

***Cryptosporidium* and *Giardia* in recreational water in Belgium**

Md. Amimul Ehsan, Stijn Casaert, Bruno Levecke, Liesbet Van Rooy, Joachim Pelicaen, Anne Smis, Joke De Backer, Bart Vervaeke, Sandra De Smedt, Filip Schoonbaert, Saskia Lammens, Thierry Warmoes, Thomas Geurden and Edwin Claerebout

ABSTRACT

The objective of this study was to investigate the presence of *Cryptosporidium* and *Giardia* in different recreational water bodies in Belgium and to estimate the infection risk associated with swimming and other recreational activities. *Cryptosporidium* oocysts and/or *Giardia* cysts were detected in three out of 37 swimming pools, seven out of 10 recreational lakes, two out of seven splash parks and four out of 16 water fountains. In the swimming pools no infection risk for *Cryptosporidium* could be calculated, since oocysts were only detected in filter backwash water. The risk of *Giardia* infection in the swimming pools varied from 1.13×10^{-6} to 2.49×10^{-6} per swim per person. In recreational lakes, the infection risk varied from 2.79×10^{-5} to 5.74×10^{-5} per swim per person for *Cryptosporidium* and from 7.04×10^{-5} to 1.46×10^{-4} for *Giardia*. For other outdoor water recreation activities the estimated infection risk was 5.71×10^{-6} for *Cryptosporidium* and 1.47×10^{-5} for *Giardia*. However, most positive samples in the recreational lakes belonged to species/genotypes that are either animal-specific or predominantly found in animals. No *Cryptosporidium* was found in splash parks and water fountains, but the presence of *Giardia* cysts suggests a risk for human infection. The infection risk of *Giardia* infection during a 3.5-minute visit to a splash park for children equalled 1.68×10^{-4} .

Key words | Belgium, *Cryptosporidium*, *Giardia*, recreational water, risk assessment

INTRODUCTION

Cryptosporidium and *Giardia* are important parasites in the aetiology of diarrhoea worldwide. In industrialised countries *Cryptosporidium* spp. are detected in up to 54.2% and *Giardia* spp. in up to 74.4% (Homan & Mank 2001; Geurden *et al.* 2009) of immunocompetent patients with diarrhoea. In non-outbreak-related cases of diarrhoea in Belgian patients, *Giardia* was identified as the second most prevalent pathogen. In the same study *Cryptosporidium* was also within the five most commonly detected pathogens, indicating that both parasites occur frequently in patients with gastro-intestinal symptoms (Geurden *et al.* 2009). In Belgium, the national incidence of cryptosporidiosis and giardiasis is 2.5 and 10.8

per 100,000 inhabitants, respectively, with the highest incidence in children (<5 years of age) and young adults (25–44 years old) (Wetenschappelijk Instituut Volksgezondheid [WIV] 2010).

Transmission of *Cryptosporidium* spp. and *Giardia* spp. occurs from either humans or animals by the faecal-oral route. In humans worldwide *C. hominis* and *C. parvum* are the most commonly detected *Cryptosporidium* species. *C. hominis* is human-specific, while *C. parvum* infects ruminants and humans. *Giardia duodenalis* assemblage A and B are responsible for giardiasis in humans, and are also found in a wide range of mammals. Within assemblage A,

Md. Amimul Ehsan[†]
Stijn Casaert[†]
Bruno Levecke
Thomas Geurden
Edwin Claerebout (corresponding author)
Laboratory for Parasitology,
Faculty of Veterinary Medicine,
Ghent University,
Salisburylaan 133, 9820 Merelbeke,
Belgium
E-mail: edwin.claerebout@ugent.be

Liesbet Van Rooy
Anne Smis
Joke De Backer
Filip Schoonbaert
Agency for Care and Health, Flemish Ministry for
Welfare, Public Health and Family,
Brussels,
Belgium

Joachim Pelicaen
Bart Vervaeke
Sandra De Smedt
Saskia Lammens
Thierry Warmoes
Flemish Environment Agency,
Erembodegem,
Belgium

[†]Contributed equally to this work.

sub-assembly AI is mostly found in animals, whereas sub-assembly AII is predominantly found in humans (Sprong *et al.* 2009). Sub-assembly AIII is almost exclusively found in wild hoofed animals. In contrast, there is no clear subgrouping within assembly B (Sprong *et al.* 2009; Feng & Xiao 2011). Next to direct contact with an infected host, *Cryptosporidium* and *Giardia* can be transmitted through faecal contamination of food or water. Because of the parasites' ability to survive in the environment, their relative resistance to disinfection and the low number of (oo)cysts needed for infection, *Cryptosporidium* and *Giardia* are considered as important waterborne infections (Smith *et al.* 2006). From 2004 to 2010, 199 outbreaks of human diseases due to the waterborne transmission of parasitic protozoa were reported worldwide. *Cryptosporidium* spp. were the aetiological agent in 60.3% of the outbreaks and *Giardia* spp. in 35.2% (Baldursson & Karanis 2011). In a third of the outbreaks, recreational water was detected as the source of infection, (Baldursson & Karanis 2011). Swimming in contaminated waters and swimming pools is now recognised as an important transmission route for *Cryptosporidium* (Karanis *et al.* 2007). Outbreaks of cryptosporidiosis have been associated with recreational water in the USA (e.g. Craun *et al.* 2005; Causer *et al.* 2006; Wheeler *et al.* 2007; Boehmer *et al.* 2009; Cantey *et al.* 2012), Canada (Hopkins *et al.* 2013), Australia (e.g. Dale *et al.* 2010; Waldron *et al.* 2011), Japan (Takagi *et al.* 2008), Sweden (Insulander *et al.* 2005; Mattsson *et al.* 2008) and the UK (e.g. Smith *et al.* 2006; Coetzee *et al.* 2008). Although few reports can be found on *Giardia* infections related to recreational water (e.g. Porter *et al.* 1988), a marked seasonality in the onset of giardiasis occurs in summer to early autumn in many countries, including Belgium (WIV 2010). This increase coincides with increased outdoor activities (e.g. swimming) (Hlavsa *et al.* 2005) and with increased travelling during summer holidays.

In Belgium, *Cryptosporidium* and *Giardia* were detected in surface water that is used for drinking water production (Ehsan *et al.* 2014), but no data are available for recreational water, and the importance of water recreation in the transmission of *Cryptosporidium* and *Giardia* in Belgium is unknown. The objective of this study was to investigate the presence of *Cryptosporidium* and *Giardia* in public

swimming pools, recreational water bodies and splash parks in Flanders (Northern Belgium) and to estimate the infection risk associated with swimming and other recreational activities. Positive samples were genotyped in an attempt to identify the source of infection (human vs. animal).

METHODS

Sampling

In total, 99 samples from recreational water bodies in Flanders, Belgium were analysed. From March to October 2010, 36 public swimming pools were sampled. The swimming pools were selected based on an increased risk for faecal accidents or external contamination (i.e. paddling pools, therapy pools frequently visited by mentally disabled persons and outdoor swimming pools). Convenience samples were collected from three types of water samples, including pool water (60 L) in 20 swimming pools, filter backwash water (2–60 L) in 16 pools and water from continuous flow centrifugation (2 L) in one swimming pool.

In August 2011, 40 convenience samples (30 L) were collected from 10 recreational lakes. Each lake was sampled four times with weekly intervals.

In July and August 2012, 23 convenience samples (30 L) were taken from neighbourhood water fountains ($n = 16$), splash parks ($n = 7$) and a water fountain in a boating lake ($n = 1$).

All samples were transported to the laboratory, stored at 4 °C and analysed within 72 hours.

Detection of *Cryptosporidium* oocysts and *Giardia* cysts

A protocol was optimised to detect *Cryptosporidium* and *G. duodenalis* in water samples, based on the United States Environmental Protection Agency (USEPA) method 1623 (USEPA 2005). Water samples were filtered through Filtamax Xpress filters (IDEXX Laboratories, Inc., Westbrook, ME, USA) with the aid of a peristaltic pump with recommended flow rates of 2 L/min. The Filtamax Xpress filters were washed with the Filtamax Xpress automated washing station for elution of the filters following the

manufacturer's instructions. The eluate was centrifuged and the volume of sediment was measured. Between 0.5 and 2 mL of sediment was used for immunomagnetic separation (IMS) of the (oo)cysts. Oocysts and cysts in the sediment were purified by IMS using *Cryptosporidium*- and *Giardia*-specific antibody-coated magnetic beads according to the manufacturer's protocol (Dynabeads® GC-Combo, Invitrogen Dynal, A.S., Oslo, Norway). IMS-purified cysts and oocysts were stained on well slides by fluorescein isothiocyanate (FITC)-conjugated anti-*Cryptosporidium* and anti-*Giardia* MAbs FITC-conjugated monoclonal antibodies (EasyStain™) (BTF Pty Ltd, Macquarie Park, Australia). Slides were examined using a Leica Leitz DMRB fluorescence microscope. The well surface was scanned at 200 or 400 times magnification using a FITC fluorescence filter (450–590 nm Chroma technology corp.). *Cryptosporidium* oocysts and *Giardia* cysts were identified and counted based on their size, morphology and fluorescence. Results were expressed as count per litre. Slides containing (oo)cysts were kept at 4 °C for DNA extraction.

Risk of *Cryptosporidium* and *Giardia* infection

Risk of *Cryptosporidium* and *Giardia* infection (P_{inf}) during swimming or other recreational water activities (fishing, boating, canoeing and rowing) was based on the equation below

$$P_{\text{inf}} = 1 - e^{-D p_m}$$

In this equation, D represents the infection dose and p_m represents the dose-response parameter. The dose D was determined by multiplying the observed number of (oo)cysts in 1 L by the volume v of water swallowed during each of the different water activities, including swimming, limited contact water recreation activities (e.g. fishing, boating, canoeing and rowing) and visiting splash parks. For swimming, we applied the v -estimates reported by Schets *et al.* (2011b): swimming in a pool: 34.0×10^{-3} L (male), 23.0×10^{-3} L (female), 51.0×10^{-3} L (children); swimming in fresh water: 27.0×10^{-3} L (male), 18.0×10^{-3} L (female), 37.0×10^{-3} L (children). For recreation activities with limited water contact, v was set at 3.7×10^{-3} L (Dorevitch *et al.* 2011). For visiting splash parks, we assumed that a

child swallows 75.7×10^{-3} L during a 3.5-minute visit (de Man *et al.* 2014). Recovery rates for *Cryptosporidium* oocysts and *Giardia* cysts obtained with the USEPA 1623 protocol for drinking water (*Cryptosporidium* 44.8%; *Giardia* 45.1%) and surface water (*Cryptosporidium* 44.1%; *Giardia* 27.7%) in Flanders (Ehsan *et al.* 2014) were used to correct D in swimming pools, splash parks (drinking water) and recreational lakes (surface water). The p_m was set at 28×10^{-5} for *Cryptosporidium* (Messner *et al.* 2001), and at 19.9×10^{-5} for *Giardia* (Teunis *et al.* 1996). Finally, we used Monte Carlo simulations (500,000 iterations) to obtain the median, mean and 95% confidence interval. To this end, we considered the variation in each of the parameters inserted in the formula above. We assumed that the dose-response parameter p_m followed a beta distribution with scale parameters α and β (expected value $p_m = \alpha / (\alpha + \beta)$, variance $p_m = \alpha \times \beta / ((\alpha + \beta)^2 \times (\alpha + \beta + 1))$). For *Cryptosporidium*, α and β were set at 0.53 and 18.45, respectively; for *Giardia* these values were set at 1.83 and 90.05. These values were derived from the estimated p_m and corresponding 90th percentile for *Cryptosporidium* (66.0×10^{-3} ; Messner *et al.* 2001) and the 97.5th percentile for *Giardia* (56.6×10^{-3} ; Teunis *et al.* 1996). For the variation of number of (oo)cysts per L and the recovery rates of (oo)cyst counts we re-sampled from the original raw data. For the variation in volume v swallowed we used distributions described by Schets *et al.* (2011a, 2011b) (swimming) and de Man *et al.* (2014) (splash parks). For the risk involving limited contact water recreation activities we did not consider the v -distribution, as this was not available (Dorevitch *et al.* 2011).

DNA extraction and molecular identification

DNA was extracted from water samples from recreational lakes that were positive by microscopy for *Cryptosporidium* and/or *Giardia*. Positive samples from indoor swimming pools, neighbourhood fountains and splash parks were not genotyped, as numbers of (oo)cysts were too low.

Genomic DNA was extracted from (oo)cysts that were scraped from the microscope slides using the QIAamp DNA Mini Kit (Qiagen GmbH, Hilden, Germany) or from sediment using the QIAamp Stool Mini Kit according to the manufacturer's instructions, incorporating an initial step of three freeze-thaw cycles (freezing in liquid nitrogen

for 5 minutes and heating at 95 °C for 5 minutes) in the protocol to maximise disruption of (oo)cysts. Previously described polymerase chain reaction (PCR) protocols were used to amplify the 18S rDNA gene (Ryan *et al.* 2003) and the heat shock protein (*hsp*)-70 gene (Morgan *et al.* 2001) of *Cryptosporidium*. For the identification of *Giardia* the β -giardin gene (Lalle *et al.* 2005) was used in a nested PCR. For assemblage-specific amplification of *Giardia*, the triose phosphate isomerase (*tpi*) gene was used (Sulaiman *et al.* 2003; Geurden *et al.* 2008; Levecke *et al.* 2009). For all PCR reactions, negative (PCR water) and positive controls (genomic DNA) were included. The PCR products were visualised in agarose gel (1.5%) stained with ethidium bromide under ultraviolet (UV) light. PCR products were fully sequenced by the BIG Dye Terminator V3.1 Cycle sequencing Kit (Applied Biosystems). Sequencing reactions were analysed on a 3100 genetic Analyzer (Applied Biosystems) and assembled with the program SeqMan II (DNASTAR, Madison, WI, USA). To determine the genotypes/assemblages the fragments were aligned with homologous sequences available in the GenBank database, using MegAlign (DNASTAR, Madison, WI, USA).

RESULTS

Backwash water samples from two swimming pools were found positive for *Cryptosporidium*, with 0.23 oocysts/L and 0.03 oocysts/L, respectively. In one of these samples, as well as in one pool water sample from another swimming pool, *Giardia* was detected (0.23 cysts/L and 0.07 cysts/L, respectively). Only the results from the pool water were used for calculating the infection risk. The risk of *Giardia* infection in the investigated swimming pools varied from 1.13×10^{-6} to 2.49×10^{-6} per swim per person (Table 1).

Table 1 | Risk of *Giardia* infection per swim in swimming pools for, respectively, men, women and children (median, mean and 95% confidence interval)

	Median	Mean	95% CI
<i>Giardia</i>			
Men	0	1.61×10^{-6}	$0 - 8.33 \times 10^{-6}$
Women	0	1.13×10^{-6}	$0 - 6.24 \times 10^{-6}$
Children	0	2.49×10^{-6}	$0 - 1.90 \times 10^{-5}$

Eight out of 10 sampled recreational lakes were positive for one or both parasites on at least one sampling occasion. In seven out of 10 lakes *Cryptosporidium* oocysts were detected once ($n = 4$) or twice ($n = 3$), with oocyst counts ranging from 0.07 to 0.60 oocysts per L (Table 2). Genotyping was only successful for two out of 10 samples. Both sequences were identified as *Cryptosporidium andersoni* (National Centre for Biotechnology Information [NCBI] accession numbers KM455082, KM455083). In the other cases either no DNA could be obtained from the slides ($n = 1$), no PCR product was obtained ($n = 6$) or no sequence was obtained from the PCR product ($n = 1$). *Giardia* cysts were detected once ($n = 5$) or twice ($n = 2$) in seven out of 10 lakes. Apart from one lake, these were the same lakes that were also positive for *Cryptosporidium*. *Giardia* cyst counts ranged from 0.23 to 0.70 cysts/L (Table 2). In seven out of nine positive samples a PCR product was obtained with the β -giardin gene and/or the *tpi* gene. Sequencing results showed *G. duodenalis* assemblage AI in six out of seven cases (NCBI accession numbers KM455069-KM455071, KM455074-KM455078, KM455080, KM455081), either as the only assemblage ($n = 4$) or in combination with assemblage AII ($n = 1$, NCBI accession number KM455079) or assemblages BIII (NCBI accession number KM455072) and E (NCBI accession number KM455073) ($n = 1$). In one positive sample assemblage 'BIV-like' was present (NCBI accession number KM455068). The infection risk for men, women and children for swimming in recreational lakes is shown in Table 3. The estimated infection risk for *Cryptosporidium* varied from 2.79×10^{-5} to 5.74×10^{-5} per swim per person, while for *Giardia* the infection risk was between 7.04×10^{-5} and 1.46×10^{-4} per swim per person. For other outdoor water recreation activities the estimated infection risk was 5.71×10^{-6} for *Cryptosporidium*, and 1.47×10^{-5} for *Giardia*.

No *Cryptosporidium* oocysts were detected in any of the sampled water fountains or splash parks. However, water samples from three fountains and two splash parks contained *Giardia* cysts. Cyst counts were 0.03, 0.07 and 0.20 cysts/L in the water fountains and 0.13 cysts/L in both splash parks. The infection risk of *Giardia* infection during a 3.5-minute visit to a splash park for children equalled 1.68×10^{-4} (95% confidence interval: $0 - 1.57 \times 10^{-3}$).

Table 2 | Number of *Cryptosporidium* oocysts (C) and *Giardia* cysts (G) per L water in recreational lakes in Flanders, Belgium in August 2011

Sampling date	02/08/11		08/08/11		16/08/11		22/08/11		28/08/11	
	C	G	C	G	C	G	C	G	C	G
Lake 1	0	0	0	0	0	0	0	0	ND	ND
Lake 2	0	0	0	0	0	0	0	0	ND	ND
Lake 3	0	0.23	0	0	0.17	0	0	0	ND	ND
Lake 4	0	0	0	0	0	0	0.07	0	ND	ND
Lake 5	0	0	0	0	0	0.70	0	0	ND	ND
Lake 6	0	0.70	0.17	0.47	0	0	0	0	ND	ND
Lake 7	0.30	0	0	0	0	0.23	0.30	0	ND	ND
Lake 8	0.17	0.47	0	0	0	0	0	0.23	ND	ND
Lake 9	0	0	0	0.23	0.17	0	0.17	0	ND	ND
Lake 10	ND	ND	0.07	0.23	0	0	0.60	0	0	0

ND = not done.

Table 3 | Risk of *Cryptosporidium* and *Giardia* infection per swim in recreational lakes for, respectively, men, women and children (median, mean and 95% confidence interval)

	Median	Mean	95% CI
<i>Cryptosporidium</i>			
Men	0	4.13×10^{-5}	0 – 1.15×10^{-4}
Women	0	2.79×10^{-5}	0 – 1.11×10^{-4}
Children	0	5.74×10^{-5}	0 – 2.35×10^{-4}
<i>Giardia</i>			
Men	0	1.08×10^{-4}	0 – 9.45×10^{-4}
Women	0	7.04×10^{-5}	0 – 6.37×10^{-4}
Children	0	1.46×10^{-4}	0 – 5.85×10^{-4}

DISCUSSION

Swimming is one of the most popular recreational activities worldwide with over 350 million person-events estimated to take place annually in the USA alone (Fayer 2004). Health risks for swimmers may arise from exposure to bathing waters of poor quality. They may suffer from various diseases such as gastro-enteritis caused by bacteria, viruses or parasites of faecal origin (World Health Organization (WHO) 2003). Among protozoan parasites, *Giardia* and *Cryptosporidium* are associated with waterborne outbreaks worldwide from recreational water, including

swimming pools and recreational lakes. In this study, three out of 37 swimming pool samples (8.1%) tested positive for *Cryptosporidium*, *Giardia* or both (two filter backwash samples and one swimming pool water sample). Similar contamination rates were reported in non-outbreak-related pools in France, the Netherlands, the USA and Egypt (Fournier et al. 2002; Schets et al. 2004; Shields et al. 2008; Abd El-Salam 2012). In Italy, a higher proportion of pools were positive for *Cryptosporidium* and/or *Giardia* (Briancesco & Bonadonna 2005; Oliveri et al. 2006), while in Greece no (oo)cysts were found in five swimming pools (Papadopoulou et al. 2008). Based on the observed (oo)cyst concentrations, an attempt was made to estimate the infection risk associated with swimming in these pools. Since oocysts were only detected in filter backwash water, no infection risk for *Cryptosporidium* could be calculated for the investigated swimming pools. Using the methodology of Schets et al. (2011b), the infection risk for *Giardia* was estimated as 1.13 to 2.49×10^{-6} per swim per person. In comparison, in a swimming pool in the Netherlands, an infection risk of $1.3\text{--}2.8 \times 10^{-5}$ for *Giardia* was estimated per swimming event per person (Schets et al. 2004, 2011b). The higher infection risk in the Dutch study was due to a faecal contamination incident and filter malfunctioning during the time of sampling. Although no recent faecal contamination incidents were reported in the swimming pools in the present study, the selection of 'high risk' swimming

pools may have introduced a bias, and the calculated infection risk may not be representative of all swimming pools in Flanders. An increased infection risk has been associated with swimming pools that are frequently visited by young children (Stafford *et al.* 2000; Hlavsa *et al.* 2005). Moreover, as cyst viability was not assessed, total cyst counts were used in our risk assessment, assuming 100% viability. Although *Giardia* cysts can survive in water for a considerable time (Olson *et al.* 1999), the infection risk is possibly overestimated because of this assumption. However, it should be noted that these are baseline contamination levels and that the infection risk can increase dramatically in the case of a faecal contamination incident or filter malfunctioning.

In seven out of 10 of the investigated recreational lakes (10 out of 40 samples) *Cryptosporidium* was detected at least once, with relatively low oocyst concentrations (0.07–0.6 oocysts/L). Similar contamination rates and oocyst counts were obtained in recreational waters in France, the Netherlands, Luxembourg and in central Spain, (Coupe *et al.* 2006; Schets *et al.* 2008; Helmi *et al.* 2011; Galvan *et al.* 2014), while higher contamination rates and oocyst counts were reported in northern Spain (Castro-Hermida *et al.* 2010) and Canada (Loganthan *et al.* 2012). In most of these studies, contamination rates with *Giardia* were slightly higher, with higher cyst counts, compared to *Cryptosporidium*. Similarly, in the present study *Giardia* cyst concentrations were 0.23–0.7 *Giardia* cysts/L. Based on the observed (oo)cyst counts, the estimated infection risk for *Cryptosporidium* varied from 2.79 to 5.74×10^{-5} per swim per person, while for *Giardia* the infection risk was between 1.46×10^{-4} and 7.04×10^{-5} per swim per person. For the limited contact water recreation activities the estimated infection risk was lower than for swimming for *Cryptosporidium* (5.71×10^{-6}), and comparable for *Giardia* (1.47×10^{-5}). Assuming only one visit to one of the recreational lakes per year, the infection risk for *Giardia* associated with swimming in recreational lakes was already above the generally accepted criterion of <1 infection per 10,000 individuals (USEPA 1989) despite the fact that all the investigated lakes had at least good water quality, according to the criteria of Directive 2006/7/EC. In this directive, bathing water quality is defined by threshold values for microbiological parameters, corresponding to four bathing qualities ('excellent', 'good', 'average' and 'poor'). As parasites are not covered by this

directive, they are not routinely monitored. The results of this study and other studies (Schets *et al.* 2008, 2011a, 2011b) suggest that infection risk for *Cryptosporidium* and *Giardia* cannot be extrapolated from the commonly used parameters for bathing water quality. However, it should be noted that the infection risk for *Cryptosporidium* and *Giardia* might be prone to bias. First, we used the total (oo)cyst concentrations to estimate this risk, assuming that all (oo)cysts were assumed to be viable in the risk assessment. Second, not all *Cryptosporidium* and *Giardia* species that were detected are infectious to humans. Regarding *Cryptosporidium*, only *C. andersoni* was identified. Although *C. andersoni* has been reported in human patients (e.g. Jiang *et al.* 2014), it is usually associated with cattle. Similarly, the frequently identified zoonotic *Giardia duodenalis* assemblage AI (6/7 samples) is mostly found in animals (Sprong *et al.* 2009). Other assemblages that were identified are either livestock-specific (E) or found predominantly in animals (BIV-like), and only in two samples were human-specific assemblages (AII, BIII) identified. Although it cannot be excluded that other species or genotypes were overlooked, e.g. due to poor recovery of DNA, these data suggest that animals, possibly livestock, were the predominant source of contamination for the investigated recreational lakes, indicating that the risk of these protozoa might be overestimated. Finally, we would like to underscore that the choice of the dose-response parameter p_m has an important impact on the final risk assessment. This is particularly the case when the dose-response varies considerably between different *Cryptosporidium* species and *G. duodenalis* (sub)assemblages. For example, within *C. parvum* a large variation in dose-response estimates was observed, ranging from 5.3×10^{-3} (Iowa-isolate) to 59.0×10^{-3} ('Ungar *C. parvum*' isolate) (Messner *et al.* 2001). In the present study, we allowed for this variation in the risk assessment of *Cryptosporidium*, but not for *Giardia* as currently little is known about the variation in dose-response between different isolates and (sub)assemblages.

Giardia cysts were detected in water samples from three out of 17 fountains and two out of seven splash parks. Although no *Cryptosporidium* oocysts were found, it cannot be excluded that low concentrations of oocysts were missed, given the limited sensitivity of the USEPA 1623 method. The infection risk during a splash park visit

equalled 1.68×10^{-4} , and hence, of all water-related activities, poses the highest risk for a *Giardia* infection. Outbreaks of cryptosporidiosis and giardiasis related to visits to water fountains have been documented in the USA (e.g. Centers for Disease Control and Prevention (CDC) 1998, 2000; Eisenstein et al. 2008; Kirian et al. 2008), suggesting that the presence of *Giardia* in fountains may constitute a real risk for waterborne infection.

CONCLUSIONS

Cryptosporidium oocysts and/or *Giardia* cysts were detected in swimming pools, recreational lakes, splash parks and water fountains in Belgium. Although in recreational lakes (oo)cysts were frequently present, most positive samples belonged to species/genotypes that are either animal-specific or predominantly found in animals, suggesting that the risk of infection during recreation is relatively low. Lower contamination rates were found in swimming pools, splash parks and water fountains, but assuming that humans are the most probable source of contamination for these water bodies, these findings suggest a risk for human infection.

REFERENCES

- Abd El-Salam, M. M. 2012 Assessment of water quality of some swimming pools: a case study in Alexandria, Egypt. *Environ. Monit. Assess.* **184** (12), 7395–7406.
- Baldursson, S. & Karanis, P. 2011 Waterborne transmission of protozoan parasites: review of worldwide outbreaks – an update 2004–2010. *Water Res.* **45** (20), 6603–6614.
- Boehmer, T. K., Alden, N. B., Ghosh, T. S. & Vogt, R. L. 2009 Cryptosporidiosis from a community swimming pool: outbreak investigation and follow-up study. *Epidemiol. Infect.* **137** (11), 1651–1654.
- Briancesco, R. & Bonadonna, L. 2005 An Italian study on *Cryptosporidium* and *Giardia* in wastewater, fresh water and treated water. *Environ. Monit. Assess.* **104** (1–3), 445–457.
- Cantey, P. T., Kurian, A. K., Jefferson, D., Moerbe, M. M., Marshall, K., Blankenship, W. R., Rothbarth, G. R., Hwang, J., Hall, R., Yoder, J., Brunkard, J., Johnston, S., Xiao, L., Hill, V. R., Sarisky, J., Zarate-Bermudez, M. A., Otto, C. & Hlavsa, M. C. 2012 Outbreak of cryptosporidiosis associated with a man-made chlorinated lake-Tarrant County, Texas, 2008. *J. Environ. Health.* **75** (4), 14–19.
- Castro-Hermida, J. A., Garcia-Preseido, I., Gonzalez-Warleta, M. & Mezo, M. 2010 *Cryptosporidium* and *Giardia* detection in water bodies of Galicia, Spain. *Water Res.* **44** (20), 5887–5896.
- Causser, L. M., Handzel, T., Welch, P., Carr, M., Culp, D., Lucht, R. & Mudahar, K. 2006 An outbreak of *Cryptosporidium hominis* infection at an Illinois recreational waterpark. *Epidemiol. Infect.* **134** (1), 147–156.
- CDC 1998 Outbreak of cryptosporidiosis associated with a water sprinkler fountain–Minnesota, 1997. *MMWR Morb. Mortal. Wkly. Rep.* **47** (40), 856–860.
- CDC 2000 Outbreak of gastroenteritis associated with an interactive water fountain at a beachside park–Florida, 1999. *MMWR Morb. Mortal. Wkly. Rep.* **49** (25), 565–568.
- Coetzee, N., Edeghere, O., Orendi, J., Chalmers, R. & Morgan, L. 2008 A swimming pool-associated outbreak of cryptosporidiosis in Staffordshire, England, October to December 2007. *Eurosurveill.* **13** (45), pii: 19028.
- Coupe, S., Delabre, K., Pouillot, R., Houdart, S., Santillana-Hayat, M. & Derouin, F. 2006 Detection of *Cryptosporidium*, *Giardia* and *Enterocytozoon bieneusi* in surface water, including recreational areas: a one-year prospective study. *FEMS Immunol. Med. Microbiol.* **47** (3), 351–359.
- Craun, G. F., Calderon, R. L. & Craun, M. F. 2005 Outbreaks associated with recreational water in the United States. *Int. J. Environ. Health Res.* **15** (4), 243–262.
- Dale, K., Kirk, M., Sinclair, M., Hall, R. & Leder, K. 2010 Reported waterborne outbreaks of gastrointestinal disease in Australia are predominantly associated with recreational exposure. *Aust. N Z. J. Public Health.* **34** (5), 527–530.
- de Man, H., Leenen, E. J., van Knapen, F. & de Roda Husman, A. M. 2014 Risk factors and monitoring for water quality to determine best management practices for splash parks. *J. Water Health* **12** (3), 399–403.
- Dorevitch, S., Panthi, S., Huang, Y., Li, H., Michalek, A. M., Pratap, P. & Wroblewski, M. 2011 Water ingestion during water recreation. *Water Res.* **45** (5), 2020–2028.
- Ehsan, Md. A., Geurden, T., Casaert, S., Paulussen, J., De Coster, L., Schoemaker, T., Chalmers, R., Grit, G. H., Vercruyse, J. & Claerebout, E. 2014 *Cryptosporidium* and *Giardia* in different water catchments in Belgium. *Environ. Monit. Assess.* **187** (2), 4157. doi:10.1007/s10661-014-4157-z.
- Eisenstein, L., Bodager, D. & Ginzl, D. 2008 Outbreak of Giardiasis and cryptosporidiosis associated with a neighborhood interactive water fountain – Florida, 2006. *J. Environ. Health.* **71** (3), 18–22; quiz 49–50.
- Fayer, R. 2004 *Cryptosporidium*: a water-borne zoonotic parasite. *Vet. Parasitol.* **126** (1–2), 37–56.
- Feng, Y. & Xiao, L. 2011 Zoonotic potential and molecular epidemiology of *Giardia* species and Giardiasis. *Clin. Microbiol. Rev.* **24** (1), 110–140.
- Fournier, S., Dubrou, S., Liguory, O., Gaussin, F., Santillana-Hayat, M., Sarfati, C., Molina, J. M. & Derouin, F. 2002 Detection of Microsporidia, cryptosporidia and *Giardia* in swimming pools: a one-year prospective study. *FEMS Immunol. Med. Microbiol.* **33** (3), 209–213.

- Galvan, A. L., Magnet, A., Izquierdo, F., Fernandez Vadillo, C., Peralta, R. H., Angulo, S., Fenoy, S. & del Aguila, C. 2014 A year-long study of *Cryptosporidium* species and subtypes in recreational, drinking and wastewater from the central area of Spain. *Sci. Total Environ.* **468–469**, 368–375.
- Geurden, T., Geldhof, P., Levecke, B., Martens, C., Berkvens, D., Casaert, S., Vercruyse, J. & Claerebout, E. 2008 Mixed *Giardia duodenalis* assemblage A and E infections in calves. *Int. J. Parasitol.* **38** (2), 259–264.
- Geurden, T., Levecke, B., Caccio, S. M., Visser, A., De Groote, G., Casaert, S., Vercruyse, J. & Claerebout, E. 2009 Multilocus genotyping of *Cryptosporidium* and *Giardia* in non-outbreak related cases of diarrhoea in human patients in Belgium. *Parasitology.* **136** (10), 1161–1168.
- Helmi, K., Skrabber, S., Burnet, J. B., Leblanc, L., Hoffmann, L. & Cauchie, H. M. 2011 Two-year monitoring of *Cryptosporidium parvum* and *Giardia lamblia* occurrence in a recreational and drinking water reservoir using standard microscopic and molecular biology techniques. *Environ. Monit. Assess.* **179** (1–4), 163–175.
- Hlavsa, M. C., Watson, J. C. & Beach, M. J. 2005 Giardiasis surveillance—United States, 1998–2002. *MMWR Surveill. Summ.* **54** (1), 9–16.
- Homan, W. L. & Mank, T. G. 2001 Human giardiasis: genotype linked differences in clinical symptomatology. *Int. J. Parasitol.* **31** (8), 822–826.
- Hopkins, J., Hague, H., Hudgin, G., Ross, L. & Moore, D. 2013 An outbreak of *Cryptosporidium* at a recreational water park in Niagara Region, Canada. *J. Environ. Health.* **75** (9), 28–33.
- Insulander, M., Lebbad, M., Stenstrom, T. A. & Svenungsson, B. 2005 An outbreak of cryptosporidiosis associated with exposure to swimming pool water. *Scand. J. Infect. Dis.* **37** (5), 354–360.
- Jiang, Y., Ren, J., Yuan, Z., Liu, A., Zhao, H., Liu, H., Chu, L., Pan, W., Cao, J., Lin, Y. & Shen, Y. 2014 *Cryptosporidium andersoni* as a novel predominant *Cryptosporidium* species in outpatients with diarrhea in Jiangsu Province, China. *BMC Infect. Dis.* **14**, 555.
- Karanis, P., Kourenti, C. & Smith, H. 2007 Waterborne transmission of protozoan parasites: a worldwide review of outbreaks and lessons learnt. *J. Water. Health* **5** (1), 1–38.
- Kirian, M. L., Meregillano, G., Gennette, D. & Weintraub, J. M. 2008 Multi-jurisdictional investigation of interactive fountain-associated cryptosporidiosis and salmonellosis outbreaks. *Epidemiol. Infect.* **136** (11), 1547–1551.
- Lalle, M., Pozio, E., Capelli, G., Bruschi, F., Crotti, D. & Caccio, S. M. 2005 Genetic heterogeneity at the beta-giardin locus among human and animal isolates of *Giardia duodenalis* and identification of potentially zoonotic subgenotypes. *Int. J. Parasitol.* **35** (2), 207–215.
- Levecke, B., Geldhof, P., Claerebout, E., Dorny, P., Vercammen, F., Caccio, S. M., Vercruyse, J. & Geurden, T. 2009 Molecular characterisation of *Giardia duodenalis* in captive non-human primates reveals mixed assemblage A and B infections and novel polymorphisms. *Int. J. Parasitol.* **39** (14), 1595–1601.
- Loganathan, S., Yang, R., Bath, A., Gordon, C. & Ryan, U. 2012 Prevalence of *Cryptosporidium* species in recreational versus non-recreational water sources. *Exp. Parasitol.* **131** (4), 399–403.
- Mattsson, J. G., Insulander, M., Lebbad, M., Bjorkman, C. & Svenungsson, B. 2008 Molecular typing of *Cryptosporidium parvum* associated with a diarrhoea outbreak identifies two sources of exposure. *Epidemiol. Infect.* **136** (8), 1147–1152.
- Messner, M. J., Chappell, C. L. & Okhuysen, P. C. 2001 Risk assessment for *Cryptosporidium*: a hierarchical Bayesian analysis of human dose response data. *Water Research* **35** (16), 3934–3940.
- Morgan, U. M., Monis, P. T., Xiao, L., Limor, J., Sulaiman, I., Raidal, S. & O'Donoghue, P. 2001 Molecular and phylogenetic characterisation of *Cryptosporidium* from birds. *Int. J. Parasitol.* **31** (3), 289–296.
- Oliveri, R., Di Piazza, F., Marsala, B., Cerame, G., Firenze, A. & Di Benedetto, M. A. 2006 Occurrence of *Giardia* cysts and *Cryptosporidium* oocysts in swimming pools in the province of Palermo, Italy. *Ann. Ig.* **18** (5), 367–374.
- Olson, M., Goh, J., Phillips, M., Guselle, N. & McAllister, T. 1999 *Giardia* cyst and *Cryptosporidium* oocyst survival in water, soil and cattle feces. *J. Environ. Qual.* **28**, 1991–1996.
- Papadopoulou, C., Economou, V., Sakkas, H., Gousia, P., Giannakopoulos, X., Dontorou, C. & Filioussis, G. 2008 Microbiological quality of indoor and outdoor swimming pools in Greece: investigation of the antibiotic resistance of the bacterial isolates. *Int. J. Hyg. Environ. Health.* **211** (3–4), 385–397.
- Porter, J. D., Ragazzoni, H. P., Buchanon, J. D., Waskin, H. A., Juranek, D. D. & Parkin, W. E. 1988 *Giardia* transmission in a swimming pool. *Am. J. Public Health.* **78** (6), 659–662.
- Ryan, U., Xiao, L., Read, C., Zhou, L., Lal, A. A. & Pavlasek, I. 2003 Identification of novel *Cryptosporidium* genotypes from the Czech Republic. *Appl. Environ. Microbiol.* **69** (7), 4302–4307.
- Schets, F. M., Engels, G. B. & Evers, E. G. 2004 *Cryptosporidium* and *Giardia* in swimming pools in the Netherlands. *J. Water Health* **2** (3), 191–200.
- Schets, F. M., van Wijnen, J. H., Schijven, J. F., Schoon, H. & de Roda Husman, A. M. 2008 Monitoring of waterborne pathogens in surface waters in Amsterdam, the Netherlands, and the potential health risk associated with exposure to *Cryptosporidium* and *Giardia* in these waters. *Appl. Environ. Microbiol.* **74** (7), 2069–2078.
- Schets, F. M., De Roda Husman, A. M. & Havelaar, A. H. 2011a Disease outbreaks associated with untreated recreational water use. *Epidemiol. Infect.* **139** (7), 1114–1125.
- Schets, F. M., Schijven, J. F. & de Roda Husman, A. M. 2011b Exposure assessment for swimmers in bathing waters and swimming pools. *Water Res.* **45** (7), 2392–2400.

- Shields, J. M., Gleim, E. R. & Beach, M. J. 2008 Prevalence of *Cryptosporidium* spp. and *Giardia intestinalis* in swimming pools, Atlanta, Georgia. *Emerg. Infect. Dis.* **14** (6), 948–950.
- Smith, A., Reacher, M., Smerdon, W., Adak, G. K., Nichols, G. & Chalmers, R. M. 2006 Outbreaks of waterborne infectious intestinal disease in England and Wales, 1992–2003. *Epidemiol. Infect.* **134** (6), 1141–1149.
- Sprong, H., Caccio, S. M. & van der Giessen, J. W. 2009 Identification of zoonotic genotypes of *Giardia duodenalis*. *PLoS Negl. Trop. Dis.* **3** (12), e558.
- Stafford, R., Neville, G., Towner, C. & McCall, B. 2000 A community outbreak of *Cryptosporidium* infection associated with a swimming pool complex. *Commun. Dis. Intell.* **24** (8), 236–239.
- Sulaiman, I. M., Fayer, R., Bern, C., Gilman, R. H., Trout, J. M., Schantz, P. M. & Das, P. 2003 Triosephosphate isomerase gene characterization and potential zoonotic transmission of *Giardia duodenalis*. *Emerg. Infect. Dis.* **9** (11), 1444–1452.
- Takagi, M., Toriumi, H., Endo, T., Yamamoto, N. & Kuroki, T. 2008 An outbreak of cryptosporidiosis associated with swimming pools. *Kansenshogaku Zasshi.* **82** (1), 14–19.
- Teunis, P. F. M., Van der Heijden, O. G., Van der Giessen, J. W. B. & Havelaar, A. H. 1996 *The Dose-Response Relation in Human Volunteers for Gastro-Intestinal Pathogens*. RIVM, Bilthoven.
- USEPA 1989 <http://water.epa.gov/lawsregs/rulesregs/sdwa/swtr/upload/SWTRPlainEnglishGuideFinal.pdf>.
- USEPA 2005 <http://www.epa.gov/microbes/1623de05.pdf>.
- Waldron, L. S., Ferrari, B. C., Cheung-Kwok-Sang, C., Beggs, P. J., Stephens, N. & Power, M. L. 2011 Molecular epidemiology and spatial distribution of a waterborne cryptosporidiosis outbreak in Australia. *Appl. Environ. Microbiol.* **77** (21), 7766–7771.
- Wheeler, C., Vugia, D. J., Thomas, G., Beach, M. J., Carnes, S., Maier, T., Gorman, J., Xiao, L., Arrowood, M. J., Gilliss, D. & Werner, S. B. 2007 Outbreak of cryptosporidiosis at a California waterpark: employee and patron roles and the long road towards prevention. *Epidemiol. Infect.* **135** (2), 302–310.
- WHO 2003 *Guidelines for safe recreational water environments (Volume 1: Coastal and Fresh Waters)*. World Health Organization, Geneva, Switzerland.
- WIV 2010 <http://www.wiv-isp.be/epidemiology/epinl/plabnl/plabannl/index10.htm>.

First received 10 November 2014; accepted in revised form 1 March 2015. Available online 8 April 2015