Quantitative risk assessment of Cryptosporidium in surface water treatment


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Abstract Quantitative microbiological risk assessment requires quantitative data to assess consumer exposure to pathogens and the resulting health risk. The aim of this study was to evaluate data sets on the occurrence of Cryptosporidium oocysts in raw water and on the removal of model organisms (anaerobic spores, bacteriophages) to perform such a risk assessment. A tiered approach was used by first calculating approximate point estimates and when the point estimate was close to the required safety level (10⁻⁴ annual risk of infection), fitting the data to probability distributions and Monte Carlo analysis to calculate the distribution of the risk of infection. Sensitivity analysis showed that the variability in the Cryptosporidium data in raw water (largely introduced by the variability of the recovery efficiency of the detection method) determined most of the variance in the risk estimate.

Keywords Cryptosporidium; drinking water; probability distributions; risk assessment; treatment efficiency

Introduction

The new Dutch Drinking Water Decree states that for pathogenic microorganisms, the health risk should be as low as 1 infection/10,000 consumers/year (VROM, 2001). As the corresponding maximum concentrations in drinking water are far too low to be determined with the current detection methods, the risk of infection is to be inferred from data on the occurrence of pathogenic microorganisms in raw water and information on the efficiency of the treatment processes in elimination of these microorganisms. The legislation does not contain a specific protocol or guidance on how to collect the required information or how to infer a risk of infection from the data on raw water and treatment efficiency. Mathematical approaches to microbiological risk assessment for drinking water have been previously published (Teunis et al., 1997; Haas et al., 2000). The aim of this study was to determine the applicability of data on raw water quality and treatment efficiency to assess the risk of infection of Cryptosporidium from drinking water produced by surface water treatment systems in the Netherlands using these mathematical approaches.

Methods

The exposure assessment was based on data on the occurrence of Cryptosporidium in raw waters and on the removal efficiency of treatment systems obtained with removal of spores of sulphite-reducing clostridia (SRC) (or bacteriophages) under full-scale conditions. These were combined with published data on the consumption of unheated drinking water in the Netherlands. To assess the potential health effects, these exposure data were fed into the dose-response relation for infection with Cryptosporidium. The combined dose-infection relation for three C. parvum strains of the bovine genotype was used (Ockhuysen et al., 1999; Teunis, pers. comm.). A tiered approach was used. Point estimates were made of the...
risk of infection through drinking water. In systems with a relatively high-risk estimate, the uncertainty in the risk estimates was determined by fitting probability distributions to the data sets. The distribution of the risk was determined with Monte Carlo analysis.

**Case study 1 – approximate assessment indicated management actions needed**

A surface water company used water from a lake as source water. The lake was fed by a river contaminated by discharges of mainly treated domestic wastewaters and by local streams. During rainfall events, additional contamination was likely to come from run-off from agricultural lands and overflows of combined sewers. The raw water was taken into a reservoir with an average residence time of one month, treated with microstrainers (pore size 35 µm), break-point chlorination (CT₁₀ = 10 min.mg/L), coagulation (Fe dose 5 mg/L, flocculant aid Wispro added in winter), sedimentation and rapid sand filtration, activated carbon filtration (10 min contact time) and chlorine dioxide (CT₁₀ = 2 min.mg/L for post-disinfection). The treated water was distributed to 100,000 consumers through a metered distribution system with water losses of <1% and back-siphon-stops.

**Source water quality**

The water company had conducted a monitoring programme of its source water for one year. The source water was sampled each month for the presence of *Cryptosporidium* (Figure 1) with samples (100 L) being filtered and concentrated by centrifugation. The concentrate (1 mL, 3–30% of the sample) was purified by Percoll sucrose flotation and FACS followed by analysis for *Cryptosporidium*.

The concentration of oocysts varied from not detectable to 1.2 oocysts/L with levels highest in winter. The recovery efficiency of the detection method was low and variable. Hence, as the measured concentrations were an underestimation of the actual concentration of oocysts in the source water, measured data were adjusted for the recovery efficiency. To include the variation in both the measured concentrations and the recovery efficiency in the adjusted concentrations, one concentration was randomly selected from the data set on measured concentrations and one recovery efficiency was drawn from the data set on the recovery efficiency of the method in surface water samples (n = 99). These were used to calculate an adjusted concentration. This was repeated 5,000 times. The resulting distribution of adjusted concentrations generated by this bootstrap analysis is shown in Figure 2. The distribution was very skewed with 42% of the adjusted measurements being 0/L, while a small fraction of the adjusted concentrations were very high due to the combination of a high measured concentration and a low recovery efficiency. The median

![Figure 1](https://iwaponline.com/wst/article-pdf/47/3/241/424030/241.pdf)
of this distribution was 1.34 oocysts/L, the average 51 oocysts/L and the 95-percentile was 128 oocysts/L.

**Treatment efficiency**

To get a first idea of the level of protection of the drinking water, an approximate assessment of the treatment efficiency was conducted. The reservoir storage had been shown to reduce the concentration of indicator bacteria by 0.5–1 log$_{10}$ unit. This was considered indicative of the reduction of the *Cryptosporidium* concentration. It was noted, however, that recontamination by wildlife might occur in the reservoir. The microstrainers were considered to have little or no effect on oocyst levels. The removal of oocysts by the coagulation-filtration step was inferred, from data on removal of anaerobic spores, as 2–2.5 log$_{10}$ units. Chlorination was considered to have no effect. In combination with the low dose of chlorine dioxide some inactivation might have been attributed but this was considered to be <0.5 log$_{10}$ units. Hence, the approximate assessment of the treatment efficiency resulted in an expected efficiency of 2.5–4 log$_{10}$.

**Concentration in drinking water and water consumption**

The concentration in drinking water, as calculated from the average adjusted concentration of oocysts in the source water and the expected treatment efficiency, was 0.0051–0.16 oocysts/L. The median consumption of drinking water in the Netherlands (without further heat treatment) was taken as 0.153 L/d (Teunis *et al*., 1997).

**Exposure assessment and approximate risk characterisation**

A point estimate of the annual exposure of the consumers, inferred from these data, was 0.28–9 oocysts/person/year. The exposure estimate was transformed into an estimate of the risk of infection using the dose response data and the best-fit exponential model (Teunis *et al*., 1999):

\[
P_{inf} = 1 - e^{-\text{dose}*0.004005}
\]  

The calculated risk of infection was $1.1 \times 10^{-3} - 3.5 \times 10^{-2}$/person/year.
Risk management

The approximate risk assessment indicated that the risk of infection of the drinking water produced by this treatment plant was above the acceptable level of a $10^{-4}$ risk of infection both in the high and the low estimate of the treatment efficiency. The assessment of the source water quality was considered as conservative since it used concentrations that (a) were adjusted for the recovery efficiency and (b) did not take into account that a proportion of the oocysts detected may have been inactive or of a genotype that was not infectious to humans. On the other hand, the guidelines on the acceptable risk of infection in the Netherlands also state that a safety factor of 10 should be incorporated into the risk assessment because of host and parasite variation. As the difference between required and actual treatment efficiencies was substantial it was felt that this gap ($1–2.5 \log_{10}$) could not be closed by refinements in source water quality assessment (viability assays, genotyping) or a more detailed assessment of the treatment efficiency. Based on this approximate risk estimate the water company concluded that additional or alternative treatment should be installed. By combining this information with the formation of toxic by-products by chlorine, the reported effectiveness of UV in inactivating Cryptosporidium and the costs of UV, the water company is to run a pilot plant to study the implications of replacing chlorine with UV treatment.

Case study 2 – approximate assessment indicated system is safe

The source water described in case study 1 was also used in another treatment system. After coagulation/filtration the water was infiltrated into a fine-grained sandy soil. After passage through the aquifer, with a residence time of 60 d and travel distance of 60 m, the water was recollected from the soil by pumping wells and, after aeration and rapid sand filtration, was distributed to the consumers in a distribution system similar to case study 1.

Treatment efficiency

In addition to the removal by reservoir storage and coagulation/filtration, removal by soil passage was assessed. From field experiments, where MS2 and PRD1 bacteriophages were inoculated into the water just prior to infiltration and breakthrough was monitored in a ray of monitoring wells, it was shown that a soil passage of 30m yielded 8 logs reduction of the phage concentration (Schijven et al., 1999). The first (centi?)metres of passage were most efficient. Subsequent passage could be described with a linear relation of 0.2 log removal per metre of soil passage. In 50 cm soil columns, containing the sand from this infiltration site, it was shown that Cryptosporidium parvum oocysts were 1.5–3× more efficiently removed than MS2 phages. By combining these data, it was inferred that the 60 m of soil passage would yield a Cryptosporidium removal of 18–36 log_{10} units. Added to the approximate efficiency of the pre-treatment of 2.5–4 \log_{10}, the treatment efficiency of this system was very high.

Concentration in drinking water

The concentration in drinking water, as calculated from the average adjusted concentration of oocysts in the source water and the expected treatment efficiency, was $<5 \times 10^{-19}$ oocysts/L.

Exposure assessment and approximate risk characterisation

The point estimate of the annual exposure of the consumers was $2.8 \times 10^{−17}$ oocysts/person/year. The exposure estimate was transformed into a risk estimate (see case study 1) and the calculated risk of infection/person/year was 0.
Risk management
The approximate risk assessment indicated that the risk of infection of the drinking water produced by this treatment plant was very far below the acceptable level of a $10^{-4}$ risk of infection, already with the low estimate of the treatment efficiency. Based on this approximate risk estimate, the water company concluded that this system was very safe with regard to *Cryptosporidium* and have provided valuable information to substantiate this claim.

Case study 3 – comprehensive risk assessment
Another water company used river water as a source which fed into three subsequent reservoirs with an average residence time of five months. In the treatment system, water received from the reservoirs was filtered through microstrainers and supplemented with ferric sulphate to induce floc formation. Flocs were removed by flotation and rapid sand filtration. The water was subsequently treated with ozone ($CT_{10} = 1.1–1.2 \text{ mg.min/L}$) followed by sand/anthracite filtration and GAC filtration with 10 min contact time. After addition of chlorine dioxide ($<0.2 \text{ mg/L}$) the water was distributed to consumers. This plant was properly designed and operated. No extraordinary events occurred in raw water quality or treatment performance.

Source water quality
The source water was monitored for *Cryptosporidium* by weekly samples for one year. Analytical methods were identical to the EPA1623 method with the exception that laser scanning cytometry (Chemscan RDI) was used to locate the stained oocysts and oocysts were stained with PI/DAPI. In addition, a PCR was performed on the water concentrates using the 18S-rRNA gene as target (Heijnen et al., 2002). The PCR-product was cloned and sequenced to determine if the *Cryptosporidium* oocysts were human or animal genotypes. The recovery efficiency of the sampling and concentration method was determined for every sample by taking an additional sample and spiking this with a known number of oocysts. Basic statistics of the occurrence data, corrected for their corresponding recovery efficiency, are presented in Table 1 where it can be seen that the distribution was skewed.

The data were fitted to a Negative Binomial distribution, yielding the parameters (i) $k = 0.131, p = 0.000226$ for all oocysts and (ii) $k = 0.063, p = 0.000219$ for PI-negative (potentially infectious) oocysts.

Of 18 samples tested with PCR, *Cryptosporidium* was detected in seven, of which five contained the bovine genotype of *C. parvum* and one contained a genotype closely related to *C. baileyi*. The other genotype was considered a new genotype of *Cryptosporidium* (Heijnen et al., 2002). Accordingly, the majority of samples that contained *Cryptosporidium* were of strains probably pathogenic to humans.

Treatment efficiency
A bench-scale study on the effectiveness of ozone against *C. parvum* oocysts in water of this treatment plant indicated that at low temperatures little to no inactivation of oocysts was to

| Table 1 *Cryptosporidium* in raw water (after off-stream storage for 5 months). Data corrected for the (individual) recovery efficiency data |
|---------------------------------|-----------------|-----------------|
| No of samples | 26 | P90% (n/1,000 L) | 1,067 |
| No of positive samples | 17 | Total number of oocysts detected | 70 |
| P10% (n/1,000 L) | 0 | PI- (non-viable) | 40% |
| P50% (median; n/1,000 L) | 47 | | |
be expected (Oppenheimer et al., 2000). Thus, attention was focussed on the effectiveness of physical removal by the coagulation/sedimentation/filtration process. Spores of sulphite-reducing clostridia (in volumes up to 100 L) were monitored before and after treatment to determine the removal efficiency (Table 2).

**Risk assessment – point estimate**

A point estimate of the concentration in drinking water was calculated from the data (median) on PI-negative *Cryptosporidium* oocysts in raw water and the average log-removal of anaerobic spores. This resulted in calculated concentrations in drinking water of $0.50/1,000$ L. The corresponding point estimate of the annual risk of infection was $1.1 \times 10^{-4}$. As this point estimate was close to the required $10^{-4}$/person/year risk of infection, more effort was dedicated to determine the uncertainty of this estimate.

**Risk assessment – stochastic modelling**

The data on PI-negative *Cryptosporidium* in raw water (corrected for the recovery efficiency), on the removal of SRC during treatment and the consumption of drinking water were fitted to statistical distributions (respectively Negative Binomial, Beta-Binomial and Log-normal). Monte Carlo analysis resulted in a distribution of exposure that was used as input for the dose-response model to calculate the risk of infection (Figure 3). This showed

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<td>Coagulation/lamellae</td>
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<tr>
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**Figure 3** Cumulative frequency distribution of the calculated annual risk of infection, using spore or diatom removal as model for *Cryptosporidium*
that the $10^{-4}$ level coincided with the 90.7% confidence limit of the risk distribution. In other words, the probability that the average risk was below $10^{-4}$ was 90.7%. With the safety factor of 10 ($10^{-5}$ risk), this probability was 82.8%. The legislation is not clear on whether this was sufficient or not. The water company is now focussing research on reducing the uncertainty in these data. Sensitivity analysis showed that 94% of the variance in the risk estimate was determined by the data on Cryptosporidium in raw water, so this is the main research topic. As anaerobic spores are smaller than oocysts, they may be a conservative model for oocyst removal by coagulation and filtration. Research will also be dedicated to compare the removal of spores and oocysts by these processes.

Conclusions
This study showed that quantitative risk assessment could be used in a tiered approach. Approximate point estimates may be sufficient in cases where the risk estimate lies clearly above or below the required safety level. More extensive risk assessment, using statistical techniques to determine the distribution of the risk estimate (providing a quantitative estimate of certainty) was used when the approximate estimate was close to the required safety level. The uncertainty in the risk estimate was primarily determined by the variability in the data on occurrence of oocysts in raw water, which was in turn primarily determined by the variability in the recovery efficiency of the detection method. Also the variability in treatment efficiency determined the uncertainty in the overall estimate. The use of model organisms to determine the efficiency of treatment systems introduced another level of uncertainty but was necessary to collect data on full-scale treatment systems rather than bench or pilot scale data. The relative removal of Cryptosporidium and these model-organisms is the subject of ongoing studies.

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References