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# 12. *Cryptosporidium* sp. in public swimming pools in Barcelona

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**Abstract.** The presence of *Cryptosporidium* oocysts in swimming pools in Barcelona was studied. The possible influence of the different parameters related to water conditions, pool structure and users has also been analyzed. *Cryptosporidium* oocysts were detected in 54% of the swimming pools analyzed and in 85% of the sport centers checked. Oocysts concentrations ranged from 0 to 30 oocysts/L. Among parameters related to water conditions, there was no relationship between oocyst presence and water pH, but the influence of temperature was statistically significant ($p<0.05$) ($\chi^2=0.024$). Among parameters related to pool structure, the filter backwash water samples revealed a higher percentage of parasitation (58.8%) than the prefilter ones (42.9%). With the medium volume pools (100-500 $m^3$), the percentage was higher (80%). Covered and uncovered pools presented similar prevalences for *Cryptosporidium* oocysts. Looking at two of the user related parameters, swimming pools with more users and children's swimming pools showed

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highest presence of oocysts, 75% and 66.7% respectively. *Cryptosporidium* oocysts were found in all the studied neighborhoods, and this factor exerted a statistically significant influence on the concentration of oocysts ($p<0.05$) ($\chi^2=0.021$).

# Introduction

Nowadays, society's concern for our physical and psychological well-being has popularized sports and therapies involving water. For this reason, many sport centers include swimming pools and Jacuzzis where the partial recirculation of water and the high concentration of people act as a perfect environment for the development and transmission of some human pathogens, such us *Cryptosporidium*, which is a significant waterborne protozoa parasite. It is a cosmopolitan human enteropathogen with fecal-oral transmission, capable of surviving in the environment for prolonged periods. Its direct life cycle includes an intracellular intestinal phase inside the host and a stage, oocyst, which is highly resilient in the face of environmental stress and standard water treatment technologies [1,2]. The oocyst is microscopic in size, already infectious at the time of shedding, its infection dose is low and it is excreted in high numbers and for a long period of time [3].

Clinical symptoms of cryptosporidiosis are variable but frequently manifest as an acute or chronic diarrhea that severely affects people with immuno-deficiencies [4]. *Cryptosporidium hominis* and *C. parvum* are the most frequent species found in humans [5].

Several waterborne outbreaks of cryptosporidiosis have been reported worldwide [6-8]. Among them, some have been related to swimming pool activities [5,9,10]. In swimming pools, several adverse conditions may exist, such as poor hygiene [11], defective filtration systems, animals and rain runoff, all of which favor oocyst occurrence. In addition, many recreational water facilities allow users of any age including, among them, groups that could suffer fecal incontinence.

The objective of this study was to achieve for the first time a qualitative and quantitative determination of presence of *Cryptosporidium* oocysts in swimming pools in the Barcelona area, as well as to analyze the influence of physical, chemical and social swimming pool parameters over the presence of *Cryptosporidium* oocysts.

# Methods

Samples were taken in collaboration with *Agència de Salut Pública de Barcelona* and collected from the filter backwash water or from the prefilter

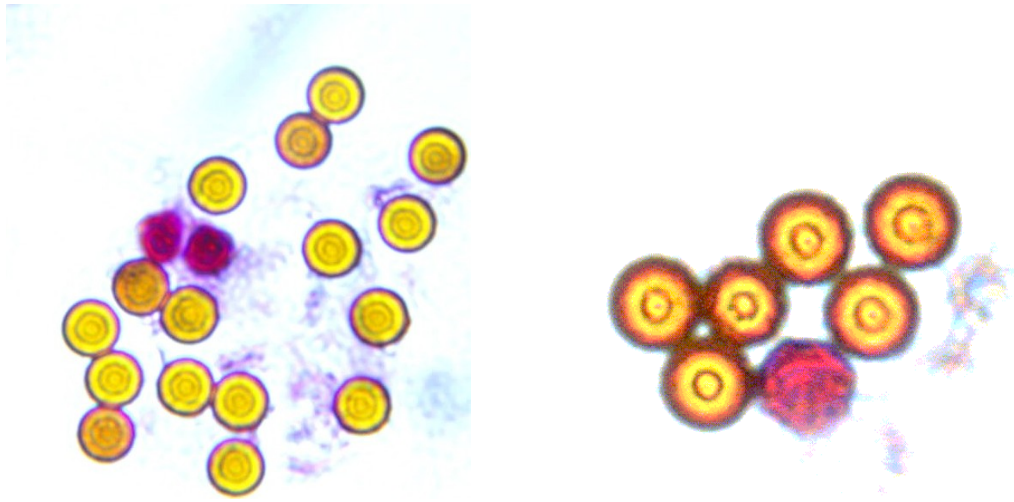

**Figure 1.** *Cryptosporidum* oocysts (red) bound to immunomagnetic spheres.

water depending on the facility conditions. Thirteen sport centers and 24 swimming pools were analyzed.

Data about water pH (6.9-7.9), chemical treatment (chlorine/bromine), temperature (~15 ºC, 26-28 ºC and >32 ºC), sampling point (filter backwash water/prefilter water), volume (<100, 100-750 and >750 $m^3$), swimming pool structure (covered/uncovered), average daily user number (<100, 100-500 and >500), user age (children/adults), nine neighborhoods and thirteen sport centers were requested. Water was collected in 10 L drums and transported to the laboratory.

The analytic technique used to detect *Cryptosporidium* oocysts included a concentration by flocculation with calcium carbonate, isolation by immunomagnetic separation and identification by Ziehl-Neelsen staining (Fig. 1).

Statistical treatment was done with SPSS v12.

## Results and discussion

*Cryptosporidium* oocysts were detected in 54% of the checked swimming pools and in 85% of the sport centers (Table 1). Although one of the swimming pools checked was temporarily out of use when the water sample was taken (number 7 in table 1), it has been considered in this study because it was still in use until one week prior to being checked for the parasite.

A study performed in Netherlands found a presence of 4.6% oocysts in 7 swimming pools analyzed over one year [12]. In Italy *Cryptosporidium* oocysts were detected in 40% of the swimming pools studied in 2004 and 28.6% in 2006 [13,14].

**Table 1.** Pools vs. temperature, volume, sampling point, daily users, user age, neighborhood and concentration of *Cryptosporidium* oocysts. (ND) not detected.

| Pools | Temp. | V($m^3$) | Sampling point | Daily users | User age | Neighbor-hood | Oocysts/L |
|---|---|---|---|---|---|---|---|
| 1 | 26-28 °C | 1400 | Filter | 400 | Adult | 1 | 30 |
| 2 | 26-28 °C | 460 | Filter | 400 | Adult, child | 2 | ND |
| 3 | 26-28 °C | 245 | Filter | 400 | Adult, child | 2 | 5 |
| 4 | 26-28 °C | 566 | Filter | 150-200 | Adult, child | 3 | 20 |
| 5 | 26-28 °C | 625 | Filter | >100 | Adult, child | 4 | ND |
| 6 | | 3500 | Filter | 1000 | Adult, child | 5 | 10 |
| 7 | 26-28 °C | 1500 | Prefilter | No users | No users | 6 | ND |
| 8 | 26-28 °C | 3500 | Prefilter | 1000 | Adult, child | 6 | ND |
| 9 | 26-28 °C | 3500 | Prefilter | 1000 | Adult, child | 6 | 2.5 |
| 10 | 26-28 °C | 250 | Filter | 200 | Adult, child | 6 | 5 |
| 11 | 26-28 °C | 450 | Filter | 700 | Adult, child | 7 | 5 |
| 12 | 26-28 °C | 60 | Filter | 100 | Child | 7 | 5 |
| 13 | 26-28 °C | 200 | Prefilter | 150 | Adult, child | 2 | 20 |
| 14 | ≥ 32 ºC | 48.26 | Filter | 240 | Adult | 8 | ND |
| 15 | ~15 ºC | 14.62 | Filter | 135 | Adult | 8 | ND |
| 16 | 26-28 °C | 346 | Filter | 80 | Adult, child | 8 | 15 |
| 17 | 26-28 °C | 28.8 | Filter | 80 | Child | 8 | 5 |
| 18 | ≥ 32 ºC | 75 | Filter | 127 | Adult,child | 9 | ND |
| 19 | 26-28 °C | 468 | Filter | 169 | Adult, child | 9 | ND |
| 20 | 26-28 °C | 142 | Filter | 197 | Adult, child | 9 | 5 |
| 21 | ≥ 32 ºC | 1.6 | Prefilter | 40 | Adult, child | 7 | ND |
| 22 | ≥ 32 ºC | 1.6 | Prefilter | 40 | Adult, child | 7 | ND |
| 23 | 26-28 °C | 430 | Filter | 50 | Adult, child | 7 | 7.5 |
| 24 | 26-28 °C | 18.9 | Filter | 50 | Child | 7 | ND |

# 1. Water parameters

Among parameters related to water (pH, chemical treatment and temperature), no statistically significant relation ($R^2$=0.692) was observed between the presence of *Cryptosporidium* oocysts and water pH. The pH average was 6.9-7.9, close to neutrality, in all pools, so this parameter is not a deciding factor in the presence of oocysts.

Oocysts were present in 60% of the chlorine treated pools and in 44% of the bromine treated ones. Consequently, no statistically significant differences have been detected related to chemical water treatment ($p<0.05$)

($\chi^2$=0.459) but bromine seems to be more efficient than chlorine. In this sense, bromine (1180 mg/L, 60 min) reduces 89% of oocysts infectivity [15], and the reduction provided by hypochlorite is lower, although it does depend on temperature [16]. *Cryptosporidium* oocysts are highly resistant to chlorine, which is the typical chemical treatment added to swimming pool waters. A concentration of 30 mg/L of chlorine during 240 min, a pH of 7 and a temperature of 25 ºC are necessary in order to inactivate the 99% of oocysts [17]. To achieve the effective level of disinfection in a standard pool, 1 ppm of chlorine is necessary [18], and it is important to note that oocysts can survive more than 6 days under these conditions [12].

The relationship between the presence of oocysts and temperature was statistically significant ($p<0.05$) ($\chi^2$=0.024). Swimming pools with temperatures above 32 ºC and below 15 ºC were negative for *Cryptosporidium* oocysts that were detected in 68% of the swimming pools at 26-28 °C. The temperature can affect the survival of *Cryptosporidium* oocysts. Then, temperatures between 5 and 15 ºC are adequate to maintain the infective capacity of oocysts [19]. Temperatures over this range seem to reduce their infective capacity and oocysts are inactivated in 72 hours with temperatures over 30 ºC [20]. In general, the average temperature in swimming pools is 26-28 °C and *Cryptosporidium* oocysts can survive 12 to 24 weeks in aquatic environments with temperatures between 20 and 25 ºC [19]. Consequently, it is not expected that swimming pools at 26-28 °C temperature reduce the presence of the parasite.

## 2. Swimming pool structure parameters

Among parameters related with pool structure (sampling point, volume and covered or uncovered facility), it was observed that samples taken from filter backwash water were more positive (58.8%) than those taken from prefilter (42.9%) although the oocysts concentration was high in both points with a maximum of 30 and 20 oocysts/L respectively.

There are no statistically significant differences regarding the sampling point and its relation to the presence/absence of oocysts, although it should be pointed out that 3 of the 7 prefilter samples were positive with concentrations ranging from 2.5-20 oocysts/L. These values should be emphasized because they represent the actual concentration of oocysts in the pool. One of the filter backwash water samples had a higher concentration (30 oocysts/L). Although this was not the real swimming pool concentration, the risk of infection could be important in the event of filter washing failure or filter washing while the swimming pool is in use, because oocysts find their way back into the re-circulated water.

The medium volume pools (100-750 $m^3$) were the most positive (80%), followed by big volume pools (50%) and small pools (25%) but the difference was not statistically significant ($p<0.05$) ($\chi^2=0.065$). Shields et al. [21] analyzed swimming pools in Atlanta with different water volumes and no oocysts were detected in swimming pools exceeding 800 $m^3$, while in Barcelona there was an important prevalence found in high volume (>1000 $m^3$) swimming pools. The authors found the protozoan in the 5.5% of the small swimming pools (<20 $m^3$), which is similar to the data found in Barcelona. The medium volume swimming pools in Atlanta showed a 1.8-2.8% of prevalence while the prevalence was 80% in Barcelona.

Covered and uncovered pools presented similar prevalence (54.5% and 50% respectively) for *Cryptosporidium* oocysts. Covered swimming pools prevent exogenous contamination from animal reservoirs such as birds and small mammals (rats, mice, etc.), that frequently carry the parasite and could contaminate the uncovered swimming pools close to their natural environments. *Cryptosporidium hominis*, a species that infects humans, has been detected even in doves from the Canary Islands, demonstrating capacity of the birds to act as reservoirs [22]. In covered swimming pools, the water contamination has a human origin, mainly due to fecal accidents.

## 3. Water user parameters

Among parameters related to users (average daily user number, ages, neighborhood location and sport centre), the results revealed that swimming pools with more users (>500) were the most positive in oocysts (75%) although without statistical significance ($p<0.05$) ($\chi^2=0.657$). This seems reasonable bearing in mind that users are the main contamination source. In Atlanta, Shields et al. [21] described the opposite situation, detecting the parasite in swimming pools with less than 10 daily users. They observed prevalence of 4.4% in low-user swimming pools while in Barcelona it was 50%. But the user number was always higher in Barcelona, with a daily minimum of 40 users.

Prevalence in children-user swimming pools was the most positive (66.7%), but the same pools also displayed the lowest oocysts concentrations (0-5 oocysts/L), whereas the opposite happened at the adult-user swimming pools (33.3% and 0-30 oocysts/L), although differences were not statistically significant. Data were different than those from Atlanta, where Shields et al. [21] found *Cryptosporidium* oocysts as follows: when entry was allowed only for children, 3.5% of pools were positive; in those for children and adults, 2.2% presented the protozoa; while they did not find oocysts in adult swimming pools.

*Cryptosporidium* oocysts were found in all the neighborhoods studied, and the oocyst concentration in them has a statistically significant relation ($p<0.05$) ($\chi^2=0.021$). Nevertheless, differences in the parasite presence/absence and in the swimming pool location were not statistically significant. In neighborhood 2, the number of positive swimming pools (2) was higher than the number of negative ones (1). Only one swimming pool was analyzed in each of neighborhoods 1, 3 and 5, and in all three cases they were positive. Neighborhoods 6 and 9 had a minimum positivity (1 of 3). As the named neighborhoods have no remarkable differences in terms of demographics, the parasite distribution is not easily explained. Neighborhood 2 stands out for the high prevalence (20 oocysts/L) detected directly in the swimming pool.

The pools are located in 13 sport centers, and among these centers only 2 tested negative for the parasite. Statistically significant differences were not observed in sport centers samples and their *Cryptosporidium* oocysts presence/absence, but these differences were close to being statistically significant ($p<0.05$) ($\chi^2=0.061$).

## 4. Conclusion

- There was a presence of *Cryptosporidium* oocysts in more than half of the checked swimming pools in Barcelona.
- There were high parasitic concentrations even in prefilter water which corresponds to the real value in the vessel. The oocyst concentration ranged from 0 to 30 oocysts/L in the filter backwash water.
- Water temperature influenced the presence of *Cryptosporidium* oocysts.
- Neighborhood location of the pool within Barcelona seems to have had an influence on the *Cryptosporidium* oocysts concentration.
- The swimming pool volume and the sport centre in which the pool is found may have influenced both the presence and concentration of *Cryptosporidium* oocysts.
- pH, treatment, structure, sampling point, age and number of users had no relation to either presence or concentration of *Cryptosporidium* oocysts.

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