Faecal contamination in public pools in Barcelona province: *Cryptosporidium* spp. and bacterial indicators

M. Gracenea, S. Castaño, J. Méndez, F. Lucena and M. S. Gómez

**ABSTRACT**

A study was conducted of the occurrence of *Cryptosporidium* in indoor heated public swimming pools and of three bacterial indicators (*Escherichia coli*, faecal enterococci and *Clostridium perfringens*) on pool surrounds. Although all examined pools adhered strictly to the Spanish regulations, the influence of several parameters related to water conditions, pool structure, users and location on the presence of protozoa and bacteria was analysed. *Cryptosporidium* was detected in 18.8% of pools in 60% of the five towns studied. The maximum concentration was 13 oocysts/L in one swimming pool and one Jacuzzi. The bacterial indicators’ prevalence on pool surrounds was higher than 50%, being present in all of the towns. Plastic surfaces presented the lowest bacterial prevalence, whereas painted surfaces were 100% positive. No differences were observed for pool surrounds with autonomous or disabled users. Risk of cryptosporidiosis in pool vessels indicated that concentrations over 1 oocyst/10 L enhance the risk of infection, even in one exposure. Guidelines for managing faecal accidents and public information on the importance of good hygiene behaviours in and around swimming pools are recommended to limit oocysts’ presence.

**Key words** | bacterial indicators, *Cryptosporidium* spp., quantitative microbial risk assessment (QMRA), swimming pools

**INTRODUCTION**

Nowadays, many sports centres in developed countries offer various sports and wellness activities involving water. To ensure the safety of pools and spas, the water is filtered, disinfected and monitored using faecal bacterial indicators, which provide information about water quality. However, some human pathogens, such as *Cryptosporidium*, a waterborne protozoan parasite, are more resistant to the standard treatment than bacterial indicators (McGuigan et al. 2006; Shields et al. 2008a). Several cryptosporidiosis outbreaks associated with swimming in public pools have been reported worldwide (Baldrusson & Karanis 2011). In some cases, *Cryptosporidium* oocysts have been detected in the pool water (Hunt et al. 1994; Lemmon et al. 1996; Furtado et al. 1998) or in the filter backwash water (McAnulty et al. 1994; Schets et al. 2004; Shields et al. 2008b), confirming the source of the outbreak. This pathogen has been highlighted as the primary causative agent of swimming pool-associated outbreaks (Hlavsa et al. 2014).

Infected individuals excrete large numbers of *Cryptosporidium* oocysts (Pond 2005). Although some cases are asymptomatic (Cotruvo et al. 2004), the most common symptom of cryptosporidiosis is watery diarrhoea that can become chronic in immunocompromised hosts. In healthy adults, the infective dose is low, at between 10 and 30 oocysts (Yoder & Beach 2010). Several cryptosporidiosis outbreaks associated with swimming in public pools have been reported worldwide (Baker et al. 1998; Louie et al. 2004; Insulander et al. 2005; Black & McAnulty 2006; World Health Organization 2006; Takagi et al. 2008). Many conditions are conducive to the occurrence of oocysts in pools, including chlorine resistance of *Cryptosporidium* (Shields et al. 2008a), defective filter systems, poor user hygiene and high bather densities.
The main microorganisms used to assess the microbial quality of swimming pools include faecal indicators such as the thermotolerant coliforms, *Escherichia coli* and *Enterococcus* spp. However, these present problems such as limited survival in water, the ability to multiply in water and sensitivity to disinfection agents, and it is therefore recommended to also use alternative anaerobic bacteria as faecal indicators, such as *Clostridium perfringens* (World Health Organization 2006). In Spain, Royal Decree 742/2013 sets out specific requirements regarding microbiological criteria and swimming pool monitoring: for every 100 mL of pool water analysed, no *E. coli* or *Pseudomonas aeruginosa* should be detected. In addition, *Legionella* spp. monitoring is mandatory in heated pools or pools with aeration in the pool vessel, and concentrations must be lower than 100 CFU/L.

When pool disinfection levels are inadequate, many microorganisms tend to form associations with other microorganisms on the surface of the pool water, creating biofilms. In some cases, these associations render it much more difficult to achieve suitable disinfection, and higher levels of disinfectant are required to penetrate and inactivate the biofilm (Bonnick 2006).

The principal objective of this cross-sectional study was to conduct a quantitative and qualitative determination of *Cryptosporidium* oocysts in swimming pools in different towns in the province of Barcelona, and of three bacterial indicators, *E. coli*, faecal enterococci and *Cl. perfringens*, on pool surrounds, in the absence of a reported cryptosporidiosis outbreak. In addition, a quantitative microbial risk assessment (QMRA) was conducted to assess the health risk of *Cryptosporidium* spp. infection associated with recreational water use in public facilities.

The results indicate the current status of the pools and the sanitary quality of both the facilities and the pool water. An analysis was conducted of the influence of physical, chemical and social parameters on the presence of these organisms. The location of the swimming pools in towns and sports facilities was also studied.

**METHODS**

*Cryptosporidium* detection was carried out in 32 indoor pools (21 swimming pools and 11 Jacuzzis) by collecting 10 L of the pool water of each pool. The pools surrounds were analysed in 28 indoor pools by collecting water accumulations in shower areas located close to the vessel entrance.

Pools were located in ten sports centres in five towns close to the city of Barcelona.

In order to study the influence of various factors, the following parameters were recorded for each sample: water pH (6.99–7.80), water temperature (<25 °C (low), 25–29 °C (medium) and >30 °C (high)), chemical treatment (chlorine, chlorine with UV and bromine), pool volume (<100 m³ (small), 100–750 m³ (medium) and >750 m³ (large)), type of pool (swimming pool or Jacuzzi), number of users per day, presence of aeration (in the vessel), pool temperature (swimming pool or Jacuzzi), and material of pool surround surface (smooth or rough/ceramic, plastic or paint), average daily number of users (<100 (low), 100–500 (medium) and >500 (high)), user age (children or adults) and user conditions (autonomous or disabled). Regarding filter system, all pools employed sand filters.

For *Cryptosporidium* detection and quantification, 10 L water samples were collected, processed by flocculation with calcium carbonate (Vesey et al. 1993) followed by immunomagnetic separation (IMS), and identified by Ziehl-Neelsen staining. According to Greinert et al. (2004), flocculation and IMS are effective tools for the detection and quantification of *Cryptosporidium* spp. in water samples.

For each bacterial indicator detection, 100 mL water samples were collected from each pool surround, transported at 4 °C and analysed within 12 hours of sampling. Faecal enterococci and *E. coli* were detected by the membrane filtration method described in ISO 7899-2:2000 and ISO 9308-1:2000, respectively (International Organization for Standardization 2000a, 2000b). *Cl. perfringens* samples were heated at (80 ± 1) °C during 10 minutes before culturing on SPS agar medium according to Bufton (1959). Bacterial results under the limit of detection were considered negative samples. Statistical analysis was performed using PAWS Statistics v17, and the influence of the compiled data was studied (*p* < 0.05).

An oocyst ingestion risk assessment was performed for each pool with a positive detection. The Monte Carlo simulation for QMRA analysis was carried out using the R-package mc2d (Pouillot & Delignette-Muller 2010). Two scenarios were defined for the possible occurrence of
The presence of the oocysts in the pool water was considered as an effect of a faecal accident. Two scenarios were considered. A recent contamination scenario in which the faecal accident occurred randomly during the swimming activity; and a second scenario, in which the faecal accident had occurred previously and the lapse of time between the accident and the exposition was the half turnover time. In this scenario, the lapse of time was modelled as a random uniform variable ranging from 90 to 150 minutes.

8. Despite the possibility that some of the detected oocysts were not infective, since no infectivity assay was carried out, all oocysts were considered as if they were infective.

**RESULTS**

*Cryptosporidium* was detected in 18.8% of the analysed pools, and the maximum concentration observed was 15 oocysts/L (Table 1). The general prevalence of bacterial indicators was 78.6%, and all three were simultaneously present on 57.1% of the pool surrounds. The maximum concentrations detected were within the limits reported in greywater studies (Table 1) (Laine 2001; Ottoson & Stenström 2003; Birks et al. 2004; Winward 2008).

The average water pH was virtually constant (6.99–7.80), and did not present a statistically significant relationship ($R^2 = 0.037$) with the presence of *Cryptosporidium* oocysts. The prevalence of *Cryptosporidium* oocysts in swimming pools with different temperatures and water disinfection treatments indicated that the protozoan was only detected in pools with temperatures above 25 °C (prevalence 20%), and it was found with all three chemical treatments, although the highest prevalence (66.7%) was observed in pools disinfected with chlorine plus UV (Table 2).
A water temperature above 25 °C was conducive to the simultaneous presence of the protozoan and the three bacterial indicators \(E. \text{ coli}\), faecal enterococci and \(Cl. \text{ perfringens}\), and this was observed in two pools. The simultaneous presence of the three bacterial indicators was observed in pools at all temperatures, being present in 6 of the 10 pools with temperatures ranging from 25 to 30 °C (60%) and in 9 of the 17 pools with temperatures above 30 °C (52.9%).

As regards water treatment, parasitic and bacterial contamination was simultaneously detected in two pools, one treated with chlorine and another with chlorine and UV. The three bacterial indicators were simultaneously present with a similar prevalence in chlorinated pools (10 out of 18 pools, 55.6%) and bromine-treated ones (5 of 8 pools, 62.5%), and these values were higher than those for pools disinfected with chlorine and UV (50% of pools).

Oocysts were more frequently detected in swimming pools than in Jacuzzis (Table 2), but the maximum oocyst concentration (13 oocysts/L) was observed in one swimming pool and one hot Jacuzzi (Table 1). In terms of pool size, large pools (>750 m³) were the most positive for \(Cryptosporidium\) oocysts (Table 2). The prevalence of bacterial indicators was quite similar for swimming pools and

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**Table 1**  | Parasitic and microbiological results in relation to temperature (T), volume (V), chemical treatment (CT), daily users, user age, user type and surface type and material

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<th>V (m³)</th>
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SC, sports centre; J, Jacuzzi; Ad, adults; C, children; A, autonomous; A&D, autonomous and disabled; EC, \(E. \text{ coli}\); FE, faecal enterococci; SRC, \(Cl. \text{ perfringens}\); (*), cfu/100 mL; <LOD, below the limit of detection; NS, no sample.
Jacuzzis (Table 2). Two swimming pools presented oocysts and bacterial indicators at the same time, but no Jacuzzis were positive for the parasite and the bacterial indicators simultaneously (Table 1).

Pool volume did not seem to influence the presence of oocysts, since the protozoan was detected at all volumes, although the highest prevalence was recorded in two large pools (33.3%), being lower in medium-sized (3 out of 11, 27.3%) and small pools (1 out of 15, 6.7%) (Table 2). The protozoan and any of the three bacterial indicators were simultaneously found in two medium-sized pools but not in large ones. In addition, the simultaneous presence of the three bacterial indicators was observed in pools at all volumes: in 2 of the 6 large pools (33.3%), in 7 of the 11 medium-sized pools (63.6%) and in 7 of the 11 small pools (63.6%), although the lowest prevalence detected was in large pools.

Smooth surfaces were positive for bacterial indicators (E. coli 50%, faecal enterococci 75% and Cl. perfringens 100%) and quite similar to rough ones (E. coli 58.3%, faecal enterococci 87.5% and Cl. perfringens 66.7%). Among the rough surfaces, bacterial indicators were more prevalent (100% prevalence for the three indicators) on painted surfaces than on plastic ones (37.5–62.5%).

The daily number of users influenced the presence of Cryptosporidium, since pools with fewer users (<100) were all negative, whereas pools with a medium user frequency (100–500) were the most positive for oocyst prevalence (25%) (Table 2). These latter were also the most positive for faecal indicators, since the three bacteria (E. coli, faecal enterococci and Cl. perfringens) were simultaneously present in 52.9% of these pools. In addition, oocysts and bacterial indicators were simultaneously present in two pools with a medium daily number of users.

The highest prevalence of Cryptosporidium was detected in pools used by adults and children (27.8%) (Table 2). Pools used exclusively by adults or children were negative and only one pool used by adults had Cryptosporidium. Although the presence of bacterial indicators was not influenced by user age,
the same type of bacteria was not detected in all pools. Pools used exclusively by children only presented faecal enterococci, whereas pools used only by adults presented the three bacterial indicators assessed (Table 2). The simultaneous presence of Cryptosporidium and bacterial indicators was only detected in one pool used by all ages (pool 10, Table 1).

Pools used by autonomous people were more positive for Cryptosporidium (26.3%) than those frequented by disabled users (Table 2). Type of user did not influence the presence of faecal indicators on pool surrounds, and bacterial prevalence was very similar for E. coli, faecal enterococci and Cl. perfringens (Table 2). Only two pools with autonomous users simultaneously presented Cryptosporidium oocysts and the three bacterial indicators (pools 10 and 17, Table 1).

Of the five towns in the province of Barcelona where the swimming pools were located, Cryptosporidium oocysts were found in three towns (α, β, and ε). The parasite was detected in 25% of pools in two of these towns, and in 14.3% of pools in the other town. The 32 pools analysed were located in ten sports centres, four of which were positive for Cryptosporidium (Table 2). All of the sports centres were positive for one or more of the three bacterial indicators, with one exception, sports centre B (Table 1). Two sports centres presented the three indicators simultaneously in all of their pools analysed (sports centres E and G) and one of these also presented Cryptosporidium oocysts (sports centre C). Only one sports centre was negative for bacterial and parasitic contamination (sports centre B) (Table 1).

No statistically significant influence on bacterial and protozoan contamination was detected for water pH or temperature, chemical treatment, volume of pool, type of pool, type and material of the pool surround, average daily number of users, user age, user conditions or location of the swimming pools in towns and sports facilities.

In the QMRA analysis, the ingestion model comprised water ingestion by child and adult swimmers. The 95% CI of water intake was 0 to 88.5 mL per child and 0 to 45 mL per adult. The modelled 95% CI of average ingestion was 1.77 to 65.8 mL per swimmer. The simulated recovery rate showed a mean value of 65%, with a standard deviation of 4.99%, and the respective 95% CI was 55.2% to 74.8%, with a minimum value of 43% and a maximum of 86.4%.

Regarding disinfection/removal processes, similar risk results were obtained regardless of whether UV disinfection was considered or not, and the main oocyst elimination process was removal by filtration.

Table 3 summarizes the results obtained in the risk simulations, and shows the mean risk values for the 97.5th percentile for each dose–response models (six models) assayed under two scenarios, scenario A, which corresponds to a recent faecal contamination and scenario B, which corresponds to a 50% of oocysts’ removal due to water turnover. In both scenarios, it was distinguished between adult and children swimmers.

It is worth noting that the risk was barely acceptable, considering the level of acceptable risk threshold of $1 \times 10^{-4}$, in the less restrictive exponential dose–response models with an oocyst concentration of 1 oocyst/10 L using the dose–response models based on Messner et al. (2001) for the Iowa isolate or the model based on DuPont et al. (1995).

Assuming a homogeneous distribution of Cryptosporidium parvum in the water, oocyst concentrations in the other dose–response models indicated an acceptable risk level for children under the most restrictive modelling assumptions: for the exponential dose–response model ($r = 0.0572$) $\leq 1$ oocysts/70 L, and for the Beta-Poisson the allowed concentration ranged from $\leq 1$ oocyst in 118 L ($\alpha = 0.145, \beta = 1.52$) to $\leq 1$ oocyst in 238 L ($\alpha = 0.270, \beta = 1.40$). The results of the sensitivity analysis based on Spearman’s correlations were quite similar for the different dose–response models. Statistically significant differences were observed for the maximum oocyst concentration and the limit of detection.

When the maximum was analysed, the main factor associated with the risk of infection was the water ingested during swimming ($p \approx 0.93$) followed by the concentration of oocysts in the water ($p \approx 0.53$). Moreover, a negligible correlation ($p \approx 0.088$) was observed between the recovery rate and the oocyst removal/disinfection rate ($p, -0.007$ to $-0.02$).

In contrast, for the limit of detection, the sensitivity analysis showed a high correlation ($p \approx 0.98$) between lower oocyst concentration values and ingestion ($p \approx 0.03$), the recovery rate ($p \approx 0.002$) and the oocyst removal/disinfection rate ($p, -0.006$ to $-0.0002$).
Table 3 | Cryptosporidium spp. risk assessment considering (a) fresh contamination due to accidental faecal release and (b) after 50% water turnover

<table>
<thead>
<tr>
<th>Exponential dose-response models</th>
<th>Beta-Poisson dose-response models</th>
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<tr>
<td></td>
<td>r – 0.05720⁺ᵇ</td>
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<td></td>
<td>1.07 × 10⁻³</td>
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<td>(a)</td>
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<tr>
<td></td>
<td>4.75 × 10⁻⁴</td>
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<td>3.36 × 10⁻³</td>
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<td></td>
<td>4.93 × 10⁻³</td>
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<td></td>
<td>8.73 × 10⁻³</td>
</tr>
<tr>
<td></td>
<td>1.11 × 10⁻²</td>
</tr>
</tbody>
</table>

*Preferred model.
*Meissner et al. (2001).
*DuPont et al. (1995).
*Chappell et al. (2006).
*Okhuysen et al. (2002).

DISCUSSION

The prevalence of Cryptosporidium oocysts observed in this study (18.8%) is similar to the 16.6% reported by Fournier et al. (2002) for pools in Paris and quite close to the 28.5% reported by Oliveri et al. (2006) in Palermo.

No significant correlation between temperature or disinfection treatment with the presence of Cryptosporidium was found. In addition, pool water did not seem to influence the presence of oocysts, since the protozoan was detected in all of the volumes considered, although the highest prevalence was observed in two large pools (33.3%), and prevalence was lower in medium-sized (3 out of 11, 27.3%) and small pools (1 out of 15, 6.7%) (Table 2). In contrast to the results obtained in the present study, Shields et al. (2008a, 2008b) did not find oocysts in large pools, but observed a similar prevalence in small pools (5.5%). In a study of the city of Barcelona, Gómez et al. (2011) found the lowest prevalence in small pools, as in the present study; however, they found the maximum prevalence in medium-sized pools.

Regarding the type and material of pool surround surface (smooth or rough/ceramic, plastic or paint), it might be expected that rough surfaces would be more conducive to the persistence of faecal microorganisms, but the results of this study indicated that smooth surfaces were equally or more positive for bacterial indicators. The pool surround surfaces analysed were ceramic, plastic or painted, and the results for bacterial indicators showed that painted surfaces were conducive to faecal contamination (100% prevalence for the three indicators), whereas plastic surfaces seemed to partially prevent it (38–63% positive). Plastic materials such as PVC and HDPE are used for water pipes because they delay the growth of pathogens and biofilm; consequently, it is to be expected that plastic pool surround surfaces probably act in the same way.

In relation to the average daily number of users, both Gómez et al. (2011) and Shields et al. (2008a) have described different results to those obtained in this study, in which only one pool with more than 500 users was positive.

With regard to user age, Gómez et al. (2011) and Shields et al. (2008a) found the maximum prevalence of Cryptosporidium in pools used exclusively by children (10.6% and 66.7%, respectively). In this study, absence of Cryptosporidium in the single pool used exclusively by children was not significant. Nevertheless, the implementation of regulations concerning the mandatory use of nappies, may lead to an improvement in child hygiene measures. In accordance with this, Furtado et al. (1998) have suggested that prevention measures should be focused on small children in order to reduce pool water contamination by Cryptosporidium oocysts. The simultaneous presence of Cryptosporidium and bacterial indicators only occurred in two pools, one used by adults and the other by all ages. Therefore, both types of contamination could be considered independent of user age.

Of the five towns studied, the parasite was only present in three of them, and in four of eight sports centres located in these towns. These results suggest that the parasite is less widespread in towns close to Barcelona than in the city itself, where all the neighbourhoods studied by Gómez et al. (2011) were positive for the parasite and it was present in 84.6% of the sport centres.

Apparently, the presence of oocysts and bacterial indicators was independent of pool volume; there is no relation between the presence of the parasite and bacterial indicators, both types of contamination seemed to be independent. The results showed that users from the towns studied presented a similar microbiota and behaviour, and consequently no geographical differences were found. Thus, no relationship was observed between sports centres and the presence of these faecal microorganisms.

The QMRA results indicated that the absence of Cryptosporidium oocysts in 10 L might not be sufficient to ensure an acceptable risk (Table 3).

Results from similar studies provided comparable situations. Pintar et al. (2010) reported a mean risk of $1.11 \times 10^{-5}$ and $2.57 \times 10^{-5}$, respectively, for adults and children in one visit to swimming pool. Schets et al. (2011) estimated a mean risk of $2.2 \times 10^{-3}$ for children swimmers, whereas the risk for adults ranged from $1.1 \times 10^{-3}$ to $1.5 \times 10^{-5}$, respectively, for women and men. Suppes et al. (2016) obtained a mean risk of $2.5 \times 10^{-4}$ for adult swimmers and $3.5 \times 10^{-4}$ for children. In the present study, mean concentration of oocysts of 1.47 oocysts/L corresponds to a risk of $9.5 \times 10^{-4}$ for adults and $1.95 \times 10^{-3}$ for children, using the preferred dose–response model of Messner et al. (2001).
Regarding the age of swimmers, children were the bathers with the highest infection risk, even assuming equal swim duration as adults, due to the increased ingestion of water. Moreover, the results of the sensitivity analysis differed according to the oocysts’ concentration; at lowest oocysts’ concentration the sensitivity was mainly influenced by the concentration of oocysts whereas at highest oocysts’ concentration ingestion was the most influential factor. That fact indicates that the increase of number of oocysts in water causes infection at low ingestion rates.

Acceptable risk levels are not achievable by conventional chemical treatments alone; although disinfectants can inactivate oocysts, their effectiveness depends on the limits approved by local regulations (0.5 to 2 mg/L \( \times \) of free chlorine or 2 to 5 mg/L \( \times \) of bromide under the Spanish regulations) and on contact time. Moreover, levels of disinfection must be maintained for sufficient time in order to be effective, and that level would be difficult to achieve in a reasonable timeframe sufficient to prevent ingestion exposures. Hence, it is essential to determine filtering removal efficiencies and pool turnover time in order to tackle oocyst-rich incidences.

Cryptosporidiosis is not a widespread disease in Spain, and Cryptosporidium tests are only performed in laboratories on specific request; thus, the actual annual number of cases is unknown. The frequency of accidental faecal releases and contamination of Spanish public swimming pools is also not known. In fact, only Galmes et al. (2003) have documented an outbreak associated with swimming pool users in Spain.

The present data on Cryptosporidium and faecal indicators constitute a timely contribution to knowledge of oocyst and microorganism prevalence in pools in five Catalonian towns. Since the study was not associated with a diarrhoeal outbreak, the results obtained indicate the current status of pools and provide evidence of faecal contamination in pools and facilities.

In this context, appropriate user behaviour and proper maintenance of facilities is crucial to reduce cryptosporidiosis outbreaks and faecal contamination in public swimming pools. The WHO (World Health Organization 2006) has provided a basis for standard swimming pool settings that represents a consensus view among experts related to the health risk posed by various recreational water media and activities, as well as the effectiveness of control measures to protect health.

On the other hand, usually, rules for sanitation and safety of swimming pools are clearly focused on the chemical and bacteriological quality of the pool water without, perhaps, paying adequate attention to pool surroundings.

**CONCLUSIONS**

The results show that the public swimming pools and spas analysed were in full compliance with Spanish standards. The detection of Cryptosporidium and the incidence of faecal indicator bacteria in swimming pool surrounds demonstrates the importance of proper maintenance and monitoring of pools and associated facilities.

The significant presence of bacterial indicators in some pool beaches indicates that swimmers do not assume the need to carry out careful personal hygiene before accessing the pool facility. Moreover, it can also indicate that Cryptosporidium was entering the water not only due to a faecal accident, but that it could be transported from outdoors by the swimmers themselves.

Risk of cryptosporidiosis associated with the presence of Cryptosporidium oocysts in pool vessels indicated that their absence in 10 L might not be sufficient to ensure an acceptable risk.

Given the resistance of \( C. \) parvum oocysts to the chemical disinfectants commonly used in pools, their presence in several pool water samples underlines the need for continued emphasis on improving pool staff and user knowledge and awareness to reduce the risk of pathogen transmission, by implementing good hygiene practices in and around swimming pools and promoting healthy swimming habits.

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