

**Occurrence of *Cryptosporidium* and *Giardia* in raw and finished drinking water in north-eastern Spain**

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## Abstract

This paper collects the first large-sample-size study on the presence of *Cryptosporidium* oocysts and *Giardia* cysts in drinking water plants at the 20 most populated towns in Aragón (north-eastern Spain). Samples of influent raw water and effluent finished water were collected from each plant during different seasons and processed according to USEPA Method 1623. *Cryptosporidium* oocysts and *Giardia* cysts were detected in samples collected from 55% and 70% plants, respectively, with nine plants being positive for both protozoa and only four plants being negative over the study period. Both parasites were identified in the raw water throughout the year, with a lower frequency in autumn and a peak in winter, at a mean concentration of  $67 \pm 38$  oocysts per 100 litres and  $125 \pm 241$  cysts per 100 litres. The turbidity of raw water was not related to the presence or concentration of (oo)cysts, and the (oo)cyst removal efficiency was not related to the type of water treatment. One or both pathogens were identified in the finished water in 7 out of 11 plants with a conventional treatment process (coagulation, flocculation, sedimentation, filtration, and disinfection processes) compared to 4 out of 9 plants that did not apply one of the pre-chlorination treatment steps. Protozoa were detected in the finished water of positive plants at a mean concentration of  $88 \pm 55$  oocysts per 100 litres and  $37 \pm 41$  cysts per 100 litres, and most of them excluded propidium iodide so were considered potentially viable. The ubiquity of these parasites in the drinking water sources and the inefficiency of conventional water treatment in reducing/inactivating them may present a serious public health issue in this geographical area.

## 1. Introduction

*Cryptosporidium* and *Giardia* are the most common parasites involved in the etiology of waterborne diarrheic outbreaks in human populations in developed countries. Outbreaks have been associated with contaminated drinking water or recreational water and were most often due to the presence of *Giardia* in the United States, while *Cryptosporidium* predominates in Europe and Australia (Craun et al., 2010; Baldurson and Karanis, 2011). Outbreaks of these parasites can be particularly large in size, for example the massive cryptosporidiosis outbreak in 2003 affecting 403,000 people in Milwaukee (Wisconsin, USA), and the giardiasis outbreak in 2004 resulting in an estimated 2,500 cases in Bergen (Norway) (Mac Kenzie et al. 1994; Nygårdk et al. 2006). In Europe, most waterborne cryptosporidiosis and giardiasis infections have been reported in the United Kingdom and Ireland, although they have also been detected in the Nordic countries, France, Germany, Italy, Greece, and Spain (Semenza and Nichols, 2007; Baldursson and Karanis, 2011; Chalmers, 2012; Guzman-Herrador et al., 2015a).

Several factors have favored the emergence of these protozoa as waterborne pathogens, including their ubiquitous distribution, the environmental resistance of *Cryptosporidium* oocysts and *Giardia* cysts, the high (oo)cyst excretion rate from infected hosts and low infectious doses, and the presence of multiple hosts for some of the human pathogenic species and genotypes (Carmena, 2010; Escobedo et al. 2010). Furthermore, both parasites and particularly *Cryptosporidium* are not effectively removed by conventional water treatment processes because of their small size and resistance to disinfection. Outbreaks have been reported through drinking water that met the legal quality standards, including the WHO Guidelines for Drinking Water Quality (WHO, 2011)

and the European Drinking Water Directive (Directive 98/83/EC) (Chalmers, 2012). *Cryptosporidium* is considered a reference pathogen for drinking water (WHO, 2006), and is included in Directive 2003/99/EC of the European Parliament and the Council of the European Union on the monitoring of zoonoses and zoonotic agents. Human cryptosporidiosis cases should therefore be collected in Member States and communicated to the European Commission, but *Cryptosporidium* diagnosis is statutorily notifiable in only a few countries (Semenza and Nichols, 2007; Chalmers, 2012).

In Spain, *Cryptosporidium* and *Giardia* are significant causative agents of diarrhoeal disease in humans and young ruminants, which are major reservoirs of human-infective *Cryptosporidium* species and may harbor some *Giardia* genotypes/assemblages known to be infectious to humans (Navarro-i-Martínez et al., 2011; Carmena et al., 2012). Previous studies in regions of Spain have revealed the common occurrence of *Cryptosporidium* and *Giardia* in water (surface water, drinking water, wastewater, reclaimed, recreational) (Montemayor et al., 2005; Carmena et al., 2007; Castro-Hermida, 2008, 2009, 2015; Galván et al., 2014), and human cryptosporidiosis outbreaks linked to contaminated drinking water supplies or recreational water have occasionally been detected (Rodríguez-Salinas et al. 2000; Galmes et al., 2003, Soler, 2004). In spite of this, the potential of both protozoa for waterborne transmission has apparently been considered of minor significance, and specific legislative bodies established in some parts of the world (e.g. UK, USA) to regulate the monitoring of water for these parasites have not been introduced in Spain (Chalmers, 2012).

The region of Aragón (north-eastern Spain) has the highest number of notifications of human cases of cryptosporidiosis according to the Spanish Microbiological Information System (MIS, 2015). Two waterborne outbreaks involving an estimated 750 and 100 human cases, respectively, were reported in this region in 2000, which was also characterized by high rates of *Cryptosporidium* and *Giardia* infections in livestock farms (Quílez et al., 1996; 2008; Causapé et al., 2002; Anonymous, 2003). This study was designed to provide information on the occurrence and concentration of *Cryptosporidium* and *Giardia* in drinking water treatment plants in this geographical area. The efficiency of treatment plants in removing the protozoans was also investigated.

## **2. Materials and Methods**

### *2.1. Site location and sampling*

Over the period 2013-2015, water samples were collected at 20 municipal drinking water treatment plants located in the most populated towns in Aragón (north-eastern Spain) (Figure 1). These plants serve local settlements ranging from 7,000 to over 660,000 inhabitants and provide potable water to nearly 1 million people in total, which represents over 75% of the total population in Aragón (Table 1). From each water treatment facility, samples of untreated raw water (influent) and treated finished water (effluent) were collected at four different times, each sampling time matching a different season (spring, summer, autumn, and winter). Turbidity of each sample was measured with a portable turbidimeter model HI93703 (Hanna Instruments, Spain) and the results were expressed in nephelometric turbidity units (NTU).

Plants had different sources for their raw water, including surface water (river, reservoir) and groundwater. Eleven facilities applied conventional water treatment including coagulation-flocculation, clarification through sedimentation, filtration and chlorination (Table 1). Two types of filtration were used in the latter facilities, including a system combining sand and activated carbon filtering (six plants), or a rapid sand filtration system where water flows through several layers of coarse-grained sand and gravel to remove particles that have been trapped during the previous flocculation (five plants). Four additional facilities included slow filtration, which combines both physical and biological properties of the sand bed previous to the chlorination. Finally, five plants were small facilities that only applied sedimentation and chlorination. None of the plants had ultraviolet systems for water disinfection. The turbidity removal efficiency achieved by each plant was calculated using the following equation:

$$\text{Turbidity removal efficiency (\%)} = [( \text{turbidity influent} - \text{turbidity effluent} ) / ( \text{turbidity influent} )] \times 100$$

## *2.2. Detection and molecular characterization of Cryptosporidium and Giardia*

A total of 160 water samples were analysed for the presence of *Cryptosporidium* oocysts and *Giardia* cysts according to the U.S. Environmental Protection Agency Method 1623 (USEPA, 2005). Briefly, samples of untreated (up to 50 litres of the raw influent) and treated (up to 100 litres of the finished effluent) water were filtered through Filt-Max filters (IDEXX Laboratories, Inc., Westbrook, ME, USA) using a motorized pump at recommended flow rates. The holding times of each step in the treatment were taking into account during sampling, in order to examine the same water

at both points in the process. The filters were transported to the laboratory in labelled and sealed plastic bags, and stored at 4°C. Elution procedures were carried out within 24 hours after collection with the Filt-Max Manual System (IDEXX Laboratories, Inc.) according to the manufacturer's instructions. After centrifugation at  $1,500 \times g$  for 15 min, the supernatant was aspirated to 20 ml and 10 ml of the resuspended pellet was subjected to immunomagnetic separation (IMS).

(Oo)cysts in concentrated samples of water were further purified from other particulates using the Dynal IMS procedure (Dynabeads GC-Combo, Invitrogen Dynal, A.S., Oslo, Norway) according to manufacturer's instructions. IMS-purified (oo)cysts were stained on well slides by fluorescein isothiocyanate (FITC)-conjugated anti-*Cryptosporidium* and anti-*Giardia* monoclonal antibodies (Crypto/Giardia Cel, Cellabs Pty Ltd, Australia). The internal structure of (oo)cysts was confirmed by staining with the nuclear fluorochrome 4',6-diamidino-2-phenylindole (DAPI) (Sigma). Slides were examined using an epifluorescence microscope and (oo)cysts showing typical, confirmatory features (size, internal contents, fluorescence) were enumerated and numbers extrapolated to concentrations of parasite per 100 litres of water. Positive and negative staining controls were routinely included. All calculations were adjusted taking into account the recovery efficiency of *Cryptosporidium* and *Giardia* reported in our laboratory and the volume of water filtered.

The potential viability of (oo)cysts was estimated by staining with the vital dye propidium iodide (PI) (Sigma-Aldrich, USA). Stock solution was prepared by dissolving PI in distilled water (1mg/ml). A volume of 10 µl of PI working solution were added to each well and incubated at room temperature in the dark for 1 minute,

and excess PI solution was removed by washing the slides in distilled water. The (oo)cysts were counted according to whether they were PI-positive (PI+: permeable and presumably dead) or PI-negative (PI–: impermeable and presumably viable) (Jenkins et al., 1997).

The recovery efficiency of the method was determined by seeding 10 litres of distilled water with different turbidity values (0, 20, and 50 NTU) with a known number of (oo)cysts according to the instructions of the USEPA 1623 method. Manually enumerated (oo)cysts stained with FITC-labelled antibodies were used due to cost restrictions. This procedure was repeated three times for each turbidity value. The mean recovery efficiency was  $19.8 \pm 4.3\%$  for *Cryptosporidium* and  $49.2 \pm 16.5\%$  for *Giardia*, which meets the acceptance criteria described in this method (McCuin and Clancy, 2003; USEPA, 2005).

The removal efficiency of (oo)cysts by each plant and sampling time was calculated as follows:

$$\text{Log removal} = \text{Log influent concentration} - \text{Log effluent concentration}$$

Molecular characterization was attempted in positive samples with a concentration higher than 10 (oo)cysts per 100 litres. *Cryptosporidium* and *Giardia* DNA was extracted from IMS-purified (oo)cysts using the QIAamp DNA Mini Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions, incorporating an initial step of three freeze-thaw cycles (freezing in liquid nitrogen for 5 min and heating at 100°C for 1 min) in the protocol. Previously described PCR protocols were



used to amplify the SSU rRNA and  $\beta$ -giardin genes of *Cryptosporidium* and *Giardia*, respectively (Xiao et al. 2001; Lalle et al. 2005).

### 2.3. Statistical analysis

Chi-squared and two-tailed Fisher's exact tests were used to evaluate possible significant differences in the seasonal pattern of *Cryptosporidium* and *Giardia* positive samples. The nonparametric Kruskal-Wallis test was used to evaluate significant differences in the (oo)cyst counts between influent and effluent water and between seasons. Analyses were performed using the SPSS statistical package for Windows version 18 (SPSS Inc.). Values of  $P < 0.05$  were considered statistically significant.

## 3. Results

*Cryptosporidium* and *Giardia* were identified in water samples of 11 (55%) and 14 (70%) drinking water treatment plants, respectively, with nine plants being positive for both protozoa and only four plants being negative over the study period (Tables 2 and 3). The analysis of results by season in the influent water samples revealed that winter showed a higher rate of positive plants than other seasons did, although differences were only statistically significant when compared to autumn, for both *Cryptosporidium* (30% versus 0%;  $P < 0.01$ ) and *Giardia* (45% vs. 15%;  $P < 0.05$ ) (Figure 2). (Oo)cyst counts in the influent samples revealed a mean concentration of  $67 \pm 38$  *Cryptosporidium* oocysts and  $125 \pm 241$  *Giardia* cysts per 100 l, with maximum values being recorded in spring in the same plant (160 oocysts and 1759 cysts per 100 l).

All water treatment facilities positive for *Cryptosporidium* in the raw water (n = 8) achieved high removal efficiencies, since no oocysts were detected in the finished water. In contrast, *Cryptosporidium* was identified in the finished water of three additional plants where samples of raw water were negative for this protozoan. The water treatment also reduced the *Giardia* cyst counts in most plants, which exhibited a removal efficiency ranging from 0.01-log to >2.96-log, although some samples from four plants were only positive in the effluent water. Nevertheless, differences in the parasite concentration between the influent and effluent samples were not significant, which was probably due to the small number of (oo)cysts identified. Protozoa were detected in the finished water of positive plants at a mean concentration of  $88 \pm 55$  oocysts per 100 l and  $37 \pm 41$  cysts per 100 l. Based on the dye permeability staining, *Cryptosporidium* oocysts identified in all positive samples excluded propidium iodide and were thus considered potentially viable. Similarly, *Giardia* cysts identified in all but four samples from either raw or finished water were also impermeable to the fluorochrome. Sources of water in the positive facilities included both surface and groundwater, and the four plants negative for both protozoa collected water from rivers or reservoirs.

The (oo)cyst removal efficiency was not related to the type of water treatment. One or both protozoa were identified in the finished water of 7 out of 11 facilities with a conventional treatment process (coagulation, flocculation, sedimentation, filtration, and disinfection processes) compared to 4 out of 9 plants that did not apply one of the pre-chlorination steps. No obvious differences were seen in the removal efficiency in relation to the type of filtration, since (oo)cysts were seen in the effluent water of plants using rapid filtration (4/5), sand plus activated carbon filtering (3/6) or slow filtration

(2/4). The mean turbidity of the raw water samples ranged from 0 to 32 NTU and was not related to the presence or concentration of (oo)cysts, as demonstrated by the low turbidity values (< 1 NTU) in some positive samples and the high value (> 5 NTU) recorded in some negative plants (Table 1). Most water treatment facilities achieved 100% turbidity removal efficiency, although a high value (186 NTU) was detected in the finished water of a plant during spring, which was related to an accidental electricity failure causing contamination with sand in the effluent water. Repeated attempts to characterize *Cryptosporidium* and *Giardia* positive samples at the species level by PCR were unsuccessful.

#### 4. Discussion

Results from this long-term study show that both *Cryptosporidium* and *Giardia* are common contaminants in the raw water of most water treatment facilities in Aragón (north-eastern Spain). The mean recovery percentage of the analytical procedure was close to 20% for *Cryptosporidium* and 50% for *Giardia*, which is considered acceptable for the USEPA method 1623 and was similar to data reported in other studies (USEPA, 2005; Ongerth, 2013). *Giardia* was identified in more than half of the facilities, being a much more prevalent protozoan than *Cryptosporidium* in all seasons, which is in agreement with observations reported for drinking water sources in Spain and other countries (Hörman et al., 2004; Carmena et al., 2007; Mons et al., 2009; Burnet et al., 2014; Castro-Hermida et al., 2015). Similarly, (oo)cyst counts revealed an overall higher concentration of *Giardia* cysts than *Cryptosporidium* oocysts, although differences were not statistically significant and contamination in the raw water rarely exceeded 100 cysts per 100 l.

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275 These results are consistent with the ubiquity of *Cryptosporidium* and *Giardia*, which  
276 have been widely reported in the human population and are common pathogens in  
277 young livestock in Spain (Navarro-i-Martínez, 2011; Carmena et al. 2012). Some  
278 epidemiological surveys in diarrhoeic suckling ruminants have revealed infection rates  
279 by *Cryptosporidium* close to 60% and 80% in calves and lambs, respectively, and  
280 prevalence values close to 100% at farm level have been reported for *Giardia* infections  
281 in cattle and sheep farms in some Spanish regions (Causapé et al., 2002; Castro-  
282 Hermida et al., 2006; Quílez et al., 2008; Gómez-Muñoz et al., 2009). In humans,  
283 cryptosporidiosis and giardiasis have been nationally notifiable diseases since 2009, but  
284 routine testing is not carried out in many laboratories, and therefore many cases may  
285 remain unnoticed, especially for cryptosporidiosis (Martín-Ampudia et al., 2012). In  
286 fact, the number of cases of giardiasis and cryptosporidiosis reported to the Spanish  
287 Microbiological Information System in 2014 were 785 and 264 respectively (MIS,  
288 2015), but studies focusing on the detection of these protozoa in symptomatic patients,  
289 mainly pediatric patients, have revealed prevalence rates up to 13-25% for giardiasis  
290 (Carmena et al., 2012). Likewise, incidence rates of human *Cryptosporidium* infections  
291 in some Spanish areas are larger than those typical of other European countries (Abal-  
292 Fabeiro et al., 2015).

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294 Several factors could account for seasonal variations in the number of (oo)cysts present  
295 in the environment, including peaks of incidence in humans and animals, rainfall,  
296 agricultural practices, humidity and temperature (Putignani and Menichella, 2010;  
297 Guzman Herrador et al., 2015b). In livestock, the occurrence of *Cryptosporidium*  
298 infections have been reported to peak corresponding with the lambing/calving season,

and consistent seasonal patterns across national boundaries have also been found for human cryptosporidiosis or giardiasis albeit with variations between different areas and countries (Xiao and Ryan, 2008; Lal et al., 2012). In this study, both protozoa were identified in the influent of drinking water facilities throughout the year, with a lower frequency in autumn and a peak in winter. This finding is in agreement with the significantly higher prevalence of human cryptosporidiosis in winter, previously observed in children aged 1-4 years in this geographical area (Clavel et al., 1996), although other studies have recorded that human cases in Spain peaked in summer (Semenza and Nichols, 2007). Diverse results in the occurrence of *Cryptosporidium* and *Giardia* in samples from river water or drinking water supplies have also been reported in other Spanish areas, with the highest frequencies in spring and autumn in the north-east (Montemayor et al., 2005), in summer and autumn in the north (Carmena et al., 2007), in spring and summer in the north-west (Castro-Hermida et al., 2008, 2009), and in winter and spring in the central area (Galván et al., 2014).

The turbidity of water has been correlated significantly with the presence of *Cryptosporidium* and *Giardia* and is considered an indicator of filtration efficiency for removal of these pathogens (Hsu and Yeh 2003; Burnet et al. 2014). Moreover, recovery rates of *Cryptosporidium* and *Giardia* by filtration are positively correlated with the turbidity of samples, with a high turbidity being considered the major contributing factor to the poor recovery of (oo)cysts from the sample concentrate (Hu et al., 2004; Kothavade, 2012). In the current study, spiking experiments revealed that recovery efficiency of our detection method was certainly related to the turbidity of water, and the treatment process in most plants was consistently efficient in reducing turbidity to values below 0.4 NTU in the finished water. However, the turbidity of the

raw water was not related to the presence and/or concentration of (oo)cysts. Most plants with a turbidity value under 5 NTU in the influent water (11/12) were positive for either *Cryptosporidium* and/or *Giardia*, while a value over 5 NTU was recorded in the raw water of three of the four plants testing negative for both protozoa. These results contrast with Spanish legislation on the quality of drinking water intended for human consumption, which does not consider the routine detection of *Cryptosporidium* or other parasites but suggests its investigation only in the presence of enteric bacteria and turbidity values over 5 NTU (RD 140/2003).

Disinfection is the final step in the conventional water treatment process, with chlorine being the most common disinfectant. Most viruses and bacteria are effectively inactivated by chlorination, but some protozoa such as *Giardia* may require prolonged contact times at high chlorine residuals to achieve 3-log inactivation, and chlorine-based disinfectants are generally not effective at inactivation of *Cryptosporidium* (Betancourt and Rose, 2004). For this reason, emphasis is placed on removing protozoa in the treatment process preceding chlorination. Nevertheless, waterborne outbreaks caused by these protozoa have occurred in systems employing conventional water treatments consisting of coagulation, flocculation, sedimentation, and filtration, revealing the inability of this technology to completely remove (oo)cysts from water (Clancy and Hargy, 2008). Several studies at pilot- and full-scale conventional water treatment plants have showed removal efficiencies ranging from 1.4 to 4.0-log for *Cryptosporidium* and 1.5-log to 4.0-log for *Giardia* (Betancourt and Rose, 2004).

In this study, the removal efficiency was similar in plants with a complete water treatment and those lacking one treatment step preceding chlorination. Drinking water

facilities were effective in retaining most (oo)cysts as demonstrated by the reduction in the number of positive plants and (oo)cyst counts in the treated water. However, some plants achieved less than 1-log removal efficiency and small numbers of *Cryptosporidium* oocysts (40-166 per 100 l) and *Giardia* cysts (12-148 per 100 l) were still identified in the finished water of three and nine facilities respectively. Additionally, (oo)cysts in most samples from both raw and finished water were found to be impermeable to propidium iodide and thus considered potentially viable, suggesting that viability is not reduced by the water treatment. Nevertheless, these findings should be taken with caution due to the small number of (oo)cysts and limitations of vital dye assays resulting in overestimation of viability (Jenkins et al. 1997). It is significant to note that none of the facilities had ultraviolet radiation, which has been reported as an alternative disinfection procedure to inactivate (oo)cysts in water at appropriate levels (Clancy and Hargy, 2008). It is also worth mentioning the apparent lack of (oo)cysts in the raw water of four plants which tested positive for the finished water, a finding previously reported in drinking water facilities, that could be related to the small volume of raw water filtered because of filter clogging, revealing the limitations of analytical methods for recovering these organisms from water samples (Castro-Hermida et al., 2008; Ongerth, 2013).

The presence of potentially viable *Cryptosporidium* oocysts and *Giardia* cysts in the finished water of some municipalities in this study may present a serious public health issue. The (oo)cyst concentrations may not be enough to induce human infections since the infectious dose (ID50) has been estimated to be 10-83 oocysts for *Cryptosporidium* (Chappell et al., 2006) and 19-50 cysts for *Giardia* (Adam, 2001), but serve as a warning with regard to the potential risk of waterborne outbreaks and the significance of

protecting water sources from contamination. Unfortunately, the presence of zoonotic *Cryptosporidium* spp / *Giardia* spp could not be confirmed, since the PCR amplification was unsuccessful for all positive specimens selected for molecular characterization, a finding which might be related to the low parasite load or the presence of PCR inhibitors in the water. In northern and central Spain, conventional water treatment plants were highly efficient and no protozoa were detected in the finished water, but a high proportion of samples of effluent water from small facilities, including rapid filtration and/or disinfection processes only, were positive at average concentrations of 2.3-7.8 oocysts / 100 l and 1.3-2 cysts / 100 l (Carmena et al., 2007; Galván et al., 2014). In contrast, conventional water treatment facilities were inefficient in reducing/inactivating these pathogens in drinking water in the north-west of the country, where up to 190 oocysts per 100 l and 330 cysts per 100 l were estimated to be present in the treated drinking water of some facilities, and 90-95% of (oo)cysts were potentially viable according to a vital-dye assay (Castro-Hermida et al., 2015).

## 5. Conclusions

The current study highlights the occurrence of *Cryptosporidium* and *Giardia* in the drinking water facilities of the most populated towns in Aragón region of Spain, and the limited efficiency of conventional water treatment processes in removing (oo)cysts. Intensive efforts should be made to prevent the contamination of water sources with these pathogens and new regulations should be implemented to evaluate the need of testing for *Cryptosporidium* and *Giardia* in water, including monitoring tools as an alternative to turbidity readings. Our results also reveal the need for improving



analytical methods to identify these protozoa and test their viability/infectivity in contaminated water.

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**Figure 1.** Geographic location of drinking water facilities sampled in Aragón (north-eastern Spain). The served population, source of water and type of water treatment for each plant are indicated in Table 1.

**Figure 2.** Number of positive plants for *Cryptosporidium* and *Giardia* in samples from 20 drinking water treatment facilities in north-eastern Spain. Samples from the influent and effluent water were collected at each plant in spring, summer, autumn and winter.

**Table 1.** Main features of drinking water treatment plants at the most populated towns in Aragón (north-eastern Spain). Average values and ranges (maximum and minimum) in the turbidity and turbidity removal efficiency are indicated. Values of turbidity are expressed in nephelometric turbidity units (NTU)

Plant <sup>a</sup>	Served population	Source of water	Water treatment	Turbidity (NTU)		Turbidity removal efficiency (%)
				Influent	Effluent	
1	664,953	River	P1A	32 (6–42)	0	100
2	12,141	River, reservoir	P1B	6 (0–12)	0	100
3	7,820	Reservoir	P1B	3 (0–9)	0	100
4	7,014	River, reservoir	P2	5 (0–18)	0	100
5	6,941	Reservoir	P3	6 (0–22)	0.1 (0–0.4)	98.3
6	16,754	Reservoir	P1B	7 (4–9)	0	100
7	19,724	Reservoir	P1A	5 (0–19)	0	100
8	7,680	River	P3	0	0	0
9	10,759	River	P1B	6 (0–11)	0	100
10	9,867	River	P3	0.2 (0–1)	0.4 (0–1)	NR
11	52,239	River	P3	3 (0–6)	0	100
12	13,088	River	P1A	0.1 (0–0.3)	46 (0–186)	-----
13	9,598	Underground river	P3	0	0	0
14	17,020	River	P1B	3 (2–3)	2 (0–7)	33.3
15	17,260	Reservoir	P1B	24 (4–49)	0	100
16	14,921	Reservoir	P2	3 (0–12)	0	100
17	9,439	Reservoir	P2	2 (0–6)	0	100
18	35,590	Groundwater	P1A	0	0	0
19	8,065	Groundwater	P2	0	0	0
20	16,230	River	P1A	4 (0–9)	0	100

- P1: conventional water treatment facilities that include coagulation, flocculation, sedimentation, filtration and chlorination processes. Two types of filtration systems were used, including rapid sand filtration (A) or a combination of sand and activated carbon filtering (B)
- P2: water treatment facilities that include slow sand filtration and chlorination only
- P3: water treatment facilities that include sedimentation and/or chlorination only

**Table 2.** *Cryptosporidium* oocyst counts [arithmetic mean and ranges (maximum and minimum)] and removal efficiency (removal log) of oocysts in drinking water treatment plants in north-eastern Spain. Samples from influent and effluent water were collected four times from each plant, each sampling time matching with a different season.

Plant	Influent		Effluent	
	Positive season <sup>a</sup>	Oocysts / 100 litres	Positive season <sup>a</sup>	Oocysts / 100 litres
1	W, SM	65 (30–101)	–	0
2	W	40	–	0
3	W	40	–	0
4	–	0	–	0
5	SM	40	–	0
6	–	0	–	0
7	SP	160	–	0
8	–	0	–	0
9	W, SP	70 (40–101)	–	0
10	W	81	–	0
11	W	40	–	0
12	–	0	A	166
13	–	0	A	40
14	–	0	–	0
15	–	0	–	0
16	–	0	–	0
17	–	0	SP	60
18	–	0	–	0
19	–	0	–	0

20	–	0	–	0	–
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<sup>a</sup> SP: Spring; SM: summer; A: autumn; W: Winter

<sup>b</sup> NR: No removal: oocyst were only detected in the finished water.



**Table 3.** *Giardia* cyst counts [arithmetic mean and ranges (maximum and minimum)] and removal efficiency (removal log) of cysts in drinking water treatment plants in north-eastern Spain. Samples from influent and effluent water were collected four times from each plant, each sampling time matching with a different season.

Plant <sup>a</sup>	Influent		Effluent	
	Positive season <sup>a</sup>	Cysts / 100 litres	Positive season <sup>a</sup>	Cysts / 100 litres
1	W, SM, SP	94 (41–142)	W	12
2	–	0	SP	24
3	SM	33	SM	16
4	–	0	–	0
5	W, SP	49 (33–65)	–	0
6	–	0	–	0
7	W, SP	915 (53–1769)	–	0
8	–	0	–	0
9	W, SM, A, SP	152 (33–407)	SP	148
10	A, SP	49 (33–65)	A	16
11	–	0	–	0
12	W, SM, A	33	–	0
13	–	0	–	0
14	W, SM	33	–	0
15	–	0	–	0
16	SM	33	A	16
17	–	0	SP	49
18	W	24	W, SM, A	33 (16–49)
19	W, SP	49 (33–65)	–	0

<b>20</b>	W, SM	33	SP	16	0.31
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<sup>a</sup> SP: Spring; SM: summer; A: autumn; W: Winter

<sup>b</sup> NR: No removal: cysts were only detected in the finished water.

Figure 1  
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