Quantitative microbial risk assessment of distributed drinking water using faecal indicator incidence and concentrations

J. Hein M. van Lieverloo, E. J. Mirjam Blokker and Gertjan Medema

ABSTRACT

Quantitative Microbial Risk Assessments (QMRA) have focused on drinking water system components upstream of distribution to customers, for nominal and event conditions. Yet some 15—33% of waterborne outbreaks are reported to be caused by contamination events in distribution systems. In the majority of these cases and probably in all non-outbreak contamination events, no pathogen concentration data was available. Faecal contamination events are usually detected or confirmed by the presence of *E. coli* or other faecal indicators, although the absence of this indicator is no guarantee of the absence of faecal pathogens. In this paper, the incidence and concentrations of various coliforms and sources of faecal contamination were used to estimate the possible concentrations of faecal pathogens and consequently the infection risks to consumers in event-affected areas. The results indicate that the infection risks may be very high, especially from *Campylobacter* and enteroviruses, but also that the uncertainties are very high. The high variability of pathogen to thermotolerant coliform ratios estimated in environmental samples severely limits the applicability of the approach described. Importantly, the highest ratios of enteroviruses to thermotolerant coliform were suggested from soil and shallow groundwaters, the most likely sources of faecal contamination that are detected in distribution systems. Epidemiological evaluations of non-outbreak faecal contamination of drinking water distribution systems and thorough tracking and characterisation of the contamination sources are necessary to assess the actual risks of these events.

Key words | *E. coli*, infection risks, outbreaks, pathogens, Quantitative Risk Assessment, thermotolerant coliforms

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INTRODUCTION

The integrity of infrastructure, such as reservoirs and mains in the distribution network, is critical for the safety of drinking water, as is hygiene during invasive operations. Especially in the case of buried mains, it is difficult for water companies, inspectorates and regulators to verify whether the efforts of safeguarding water safety are sufficient. Although the health impact of reported waterborne outbreaks usually is relatively well known, the *potential* health impact of the more frequently occurring non-outbreak contamination events is

not. The purpose of this study was to provide and demonstrate a method of estimating the probability of faecal contamination of distributed drinking water and the pathogen concentrations in faecally contaminated water.

Outbreaks associated with contamination events in distribution systems

There have been many reports of waterborne outbreaks through drinking water that is contaminated within

the distribution system. In a study by Risebro *et al.* (2005), outbreaks through public water supplies in Europe, from 1990–2004, are reviewed and analysed by fault tree analysis. Some 86 outbreaks were reported, with a total of 72,546 cases of illness, of which 341 people were hospitalised and 1 died. In 33% of these outbreaks, contamination during distribution was the dominant cause of the outbreak. The fault tree analysis showed that events that have contributed to outbreaks through contamination of distributed water were:

- cross connections/backflow
- construction or repair
- damaged/old mains
- low pressure
- cleaning
- reservoir contamination.

From the review of outbreaks through drinking water of Hrudey & Hrudey (2004) it is clear that, in many distribution-related outbreaks, lack of or non-compliance to adequate hygiene procedures to maintain the integrity of the network or to ensure safety during and after breaks and repairs have led to gross contamination of mains water which resulted in people falling ill and even in fatalities.

Previous studies also show that outbreaks often are caused by failures during distribution:

- Hunter (1997): 15 of 57 outbreaks (26%) in the UK (1911–1995)
- Stenström *et al.* (1994): 18–20% of the outbreaks in Nordic countries (1975–1991)
- Craun & Calderon (2001): 18% of 619 outbreaks in the USA (1971–1998).

In the Netherlands, only three outbreaks have been reported for public drinking water systems since the end of World War II. The first of these occurred in 1962, when five cases of typhoid fever were reported in Amsterdam, probably as a result of a contamination of a drinking water main with sewage (Anon. 1962). The second reported outbreak occurred in 1981 in Rotterdam, when sewage and wastewater from a foreign marine vessel were pumped into the distribution system via a drinking water supply valve for marine vessels. This event led to 609 reported cases, mainly of gastroenteritis. Pathogens isolated from stool samples included *Giardia* (8%), *Campylobacter* (5%), *Entamoeba histolytica* (2.3%) and *Salmonella* (1.2%) (Huisman & Nobel 1981). The third reported outbreak occurred more recently and is evaluated in this study for the purpose of estimating the expected infection risks from pathogens, based on the concentrations of thermotolerant coliforms found in tap water samples during the outbreak.

Non-outbreak contamination events in distribution systems

Outbreaks are known to be underreported (Craun *et al.* 1996). Outbreaks are not always detected and contaminations may even lead to illness in the community supplied without a link being made to the water system. Evidence that contamination events occur much more frequently than outbreaks is provided by the statutory monitoring of drinking water for *E. coli* (formerly also determined as thermotolerant coliforms). Bartram *et al.* (2002) evaluated the results of monitoring of thermotolerant coliforms in drinking water samples in European countries and suggested that, on average, the percentage of samples showing the presence of thermotolerant coliforms in drinking water from public systems is around 1–2% (range 0–12%). Although these levels appear high compared to most data in other studies (Van der Kooij *et al.* 2003; Mendez *et al.* 2004; Van Lieverloo *et al.* 2006*a, b*; Hambsch *et al.* 2007), most studies show that faecal contamination (as suggested by thermotolerant coliforms or *E. coli* detection) is more frequent than outbreaks would suggest. Mendez *et al.* (2004) showed that other indicators of faecal contamination (*Clostridium* spores, somatic coliphages, F-RNA coliphages and *Bacteroides fragilis* phages) may be present in (chlorinated) tap water in which no *E. coli* is detected in 100 ml samples. Several outbreaks of viral and protozoan illness have been reported from water that met the *E. coli* standard of absence in 100 ml (Craun & Calderon 2001; Anderson & Bohan 2001). *E. coli* is more sensitive to chlorine than viral and protozoan pathogens (Payment 1999). So, especially in chlorinated tap water, the frequency of *E. coli* detection is likely to underestimate the frequency of faecal contamination.

Event reports from water companies also illustrate that contamination events are far more common than outbreaks.

Van Lieverloo *et al.* (2003) evaluated contamination events reported by eight water utilities in the Netherlands in 1995–2000. In 9 of the 27 events reported, thermotolerant coliforms or *E. coli* were detected in these system's drinking water on several occasions. Furthermore, the incident frequency was considered by the water companies to underestimate the actual frequency. No waterborne outbreak was reported in the same period as the events. Of these events, five were associated with a contamination in the distribution network due to cross-connection, open connection and mains breaks and three with infiltration into a reservoir.

Other studies suggest that contamination may occur during standard operating conditions. LeChevallier *et al.* (2003) studied the impact of transient pressure events in distribution networks. Negative-pressure events occur due to power failures or sudden pump/valve shutdowns. LeChevallier *et al.* (2003) have shown that (i) these events occur in practice, (ii) during these events, leaks provide a portal of entry for groundwater to enter distribution systems and (iii) faecal indicators and human viruses may be present in the groundwater surrounding drinking water mains. They could not determine if this contamination route may lead to significant contamination of drinking water, because of insufficient data. Negative-pressure events were usually short-term (<1 min) and outside of the periods of intrusions, drinking water would be flowing out of the main. The level of contamination of water entering the distribution network during short negative-pressure events is therefore difficult to assess.

A recent case-control study on sporadic cryptosporidiosis in the UK reported an association between gastrointestinal illness and the loss of water pressure in the distribution network (Hunter *et al.* 2005). Some 28 of 423 people surveyed reported diarrhoea in the two weeks before the questionnaire. Analysis of possible risk factors showed a 12-fold increase in gastrointestinal illness associated with the loss of water pressure at the household tap. Most of these pressure losses were associated with reported events in the distribution network, such as a burst in water mains. Hunter *et al.* suggest that failures in the distribution network could have a significant contribution (around 15%) to the overall rate of gastro-enteritis in the population.

Quantitative Microbial Risk Assessment in drinking water supply

Quantitative Microbial Risk Assessment (QMRA), as described by Haas *et al.* (1999), can be used to estimate human health risks from the presence of pathogens in drinking water. In the Netherlands, water companies already apply this approach to estimate infection risks from primary contamination, i.e. contamination from insufficient treatment of source water (Dechesne *et al.* 2006; Smeets *et al.* 2006).

No pathogen data, however, has been reported for the estimation of infection risks arising from faecal contamination of distribution waters (secondary contamination). There are various reasons, including the very low probability of detecting faecal contamination (Van Lieverloo *et al.* 2007) and the uncertainty about the actual frequency and duration of such contaminations. Quantification of pathogen concentrations is not only costly, but more importantly detection limits are high compared to acceptable concentrations (Teunis *et al.* 1997). Furthermore, in most contamination events the signs of faecal contamination may quickly disappear. Therefore, drinking water is seldomly tested for the presence of pathogens after a faecal contamination is detected, e.g. by finding *E. coli* in routine samples. In most cases, even *E. coli* is no longer detectable in the required repeat sample usually taken the following day (Van Lieverloo *et al.* 2006b). In the absence of quantitative data to assess the effect of detected faecal contamination of drinking water, pathogen concentrations must be estimated per case of contamination. Westrell *et al.* (2003) assumed effects for each recorded failure in a treatment plant and the distribution system of the city of Gothenburg, Sweden. The coliform concentrations detected in drinking water during events were used and related to the coliform concentrations in sewage to estimate the possible level of sewage contamination. In addition, the pathogen concentration in sewage was used to calculate their subsequent pathogen concentrations in drinking water during the contamination event. The pathogen concentrations in the drinking water were translated to a risk of infection to the exposed consumers, taking the size of the affected areas and the duration of the contamination event into account. The resulting annual risk of infection from contamination events in the distribution system was found

to be lower than the risks resulting from normal operation of the Gothenburg system. The basics of the method presented by Westrell *et al.* (2003) were applied in this study, using other data on pathogen occurrence in contamination sources, daily consumption and dose–response models. Furthermore, actual *E. coli* or thermotolerant coliform concentrations found in drinking water during contamination events were used to estimate pathogen concentrations.

Statutory monitoring of drinking water includes testing for the presence of *E. coli* (The Council of the European Union 1998), an indicator of contamination with faecal matter from warm-blooded animals or humans and therefore the possible presence of pathogenic micro-organisms (Ashbolt *et al.* 2001). Over 50,000 drinking water samples are analysed for the presence of *E. coli*[1] yearly in the Netherlands with a population of 16.3 million, and it is likely that over a million samples are collected yearly in the European Union (population ca. 460 million). The results, in most cases showing the absence of *E. coli*, are used only as a qualitative indication of the safety of drinking water and to verify the effects of corrective measures after detection of a contamination event. This study quantitatively evaluates thermotolerant coliform and *E. coli* data, presenting a method to quantify health risks from *E. coli* data collected during faecal contamination events. The method is described and applied to data collected during an outbreak and faecal contamination events. The applicability of the method is shown as well as its limitations and sensitivity to variability and uncertainty.

METHODS AND MATERIALS

Incidence and concentrations of thermotolerant coliforms/*E. coli*

Coliform data was obtained from one outbreak and from 49 non-outbreak faecal contamination events in non-chlorinated Dutch distribution systems described below.

Most water companies in the Netherlands used the Laurylsulphate agar (LSA) method after membrane

filtration (Anon. 1982) for incubation of thermotolerant coliforms up to 2002. Typical yellow colonies were confirmed in Brilliant Green Bile Lactose Broth (BBLB) at $44 \pm 1°C$. Yellow colonies that produced gas in BBLB after $22 \pm 2 h$ or $44 \pm 4 h$ were considered thermotolerant coliforms. Starting in 2002, *E. coli* was determined using the ISO 9308-1 method (ISO 2000), but with the LSA medium (not the LTTC medium) as the selectivity of this medium was proven to be higher (Schets *et al.* 2002).

Outbreak data

In 2001, an outbreak of waterborne gastroenteritis occurred in the Netherlands as a result of an accidental cross-connection between the drinking water distribution system and a greywater distribution system, intended for flushing toilets, washing cloths and watering gardens in a new residential area (Anon. 2003).

On 3 December, probably two days after the contamination started, the first consumer complaints about drinking water taste were received by the water company. On 4 December samples were collected at these premises. On the evening of 5 December, a boiling advisory was issued for the ca. 900 premises in the southern part of the residential area, while on the morning of 6 December this was issued for ca. 100 premises in the northern part. Later on 6 December, the cross-connection was removed. From 7 December on, no thermotolerant coliforms were found in the drinking water samples collected.

In the two drinking water samples taken on 4 December, some of the total coliforms found were later identified as *E. coli* (of faecal origin) and *Enterobacter cloacae* (possibly of faecal origin, Camper *et al.* (1991)). The samples were taken after consumer complaints from two premises in two streets in the same area. Nine out of twelve repeat samples collected the same day contained thermotolerant coliform bacteria. The concentrations of thermotolerant coliforms on 4 December were estimated from total coliform numbers the same day (16 and 19 CFU per 100 ml) assuming the ratios of thermotolerant coliforms to total coliforms from data collected the following day (4:5 and 2:12) in samples from the same street and address, respectively. The concentration curve of thermotolerant coliforms is presented in Figure 1.

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[1] Before 2002, samples were tested for the presence of thermotolerant coliforms.
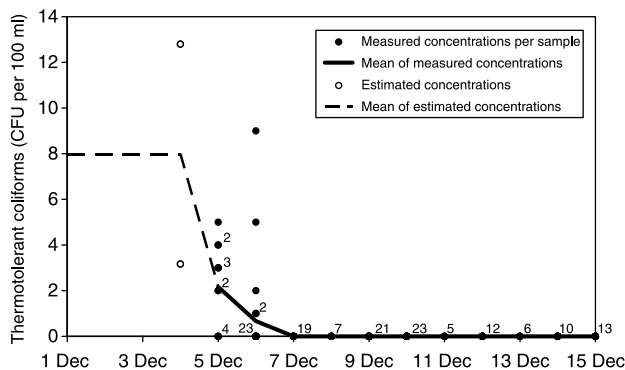
Figure 1 | Concentrations of thermotolerant coliforms in 158 drinking water samples collected from premises in a residential area of a city in the Netherlands in December 2001, after consumer complaints (taste) on 3 December. Concentrations on 1–4 December were estimated from total coliform numbers on 4 December (16 and 19 CFU per 100 ml) and the ratios of thermotolerant coliforms to total coliforms on 5 December (4:5 and 2:12) in samples from the same streets. Labels near markers indicate the number of samples with identical concentrations.

Based on monitored pressure differences between trunk mains of both systems, exposure was assumed to start 1 December, when at the end of that day all households in the contaminated area were assumed to receive undiluted greywater. According to the results of the questionnaire, 82% of the households started boiling drinking water before consumption and after receiving the boiling advisory. On the evening of 5 December 900 premises received this advisory, while the remaining 100 premises received it on the morning of 6 December. On 6 December, flushing of the mains was started and the cross-connection was discovered and closed. Presumably, exposure of the persons not complying with the boiling advisory lasted throughout 6 December before the drinking water mains were clean. Therefore, the maximum period of exposure was from 1 December through 6 December and the minimum exposure period was from 2 December through (the evening of) 5 December.

Data from 50 non-outbreak faecal contamination events

Water companies in the Netherlands were asked to supply records of events that had occurred in the period from 1994 through 2003. For this survey, events were defined as cases of water quality degradation, as determined by repeated detection of total coliforms and/or indicators of faecal contamination, during which event at least one sample contained an indicator of faecal contamination. The survey resulted in reports of 50 events (including the outbreak in 2001) from seven water companies together supplying ca. 11 million inhabitants. The estimated number of inhabitants affected by the contaminations varied from 5 to ca. 50,000, with 9 events affecting over 1,000 and a total number of ca. 185,000. The reporting water companies stressed in their contributions that, although all events have been reported to the national inspectorate, event reports have not been archived separately and were not all retrievable. Therefore, the survey resulted in an incomplete overview of both frequency as well as impact (inhabitants in contaminated area) and circumstances (cause, source, countermeasures, etc.). Based on these data, for the ca. 11 million inhabitants of the participating water companies in the Netherlands, the probability of being affected by a contamination event would be at least 185,000/11 million in 10 years, i.e. 1.7×10^{-3} per person per year.

The median duration of the events from detection to the end (defined as no further detection of *E. coli* or coliforms) is 8 d with a 95-percentile of 30 d (Figure 2). The real duration is longer, as events usually are not immediately detected at the onset (shown by 1–4 negative days for some events in Figure 2). During 26 events, no boiling water advisory was issued nor was any disinfectant dosed. Flushing was the standard response to detection of contamination events, but did not always remove the contamination. In most cases, however, flushing results in a rapid decrease of concentrations of faecal indicators and total coliforms (in 100 ml samples), after which the contamination is considered to have been removed.

The mean concentration of thermotolerant coliforms/*E. coli* (membrane filtration method), found in samples (mostly) collected from taps during the event, ranged from 0.055 CFU per 100 ml to 210 CFU per 100 ml (Figure 3). The maximum concentration of 900 CFU per 100 ml was reported on the second day of the event, with the highest initial (37 CFU per 100 ml) and mean concentration (210 CFU per 100 ml), lasting 10 d. During 17 events, the highest concentration of *E. coli* was measured in the first sample that was collected, so in the majority of the cases the peak concentration followed after the initial detection.
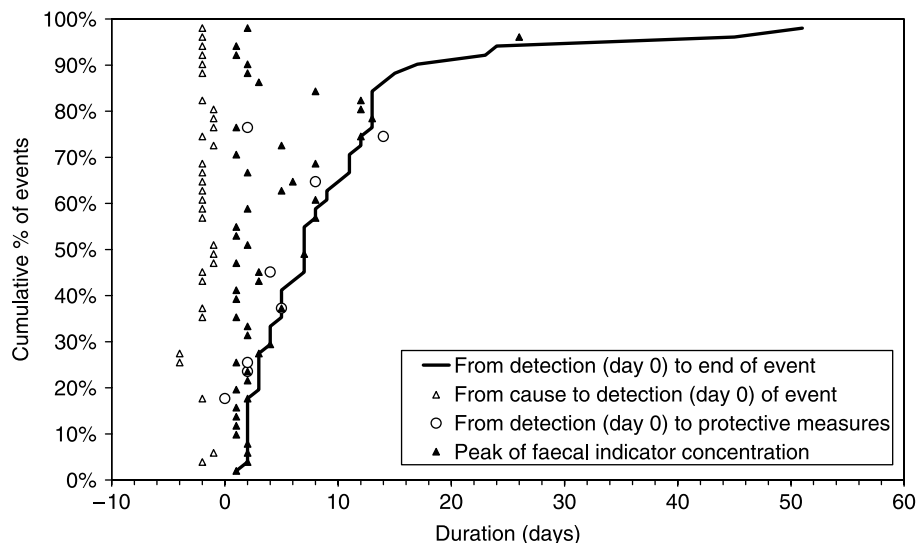
Figure 2 | Duration of 50 faecal contamination events reported in the Netherlands from 1994 through 2003 by 7 water companies supplying ca. 11 million inhabitants, affecting ca. 185,000 inhabitants. The end of the event is the second day when no indicator bacteria are found in 100 ml samples. If no protective measures (boiling water advisory, disinfectant dose) were taken, duration is not presented.

Pathogen to *E. coli* ratios

Choice of reference pathogens

For each group of pathogens, one or more representatives were chosen that both commonly occur in faecally polluted water and are common causes of waterborne outbreaks. These so-called reference-pathogens were *Cryptosporidium*

parvum and *Giardia lamblia* for protozoan parasites, *Campylobacter jejuni* for bacteria and enterovirus for viruses (Westrell *et al.* 2003).

Pathogen to *E. coli* ratios during the outbreak

Prior to the outbreak, pathogen and indicator data had been collected from the source water and after treatment (Hijnen
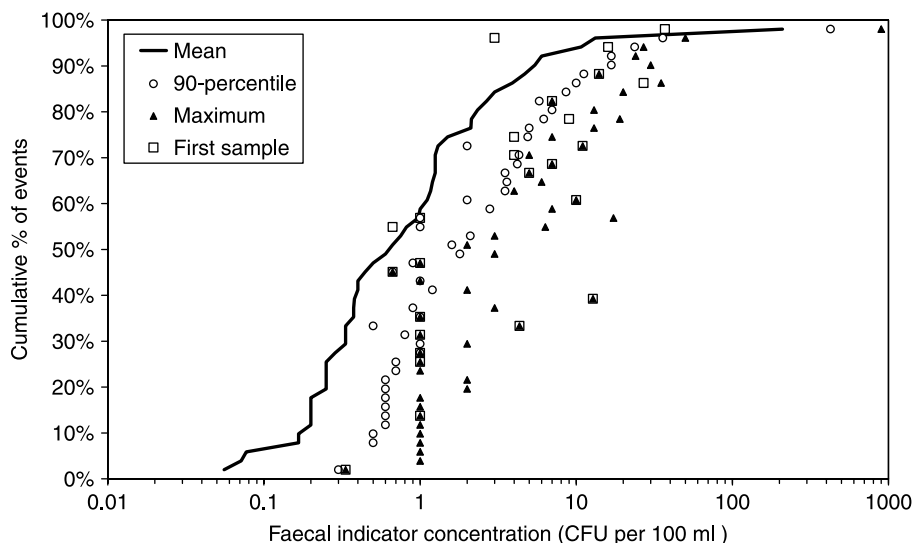

Figure 3 | Thermotolerant coliform and *E. coli* concentrations per 100 ml in water samples collected from finished water (300 ml) or distributed water (100 ml) during 50 faecal contamination events reported in the Netherlands from 1994 through 2003 by 7 water companies supplying ca. 11 million inhabitants, affecting ca. 185,000 inhabitants. Individual samples (90-percentiles, maximum or first) containing no thermotolerant coliforms or *E. coli* are not included in the figure.

et al. 2003). The treatment consisted of screening, coagulation, flocculation, sedimentation and rapid sand filtration of surface water from a canal that connects the Lek River (lower part of the Rhine River) with Amsterdam. This partially treated water is also used for drinking water production by another water company. The concentrations of reference protozoa and *Campylobacter* were estimated from concentrations in the source water and the mean elimination capacity for pathogens and *E. coli* in the same period or in the same seasonal period of another year. The estimated concentrations of *E. coli* or thermotolerant coliforms matched measured concentrations in the finished water of the pretreatment plant well[2]. Enterovirus concentrations were available from the partially treated water and were divided by *E. coli* concentrations determined in this water to calculate the ratios. A table with the actual ratios used was published earlier (Van Lieverloo *et al.* 2006b).

Pathogen to *E. coli* ratios during the non-outbreak faecal contamination events

In order to calculate possible pathogen concentrations as accurately as possible, the pathogen to thermotolerant coliforms or *E. coli* ratios used in the calculations need to be estimated in samples of the contamination source as soon as possible after the start of the contamination events. However, neither pathogen nor thermotolerant coliforms or *E. coli* in the (most likely) contamination sources were determined in any of the presented non-outbreak contamination events. Therefore, to calculate possible pathogen exposures, pathogen to thermotolerant coliform ratios in three common sources of contaminations were used: sewage, surface water and soil and shallow groundwater close to distribution mains.

Sewage as presumed source. In 1997 and 1998 pathogen and thermotolerant coliform concentrations were determined in 11 samples collected in a period of 12 months from the untreated influent of a sewage treatment plant in the Netherlands (Medema *et al.* 2001). *Campylobacter* as well as *E. coli* concentrations in samples of untreated sewage were obtained from Höller (1988).

Surface water as presumed source. In 1997 and 1998 pathogen and thermotolerant coliform concentrations were determined from 26 samples collected in a period of 12 months from the Rhine and Meuse rivers at the border of the Netherlands (Medema *et al.* 2001).

Soil and shallow groundwater as presumed source. Only one dataset is known for soil and shallow groundwater near distribution mains (Karim & LeChevallier 2000; Karim *et al.* 2003; LeChevallier *et al.* 2003) and was used to make three sets of enterovirus to thermotolerant coliform ratios:

1. ratios of culturable enteric viruses vs. thermotolerant coliforms from data pairs in which thermotolerant coliforms were detectable;
2. ratios of culturable enteric viruses and enteroviruses detectable with PCR (with unclear viability) vs. *detectable* thermotolerant coliform concentrations;
3. ratios of both culturable enteric viruses as well as enteroviruses detectable with PCR vs. thermotolerant coliforms from all data pairs. When thermotolerant coliforms were not detectable, their concentration was estimated to be half the detection limit in order to be able to calculate a ratio.

Figure 4 shows the variation of ratios between and within these matrices. Tables with the actual ratios used were published earlier (Van Lieverloo *et al.* 2006b).

Consumption of unheated drinking water

Based on a recent evaluation (Mons *et al.* 2006), the mean consumption of unheated drinking water in the Netherlands was estimated at 0.177 litres per person per day. The same evaluation showed that a Poisson distribution best fitted the variation of the daily consumption.

Probability of infections

There are two kinds of infection risk that can be calculated using *E. coli* or thermotolerant coliform data collected during detected contamination events:

- *Risk levels per event*, i.e. the possible risk of infections for the inhabitants of the area of a distribution system that was contaminated.

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[2] Means ± SD in finished water were (estimated vs. measured): 44 ± 29 vs. 35 ± 33 CFU coli44/l (for ratios of *Cryptosporidium* and *Giardia* to coli44); 67 ± 27 vs. 51 ± 102 (for *Campylobacter*) and 61 ± 61 vs. 40 ± 55 (for enterovirus).
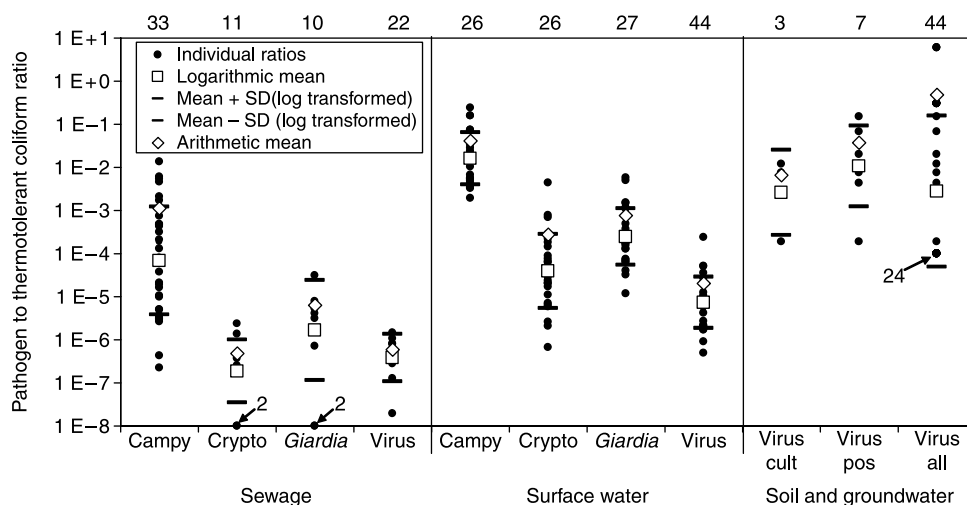
Figure 4 | Pathogen to thermotolerant coliform ratios in environmental samples collected from sewage (Höller 1988), surface water (Medema *et al.* 2001) and groundwater (Karim & Le Chevallier 2000). Campy = *Campylobacter*; Crypto = *Cryptosporidium*; virus cult = ratios of culturable enteric virus vs. thermotolerant coliforms from data pairs in which thermotolerant coliforms were detectable; virus pos = ratios of both culturable enteric viruses and enteroviruses detectable with PCR vs. detectable thermotolerant coliform concentrations; virus all = all ratios (when thermotolerant coliforms were not detectable, their concentration was estimated to be half the detection limit in order to be able to calculate a ratio). Two samples in sewage and 24 samples in soil or shallow groundwater did not contain detectable concentrations of pathogens and ratios were set to 1×10^{-8} and 1×10^{-4}, respectively, for the purpose of presentation in this graph only (indicated with arrows), including calculations of means and standard deviations. The number of data pairs per pathogen is indicated over the graphs.

- *Yearly risk levels* due to contamination events. As the uncertainties in quantitative data available about the probability of detecting a contamination incident are still high (Van Lieverloo *et al.* 2007), the calculations in this paper are limited to infection risks due to detected contamination events.

The following steps are taken to calculate these infection risks:

- infection risks per day of an outbreak or non-outbreak contamination event.
- infection risks during an outbreak or non-outbreak contamination event,
- infection risks due to non-outbreak contamination events per year

Infection risks per day of the outbreak or event

First, the pathogen exposure per person per day of the event is estimated (formula (1)):

$$P_{\exp,d} = P_{E,d}P_RP_{Cd} \tag{1}$$

where

- $P_{\exp,d}$ = the daily PDF of an inhabitant of the contaminated area being exposed to a pathogen or (when the probability is higher than 1) the expected number of pathogens consumed per person,
- $P_{E,d}$ = the empirical PDF of all *E. coli* or thermotolerant coliform concentrations in drinking water during the day,
- P_R = The empirical PDF of the pathogen to *E. coli* ratios in the contamination source,
- $P_{C,d}$ = the PDF of the daily consumption of unboiled drinking water, fitted to a Poisson distribution,
- PDF = Probability Density Function.

An empirical PDF consists of all values actually determined and is used when the available data is too limited to be fitted to a statistical distribution function with sufficient confidence. The PDFs (*E*, *R*, *C*) are multiplied by bootstrapping using MatLab® 7.0.4 (100,000 random draws).

Each value in the resulting PDF of daily exposures is used to estimate a daily infection risk, using the following dose–response models, selected by Petterson *et al.* (2006):

Campylobacter
$$P_{\inf,d} = 1 - (1 + (P_{\exp,d}/0.011))^{-0.024} \tag{2}$$

(Teunis *et al.* 2005)
(original data: Van den Brandhof *et al.* (2003); Evans *et al.* (1996))

Cryptosporidium

$$P_{\text{inf},d} = 1 - (1 + (P_{\text{exp},d}/0.176))^{-0.115} \qquad (3)$$

(Teunis *et al.*, 2002)

enterovirus

$$P_{\text{inf},d} = 1 - (1 + (P_{\text{exp},d}/0.422))^{-0.253} \qquad (4)$$

(Teunis *et al.* 1996)
(original data: Schiff *et al.* (1984))

Giardia

$$P_{\text{inf},d} = 1 - \exp(-0.0199 P_{\text{exp},d}) \qquad (5)$$

(Teunis *et al.*, 1996)
(original data: Rendtorff (1954))

where

- $P_{\text{inf},d} =$ PDF of infection risks per person per day,
- $P_{\text{exp},d} =$ PDF of exposure to the pathogen per person per day.

Infection risks during the outbreak or an event

The (retrospectively expected) total infection risk per pathogen per person during the events in this paper are calculated using formula (6):

$$P_{\text{inf},e} = 1 - \prod_{d=1}^{D} (1 - P_{\text{inf},d}) \qquad (6)$$

where

- $P_{\text{inf},e} =$ PDF of infection risks per person per outbreak or event,
- $P_{\text{inf},d} =$ PDF of infection risks per person per day.

The daily PDFs of infection risks were multiplied by bootstrapping using MatLab® 7.0.4 (10,000 random draws).

Infection risks due to non-outbreak contamination events per year

The expected infection risk per pathogen per person per year, due to detected non-outbreak faecal contamination events, assuming unchanged conditions, is estimated

using formula (8):

$$P_{\text{inf},y} = f_e P_{\text{inf},e} \qquad (8)$$

where

- $P_{\text{inf},e} =$ PDF of infection risks per person per day,
- $f_e =$ yearly fraction of the population of the Netherlands exposed during non-outbreak contamination events,
- $P_{\text{inf},d} =$ PDF of infection risks per person per outbreak or event.

RESULTS

Estimated infection probabilities from 1 CFU of thermotolerant coliforms per 100 ml

Table 1 shows the estimated infection risk at a mean concentrations of thermotolerant coliforms of 1 CFU per 100 ml during one day, e.g. represented by a single sample found positive at the detection limit. It is clear that the infection risks are much lower when assuming sewage as the contamination source than when assuming surface water or soil and shallow groundwater as a source. Incidence of single samples containing thermotolerant coliforms or *E. coli* was presented elsewhere (Van Lieverloo *et al.* 2006b; Hambsch *et al.* 2007).

Estimated infection probabilities during the outbreak

The first samples tested for the presence of thermotolerant coliforms (coli44) were collected on 4 December, a day after the taste and odour complaints. As the contamination probably started on 1 December, the coli44 concentrations through 3 December were assumed to be identical to those determined on 4 December. The mean estimated infection risks from the four index pathogens during outbreak are over 1×10^{-4} per person on each day of the contamination lasting from 1 December through 6 December 2001. The pathogen to thermotolerant coliform ratios were estimated from ratios found earlier in the contamination source. Risk levels from the four pathogens were quite similar, but the levels of the infection risks from viruses were highest, up to a maximum of 0.32 per person per day. The statistical index numbers of each daily PDF of 100,000 daily infection risks are presented in Figure 5. The results are compared with infection risks from non-outbreak events in the next subsection.

Table 1 | Estimated mean infection risks per person per day when exposed to a mean concentration of thermotolerant coliforms of 1 CFU per 100 ml. For enteroviruses in soil or shallow groundwater, three selections of the available ratios were used: Culturable = only ratios of culturable enteric virus to positive (> 0) thermotolerant coliforms (coli44); positive data = ratios of positive enteroviruses (culturable and PCR) vs. coli44; all data = all ratios, including data pairs with one or both values below the detection limit (coli44 concentrations below the detection limit were set to 50% of the detection limit)

	Assuming P/E [a] ratios from sewage	Assuming P/E [a] ratios from surface water	Assuming P/E [a] ratios from soil and shallow groundwater
Cryptosporidium	5.6×10^{-7}	3.2×10^{-4}	–
Giardia	2.2×10^{-7}	2.7×10^{-5}	–
Campylobacter	4.0×10^{-3}	4.8×10^{-2}	–
Enterovirus			
–culturable	6.3×10^{-7}	2.2×10^{-5}	6.8×10^{-3}
–positive data	–	–	3.6×10^{-2}
–all data	–	–	0.24

[a] P/E ratio: pathogen to *E. coli* or thermotolerant coliform ratio.
– no data on pathogen to thermotolerant coliforms or *E. coli* ratios available

Infection risk levels during the non-outbreak faecal contamination events

Cumulative Probability Density Functions

Figure 6 shows the means as well as the 2.5 and 97.5 percentiles of the infection risks per event, based on pathogen to thermotolerant coliform or *E. coli* ratios in surface water. These statistical index numbers per event are sorted by the mean infection risks and presented in cumulative probability density functions (Cumulative Density Function, CDF). The *y*-axis increments per event are the percentages of affected inhabitants during that event relative to the total of 185,000 inhabitants affected in all 50 events (see the step-by-step explanation in Box 1). In Figure 7, the CDFs of the mean infection risks are presented for all three evaluated possible contamination sources; sewage, surface water and soil or shallow groundwater.
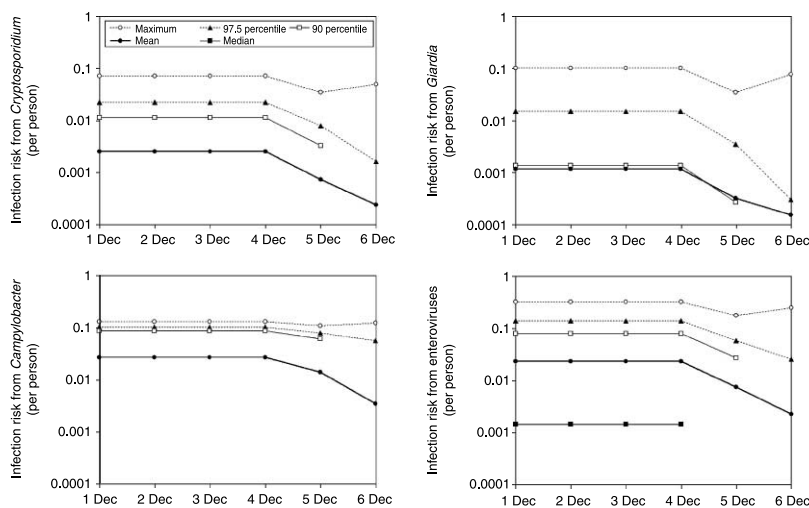

Figure 5 | Daily statistics of infection risks from four index pathogens during the 2001 outbreak estimated from thermotolerant coliform concentrations and pathogen to thermotolerant coliform ratios in the presumed contamination source (partially treated surface water).
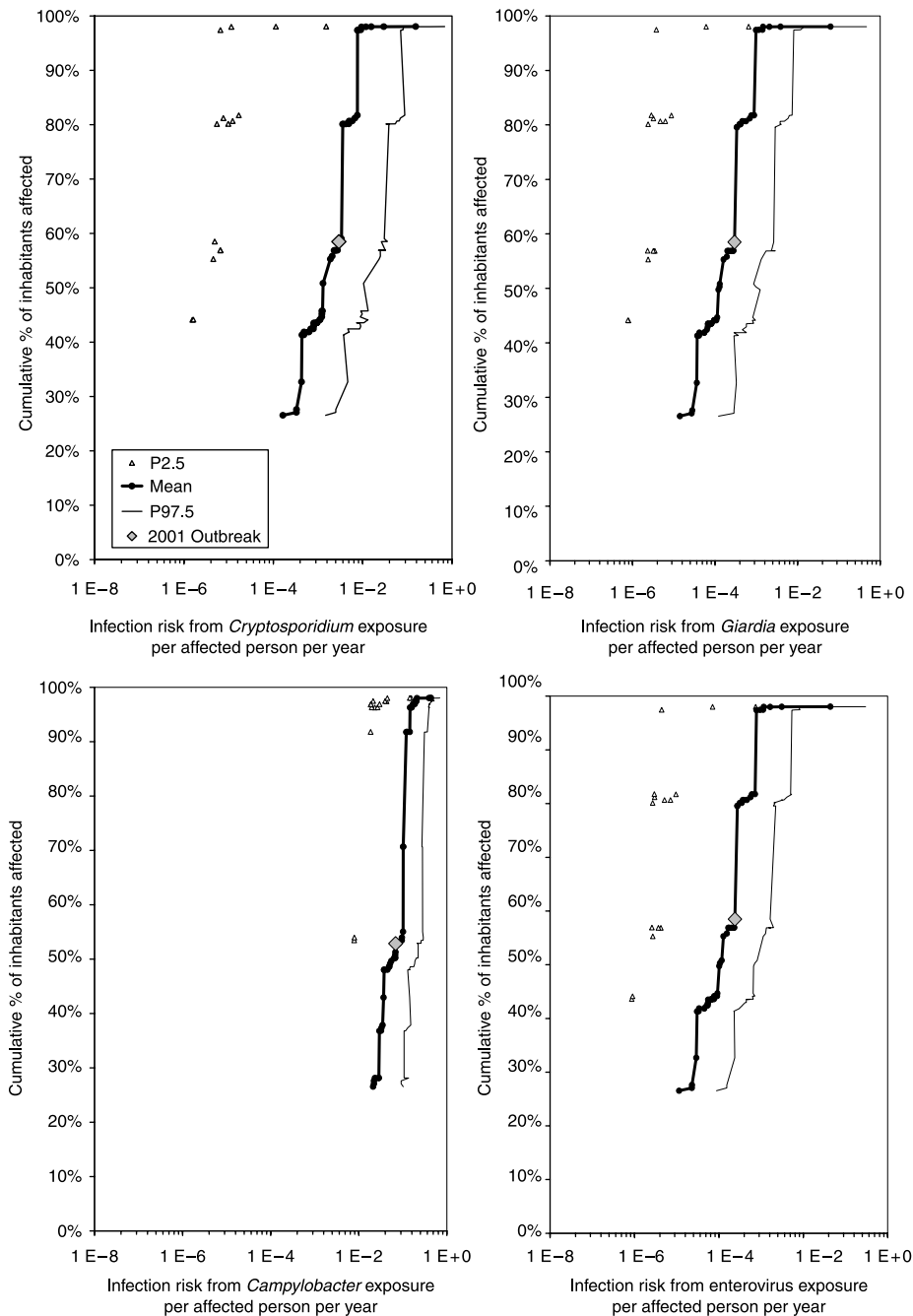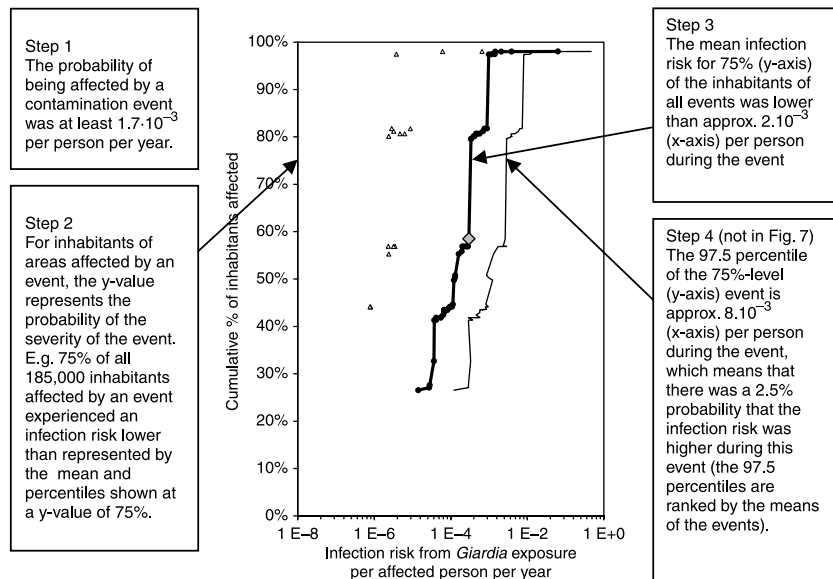
Figure 6 │ Cumulative density functions of the 2.5 percentile, mean and 97.5 percentile of total infection risks per person per event from four index pathogens during 50 mostly non-outbreak contamination events affecting a total of 185,000 inhabitants (events sorted by the mean). Infection risks were estimated from pathogen to *E. coli* or thermotolerant coliforms ratios in surface water. The mean infection risk estimated for the 2001 outbreak is marked for comparison, assuming identical ratios from surface water as in the other events. CDF percentiles (*y*-axis levels) are based upon the number of inhabitants affected per event relative to the total number of inhabitants affected by the 50 events. For most events, the 2.5 percentiles of the PDF of infection risks could not be estimated, when at least 2.5% of the thermotolerant coliform concentrations during the event were below the detection limit of 1 per 100 ml. The lowest infection risks were found for the event affecting the largest number of inhabitants (ca. 50,000). Therefore, the infection risks for this event are presented at the 27-percentile of the affected population (50,000/185,000).

Extended legend to the Cumulative Density Function (CDF) graphs in Figures 6 and 7

Step 1
The probability of being affected by a contamination event was at least $1.7 \cdot 10^{-3}$ per person per year.

Step 2
For inhabitants of areas affected by an event, the y-value represents the probability of the severity of the event. E.g. 75% of all 185,000 inhabitants affected by an event experienced an infection risk lower than represented by the mean and percentiles shown at a y-value of 75%.

Step 3
The mean infection risk for 75% (y-axis) of the inhabitants of all events was lower than approx. 2.10^{-3} (x-axis) per person during the event

Step 4 (not in Fig. 7)
The 97.5 percentile of the 75%-level (y-axis) event is approx. 8.10^{-3} (x-axis) per person during the event, which means that there was a 2.5% probability that the infection risk was higher during this event (the 97.5 percentiles are ranked by the means of the events).

Box 1 | Step-by-step explanation of the cumulative density function (CDFs, cumulative PDFs) in Figures 6 and 7.

In Figure 6, a diamond marks the infection risks during the outbreak that occurred in 2001, calculated from the same pathogen to thermotolerant coliform ratios in surface water that were used to calculate infection risks for the 49 non-outbreak events. These infection risks are far lower than the infection risks calculated from the pathogen to thermotolerant coliform ratios that were estimated for the river water that was the actual contamination source (Table 2). Estimated infection risks from *Cryptosporidium*, *Giardia* and enterovirus were higher in 48% of the non-outbreak contamination events (percentage weighted for the number of inhabitants affected). Infection risks from *Campylobacter* were higher in 76% of the events (Table 2).

An epidemiological evaluation showed that, during the 2001 outbreak, cases of gastroenteritis occurred with a clear dose–response relationship with drinking tap water. The evaluation did not reveal the pathogen/pathogens that was the most likely cause of the cases of gastroenteritis, nor was it possible to assess an accurate attack rate for the affected population. From the affected area, 19.8% of 1,866 exposed persons were diagnosed in general practice with gastroenteritis, compared to an attack rate of 7.0% in the area that was not affected (Fernandes *et al.* 2006). The total mean infection risk from all four pathogens estimated from pathogen to thermotolerant coliform ratios in the pathogen source was ca. 0.23 per person, mostly from *Campylobacter* and enteroviruses (Table 2). These pathogens were also hypothesised to be the most likely causes of the gastroenteritis by Fernandes *et al.* As not all infected persons are likely to fall ill (Haas *et al.* 1999), the mean infection risk of 0.23 per person is an underestimation, probably because no data on thermotolerant coliform concentrations are available from the first days of the outbreak (Figure 1).

Importantly, the infection risks, based on general pathogen to faecal indicator ratios in surface water, of over 40% of the non-outbreak events were higher than calculated for the outbreak, based on the same ratios (Table 2) and with a comparable lack of data between the start and the detection of the event.

Annual risk of infection

The probability of an inhabitant of the Netherlands being affected by an incident is at least 1.7×10^{-3} (see section on Methods and materials). In Figures 6 and 7, the y values indicate the cumulative probability of the severity of the
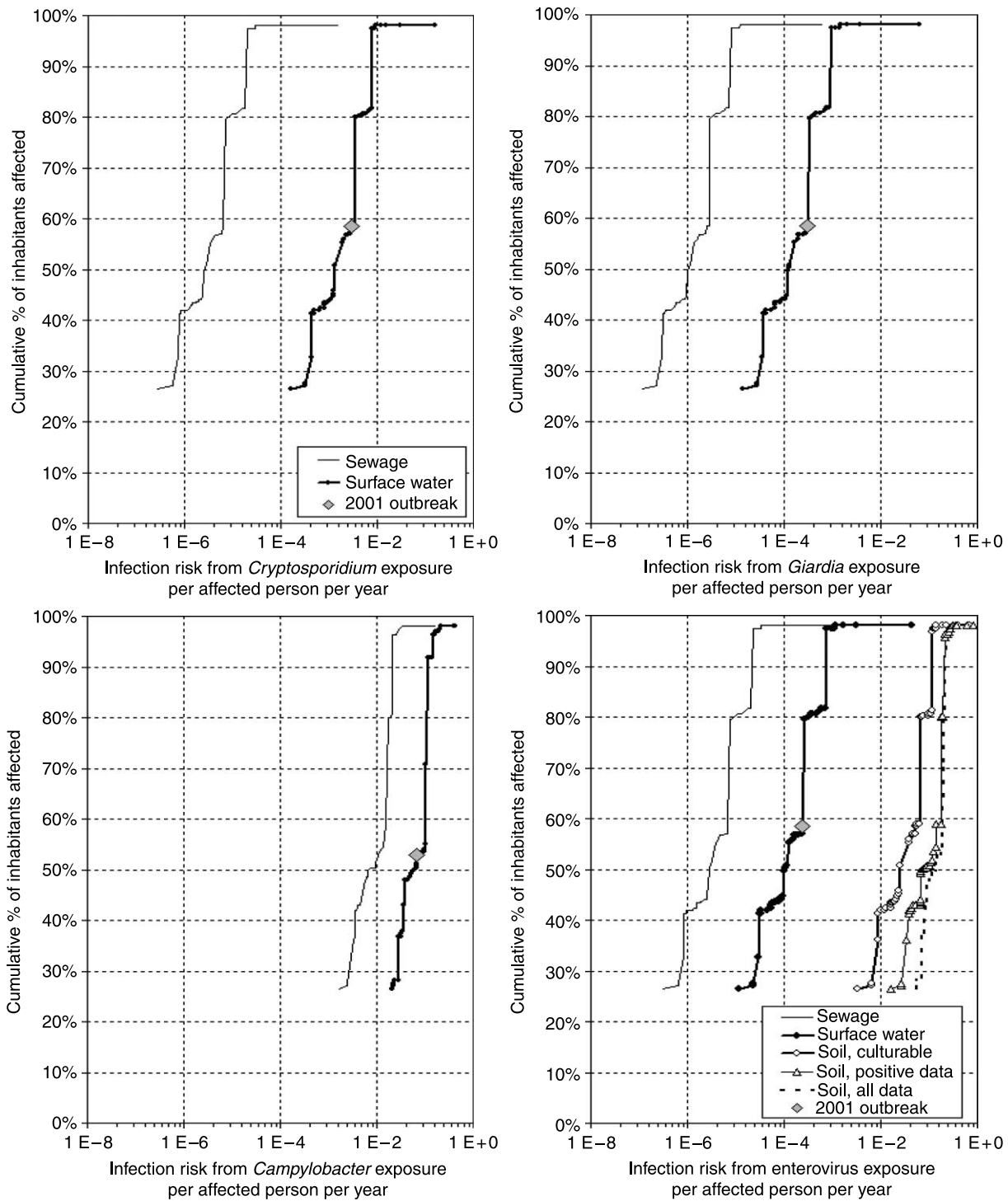
Figure 7 | Cumulative density functions of the mean total infection risks per person per event from four index pathogens during 50 mostly non-outbreak contamination events. Infection risks were estimated from pathogen to *E. coli* or thermotolerant coliforms ratios in sewage, surface water and soil. The mean infection risk estimated for the 2001 outbreak is marked for comparison, assuming identical ratios from surface water as in the other events. For enteroviruses in soil or shallow groundwater, three selections of the available ratios were used: Culturable = only ratios of culturable enteric virus to positive (> 0) thermotolerant coliforms (coli44); positive data = ratios of positive enteroviruses (culturable and PCR) vs. coli44; all data = all ratios, including data pairs with one or both values below the detection limit (coli44 concentrations below the detection limit were set to 50% of the detection limit).

Table 2 | Mean total infection risks from four index pathogens during the 2001 outbreak estimated from pathogen to thermotolerant coliform ratios in the presumed contamination source (partially treated surface water) compared to the infection risk estimated from ratios assumed for non-outbreak events (as found in surface water)

| | Mean total infection risks during the outbreak (per person) | | Mean total infection risks during the events (per person) | |
| | | | Based on P/E[a] ratios in surface water | |
	Based on P/E[a] ratios in actual source water	Based on P/E[a] ratios in surface water (% events higher[b])	95-percentile of all events	Maximum of all events
Cryptosporidium	1.1×10^{-2}	2.9×10^{-3} (42%)	7.7×10^{-3}	0.15
Giardia	5.2×10^{-3}	3.0×10^{-4} (42%)	9.8×10^{-4}	6.3×10^{-2}
Campylobacter	0.12	6.9×10^{-2} (47%)	0.15	0.44
Enterovirus	0.10	7.3×10^{-4} (42%)	7.8×10^{-4}	4.4×10^{-2}
Sum	0.23	7.2×10^{-2}	0.16	0.70

[a] P/E ratio: pathogen to *E. coli* or thermotolerant coliform ratio.
[b] % of non-outbreak events with higher infection risk levels, weighted for the number of inhabitants affected.

event for affected inhabitants. The x values indicate the probability of the infection risk of the affected inhabitants. To get an estimate of the yearly infection risk per inhabitant of the Netherlands, the risk levels in Figures 6 and 7 should be multiplied by 1.7×10^3. In the Netherlands Drinking Water Act (Anon., 2001) a preliminary maximum infection risk for finished water of surface water treatment plants is set to 1×10^{-4} per person per year. The mean infection risks from distributed drinking water in the Netherlands, especially from *Campylobacter* (assuming contamination with sewage or surface water) or enteroviruses (assuming soil or shallow groundwater), may very well be higher than this level (Table 3), in some cases much higher (Figure 7). As the incidence of thermotolerant coliforms and *E. coli* in the Netherlands was comparable to the incidence in other companies (Van Lieverloo *et al.* 2006b; Hambsch *et al.* 2007), these results show that infection risks from drinking water may be comparable in other countries as well.

DISCUSSION

The results in Figure 7 show that the risks from *Campylobacter* during faecal contaminations are relatively high both when assuming sewage as well as when assuming surface water to be the contamination source. Infection risks may be comparable or even higher from exposure to enteroviruses

when assuming contamination with soil or shallow groundwater. However, the method for estimating infection risks is fraught with uncertainties that currently limit its applicability. Future research is required to better estimate pathogen concentrations in distribution waters and the frequencies and durations of faecal intrusion events. Specific issues are now discussed, limited to possibly major factors.

Uncertainty of infection risks per event

Period between the start and the detection of the contamination

After detecting a contamination of periodical samples, the first sample contains 1 CFU of *E. coli* per 100 ml and the repeat sample does not contain any indicator bacteria ('single hit'). These results may represent small contamination events (if it was collected at the start of the event) or large contamination events (if it was collected at the end or the spatial periphery of an event). As concentrations usually subside quickly after contamination has occurred (e.g. during the outbreak described, see Figure 1), the effect on the estimated infection risks may be significant. After operations, samples are collected within the first day after the mains (or reservoir) were cleaned. Therefore, a contamination event after operations, if large enough to be detected in a 100 ml sample, is monitored from the first day.

Table 3 │ Estimated probabilities of the mean infection risks per person per year of distributed drinking water exceeding the preliminary maximum infection risk of 1×10^{-4} per person per year set for finished water of treatment plants in the Netherlands (Anon. 2001). The probabilities for exposed persons are 100% minus the percentiles at a risk level of 10×10^{-4} in Figure 7 of the estimated total population affected by a faecal contamination event (185,000 of 11 million in 10 years $= 1.7 \times 10^{-3}$ persons per year). The probabilities for the total population are 100% minus the percentiles at a risk level of 0.06 ($= 1 \times 10^{-4}$ $(1.7 \times 10^{-3})^{-1}$) per person per year (thus including the risk of being affected by a faecal contamination event)

	Assuming P/E[a] ratios from sewage		Assuming P/E[a] ratios from surface water		Assuming P/E[a] ratios from soil and shallow groundwater	
	Exposed	Total	Exposed	Total	Exposed	Total
Cryptosporidium	0.02	0	1	0.02	–	–
Giardia	0.02	0	0.44	0.02	–	–
Campylobacter	1	0.02	1	0.5	–	–
Enteric virus (culturable)	0.02	0	0.5	0	1	0.2

[a]P/E ration: pathogen to *E. coli* or thermotolerant coliform ratio.
–no data on pathogen to thermotolerant coliform or *E.coli* ratios available.

Unknown effect of protective measures

When the start of protective measures, such as boiling water advisories and dosage of disinfectants, was documented in event reports, usually there was little known about the effect of these measures on the consumption of contaminated water. Boiling water advisories are not followed by all inhabitants (Fernandes *et al.* 2006) and disinfectant residuals will inactivate chlorine-resistant pathogens (e.g. *Cryptosporidium*) much less effective than *E. coli* (Payment 1999). Therefore, infection risks should be estimated in worst case conditions, i.e. assuming no protective effect from protective measures.

Uncertainty of yearly infection risks

Probability of detecting contaminations

The analysis of large volumes of drinking water in the United Kingdom (182 samples of 10 l), Germany (130 samples of 10 l) and the Netherlands (44 samples of max. 200 l, total 7,062 l, Nobel & Oesterholt 2005) for the

presence of *E. coli* has shown that this indicator of faecal contamination is not present in detectable background concentrations, even when disinfectant residuals are absent (Van Lieverloo *et al.* 2006b; Hambsch *et al.* 2007). These results corroborate the hypothesis that faecal contaminations occur as temporary and often local events.

A preliminary simulation study in the Netherlands revealed that the probability of detecting *E. coli* in 100 ml samples after contamination of a distribution main in a small town with 16 l of sewage ranged from 0 to 15% ($n = 9$) with a mean of ca. 5% (Van Lieverloo *et al.* 2007).

Underreporting of non-outbreak contamination events

As water companies in the Netherlands are rarely confronted with an outbreak (after WWII, occurring in 1962, 1981 and 2001 only), information on small events usually is not documented and archived. In many cases, *E. coli* concentrations from old events could be retrieved from the laboratory database, but data on causes and response measures during the event were lacking.

Contamination in piping systems of connected premises

Standard sampling procedures include flushing of the piping systems of connected premises until water temperature is constant. In the Netherlands, a large company, up to 2002, flushed the tap and piping with only 250 ml. Incidence of thermotolerant coliforms was 10-fold higher than found by other water companies (1% vs. 0.1%), whereas incidence in repeat samples was 0 (compared to a mean of 0.004% in other companies). Repeat samples were always collected after flushing taps and piping according to standard sampling procedures This indicates a possible contamination of piping systems of connected premises (Van Lieverloo *et al.* 2003).

Uncertainty of pathogen to *E. coli* or thermotolerant coliform ratios

There is a large variation in pathogen to *E. coli* ratios, rendering this variable the most sensitive part of the model. Sources of variation are (partly from Pond *et al.* 2004):

Conditions in the host:

- infections and illnesses of the animal or human the faeces originate from (most likely the primary factor in the pathogen to indicator ratios),
- species of warm-blooded animal or human the faeces originates from.

Conditions in nature influence survival and therefore the ratios:

- age of the faecal material since defecation,
- matrix (surface water, soil, groundwater, man-made surfaces) and the resulting environmental conditions (presence of predators, temperature, moisture, UV radiation in sunlight).

Conditions in the drinking water distribution system also influence survival:

- temperature,
- disinfectant residual,
- flow and flush-out,
- predation in biofilm and sediments.

Unknown sources of contamination

It is usually hard to identify the source of contaminations leading to faecal contamination events and outbreaks. It is almost impossible to identify the source of 'single hits' (when repeat samples do not contain faecal indicators). Therefore, estimations of pathogen to thermotolerant coliform or *E. coli* ratios may vary from those in sewage to those in shallow groundwater (Figure 4) causing large uncertainty in estimates of infection risks (Figure 7).

Sensitivity to uncertainty of pathogen to *E. coli* ratios

The effect of the uncertainty of the contamination source probably is even higher than the uncertainty of the pathogen to thermotolerant coliform or *E. coli* ratios within a contamination source (Figures 4, 6 and 7). The highest infection risks from enteroviruses are found when assuming soil or shallow groundwater as the contamination source. A considerable part of the enterovirus to thermotolerant

coliform ratios in these sources were very much higher than in surface water or untreated sewage.

Most of the 50 recorded events were considered to be caused by operations (30) and, in most cases (26), soil was the most likely source of contamination (Van Lieverloo *et al.* 2006b). Contamination events with unknown causes and sources (19 out of 50) are likely to be caused by leaking mains in combination with loss of pressure or by unrecorded operations as well. Therefore, in most cases the entry of soil or shallow groundwater is very likely. Although the data on ratios of pathogen to thermotolerant coliforms or *E. coli* in soil or shallow groundwater available is very limited, the available data suggest that every *E. coli* found, when from such a contamination source, could be an indication of very high enterovirus concentrations in drinking water and resulting high infection risks to consumers.

CONCLUSIONS

The variation of the pathogen to thermotolerant coliform or *E. coli* ratio between and within possible contamination sources such as untreated sewage, surface water and soil or shallow groundwater is very large. This leaves a very limited applicability for estimating infection risks to consumers during short or prolonged faecal contamination events. As the most likely source of contamination, soil or shallow groundwater, results in a very high estimation of infection risk, even the presence of a single *E. coli* in a sample of 100 ml under these conditions is indicative of possibly very high infection risks.

Although the presented method is flawed, the current absence of a better index of pathogen exposure and infection risks during faecal contamination events leaves it the only method available for water companies, inspectorates and regulators to estimate the possible health effects for consumers and the need for reducing risks. The method is applicable to all secondary faecal contaminations occurring in groundwater wells, (groundwater) treatment plants and distribution systems, detectable by the presence of *E. coli*.

Epidemiological evaluations of non-outbreak faecal contamination events, including tracking down and characterising contamination sources, could validate the presented possibility of high infection risks during these events.

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