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

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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

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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 (Baldursson & 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.

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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 to 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 m^3 (small), 100–750 m^3 (medium) and >750 m^3 (large)), type of pool (swimming pool or Jacuzzi), type 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

oocysts: fresh contamination due to poor swimmer hygiene or accidental faecal release (scenario A), and after a 50% pool water turnover (scenario B).

The following assumptions were used for QMRA modelling:

1. The dose–response models and exposure models of water ingestion by swimmers were obtained from an analysis of the published literature on the Michigan State University QMRA online wiki: http://qmrawiki.msu.edu/index.php?title=Quantitative_Microbial_Risk_Assessment_%28QMRA%29_Wiki.
2. The water ingestion model was based on Dufour *et al.* (2006), and the dose–response models assessed were based on DuPont *et al.* (1995), Messner *et al.* (2001), Okhuysen *et al.* (2002) and Chappell *et al.* (2006). For the Monte Carlo simulations, we assumed a single exposure during swimming activity.
3. The probability density function of the *Cryptosporidium* concentration was considered uncertain with a Poisson density function with a mean equivalent to the oocyst value detected in each pool. Zero-values were substituted by the detection limit of the method (1 oocyst/10 L).
4. The recovery efficiency for *Cryptosporidium* was taken as normally distributed with a mean value of 65%, which was similar to the value reported by Karanis & Kimura (2002). Uncertainty was not measured either at the recovery step or at the microscopic identification phase; consequently, uncertainty associated with both steps in the modelling was dropped.
5. Regarding disinfection and removal processes that affect concentration or infectivity of the *Cryptosporidium* oocysts, the effect of chlorine was not considered. The filtration efficiency on the oocysts removal depends on diverse factors such as the use of coagulants and the filtration rate (Gregory 2002). Regarding UV irradiation Morita *et al.* (2002) showed that the infectivity of the oocysts decreased exponentially as the UV dose increased, and a dose of 1.0 mWs/cm^2 at 20 °C is enough to reach 2-log10 reduction in infectivity. In the QMRA, as a conservative approximation, it was considered that filtration and UV irradiation was used in all the analysed premises. The log-removal achieved by filtration and UV irradiation were modelled using triangular functions, filtration removal was defined with a minimum, mode and maximum log-reduction values, respectively, of 2, 3 and 4; whereas UV irradiation function was defined with log-reduction values of 4, 5 and 6, respectively, for minimum, mode and maximum.
6. Although water turnover depends on pool type and design, in the QMRA the water turnover time was considered as a random uniform variable ranging from 150 to 300 minutes.
7. 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 13 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).

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

Towns	SC	Pool	T (°C)	V (m^3)	CT	Daily users	User age	User type	Type/mat. surface	Weasel *Cryptos*. (oocy./L)	Beach		
											EC (*)	FE (*)	SRC (*)
α	A	1	28	2,500	Cl	900	All	A&D	Rough/Plastic	<LOD	9,275	1,900	275
		2	30	1,000	Cl	800	All	A	Rough/Plastic	5	<LOD	<LOD	<LOD
		3	33	310●	Br	100	Ad	A	Rough/Plastic	13	<LOD	<LOD	<LOD
		4	32	60	Cl	500	Ad	A	Rough/Plastic	<LOD	<LOD	145	30
		5	32	40	Cl	50	C	A	Rough/Plastic	<LOD	<LOD	40	<LOD
	B	6	29	594	Cl	400	All	A&D	Rough/Plastic	<LOD	<LOD	<LOD	<LOD
		7	35	1●	Br	100	Ad	A	Rough/Plastic	<LOD	NS	NS	NS
		8	35,1	1●	Br	100	Ad	A	Rough/Plastic	<LOD	NS	NS	NS
	C	9	29	264	Cl	150	All	A	Rough/Plastic	<LOD	280	165	25
		10	32	169	Cl	150	All	A	Rough/Plastic	3	154	325	25
		11	35.5	6●	Br	100	Ad	A	Smooth/Ceramic	<LOD	<LOD	2,700	180
		12	35.5	6●	Br	100	Ad	A	Smooth/Ceramic	<LOD	<LOD	<LOD	90
β	D	13	28.5	800	Cl	750	All	A&D	Rough/Ceramic	<LOD	44	30	220
		14	32	153.6	Cl	240	All	A	Rough/Ceramic	<LOD	<LOD	<LOD	<LOD
		15	31	90	Cl	225	All	A&D	Rough/Ceramic	<LOD	40	5,500	35
		16	29	469	Cl	410	All	A&D	Rough/Ceramic	<LOD	27	460	200
	E	17	28	408	Cl + UV	225	All	A	Rough/Paint	13	22	360	200
		18	28	48,3	Cl + UV	150	All	A	Rough/Paint	10	NS	NS	NS
		19	32	8●	Br	50	Ad	A	Smooth/Ceramic	<LOD	4,419	960	100
		20	32	8●	Br	50	Ad	A	Smooth/Ceramic	<LOD	114	510	125
γ	F	21	28	850	Cl	600	Ad	A&D	Rough/Ceramic	<LOD	<LOD	270	10
		22	32	300	Cl	175	All	A&D	Rough/Ceramic	<LOD	70	270	40
		23	15	1●	Br	600	Ad	A	Rough/Ceramic	<LOD	96	50	10
		24	34	50●	Br	600	Ad	A	Rough/Ceramic	<LOD	96	50	10
δ	G	25	30.2	470	Cl	450	All	A&D	Rough/Paint	<LOD	36	417	167
ε	H	26	28.8	537	Cl	800	All	A&D	Rough/Ceramic	<LOD	9	1,100	290
		27	31.8	204	Cl	450	All	A&D	Rough/Ceramic	<LOD	<LOD	<LOD	<LOD
		28	17.5	2.25●	Cl	30	Ad	A	Rough/Ceramic	<LOD	NS	NS	NS
		29	32.5	25.2●	Br	250	Ad	A	Rough/Ceramic	<LOD	346	21,200	103
	J	30	28.5	850	Cl	300	All	A&D	Rough/Ceramic	3	<LOD	<LOD	<LOD
		31	31.5	56	Cl	100	All	A&D	Rough/Ceramic	<LOD	16,000	80	110
	K	32	29	725	Cl + UV	1,000	All	A&D	Rough/Ceramic	<LOD	<LOD	120	<LOD

SC, sports centre; ●, Jacuzzis; Ad, adults; C, children; A, autonomous; A&D, autonomous and disabled; EC, *E. coli*; FE, faecal enterococci; SRC, *Cl. perfringens*; (*), cfu/100 mL; <LOD, below the limit of detection; NS, no sample.

A water temperature above 25 °C was conducive to the simultaneous presence of the protozoan and the three bacterial indicators *E. coli*, faecal enterococci and *Cl. 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 the pools with a low temperature, as well as 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 m^3) were the most positive for *Cryptosporidium* oocysts (Table 2). The prevalence of bacterial indicators was quite similar for swimming pools and

Table 2 | Prevalence of *Cryptosporidium*[a], *E. coli* (EC)[b], faecal enterococci (FE)[b] and *Cl. perfringens* (SRC)[b] in relation to water, structural and user parameters

Parameters			*Cryptosporidium* (%)	EC (%)	FE (%)	SRC (%)	EC + FE + SRC (%)
Water parameters	Temperature	High	15.8	52.9	70.6	70.6	52.9
		Medium	27.3	60.0	80	70.0	60.0
		Low	0	100	100	100	100
	Chemical treatment	Cl	15.8	55.6	72.2	66.7	55.6
		Br	10.0	62.5	75.0	87.5	62.5
		Cl + UV	66.7	50.0	100	50.0	50.0
Structural parameters	Type of pool	Swimming pool	23.8	55.0	75.0	65.0	55.0
		Jacuzzi	9.1	62.5	75.0	87.5	62.5
	Volume	Large	40.0	40.0	60.0	60.0	40.0
		Medium	25.0	58.3	66.7	58.3	58.3
		Small	6.7	63.6	90.9	90.9	63.6
	Type of surface	Smooth	0	50.0	75.0	100	50.0
		Rough	21.4	58.3	87,5	66.7	58.3
	Material of surface	Ceramic	5.3	61.1	77.5	77.8	61.1
		Plastic	30.0	37.5	62.5	50.0	37.5
		Paint	66.7	100	100	100	100
User parameters	Daily user number	High	12.5	62.5	87.5	75.0	62.5
		Medium	25.0	52.9	64.7	70.6	52.9
		Low	0	66.7	100	66.7	66.7
	User age	Adult	7.7	50.0	80.0	90.0	50.0
		Child	0	0	100	0	0
		Adults and children	27.8	64.7	70.6	64.7	64.7
	Type of user	Autonomous	26.3	53.3	73.3	73.3	53.3
		Disabled	7.7	61.5	76.9	69.2	61.5

[a]In pool waters.
[b]in pool surround water accumulations.

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 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 (sport 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 43 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 1×10^{-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 ($r = 0.0572$) ≤ 1 oocysts/70 L, and for the Beta-Poisson the allowed concentration ranged from ≤ 1 oocyst in 118 L ($\alpha = 0.145$, $\beta = 1.52$) to ≤ 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 ($\rho \approx 0.93$) followed by the concentration of oocysts in the water ($\rho \approx 0.33$). Moreover, a negligible correlation ($\rho \approx 0.088$) was observed between the recovery rate and the oocyst removal/disinfection rate (ρ, -0.007 to -0.02).

In contrast, for the limit of detection, the sensitivity analysis showed a high correlation ($\rho \approx 0.98$) between lower oocyst concentration values and ingestion ($\rho \approx 0.03$), the recovery rate ($\rho \approx 0.002$) and the oocyst removal/disinfection rate (ρ, -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

	Exponential dose–response models						**Beta-Poisson dose–response models**					
	r = 0.05720[a,b]		**r = 0.00526[b]**		**r = 0.00491[c]**		**$\alpha = 0.270$, $\beta = 1.40$[d]**		**$\alpha = 0.114$, $\beta = 1.04$[e]**		**$\alpha = 0.145$, $\beta = 1.52$[b]**	
(oocy./L)	**Adults 97.5th p.**	**Children 97.5th p.**	**Adults 97.5th p.**	**Children 97.5th p.**	**Adults 97.5th p.**	**Children 97.5th p.**	**Adults 97.5th p.**	**Children 97.5th p.**	**Adults 97.5th p.**	**Children 97.5th p.**	**Adults 97.5th p.**	**Children 97.5th p.**
(a)												
DL	1.07×10^{-3}	1.83×10^{-3}	9.93×10^{-5}	1.69×10^{-4}	9.24×10^{-5}	1.58×10^{-4}	3.60×10^{-3}	6.12×10^{-3}	2.05×10^{-3}	3.45×10^{-3}	1.78×10^{-3}	3.02×10^{-3}
3	7.50×10^{-3}	1.52×10^{-2}	6.98×10^{-4}	1.41×10^{-3}	6.46×10^{-4}	1.31×10^{-3}	2.40×10^{-2}	4.61×10^{-2}	1.36×10^{-2}	2.57×10^{-2}	1.20×10^{-2}	2.33×10^{-2}
5	1.11×10^{-2}	2.30×10^{-2}	1.03×10^{-3}	2.15×10^{-3}	9.66×10^{-4}	1.99×10^{-3}	3.49×10^{-2}	6.65×10^{-2}	1.96×10^{-2}	3.69×10^{-2}	1.75×10^{-2}	3.38×10^{-2}
10	1.99×10^{-2}	4.17×10^{-2}	1.84×10^{-3}	3.91×10^{-3}	1.72×10^{-3}	3.65×10^{-3}	5.84×10^{-2}	1.08×10^{-1}	3.25×10^{-2}	5.96×10^{-2}	2.96×10^{-2}	5.61×10^{-2}
13	2.48×10^{-2}	5.29×10^{-2}	2.31×10^{-3}	4.96×10^{-3}	2.15×10^{-3}	4.63×10^{-3}	7.09×10^{-2}	1.30×10^{-1}	3.93×10^{-2}	7.09×10^{-2}	3.61×10^{-2}	6.76×10^{-2}
(b)												
DL	4.75×10^{-4}	8.17×10^{-4}	4.34×10^{-5}	7.39×10^{-5}	4.08×10^{-5}	6.90×10^{-5}	1.58×10^{-3}	2.69×10^{-3}	9.07×10^{-4}	1.55×10^{-3}	7.84×10^{-4}	1.33×10^{-3}
3	3.36×10^{-3}	6.68×10^{-3}	1.16×10^{-4}	6.16×10^{-4}	2.83×10^{-4}	5.75×10^{-4}	1.08×10^{-2}	2.14×10^{-2}	6.26×10^{-3}	1.23×10^{-2}	5.38×10^{-3}	1.07×10^{-2}
5	4.93×10^{-3}	1.01×10^{-2}	4.54×10^{-4}	9.37×10^{-4}	4.24×10^{-4}	8.75×10^{-4}	1.60×10^{-2}	3.16×10^{-2}	9.02×10^{-3}	1.78×10^{-2}	7.96×10^{-3}	1.59×10^{-2}
10	8.73×10^{-3}	1.85×10^{-2}	8.07×10^{-4}	1.72×10^{-3}	7.53×10^{-4}	1.60×10^{-3}	2.75×10^{-2}	5.45×10^{-2}	1.55×10^{-2}	3.03×10^{-2}	1.38×10^{-2}	2.76×10^{-2}
13	1.11×10^{-2}	2.34×10^{-2}	1.01×10^{-3}	2.18×10^{-3}	9.44×10^{-4}	2.20×10^{-3}	3.39×10^{-2}	6.68×10^{-2}	1.90×10^{-2}	7.82×10^{-2}	1.70×10^{-2}	3.40×10^{-2}

[a]Preferred model.
[b]Messner *et al.* (2001).
[c]DuPont *et al.* (1995).
[d]Chappell *et al.* (2006).
[e]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 volume 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 1.11×10^{-5} and 2.57×10^{-5}, respectively, for adults and children in one visit to swimming pool. Schets *et al.* (2011) estimated a mean risk of 2.2×10^{-3} for children swimmers, whereas the risk for adults ranged from 1.1×10^{-3} to 1.5×10^{-3}, respectively, for women and men. Suppes *et al.* (2016) obtained a mean risk of 2.5×10^{-4} for adult swimmers and 3.5×10^{-4} for children. In the present study, mean concentration of oocysts of 1.47 oocysts/L^{-1} correspond to a risk of 9.5×10^{-4} for adults and 1.95×10^{-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^{-1} of free chlorine or 2 to 5 mg/L^{-1} 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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REFERENCES

Baker, M., Russell, N., Roseveare, C., O'Hallahan, J., Palmer, S. & Bichan, A. 1998 Outbreak of cryptosporidiosis linked to Hutt Valley swimming pool. *New Zeal. Health Rep.* **5**, 41–45.

Baldursson, S. & Karanis, P. 2011 Waterborne transmission of protozoan parasites: review of worldwide outbreaks – an update 2004–2010. *Water Res.* **45**, 6603–6614. doi:10.1016/j.watres.2011.10.013.

Birks, R., Colbourne, J. & Hobson, R. 2004 Microbiological water quality in a large in-building, water recycling facility. *Water Sci. Technol.* **50** (2), 165–172.

Black, M. & McAnulty, J. 2006 The investigation of an outbreak of cryptosporidiosis in New South Wales in 2005. *NSW Public Health Bull.* **17**, 76–79.

Bonnick, D. M. 2006 Swimming pool disinfection – techniques and pitfalls [WWW document]. *Water Cond. Purif.* http://coloradoedu.org/lib/S/168C/swimming-pool-disinfection-sie (accessed 29 January 16).

Bufton, A. W. J. 1959 A note on the enumeration of thermophilic sulphate-reducing bacteria (Clostridium nigrificans). *J. Appl. Bacteriol.* **22**, 278–280. doi:10.1111/j.1365-2672.1959.tb00162.x.

Chappell, C. L., Okhuysen, P. C., Langer-Curry, R., Widmer, G., Akiyoshi, D. E., Tanriverdi, S. & Tzipori, S. 2006 Cryptosporidium hominis: experimental challenge of healthy adults. *Am. J. Trop. Med. Hyg.* **75**, 851–857. doi:75/5/851[pii].

Cotruvo, J. A., Durfour, A., Rees, G., Bartram, J., Carr, R., Cliver, D. O., Craun, G. F., Fayer, R. & Garcia-Presedo, I. 2004 *Waterborne Zoonosis: Identification, Causes and Control*. World Health Organization, IWA Publishing, London.

Dufour, A. P., Evans, O., Behymer, T. D. & Cantú, R. 2006 Water ingestion during swimming activities in a pool: a pilot study. *J. Water Health* **4**, 425–430. doi:10.2166/wh.2006.017.

DuPont, H. L., Chappell, C. L., Sterling, C. R., Okhuysen, P. C., Rose, J. B. & Jakubowski, W. 1995 The infectivity of Cryptosporidium parvum in healthy volunteers. *N. Engl. J. Med.* **332**, 855–859. doi:10.1056/NEJM199503303321304.

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**, 209–213. doi:10.1016/S0928-8244(02)00322-X.

Furtado, C., Adak, G. K., Stuart, J. M., Wall, P. G., Evans, H. S. & Casemore, D. P. 1998 Outbreaks of waterborne infectious intestinal disease in England and Wales, 1992–5. *Epidemiol. Infect.* **121**, 109–119.

Galmes, A., Nicolau, A., Arbona, G., Smith-Palmer, A., Hernández Pezzi, G. & Soler, P. 2003 Cryptosporidiosis outbreak in British tourists who stayed at a hotel in Majorca, Spain. *Eurosurveillance* **7** (33), 2275.

Gómez, M. S., Gracenea Zugarramurdi, M., Ángel Ripoll, L. & Beneyto, V. 2011 Cryptosporidium sp. in public swimming pools in Barcelona. In: *Recent Advances in Pharmaceutical Sciences* (D. Muñoz-Torrero, ed.). Transworld Research Network, Kerala, India, pp. 275–282.

Gregory, R. 2002 Bench-marking pool water treatment for coping with Cryptosporidium. *J. Environmental Health Res.* **1**, 11–18.

Greinert, J., Furtado, D., Smith, J., Monte Barardi, C. & Simões, C. 2004 Detection of Cryptosporidium oocysts and Giardia cysts in swimming pool filter backwash water concentrates by flocculation and immunomagnetic separation. *Int. J. Environ. Health Res.* **14**, 395–404. doi:10.1080/09603120400012892.

Hlavsa, M. C., Robinson, T. J., Collier, S. A. & Beach, M. J. 2014 Pool chemical-associated health events in public and residential settings – United States, 2003-2012, and Minnesota, 2013. *MMWR. Morb. Mortal. Wkly Rep.* **63**, 427–430.

Hunt, D. A., Sebugwawo, S., Edmondson, S. G. & Casemore, D. P. 1994 Cryptosporidiosis associated with a swimming pool complex. *Commun. Dis. Rep. CDR Rev.* **4**, R20–R22.

Insulander, M., Lebbad, M., Stenström, T. A. & Svenungsson, B. 2005 An outbreak of cryptosporidiosis associated with exposure to swimming pool water. *Scand. J. Infect. Dis.* **37**, 354–360. doi:10.1080/00365540410021072.

International Organization For Standardization 2000a ISO 7899-2:2000. *Water Quality – Detection and Enumeration of Intestinal Enterococci – Part 2: Membrane Filtration Method*.

International Organization For Standardization 2000b ISO 9308-1:2000. *Water Quality – Detection and Enumeration of Escherichia Coli and Coliform Bacteria – Part 1: Membrane Filtration Method*.

Karanis, P. & Kimura, A. 2002 Evaluation of three flocculation methods for the purification of Cryptosporidium parvum oocysts from water samples. *Lett. Appl. Microbiol.* **34**, 444–449. doi:10.1046/j.1472-765X.2002.01121.x.

Laine, A. T. 2001 *Technologies for Greywater Recycling in Buildings*. School of Applied Sciences, Cranfield, UK.

Lemmon, J. M., McAnulty, J. M. & Bawden-Smith, J. 1996 Outbreak of cryptosporidiosis linked to an indoor swimming pool. *Med. J. Aust.* **165**, 613–616.

Louie, K., Gustafson, L., Fyfe, M., Gill, I., MacDougall, L., Tom, L., Wong, Q. & Isaac-Renton, J. 2004 An outbreak of Cryptosporidium parvum in a Surrey pool with detection in pool water sampling. *Canada Commun. Dis. Rep. Relev. des Mal. Transm. au Canada* **30**, 61–66.

McAnulty, J. M., Fleming, D. W. & Gonzalez, A. H. 1994 A community-wide outbreak of cryptosporidiosis associated with swimming at a wave pool. *JAMA* **272**, 1597–1600. doi: DOI 10.1001/jama.272.20.1597.

McGuigan, K. G., Méndez-Hermida, F., Castro-Hermida, J. A., Ares-Mazás, E., Kehoe, S. C., Boyle, M., Sichel, C., Fernández-Ibáñez, P., Meyer, B. P., Ramalingham, S. & Meyer, E. 2006 Batch solar disinfection inactivates oocysts of Cryptosporidium parvum and cysts of Giardia muris in drinking water. *J. Appl. Microbiol.* **101**, 453–463. doi:10.1111/j.1365-2672.2006.02935.x.

Messner, M. J., Chappell, C. L. & Okhuysen, P. C. 2001 Risk assessment for Cryptosporidium: a hierarchical Bayesian analysis of human dose response data. *Water Res.* **35**, 3934–3940. doi:10.1016/S0043-1354(01)00119-1.

Morita, S., Namikoshi, A., Hirata, T., Oguma, K., Katayama, H., Ohgaki, S., Motoyama, N. & Fujiwara, M. 2002 Efficacy of UV irradiation in inactivating Cryptosporidium parvum

oocysts. *Appl. Environ. Microbiol.* **68** (11), 5387–5393. doi:10.1128/AEM.68.11.5387-5393.2002.

Okhuysen, P. C., Rich, S. M., Chappell, C. L., Grimes, K. A., Widmer, G., Feng, X. & Tzipori, S. 2002 Infectivity of a Cryptosporidium parvum isolate of cervine origin for healthy adults and interferon-gamma knockout mice. *J. Infect. Dis.* **185**, 1320–1325. doi:10.1086/340132.

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. di Ig. Med. Prev. e di comunità* **18**, 367–374 (in Italian).

Ottoson, J. & Stenström, T. A. 2003 Faecal contamination of greywater and associated microbial risks. *Water Res.* **37**, 645–655. doi:10.1016/S0043-1354(02)00352-4.

Pintar, K. D., Fazil, A., Pollari, F., Charron, D. F., Waltner-Toews, D. & McEwen, S. A. 2010 A risk assessment model to evaluate the role of fecal contamination in recreational water on the incidence of cryptosporidiosis at the community level in Ontario. *Risk Anal.* **30** (1), 49–64. doi:10.1111/j.1539-6924.2009.01321.

Pond, K. 2005 *Water Recreation and Disease. Plausibility of Associated Infections: Acute Effects, Sequelae and Mortality*. World Health Organization, Geneva.

Pouillot, R. & Delignette-Muller, M. L. 2010 Evaluating variability and uncertainty separately in microbial quantitative risk assessment using two R packages. *Int. J. Food Microbiol.* **142**, 330–340. doi:10.1016/j.ijfoodmicro.2010.07.011.

Schets, F. M., Engels, G. B. & Evers, E. G. 2004 Cryptosporidium and Giardia in swimming pools in the Netherlands. *J. Water Health* **2**, 191–200.

Schets, F. M., Schijven, J. F. & Husman, A. M. D. 2011 Exposure assessment for swimmers in bathing waters and swimming pools. *Water Res.* **45**, 2392–2400.

Shields, J. M., Hill, V. R., Arrowood, M. J. & Beach, M. J. 2008a Inactivation of Cryptosporidium parvum under chlorinated recreational water conditions. *J. Water Health* **6**, 513–520. doi:10.2166/wh.2008.068.

Shields, J. M., Gleim, E. R. & Beach, M. J. 2008b Prevalence of Cryptosporidium spp. and Giardia intestinalis in swimming pools, Atlanta, Georgia. *Emerg. Infect. Dis.* **14**, 948–950. doi:10.3201/eid1406.071495.

Suppes, L. M., Canales, R. A., Gerba, C. P. & Reynolds, K. A. 2016 Cryptosporidium risk from swimming pool exposures. *Int. J. Hyg. Environ. Health* **219** (8), 915–919. doi:10.1016/j.ijheh.2016.07.001.

Takagi, M., Toriumi, H., Endo, T., Yamamoto, N. & Kuroki, T., 2008 An outbreak of cryptosporidiosis associated with swimming pools. *Kansenshōgaku zasshi. J. Japanese Assoc. Infect. Dis.* **82**, 14–19 (in Japanese).

Vesey, G., Slade, J. S., Byrne, M., Shepherd, K. & Fricker, C. R. 1993 A new method for the concentration of Cryptosporidium oocysts from water. *J. Appl. Bacteriol.* **75**, 82–86. doi:10.1111/j.1365-2672.1993.tb03412.x.

Winward, G. 2008 A study of the microbial quality of grey water and an evaluation of treatment technologies for reuse. *Ecol. Eng.* **32**, 187–197. doi:10.1016/j.ecoleng.2007.11.001.

World Health Organization 2006 *Guidelines for Safe Recreational Water Environments*. Volume 2: Swimming pools and similar environments. WHO, Geneva.

Yoder, J. S. & Beach, M. J. 2010 Cryptosporidium surveillance and risk factors in the United States. *Exp. Parasitol.* **124**, 31–39. doi:10.1016/j.exppara.2009.09.020.

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