Cryptosporidium and *Giardia* in swimming pools in the Netherlands

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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

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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 of 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.* 2001*a*, *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 ($CT_{99} = 15$; Jarroll *et al.* 1981). Chlorine concentrations used for swimming pool disinfection have no rapid inactivating effect on *Cryptosporidium* oocysts. CT_{99} 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 $1,050 \times G$ was followed by partial aspiration of the supernatant (rest volume *c*. 50–100 ml) and resuspension of the pellet; next, the concentrate was centrifuged for 10 min at $1,080 \times G$, 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 *c*. 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, $1,080 \times G$, 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 $10 \times$ SL buffer A and 1 ml of $10 \times$ 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 $1 \times$ 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 $250 \times$ using a Zeiss Axioskop. Magnification $1,000 \times$ 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

(mCP; Bisson and Cabelli 1979), respectively. 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: $P_{inf} = 1 - e^{-r\mu}$ 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 ($\leq$100 cfp ml^{-1}) by more than tenfold, indicating the poor hygienic quality of the pool water. The turbidity standard ($\leq$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

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

Sample date	Filter code	Pool type	Volume examined (l)	Number		Concentration (n l^{-1})	
				Cryptosporidium	*Giardia*	*Cryptosporidium*	*Giardia*
220501	I	L,T,W	1.0	12	24	12	24
170700	II	R,S,T,W	0.5	10	0	20.8	< 2.1
240401			0.2	0	1	< 4.0	4.0
091000	IIIa	R,S	4.0	0	1	< 0.2	0.2
231000			4.0	0	1	< 0.2	0.2
051200			4.0	0	2	< 0.2	0.5
210501			2.0	0	1	< 0.5	0.5
050601			2.0	0	1	< 0.5	0.5
270201	IIIb	T,W	5.0	3	1	0.6	0.2
070800	IVa	S,T	0.5	17	0	34	< 2.0
050201			0.2	1	0	5.2	< 1.0
030401			1.0	0	2	< 1.0	2.0
010501			0.5	0	1	< 2.0	2.0
290500	IVb	L	2.0	1	0	0.5	< 0.5
290501			17	2	1	0.1	0.06
050201	V	L	1.0	1	0	1.0	< 1.0
030401			1.0	1	0	1.0	< 1.0
010501			0.5	0	1	< 2.0	2.0

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 per cent 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 2 | Concentration of *Cryptosporidium* oocysts and *Giardia* cysts in the pool water of a learner pool; A: before opening hours, B: during therapeutic swimming class, C: during parent-and-child swimming classes, E: 3 hours after termination of swimming classes

| Sample date | Concentration (n l^{-1}) | | pH | Turbidity (NTU) |
	Cryptosporidium	*Giardia*		
240901	2.31	0.12	6.9	1.64
011001	0.23	0.10	7.4	0.74
081001A	0.01	< 0.002	7.1	0.00
081001B	0.04	0.005	7.2	0.00
081001C	0.06	< 0.001	7.3	0.00
081001E	0.04	< 0.002	7.4	0.50
151001	0.004	0.001	7.0	0.00
221001	< 0.001	< 0.001	7.2	0.00
291001	< 0.001	< 0.001	7.2	0.00

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

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%,

Table 3 | *Cryptosporidium* and *Giardia* concentrations in swimming pool water that result in a yearly infection risk of 1 in 10,000 persons, as a function of ingestion volume. The dose-response model of Haas (1983) and the dose-response parameters of Teunis *et al.* (1996) were used and one swimming pool visit per year was assumed

Dose response parameters		*Cryptosporidium*	*Giardia*
	r	0.00401	0.0199
	μ	0.0249	0.00503
Ingestion volume (ml)		Concentration ($n\,l^{-1}$)	Concentration ($n\,l^{-1}$)
1		24.9	5.03
10		2.49	0.503
25		0.998	0.201
100		0.249	0.050

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

Ingestion volume (ml)	Dose		Infection risk	
	Cryptosporidium	*Giardia*	*Cryptosporidium*	*Giardia*
1	0.0128	0.00003	5.1×10^{-5}	6.3×10^{-7}
10	0.1284	0.00032	5.1×10^{-4}	6.3×10^{-6}
25	0.3211	0.00080	1.3×10^{-3}	1.6×10^{-5}
100	1.284	0.00319	5.1×10^{-3}	6.3×10^{-5}

pool, whereby the highest numbers of (oo)cysts detected were used.

2. (Oo)cyst numbers in swimming pool water will vary between different contamination incidents and not all *Cryptosporidium* oocysts and *Giardia* cysts considered viable and possibly infectious on the basis of PI staining and DIC microscopy will in fact be infectious. Detailed data on (oo)cyst infectivity in swimming pool water are, however, lacking.

3. One visit per year to a contaminated swimming pool was assumed and the range of ingestion volumes was more or less arbitrarily chosen.

4. Individuals behave differently while swimming, resulting in different exposure to infectious (oo)cysts.

5. Individual differences (e.g. in physical condition) will influence the sensitivity to infection with parasites such as *Cryptosporidium* and *Giardia*.

6. Human feeding studies have shown that variability in infectivity between different *Cryptosporidium* isolates of the same species exists (Teunis *et al.* 2002). The estimated infection risks should therefore be considered as a first indication of the extent of the health impact that *Cryptosporidium* and *Giardia* may have through the use of indoor swimming pools in the Netherlands.

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.

CONCLUSIONS

Cryptosporidium oocysts and *Giardia* cysts have been detected in both the filter backwash water and the pool

ACKNOWLEDGEMENTS

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
CT_{99} — (concentration of disinfectant, mg l^{-1}) (contact time, min) resulting in 99% inactivation
DIC — differential interference contrast
EDTA — ethylenediamine tetraaceticacid
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
P_{inf} — 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)

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