Cryptosporidium and Giardia
- the Dutch perspective
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INTRODUCTION

A number of waterborne outbreaks of cryptosporidiosis and giardiasis in the USA, Canada and the UK have shown that water can be an important transmission route for Cryptosporidium parvum and Giardia intestinalis. In the USA, Giardia is the most frequent cause of waterborne outbreaks of intestinal illness with over 95 outbreaks (18% of all outbreaks) in the past 25 years, and a total of over 25000 cases of disease (Craun, 1990). 'Waterborne' Cryptosporidium parvum outbreaks are less frequent (1% of all outbreaks), but the number of cases that is involved is relatively large, especially in the Milwaukee outbreak with an estimated 403,000 cases (Craun et al., 1998). The waterborne outbreaks of giardiasis and cryptosporidiosis that occurred in the US and UK raised concern over the safety of Dutch drinking water with regard to Cryptosporidium and Giardia. The cause of drinking water contamination with these parasites that led to the reported outbreaks was not limited to obvious treatment inadequacies or post treatment contamination, but also occurred in apparently well-treated water (Craun et al., 1990, 1998). Moreover, coliforms, the warning-parameter used to determine the microbiological safety of the drinking water, were not detected in many of the drinking waters that were the suspected cause of an outbreak.

Several characteristics of the parasites facilitate their waterborne transmission: they are shed in high numbers by infected persons or animals, they are very resistant to environmental stress and to chemical disinfection, they can be transmitted from livestock and wildlife to man and their infectivity is high: even a dose of 1 (oo)cyst gives a discrete probability of infection. These findings make Cryptosporidium and Giardia critical pathogens for the safety of drinking water. Hence, the drinking water companies and government agencies (Directorate of the Environment, Inspectorate of Environmental Hygiene) in the Netherlands need information on the occurrence of these parasites in water to be able to determine the (im)probability of transmission of Cryptosporidium and Giardia through drinking water.

PUBLIC HEALTH DATA

Data on the occurrence of Cryptosporidium and Giardia in the general population are scarce in the Netherlands. Several incidental surveys have been conducted to determine the prevalence of cryptosporidiosis and giardiasis. Bänffer (1990) could isolate Cryptosporidium in 1.2% of 2000 stool samples of patients with diarrhoea and van Knapen et al. (1984) found Cryptosporidium in 0.88% and Giardia in 7.9% of diarrhoeal stools. A study in patients that visited the general practitioner with gastro-enteritis indicated a prevalence of 1-2% for Cryptosporidium and 3% for Giardia (Hoogenboom-Verdegaal et al. 1989). A study with random samples of the human population from four different regions in the Netherlands showed that Cryptosporidium was present in stools of patients with diarrhoea at a rate of 1.6%. The isolation frequency was highest
in young children and in the age group of 25-35 years. Asymptomatic carriage was rare. Giardia on the other hand, was found in 3-5% of the stool samples of both symptomatic and asymptomatic persons of all ages (Kortbeek et al., 1994). Giardia has been reported as the cause of an outbreak of gastro-enteritis in a day-care centre in Tilburg (Bosch, 1991) and is probably the cause of more, unreported outbreaks (Kortbeek, pers. comm.).

These isolation frequencies are similar to the frequencies reported in other industrialised countries. The isolation frequency of Cryptosporidium ranges from 1 to 3% and is also relatively high (4%) in children from 1 to 15 (Casemore, 1990, 1997). For Giardia, the isolation frequency in all age groups ranges from 2 to 15% and rises to 8-39% in young children, with day-care attendance as an important risk factor (Healy, 1979).

Recent data are available from (only) one of the Dutch regional health laboratories (Stichting Artsenlaboratorium Haarlem) that routinely monitors stool samples of patients with diarrhoea for the presence of Cryptosporidium and Giardia (Mank, 1997). Other laboratories only monitor for these parasites on specific request, which mainly occurs when the symptoms are persistent and no other pathogens can be found. The monitoring data from the Artsenlaboratorium Haarlem show that Cryptosporidium could be isolated from 3.3% of patients with persistent (> 7 days) diarrhoea and 5.4% in patients with acute (1-5 days) diarrhoea. Both groups were comprised of patients that had visited a General Practitioner for their symptoms. Asymptomatic carriage was reported in 0.5% of the persons tested (Mank, 1997). Giardia intestinalis was found in 14.6% of the patients with persistent diarrhoea, in 1.8% of patients with acute diarrhoea and in 2.0% of the stools of asymptomatic persons (Mank, 1997). Symptomatic infections with both parasites were most prevalent in the age group of 0-14 years.

The prevalence data obtained by Mank (1997) are high compared to other studies in west-European countries. This may be caused by the selection of patients with persistent diarrhoea and by the optimised protocol that was used for protozoa detection in stool samples. Seasonality was observed in the isolation of Cryptosporidium with relatively high frequencies in August and September and to a lesser extend in March/April. Also Giardia was most frequently isolated in late summer and autumn. Mank (1997) suggested that the increased prevalence of both Cryptosporidium and Giardia in summer is related to exposure to these parasites in recreational water.

In the summer of 1995, a sudden increase in the isolation frequency of Cryptosporidium (up to 17%) was noted in the west of the Netherlands (Asperen et al., 1996). A case control study indicated that the major risk factors were a household member with diarrhoea and a visit to a swimming pool. No particular pool was implicated. Drinking water, surface water recreation and holidays abroad were not associated to this epidemic. The data from the Artsenlaboratorium Haarlem showed that an increase of isolation frequency of Cryptosporidium occurs each summer (Mank, pers. comm.).
Although no survey-data are published, cryptosporidiosis is known to cause severe infections in immunocompromised individuals (patients with AIDS or chemotherapy) in the Netherlands. The effect of various therapeutic strategies is tested on these patients. No specific warning is given to this group with respect to prevention of waterborne infection with Cryptosporidium and Giardia.

**VETERINARY DATA**

Numerous reports show that Cryptosporidium and Giardia infection is prevalent in farm, pet and wild animals (Panciera et al., 1971; Davies & Hibler, 1979; Mann et al., 1986; Erlandsen & Bemrick, 1988; Angus, 1990; Casemore, 1990; Xiao, 1994). Both parasites can commonly be isolated from cattle and sheep, especially newly-born and young animals. Pigs, goats and horses are also frequently infected. Few data are available on the occurrence of Cryptosporidium and Giardia in farm, pet or wild animals in the Netherlands. A study in 11 herds showed that 55% of the 375 diarrhoeic calves sampled excreted Cryptosporidium oocysts (Leeuw et al., 1984). Cryptosporidium is commonly seen in stool from diarrhoeic calves that are presented to the Faculty of Veterinary Health of the Utrecht University (Breukink, pers. comm.) and has been isolated in low numbers from 50% of 44 young foals (Göhring, 1993) and 100% of adult horses (Medema, pers. obs.). No Cryptosporidium oocysts were found in a survey of 20 lambs (Feberwee & Wipkink, 1992). Cryptosporidium baileyi is also identified as the cause of respiratory infections in commercially raised chickens in the Netherlands (Heijmans, pers. com.). No data on the prevalence of Giardia infections in animals in the Netherlands are present.

**ENVIRONMENTAL DATA**

In 1991, ubiquitous presence of Cryptosporidium and Giardia in domestic waste water and in surface water had been shown in the USA (Rose, 1988; Rose et al., 1991a; LeChevallier et al., 1991), Canada (Hansen & Ongerth, 1991; Ongerth & Stibbs, 1987), the UK (Smith et al., 1991; Gilmour et al., 1991; Badenoch, 1990; Poulton et al., 1991) and Germany (Exner & Gornik, 1990). In a first survey of the occurrence in surface water that was initiated by the water industry together with government agencies in the Netherlands in 1991, Giardia was detected in only 2 of 12 surface water samples and none of the post-treatment samples. No Cryptosporidium was detected in any of the samples. The low isolation frequency was partly due to the insensitivity and poor recovery efficiency of the methods used at that time (Medema, 1992). A subsequent, more detailed study in 1993 at the abstraction point of the Water Storage Company Brabantse Biesbosch in the river Meuse and at the outlet of the Biesbosch reservoirs showed the presence of Cryptosporidium in river water in 12% of 52 samples and Giardia in 60% of 52 samples. High levels were observed during the winter months. Reservoir storage with an average residence
time of 5 months markedly reduced the levels of both parasites, but both were detected occasionally and in low numbers at the outlet of the reservoirs (Ketelaars et al., 1995).

**POTENTIAL FOR WATERBORNE TRANSMISSION**

The first inventory of the available knowledge in the early 1990’s showed that Giardia and Cryptosporidium are present in the human and farm animal population in the Netherlands. Isolation frequencies were comparable to other industrialised countries. They are the causative agents in a proportion of the cases of gastro-enteritis that occur in the human population. With the reported isolation frequencies and the estimated annual number of all cases of gastro-enteritis in the Netherlands ($2 \times 10^6$, Hoogenboom-Verdegaal et al., 1989, 1990), an estimated number of 20,000-30,000 symptomatic Cryptosporidium infections and 60,000-100,000 symptomatic Giardia infections occur annually in the human population in the Netherlands.

The Netherlands is a country with a high population density, both in humans and in livestock, and Cryptosporidium and Giardia are present in these populations. Hence, the sources of environmental contamination are present. In addition, both protozoa have been detected in surface water. The combination of the available information led to the conclusion that there is a clear potential for waterborne transmission of Cryptosporidium and Giardia in the Netherlands. This potential is largest for water recreation, because of the direct contact with surface water. If Cryptosporidium and Giardia can be transmitted through drinking water depends on the concentration of (oo)cysts found in source water and the efficiency of the treatment systems. Both need to be evaluated.

**PREVENTION OF TRANSMISSION THROUGH DRINKING WATER**

No outbreaks or cases of cryptosporidiosis or giardiasis through drinking water have been reported in the Netherlands. The absence of active surveillance of these parasites in stool samples makes outbreaks difficult to detect, so no firm information on the safety of drinking water can be derived from the absence of cases. The attention is primarily focussed on prevention of waterborne transmission. Several barriers in the water route reduce the risk of waterborne transmission:

- 98% of the domestic waste water is biologically treated before it is discharged into surface water. However, the river Meuse receives a high quantity of untreated sewage in Belgium, before it enters the Netherlands,
- 84% of the drinking water is produced from ground water or by soil passage of surface water (bank filtration, dune infiltration),
- multiple barriers for pathogens are installed in surface water treatment systems,
- strict hygienic procedures are used for distribution system maintenance and repair.
To be able to evaluate the effectiveness of these preventive measures, information is needed on the sources of surface water contamination, the occurrence and fate of Cryptosporidium and Giardia in surface water and the elimination capacity of the treatment systems.

**DRINKING WATER TREATMENT - MULTIPLE BARRIERS**

In the Netherlands, 64% of the drinking water is produced from (deep) ground water. This is mainly located in the north, south and east of the country. In the west, the sodium chloride-concentration in groundwater is too high for the production of drinking water, because of the intrusion of sea water. Abstracted groundwater is generally free from faecal contamination. No chemical disinfection is applied. The groundwater is only treated with aeration and filtration to remove methane, ammonium, iron and manganese.

Four basic treatment schemes are used for the production of drinking water from surface water in the Netherlands (Table 1). The most commonly used schemes are 1) storage in open reservoirs, followed by coagulation, filtration (rapid sand, dual media, GAC) and disinfection (chlorine, ozone) and 2) dune infiltration. In this latter scheme, water is pre-treated by coagulation and filtration, transported to the dunes and artificially recharged into the sand dunes along the coastal area in almost all of the west of the Netherlands. The water is pumped through the dune-sand and recovered from the dunes. The average travel time through the dune-sand is two months, but there is pressure to reduce the residence times since this would reduce the area needed for drinking water production. After soil passage, the water is either treated by filtration followed by UV or stored in an open reservoir and treated more extensively by rapid filtration (with ozone and GAC filtration) and slow sand filtration. A small but increasing percentage of surface water is treated by bank filtration along the river Meuse and Rhine. Also here, residence times are usually two months or more. After soil passage, water is treated with filtration (sand, dual media, GAC) and in some cases ozonation or UV. Another small percentage of the surface water treatments use a direct treatment with coagulation/filtration or ozonation, both followed by GAC filtration and slow sand filtration. In total, 50% of the Dutch surface water supplies use passage of two months through the soil (fine-grained sand, loamy sand), which is very effective in removing pathogenic micro-organisms.

**ASSESSMENT OF THE SAFETY OF DRINKING WATER**

**Current approach: the use of indicator bacteria**

The current approach to check the microbiological quality of drinking water leaving surface waterworks is monitoring for the presence of total and thermotolerant coliforms, faecal enterococci and spores of sulphite reducing bacteria. The frequency and volume of sampling are regulated in the Dutch
Table 1. Surface water treatment in the Netherlands

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Volume (Mm³/yr)</th>
<th>Percentage of total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bank filtration</td>
<td>80</td>
<td>6</td>
</tr>
<tr>
<td>Artificial recharge (dunes)</td>
<td>180</td>
<td>14</td>
</tr>
<tr>
<td>Storage reservoirs</td>
<td>160</td>
<td>12</td>
</tr>
<tr>
<td>Direct treatment</td>
<td>50</td>
<td>4</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>470</strong></td>
<td><strong>36</strong></td>
</tr>
</tbody>
</table>

Drinking Water Decree (1984). The standards for indicator bacteria in this Decree (Table 2) are more stringent than the standards in the current European Drinking Water Directive (1980), particularly for the spores of sulphite reducing clostridia. A stricter standard in terms of frequency and volume was felt necessary, since this parameter could indicate the breakthrough of pathogenic micro-organisms that were more resistant to oxidation processes than coliforms and faecal streptococci, such as viruses and protozoa. It was also feasible, since only a very small fraction of 1 litre samples of treated water contained these spores (Havelaar, 1981). In the current revision of the EU-Directive, the proposed new standard for Clostridium perfringens is now similar to the Dutch standard for clostridial spores.

Table 2. Dutch and EU standards for indicator bacteria in treated water leaving the treatment facility.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Dutch Drinking Water Decree</th>
<th>EU Drinking Water Directive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total coliforms</td>
<td>&lt; 1/300 ml daily</td>
<td>0/100 ml Daily</td>
</tr>
<tr>
<td>Thermotolerant Coliforms</td>
<td>&lt; 1/300 ml daily</td>
<td>0/100 ml Daily</td>
</tr>
<tr>
<td>Faecal Enterococci</td>
<td>&lt; 1/100 ml weekly</td>
<td>0/100 ml Undefined</td>
</tr>
<tr>
<td>Spores of sulph. red. clostridia</td>
<td>&lt; 1/100 ml weekly</td>
<td>0/20 ml Undefined</td>
</tr>
</tbody>
</table>

a MAC: Maximum Acceptable Concentration  
b Weekly for ground water supplies  
c Not defined for ground water supplies

New approach: guidelines for pathogens?
The occurrence of outbreaks of waterborne disease through drinking water that complies with the coliform standard, implies that this standard is inadequate to safeguard the microbiological quality of drinking water under all circumstances.
The development of dose-response assessments for microbial pathogens has made it possible to design a risk-based approach, analogous to the approach taken against the risk of toxic chemicals in drinking water (Medema & Havelaar, 1992; van der Kooij et al., 1995). Maximum acceptable concentrations of pathogens in drinking water can be determined based on a maximum acceptable risk level, in much the same way as maximum acceptable concentrations are developed for toxic compounds. The Netherlands is in the process of adopting the risk strategy that was developed by the researchers in the US (Haas, 1983; Rose et al., 1991; Regli et al., 1991) in conjunction with the US Environmental Protection Agency. In this approach, a risk level of one infection per 10,000 persons per year is regarded as maximum acceptable for pathogens in drinking water. The Ministry of the Environment has issued draft guidelines for maximum acceptable pathogen concentrations in drinking water, based on the $10^{-4}$ infection risk level, the exposure of consumers to unheated drinking water (average: 0.25 l/day) and a safety factor of 10 for interspecies variation in both pathogen virulence and host susceptibility to infection (Table 3).

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>MAMC (n/l)</th>
<th>MAMC (absence in m$^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryptosporidium</td>
<td>$2.6 \times 10^{-5}$</td>
<td>38</td>
</tr>
<tr>
<td>Giardia</td>
<td>$5.5 \times 10^{-6}$</td>
<td>180</td>
</tr>
</tbody>
</table>

* MAMC values for other pathogens (bacteria, viruses) are issued, but not presented here.

These maximum acceptable mean concentrations (MAMC) are way below the lower detection limit of the protozoa methods, so monitoring for the presence of protozoa in drinking water cannot be used to show compliance with these MAMC-values. Compliance with the MAMC-values can be assessed by combining data on source water quality with the elimination capacity of treatment systems. Or, in other words, the difference between the concentration in the source water and the MAMC-value is the required elimination capacity of the treatment processes. Currently, this approach is used to evaluate the microbiological safety of newly designed treatment systems.

**RESEARCH NEEDS**

**Sources of surface water contamination**

The design of surface water treatment systems is determined, at least in part, by the concentration of Cryptosporidium and Giardia in source water. Hence, the cost of the production of drinking water from surface water is related to the (oo)cyst concentration level in source water: the lower the concentration, the lower the costs for installation and operation of an adequate treatment system.
Reduction of the environmental load of (oo)cysts by installing source water protection measures would reduce the costs of water treatment. Similarly, these measures would reduce the health risk of water recreation. Potentially important sources for contamination of surface water with Cryptosporidium oocysts and Giardia cysts are treated and untreated sewage discharges, run-off from agricultural lands and, in more pristine waters, wildlife. Hence, the occurrence of Cryptosporidium and Giardia in several domestic waste waters and in effluents from activated sludge systems for sewage treatment was monitored. These data were used to determine the relative significance of treated and untreated sewage discharges. Also, the applicability of an emission model to determine the relative significance of treated and untreated discharges was determined (chapter 3).

Since most surface waters in the Netherlands are influenced by the international rivers Rhine and Meuse, it was important to determine the Cryptosporidium and Giardia load presented by these rivers (chapter 3). The significance of wildlife and especially waterfowl for the contamination of water in pre-treatment reservoirs was determined by measuring the occurrence of (oo)cysts in animal faeces and calculating the load of (oo)cysts to reservoir water, using information on prevalence of the animals on and around the reservoirs (chapter 4).

**Environmental ecology**

To determine the probability that (oo)cysts that are discharged into rivers and streams arrive at an abstraction point for drinking water production or at a bathing area, information is needed on the fate of (oo)cysts in the aquatic environment. Important processes that determine this fate are die-off and sedimentation. Therefore, both the kinetics of survival of Cryptosporidium oocysts in surface water under natural conditions (chapter 5) and the kinetics of sedimentation of free and attached Cryptosporidium oocysts and Giardia cysts in water (chapter 6) were assessed. This information is integrated into a quantitative descriptive model of the emission and dispersion of Cryptosporidium and Giardia in surface water in the Netherlands (chapter 3), identifying the significance of the different (oo)cyst sources and processes in surface water (transport and survival) in determining the concentration of Cryptosporidium and Giardia in surface waters.

**Quantitative description of protozoa-occurrence in source waters**

The new approach towards microbiologically safe drinking water requires quantitative information on the occurrence of Cryptosporidium oocysts and Giardia cysts at the sites where water is abstracted for the production of drinking water. These were determined with the emission and dispersion model, but also by monitoring of the concentration Cryptosporidium oocysts and Giardia cysts at several abstraction sites (chapter 3). The largest abstraction site was intensively monitored for both protozoa to determine the variation in source water quality and the relation of protozoa occurrence with other water quality variables (chapter 7).
Sensitive detection methods
The recovery efficiency of the methods to concentrate and enumerate (oo)cysts of Cryptosporidium and Giardia in water was very low. The methods that are used to concentrate cysts and oocysts from water also concentrate the majority of other biological particles > 1 µm in the water sample (i.e. algae). These particles interfered with the detection of (oo)cysts by immunofluorescence microscopy. Flow cytometry with cell sorting was applied to facilitate sensitive detection by isolating Giardia cysts and Cryptosporidium oocysts from other particles in the water concentrates and to facilitate application of vital dyes to evaluate the viability of Cryptosporidium oocysts and Giardia cysts in these samples was studied (chapter 8).

Quantitative description of treatment efficiency
To determine if treatment is adequate, methods are needed to assess treatment efficiency. Monitoring the concentration of Cryptosporidium oocysts and Giardia cysts before and after the treatment process is a method that can be used only to assess the removal capacity of the initial treatment processes (chapter 5). After the initial treatment, the concentration of (oo)cysts in the water is too low to be able to assess the removal in subsequent treatment processes. Since spores of sulphite-reducing clostridia are persistent in water, very resistant to chemical oxidising agents and small (approximately 1 µm), they could be a useful surrogate parameter to determine treatment efficiency for Cryptosporidium oocysts and Giardia cysts (Payment & Franco, 1993; Hijnen et al., 1997). If clostridial spores and (oo)cysts behave similarly during treatment, adequate removal of spores would imply adequate removal of (oo)cysts. In chapter five, these spores are applied to describe the removal capacity of subsequent treatment processes (coagulation/filtration, disinfection). Since both the concentration of Cryptosporidium and Giardia in source water and the efficiency of spore-removal by treatment processes showed considerable variation, statistical methods had to be developed to describe the survey data.

Risk assessment
The ultimate goal, description of the safety of the drinking water treatment, can be achieved by performing a risk assessment. This requires quantitative knowledge of all the factors that contribute to the health risk of consumption of drinking water from a surface water source: concentration of Cryptosporidium oocysts and Giardia cysts in source water, the recovery efficiency of the detection method, the viability of (oo)cysts in water, the removal or inactivation of (oo)cysts by water treatment, the consumption of (unheated) drinking water by the community and the dose-response relation of Cryptosporidium and Giardia. Chapter nine describes the use of data on all these factors to analyse the risk of infection by Cryptosporidium and Giardia in drinking water from a surface water source and the contribution of the uncertainty in these data-sets to the overall uncertainty of the risk estimate. The latter is useful information to focus future research efforts to reduce both the health risk itself and the uncertainty of the risk estimate.
REFERENCES


