Impact of a wastewater treatment plant on Cryptosporidium oocysts and Giardia cysts occurring in a surface water

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Abstract The aim of this study was to assess the impact of a municipal wastewater treatment plant (MWTP) on the occurrence of Cryptosporidium oocysts and Giardia cysts in the receiving water. All MWTP effluent samples were Giardia and Cryptosporidium contaminated, although low mean values were found for both parasites (0.21±0.06 oocysts/L; 1.39±0.51 cysts/L). Otherwise, in the raw sewage a greater concentration was detected (4.5±0.3 oocysts/L; 53.6±6.8 cysts/L). The major occurrence of Giardia over Cryptosporidium, both in the influent and in the effluent of the MWTP, is probably related to the human sewage contribution to the wastewater. Data on protozoa contamination of the receiving water body demonstrated similar concentrations in the samples collected before (0.21±0.07 oocysts/L; 1.31±0.38 cysts/L) and after (0.17±0.09 oocysts/L and 1.01±1.05 cysts/L) the plant effluent discharge. The results of this study suggest that the MWTP has no impact related to Giardia and Cryptosporidium river water contamination, and underline the need for investigation into the effectiveness of these protozoa removal by less technologically advanced MWTPs which are the most widespread and could probably show a lower ability to reduce protozoa.

Keywords Cryptosporidium; Giardia; monitoring; sewage; surface water; wastewater treatment plant

Introduction Many important factors can affect environmental spreading and consequently the circulation in humans of the pathogens Cryptosporidium and Giardia (Craun, 1990; Smith et al., 1995; Meinhardt et al., 1996; Casemore et al., 1997; Rose, et al., 1997a). The large number of possibilities of watersheds pollution (point and non point sources) have an important role in these protozoa circulation. The direct pollution of watersheds derives from human population presence on the land through municipal sewage discharge, direct faecal contamination, infiltration from septic tanks and from human activities such as intensive livestock breeding on the land, disposal of slurry from animal breeding farms, accidental shedding from tanks for slurry and muck storage, and agricultural manuring. The animal contribution to the spreading of these parasites is determined by infected livestock grazing and watering, infected wild animal movement, infected domestic animals and pets. The watershed pollution occurs also in an indirect way, through the run-off and leaching of wild and agricultural contaminated lands (Madore et al., 1987; Sykora et al., 1991; Smith et al., 1995; Rose, 1997b).

One of the most useful approaches to the problem of these pathogens spreading in the water bodies is to know the specific sources of contamination and their quantitative value in different lands and watersheds, in order to limit the routes of transmission.

Since waterborne outbreaks investigations frequently report sewage as the contamination source, it is fundamental to evaluate the role of municipal wastewater treatment plants (MWTP) in Cryptosporidium and Giardia water pollution with the aim of developing treatment processes with lower contamination activity.
Investigations on cyst and oocyst concentrations in raw and treated sewage reveal that the occurrence of these organisms appears to be highly variable especially in the influent samples. Values range from $10^2$ to $10^4$ oocyst/L and from $10^3$ to $10^5$ cyst/L (Madore et al., 1987; Sykora et al., 1991; Jakubowski et al., 1991; Gassmann and Schwartzbrod, 1991; Chauret et al., 1995; Vilagines et al., 1996; Mayer and Palmer, 1996; Bukhari et al., 1997) in the raw sewage, while lower numbers have been recovered in treated sewage in the order of $10$ to $10^3$ (Madore et al., 1987; Sykora et al., 1991; Chauret et al., 1995; Robertson et al., 1995; Vilagines et al., 1996; Mayer and Palmer, 1996; Bukhari et al., 1997) for both the protozoa. A general consideration is that Giardia cysts were detected in higher concentrations than Cryptosporidium oocysts in sewage influents (Vilagines et al., 1996; Mayer and Palmer, 1996; Bukhari et al., 1997). However, a comparison between these data is very difficult because different methods of sampling, purification and determination have been used, which included microscopical evaluation without IFA (Harry Mc, 1984) and detection using PCR technique (Mayer and Palmer, 1996).

Considering the removal efficiency of sewage treatment processes the parasite concentrations in the effluents of MWTP are variable, depending upon their concentration in the influent and the processes applied. The removal by typical sewage treatments is critical, and never guarantees a complete abatement of these protozoa. Primary and secondary sedimentation allow a variable removal due to the difference between the sedimentation velocity of cysts and oocysts with respect to the process (Enriquez et al., 1995; Withmore and Robertson, 1995). A recent research carried out by Medema et al. (1998) pointed out that the ability of sedimentation to settle cysts and oocysts is related to their attachment to particles. The removal efficiency of the activated sludge process for these pathogens is related to the clarification process: the flocculated material in the activated sludge was believed to contribute to the removal acting as a “settling blanket” (Villacorta-Martinez et al., 1992; Enriquez et al., 1995; Stadterman et al., 1995; Withmore and Robertson, 1995). The adoption of a filtration treatment in the tertiary phase seems to favour a further reduction of the oocyst and cyst concentration that has been evaluated with flocculant addition to be approximately 99% for Giardia and 90% for Cryptosporidium (Madore et al., 1987; Enriquez et al., 1995).

The aim of this study was to examine the impact of the effluent of a large MWTP, with technologically advanced treatment process, on Giardia cysts and Cryptosporidium oocysts in the receiving water body.

**Methods**

**Sampling**

The study was developed at the Azienda Po-Sangone (APS) sewage treatment plant, which collects sewage from a 450 km$^2$ area, which includes Turin and surrounding areas, with a population of 1.5 million inhabitants and 1,800 industries, representing 3 million equivalent inhabitants. The plant treats a yearly wastewater load of more than 200 million cubic metres and discharges the treated effluent into the Po river. Turin and the surrounding towns are the first urban centres to discharge effluents into the Po. The Turin area represents only 1.2% of the Po river basin and is responsible for about 10% of the total pollution load discharged into the Po from urban and industrial sources.

The APS plant treats mixed sewage (70% municipal and 30% industrial) and is divided into a “water line” (average daily flow 550,000 m$^3$) and a “sludge line” (average daily flow 6,000 m$^3$). The “water line” in the plant, where the study was carried out, is divided into three equivalent units and consists of primary treatment with a degritting and de-oiling phase and primary sedimentation (total surface 25,488 m$^2$, ascensional speed 1.5 m/h); secondary treatment with biological oxidation with activated sludge (capacity 153,000 m$^3$, sludge load 0.15–0.2 Kg BOD/kg SST/die), secondary clarification (total surface 41,200 m$^2$,
ascensional speed 0.9 m/h); and tertiary treatment with chemical dephosphorization and effluent filtration through multi-layer (gravel, sand and carbon) filters (total surface 2,700 m², maximum filtration speed 10 m/h).

The study was carried out in the years 1995 and 1997. Cyst and oocyst assessments were performed on effluents from the APS plant (n=11) and on water samples from the Po river collected before (n=11) and after (n=11) the plant effluent discharge. In addition, to evaluate the effectiveness of the treatment in removing protozoa, three samples of wastewater influent were also analysed to ascertain the initial level of contamination.

Sample collection and protozoa analysis
Cyst and oocyst concentration was investigated according to the U.S.E.P.A. (USEPA, 1996) procedure. Plant effluent (500 L) and influent sewage (10 L) samples and Po river water samples (500 L) were collected the same day at fixed hours, using an electric driven water pump and filtered through wound polypropylene cartridges (Fluxa, Micro-Wind II, nominal porosity 1 µm) at a flow rate of 5–10 litres per minute. Samples were processed within 24–48 h. Cartridges were cut down to the core by using a bistoury, teased and washed with three litres of PBS eluting solution with 0.1 SDS (Sigma Chimica) and 0.1% Tween-80 (Fluka Chemika-BioChemika). Solution was concentrated into a single pellet by centrifugation (1050 G, 15 min., 4ºC) and purified by flotation on Percoll-sucrose gradient (Sigma, Chimica; specific gradient 1.10) (1050 G, 15 min., 4ºC). Samples washed with PBS Tween-20 were analysed using an indirect immunofluorescence antibody assay (Hydrofluor-Combo, Ensys Inc.).

Microscopic examination was performed using a microscope equipped with phase contrast and epifluorescence optics (exciter wavelength: 450–490 nm). The screening examination was carried out at 400, confirming the proper shaped apple-green labelled structures at 1000, with the aid of a linear micrometer. Presumptive viability was evaluated by the examination of cysts and oocysts in phase-contrast microscopy.

Total coliforms, faecal coliforms and faecal streptococci were also enumerated for each sample in the treated effluent and in the Po river water samples following the APHA-AWWA-WEF Standard methods (19º ed., 1995).

Results and discussion
All the MWTP effluent samples were found to be positive for both *Cryptosporidium* oocysts and *Giardia* cysts (Table 1). However, a low level of contamination for the parasites, especially for *Cryptosporidium*, was encountered with an average value of 0.2 oocysts/L and 1.4 cysts/L. The trend reveals a variability of the protozoa concentration within a limited range from 0.1 to 0.3 oocyst/L for *Cryptosporidium* and from 0.8 to 2.4 cyst/L for *Giardia*.

Since low numbers of parasites were found in the plant effluent, three samples of raw sewage were examined to evaluate the baseline level of contamination and to point out the removal performed by the treatment process.

Taking into account the low number of samples analysed, an approximately 10-fold higher number of both the protozoa was recovered in the influent sewage than in the APS plant effluent (Table 2).

The level of parasites found was low in comparison to the concentrations reported in other investigations (Madore et al., 1987; Sykora et al., 1991; Jakubowski et al., 1991; Gassmann and Schwartzbrod, 1991; Chauret et al., 1995; Vilagines et al., 1996; Mayer and Palmer, 1996; Bukhari et al., 1997). In fact, the concentration of *Cryptosporidium* and *Giardia* in sewage influents is dependent upon both the size of the contributing community and the levels of infection and disease within that community. As reported by other authors...
(Vilagines et al., 1996; Mayer and Palmer, 1996; Bukhari et al., 1997), also in this study Giardia cysts were detected at higher concentrations than Cryptosporidium oocysts in both raw and treated sewage. This result could be related to the human sewage contribution to the wastewater.

Although the number of the influent samples in comparison with the number of effluent samples is limited, an effective reduction of the parasite concentration by the treatment was observed. The removal was approximately 77.5% for oocysts and 74% for cysts.

Evaluating the presumptive viability of the protozoa, a mean value of 49.5% for oocysts and 45.5% for cysts containing internal structures was observed in the effluent, while in the influent a greater percentage of full oocysts and cysts were recovered (65.6% for Cryptosporidium and 69.7% for Giardia). Despite the limitations of this evaluation method, the treatment process seems to favour the excystation and to reduce the potential viability of cysts and oocysts.

The analysis of the Po river before and after the APS plant effluent discharge shows the presence of both cysts and oocysts in all water samples (Table 3). The oocyst concentrations were similar in the upstream (mean value=0.2 oocyst/L) and downstream (mean value=0.17 oocyst/L) Po river samples. Also Giardia cyst concentrations demonstrated analogous values in the Po river water collected upstream (mean value=1.3) and downstream (mean value=1.0).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Results of the microbiological analyses in the MWTP effluent</th>
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<tbody>
<tr>
<td>Units</td>
<td>1</td>
</tr>
<tr>
<td>Giardia cyst/L</td>
<td>1.7</td>
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<tr>
<td>cyst viability</td>
<td>%</td>
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<tr>
<td>Cryptosporidium oocysts/L</td>
<td>0.2</td>
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<tr>
<td>oocyst viability</td>
<td>%</td>
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<th>Table 2</th>
<th>Results of the microbiological analyses in the MWTP influent (raw sewage)</th>
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<tbody>
<tr>
<td>Units</td>
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<tr>
<td>Giardia cysts/L</td>
<td>52.5</td>
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<tr>
<td>cyst viability</td>
<td>%</td>
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<tr>
<td>Cryptosporidium oocysts/L</td>
<td>4.3</td>
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<tr>
<td>oocyst viability</td>
<td>%</td>
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<tr>
<th>Table 3</th>
<th>Results of the microbiological analyses in the Po river water before (upstream) and after (downstream) the MWTP effluent discharge</th>
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<tbody>
<tr>
<td>Upstream Units</td>
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<tr>
<td>Giardia cyst/L</td>
<td>1.68</td>
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<tr>
<td>cyst viability</td>
<td>%</td>
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<tr>
<td>Cryptosporidium oocysts/L</td>
<td>0.24</td>
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<tr>
<td>oocyst viability</td>
<td>%</td>
</tr>
<tr>
<td>Downstream Units</td>
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<tr>
<td>Giardia cyst/L</td>
<td>2.55</td>
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<tr>
<td>cyst viability</td>
<td>%</td>
</tr>
<tr>
<td>Cryptosporidium oocysts/L</td>
<td>0.42</td>
</tr>
<tr>
<td>oocyst viability</td>
<td>%</td>
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</table>
Moreover, *Giardia* cysts concentrations were found always higher than *Cryptosporidium* oocysts, both in upstream and downstream samples. Little variation among oocysts concentration was recorded at the upstream site, whereas the concentration of cysts and oocysts was more variable at the downstream site.

The concentrations of both parasites are low if compared to other data regarding surface waters reported in literature (Smith *et al.*, 1995), but they are in agreement with our studies performed on Po river water samples collected at a sampling site 30 Km upstream of the sampling sites of this study (Bertolucci *et al.*, 1998).

The mean percentage of oocysts and cysts presumed viable was found to range from 60 to 66% for both protozoa in the upstream and downstream Po river samples. These percentage values could suggest that the oocysts and cysts recovered in these sites come from the same source of contamination. Moreover, the mean percentage of oocysts and cysts with internal content recovered in the Po river was higher than that encountered in the plant effluents, while it is similar to the percentage assessed in the raw sewage; this result could enhance the absence of any contribution to river pollution by the effluent discharged by the APS facility.

Results obtained from this study (Figure 1) show that the parasite concentration is comparable in the effluent of the APS plant and in the Po river before and after the plant effluent discharges. This finding, combined with results previously described regarding the presumptive viability of the protozoa, confirms that the APS plant does not contribute to *Cryptosporidium* and *Giardia* contamination of the Po river.

Further support for this hypothesis comes from the results of the other microbiological parameters evaluated (data not show). In fact, while the parasite concentrations were comparable in the effluent and in the Po river, the number of total coliforms, faecal coliforms and faecal streptococci were higher in the treatment effluent than in the Po river water. Furthermore, no significant association was found between *Cryptosporidium* and *Giardia* presence and these bacteriological parameters. So, it is probable that the contamination encountered in the Po river derives from multiple sources such as other raw or treated discharges of municipal sewage within the above area, woodlands, grazing and watering areas and agricultural run-off and shedding. Moreover, the lack of correlation between oocysts

![Graph](https://iwaponline.com/wst/article-pdf/41/7/31/427561/31.pdf)

**Figure 1** *Cryptosporidium* oocysts and *Giardia* cysts in the APS treatment plant effluent and in the Po river water before and after the effluent discharge.
and cysts concentration in the sampling sites suggests a different contamination origin of both protozoa.

In this investigation a low spreading of the parasites was found. In fact, low concentrations of cysts and oocysts were encountered in both influent and effluent from the sewage treatment facility and in the Po river when compared to the levels of contamination reported in other studies. This difference could be due to an effective low spreading of these pathogens in the analysed area and to their poor circulation in the human population. As a result, no waterborne outbreak has ever been notified either in the Piedmont region or in the whole of Italy. Moreover, it could depend on other important factors, such as geographical extension of the area served by the treatment plant, served population size, proportional composition of the raw sewage, precipitation amount and frequency during the investigation and the sampling hours.

Conclusions

The ubiquity of Cryptosporidium oocysts and Giardia cysts in sewage is a clear warning against the discharge of untreated sewage to any water that may be used as a drinking water source or is inhabited by animals that may become infected. This can cause both the spreading of the protozoa and the magnification of their concentration.

Extended monitoring in Italy must be started with the aim of making a screening of surface water Cryptosporidium and Giardia contamination for their dual aspect role of receptor body and of water source for human consumption. Monitoring is important because of the variability in different areas of the factors affecting these protozoa spreading.

Additional researches on removal ability and contamination power of sewage treatment plants are needed. Moreover, the sewage entering the plant must be examined in order to evaluate the major sources of protozoa contamination. Attention has to be paid particularly to the less technologically advanced MWTPs, which are the most widespread and will probably have a lower ability to reduce protozoa related to the inconstancy of treatment efficiency. Finally, investigations of digested sewage sludge contamination are needed to evaluate cyst and oocyst presence and viability for their use in soil fertilisation.

In conclusion, by surveying the occurrence of Giardia and Cryptosporidium in raw sewage, information about the level of circulation of these parasites in the human population could be obtained.

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References


