td>
</tr>
<tr>
<td>081001A</td>
<td>0.01</td>
<td>7.1</td>
<td>0.00</td>
</tr>
<tr>
<td>081001B</td>
<td>0.04</td>
<td>7.2</td>
<td>0.00</td>
</tr>
<tr>
<td>081001C</td>
<td>0.06</td>
<td>7.3</td>
<td>0.00</td>
</tr>
<tr>
<td>081001E</td>
<td>0.04</td>
<td>7.4</td>
<td>0.50</td>
</tr>
<tr>
<td>151001</td>
<td>0.004</td>
<td>7.0</td>
<td>0.00</td>
</tr>
<tr>
<td>221001</td>
<td>&lt;0.001</td>
<td>7.2</td>
<td>0.00</td>
</tr>
<tr>
<td>291001</td>
<td>&lt;0.001</td>
<td>7.2</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Cleaning of the pool walls and ‘dead corners’ (which might have served as an (oo)cyst reservoir) with bristles, and backwashing of the pool filter daily, did not result in complete elimination of (oo)cysts, although concentrations declined gradually (Table 2). The pool operator reported large cracks in the filter bed on 12 October, which confirmed the supposed malfunctioning of the pool filter. The cracks allowed circulation of unfiltered pool water. A more stringent backwash procedure was applied, resulting in rearrangement of the filter material and consequent closing of the cracks. From 22 October onwards, Cryptosporidium and Giardia concentrations declined to below the detection limit.

The local public health service did not observe any clustered gastro-enteritis complaints with Cryptosporidium or Giardia involved in October 2001, when viable and potentially infectious (oo)cysts were detected in the learner pool. A cluster of Cryptosporidium or Giardia infections was not observed in November 2001 either. Individual cases associated with this contaminated pool may have gone unnoticed, because no attempts were made to trace and question individuals who visited their general practitioner with gastro-enteritis complaints. Small outbreaks may also go undetected because in the Netherlands clinical faecal samples are not routinely examined for Cryptosporidium and Giardia.

The pool water quality in the learner pool complied with bacteriological standards at the time of the incident, but the turbidity standard was exceeded on 24 September. An initial turbidity increase was observed on 1 October at the onset of the swimming class, but turbidity decreased to an acceptable level within 1 hour. In other samples that contained (oo)cysts, pool water turbidity complied with the standard; therefore this parameter is an unreliable indicator for the presence of protozoan (oo)cysts. Analyses of the pool water quality from all pools included in this study, by certified commercial laboratories, showed that 73% of the pool water samples (n = 128) fully complied with the legal standards during this study. In most samples that did not comply (21%), the concentration of free available chlorine exceeded the upper limit of 1.5 mg l⁻¹, indicating that pool water disinfection with regard to bacteria and viruses was sufficient. It is clear that compliance of pool water quality with legal standards does not guarantee the absence of pathogenic protozoa, although Giardia cysts will be rapidly inactivated at the temperatures and chlorine concentrations applied in swimming pools (Jarroll et al. 1981).

The learner pool incident illustrates the importance of a properly functioning coagulation and filtration process and stresses the need for guarding the efficiency of this process. Continuous monitoring of the turbidity of the pool filter filtrate and regular visual inspection of the condition of the filter bed may be useful tools that result in early notice of poor filtration.

### Faecal accidents

All pool operators regularly observed formed stools in most pool types, not particularly in risk pools like toddler pools or learner pools. The frequency varied from about once every two months to twice a month. In all cases the solid parts were removed from the pools with a scoop or a landing net. Small toddler pools were sometimes emptied into the sewer system, cleaned and refilled with circulating water.
pool water. There is no temporal relationship between faecal accidents and positive protozoa findings. The sampling scheme was not changed following a reported faecal accident and sampling and faecal accidents did not coincide.

Formed stools in swimming pool water are probably of minor importance for Cryptosporidium transmission. Research by the CDC Recreational Waterborne Disease Working Group (2001) showed that Cryptosporidium oocysts were absent in formed stools (n = 293) found in swimming pool water. Giardia cysts were, however, detected in 4.4% of these samples. The frequency of these formed stools in pool water observed in our study may be indicative of the frequency of loose stools that are the most likely source of Cryptosporidium contamination of swimming pool water.

Loose stools are difficult to detect and pool operators are almost completely dependent upon the preparedness of the public to notify them in case of a faecal accident. This implies that the public should be well informed on the risk of transmission of infectious diseases through swimming pools and the importance of hygiene in and around the pools. Besides proper training, it is important to provide pool operators with guidelines for management of faecal accidents: for example, based on guidelines that are already used in the USA (Kebabjian 1995; MMWR Weekly 2001).

**Risk assessment**

Assuming one swimming pool visit per year, Cryptosporidium and Giardia concentrations in swimming pool water which on average result in one infection in 10,000 persons per year were calculated as a function of ingesting volume using the dose-response model of Haas (1983) (Table 3). The concentration of viable and potentially infectious (oo)cysts in the contaminated learner pool on 24 September was calculated from the detected number of (oo)cysts, the fraction of viable (oo)cysts as determined by PI staining (61% for Cryptosporidium, 9.9% for Giardia) and recovery data from laboratory seeding experiments (n = 5) using Envirochek HV filtration at a filtration rate of 10 l min⁻¹. These seeding experiments resulted in an average recovery for Cryptosporidium oocysts of 11.0%, whereas an average recovery of 37.1% for Giardia cysts was obtained. The concentration of viable Cryptosporidium oocysts (12.8 l⁻¹) was within the range of concentrations giving a yearly infection risk of 10⁻⁴ at the considered ingestion volumes, as shown in Table 3.

Calculation of the infection risk, using the same model as described above and assuming one visit to a contaminated swimming pool per year, showed that ingestion of 10 ml or more contaminated pool water resulted in an estimated risk of infection with Cryptosporidium that exceeds the generally accepted infection risk of one infection per 10,000 persons a year (Table 4). The much lower concentration of viable Giardia cysts (0.032 l⁻¹) resulted in a minor risk of infection that did not exceed the 10⁻⁴ infection risk even at ingestion of 100 ml contaminated learner pool water.

These results agree with data on poor inactivation of Cryptosporidium oocysts (Korich et al. 1990) and rapid inactivation of Giardia cysts (Jarroll et al. 1981) in swimming pool water conditions, but do not fully agree with results of epidemiological studies (de Wit et al. 2001a, b) for Giardia, which indicate swimming in a swimming pool as a risk factor for Giardia infections. However, the following should be taken into account concerning the risk calculations:

1. The estimation of the infection risks in this study was based on one contamination incident in one

<table>
<thead>
<tr>
<th>Dose response parameters</th>
<th>Cryptosporidium</th>
<th>Giardia</th>
</tr>
</thead>
<tbody>
<tr>
<td>r</td>
<td>0.00401</td>
<td>0.0199</td>
</tr>
<tr>
<td>f</td>
<td>0.0249</td>
<td>0.00503</td>
</tr>
<tr>
<td>Ingestion volume (ml)</td>
<td>Concentration (n l⁻¹)</td>
<td>Concentration (n l⁻¹)</td>
</tr>
<tr>
<td>1</td>
<td>24.9</td>
<td>5.03</td>
</tr>
<tr>
<td>10</td>
<td>2.49</td>
<td>0.503</td>
</tr>
<tr>
<td>25</td>
<td>0.998</td>
<td>0.201</td>
</tr>
<tr>
<td>100</td>
<td>0.249</td>
<td>0.050</td>
</tr>
</tbody>
</table>
CONCLUSIONS

Cryptosporidium oocysts and Giardia cysts have been detected in both the filter backwash water and the pool water of learner and toddler pools. Although most detected (oo)cysts were dead as determined by vital dye staining, and precise data on oocyst infectivity are lacking, it was shown that swimmers were exposed to a small viable and potentially infectious fraction of the (oo)cysts present. The result of the preliminary estimation of the infection risk indicates that indoor swimming pools cannot be excluded as a source of cryptosporidiosis in the Netherlands. Giardia cysts are more rapidly inactivated and form a minor risk. With supplementary data on, for example, oocyst infectivity in swimming pool water, the percentage of the Dutch swimming pools that are contaminated, the frequency of swimming pool visits, water ingestion during swimming and epidemiological data on host sensitivity, the calculation of the infection risk can be refined. Present knowledge indicates that public information and adequate training of the pool operators are useful tools for reducing the risk. This study also showed that compliance of pool water quality with legal standards does not guarantee the absence of protozoan parasites. Regular inspection of the condition of the swimming pool filters and continuous checking of the effectiveness of the filtration process will help to maintain parasitologically safe swimming pool water.

ACKNOWLEDGEMENTS

This research was performed by order and for the account of the Board RIVM and the Directorate General for

Table 4 Infection risks at once-only exposure to various doses of Cryptosporidium and Giardia in a contaminated learner pool

<table>
<thead>
<tr>
<th>Ingestion volume (ml)</th>
<th>Dose</th>
<th>Infection risk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cryptosporidium</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.0128</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.1284</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.3211</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.284</td>
</tr>
</tbody>
</table>
Environmental Protection, Ministry of Housing, Spatial Planning and the Environment, the Netherlands, within the framework of projects S/250931 and M/252202.

ABBREVIATIONS AND NOTATIONS

C measured concentration (oo)cysts in water sample
cfp colony forming particles
CT99 (concentration of disinfectant, mg l⁻¹) (contact time, min) resulting in 99% inactivation
DIC differential interference contrast
EDTA ethylenediamine tetraacet acid
FITC fluorescein-isothiocyanate
G gravity force
HCl hydrochloric acid
I fraction of (oo)cysts that are viable
IMS immuno magnetic separation
µ dose in the exponential dose-response model
mM millimolar
N normal
NaOH sodium hydroxide
NTU nephelometric turbidity unit
PI propidium iodide
Pinf probability of infection
Pint probability of infection
PBS phosphate buffered saline
R recovery of detection method
r dose-response parameter of the exponential dose-response model
rpm revolutions per minute
V individual consumption of water (l)

REFERENCES


Dutch Normalisation Institute 1987 Bacteriologisch onderzoek van water – Onderzoek met behulp van membraanfiltratie naar de aanwezigheid van thermotolerante bacteriën van de coligroep (NEN 6573). Dutch Normalisation Institute, Delft, The Netherlands (in Dutch).


MMWR Weekly 2001 Notice to readers: responding to fecal accidents in disinfected swimming venues. MMWR Weekly 50(20), 416–417.


