Estimation of pathogen concentrations in a drinking water source using hydrodynamic modelling and microbial source tracking

Ekaterina Sokolova, Johan Åström, Thomas J. R. Pettersson, Olof Bergstedt and Malte Hermansson

ABSTRACT

The faecal contamination of drinking water sources can lead to waterborne disease outbreaks. To estimate a potential risk for waterborne infections caused by faecal contamination of drinking water sources, knowledge of the pathogen concentrations in raw water is required. We suggest a novel approach to estimate pathogen concentrations in a drinking water source by using microbial source tracking data and fate and transport modelling. First, the pathogen (norovirus, *Cryptosporidium*, *Escherichia coli* O157/H7) concentrations in faecal contamination sources around the drinking water source Lake Rådasjön in Sweden were estimated for endemic and epidemic conditions using measured concentrations of faecal indicators (*E. coli* and *Bacteroidales* genetic markers). Afterwards, the fate and transport of pathogens within the lake were simulated using a three-dimensional coupled hydrodynamic and microbiological model. This approach provided information on the contribution from different contamination sources to the pathogen concentrations at the water intake of a drinking water treatment plant. This approach addresses the limitations of monitoring and provides data for quantitative microbial risk assessment (QMRA) and risk management in the context of faecal contamination of surface drinking water sources.

Key words | *Bacteroidales* markers, *Cryptosporidium*, *E. coli* O157/H7, faecal contamination, norovirus, QMRA

Ekaterina Sokolova (corresponding author)
Thomas J. R. Pettersson
Olof Bergstedt
Water Environment Technology,
Chalmers University of Technology,
SE-412 96 Gothenburg,
Sweden
E-mail: *ekaterina.sokolova@chalmers.se*

Johan Åström
Tyréns AB,
Lilla Badhusgatan 2,
SE-411 21 Gothenburg,
Sweden

Olof Bergstedt
Gothenburg Water,
City of Gothenburg,
Box 123,
SE-424 23 Angered,
Sweden

Malte Hermansson
Department of Cell and Molecular Biology,
 Microbiology,
University of Gothenburg,
SE-405 30 Gothenburg,
Sweden

INTRODUCTION

The faecal contamination of drinking water sources can lead to waterborne disease outbreaks (MacKenzie *et al.* 1994; Hrudey & Hrudey 2004). During two recent outbreaks (2010–2011) caused by faecal contamination of drinking water sources, tens of thousands of people in Sweden in the cities Östersund and Skellefteå suffered from waterborne *Cryptosporidium* infections (Skellefteå Municipality 2011; SMI 2011a). To estimate the potential risk for waterborne disease infections caused by faecal contamination of drinking water sources, a quantitative microbial risk assessment (QMRA) is often used (e.g. Westrell *et al.* 2003; De Roda Husman *et al.* 2009). QMRA involves hazard identification, exposure assessment, dose-response assessment and risk characterisation (Haas *et al.* 1999). Generic QMRA tools have been developed (Petterson & Stenström 2007; Schijven *et al.* 2011) to perform QMRA for specific water treatment plants (WTPs) with different treatment trains and varying pathogen levels in the raw water.

To perform QMRA, data on pathogen concentrations at the water intake to the WTP are needed. However, pathogen concentrations in water sources are rarely measured due to the complexity and cost of the analytical tests (e.g. Brookes *et al.* 2005; Field & Samadpour 2007). Instead of pathogens, faecal indicators are used by water producers to monitor microbial water quality. However, concentrations of faecal indicators are not directly related to the concentrations of

pathogens in the environment (Ashbolt et al. 2001; Brookes et al. 2005; Harwood et al. 2005). Pathogen concentrations at the water intake to the WTP depend on the pathogen load that enters the water body from different contamination sources (e.g. wastewater treatment plants, on-site sewer systems, emergency sewer overflows as well as surface runoff from urban and agricultural areas) and on the pathogen fate and transport within the water body.

The pathogen load to the water body from different contamination sources varies strongly with time, often due to the prevalence of the disease in the population. Under epidemic conditions, pathogens are excreted from many more human or animal hosts than under endemic conditions. An increased pathogen load, which enters the water source with wastewater discharges or surface runoff, implies higher pressure on water treatment and, as a result, increased risk of waterborne infections. The pathogen load to the water body under both endemic and epidemic conditions can be estimated based on the assumption that the ratio of pathogens to faecal indicators in fresh faecal matter is the same as in the contaminated discharges to the water source.

The estimation of pathogen load to the water body can be performed using traditional faecal indicators (such as Escherichia coli) or indicators that are applied in microbial source tracking to determine the origin of faecal contamination, such as Bacteroidales genetic markers (Field & Samadpour 2007). Several Bacteroidales assays have been proposed for the identification of host-specific faecal matter from humans and cattle (Bernhard & Field 2000; Layton et al. 2006; Reischer et al. 2006, 2007; Kildare et al. 2007; Stricker et al. 2008; Converse et al. 2009). While E. coli bacteria represent the total faecal contamination from all warm-blooded animals (humans, livestock, domestic pets, wild animals) and birds, Bacteroidales genetic markers indicate the contribution from the pathogen host groups of interest, i.e. humans and cattle. It is therefore posited that the estimation of pathogen concentrations using Bacteroidales genetic markers is more accurate than the estimation using E. coli.

The fate and transport of microbial contamination within the water source can be simulated using a coupled hydrodynamic and microbiological modelling approach. This approach has been widely applied to simulate the fate and transport of traditional faecal indicators (e.g. Sanders et al. 2005; Kashefipour et al. 2006; Liu et al. 2006; Riou

et al. 2007; Thupaki et al. 2010). However, relatively few studies have focused on modelling the fate and transport of the actual pathogens within the water source (e.g. Hipsey et al. 2004; Brookes et al. 2006; Hipsey et al. 2008), mainly due to the lack of data on pathogen concentrations.

In this study we have estimated concentrations of Cryptosporidium, norovirus and E. coli O157/H7 in contamination sources around a drinking water source, Lake Rådasjön, Sweden and simulated fate and transport of these pathogens within the lake. The concentrations in the contamination sources under endemic and epidemic conditions were estimated using the ratio of pathogens to faecal indicators (E. coli, human and ruminant Bacteroidales genetic markers) in fresh faecal matter. The spread of the pathogens within the lake from the contamination sources to the point of the WTP water intake was simulated using a three-dimensional hydrodynamic and microbiological model that takes into account pathogen inactivation in the environment. We suggest a novel approach to provide input data on pathogen concentrations in a drinking water source for QMRA by combining microbial source tracking data with hydrodynamic and microbiological modelling. Moreover, this approach provides information on the contribution from different contamination sources to the pathogen concentrations at the WTP water intake and enables prioritisation of mitigation measures.

METHODS

Study area

Lake Rådasjön (Figure 1) is located on the west coast of Sweden and constitutes the main water source for the city of Mölndal (60 000 consumers) and is a reserve water supply for the city of Gothenburg (500 000 consumers). The surface area of the lake is approximately 2.0 km², the catchment area of the lake is 14.9 km² and the maximum water depth is 23 m. The main inflow to the lake is the river Mölndalsån with a water flow in the range 1–20 m³ s⁻¹. The river Mölndalsån enters the lake in the southeast and drains the lake in the west to Lake Stensjön. The water intake for the city of Mölndal is located in the north-western part of the lake at 15 m depth (Figure 1).
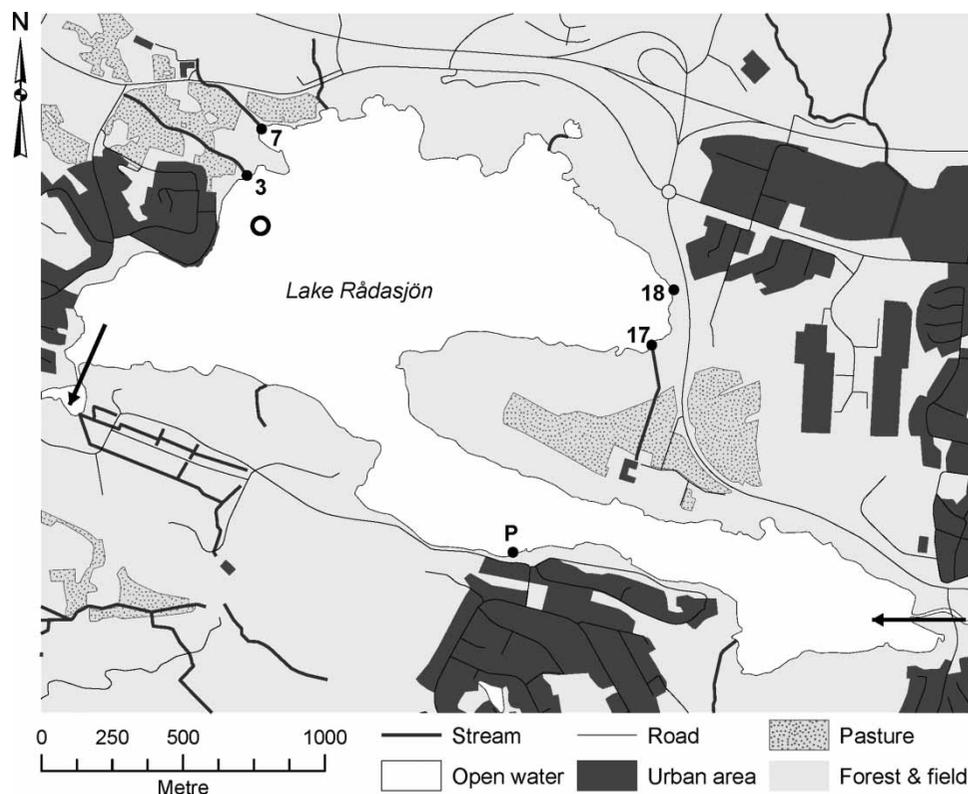
Figure 1 | Map of Lake Rådasjön and sources of microbial contamination from humans (sites 3, 7, 18 and P) and cattle (site 17). The location of the raw water intake is indicated with a circle. Inflow to and outflow from the lake are shown by arrows.

Lake Rådasjön is subjected to faecal contamination from various sources located in the catchment of the lake. In terms of the risks of waterborne disease outbreaks, the sources of human and ruminant faecal contamination are assumed to be the most relevant. Human faecal contamination at the water intake may originate from on-site sewers located to the north of the lake. On-site sewers release partly treated effluents into streams that enter the lake close to the water intake (Figure 1, sites 3 and 7). Another source of human faecal contamination is an emergency sewer overflow at a pumping station in a separate sewer system located south of the lake (Figure 1, site P). Discharges of untreated wastewater from this source occur several times a year during periods of heavy rainfall after intrusion of stormwater into the sewer network. In addition, human faecal contamination can potentially enter the lake with untreated stormwater runoff from an urban area located to the east of the lake (Figure 1, site 18). Furthermore, ruminant faecal contamination can be released from a cattle grazing area on the eastern side of the lake (Figure 1,

site 17). Faecal matter from the grazing area may reach the lake after short transport in a small stream (Figure 1, site 17). Concentrations of faecal indicators in the contamination sources were measured during a monitoring campaign performed in 2008 (Sokolova *et al.* 2012).

Norovirus can enter Lake Rådasjön from the sources of human faecal contamination (sites 3, 7, 18 and P), while *E. coli* O157/H7 can enter the lake from the source of ruminant faecal contamination (site 17; WHO 2008). Furthermore, *Cryptosporidium* can enter the lake from the sources of both human and ruminant faecal contamination (WHO 2008), i.e. all the sources mentioned above.

Estimation of norovirus, *Cryptosporidium* and *E. coli* O157/H7 concentrations in the contamination sources

To estimate the concentrations of norovirus, *Cryptosporidium* and *E. coli* O157/H7 in the discharges from contamination sources to Lake Rådasjön (Figure 1), the ratio of pathogens to faecal indicators was defined according

to Equation (1):

$$\left(\frac{C_P}{C_I}\right)_{\text{source}} = p\left(\frac{C_P}{C_I}\right)_{\text{faecal matter}} \tag{1}$$

where C_P is the pathogen concentration; C_I is the faecal indicator concentration; and p is the prevalence of a disease among people and animals present in the catchment of the lake. Prevalence of a disease was defined according to Equation (2):

$$p = \frac{I \times t}{365} \tag{2}$$

where I is the incidence of disease (the number of new cases within a specified time period divided by the size of the population initially at risk) and t is the duration of excretion (days).

The parameters in Equation (1) were described by probability distributions in order to take into account their variability. Faecal indicator concentrations in the contamination sources were described by lognormal distributions with location, 50 and 95 percentiles (hereafter denoted as %). The faecal indicator and pathogen concentrations in faecal matter, as well as the data for incidence and duration of excretion, were obtained from the literature and described by probability distributions (Table 1). The estimation of the pathogen concentrations in the contamination sources was performed for endemic and epidemic conditions. It was assumed that under epidemic conditions on average half of the population of humans or animals excrete pathogens, i.e. the prevalence of a disease under epidemic conditions was described by a Beta distribution with min = 0; 5% = 0.3; 50% = 0.5; max = 1.

The concentrations of pathogens in the contaminated discharges to the lake were calculated according to Equation (1) using Monte Carlo simulations (10 000 iterations) and presented as percentiles (5, 50 and 95%). The pathogen concentrations in the human faecal contamination

Table 1 | Literature data used for estimation of norovirus, *Cryptosporidium* and *E. coli* O157/H7 concentrations in the contamination sources

Pathogen/faecal indicator	Type of data (unit)	Distribution (values)[b]	Reference
Norovirus	Duration of excretion (log days)	Lognormal 1 (0.30; 0.60; 1.48)	Atmar *et al.* (2008)
	Incidence[a](–)	Beta (0.00; 1.00; 0.05; 0.19)	Hedlund (pers. comm. 2011)
	Concentration in faecal matter (log gene copy numbers/g)	Lognormal 1 (5.30; 7.08; 9.46)	Nordgren *et al.* (2009)
Cryptosporidium human	Duration of excretion (log days)	Normal (1.48; 0.17)	Stehr-Green *et al.* (1987)
	Incidence[a](–)	Beta (0.00; 1.00; 0.00003; 0.00005)	SMI (2011b)
	Concentration in faecal matter (log oocysts/g)	Normal (7.00; 1.00)	Girdwood & Smith (1999)
Cryptosporidium ruminant	Prevalence[a](–)	Beta (0.00; 1.00; 0.10; 0.17)	Enemark *et al.* (2002) and samples[c]
	Concentration in faecal matter (log oocysts/g)	Lognormal 2 (0,00; 2.00; 4.66)	Silverlås *et al.* (2010)
E. coli O157/H7	Prevalence[a](–)	Beta (0.00; 1.00; 0.10; 0.11)	Kistemann *et al.* (2004)
	Concentration in faecal matter (log CFU/g)	Lognormal 2 (0.00; 6.46; 8.41)	Hutchison *et al.* (2004)
Human marker	Concentration in faecal matter (log ME[d]/g)	Triangular (9.82; 10.50; 10.94)	Reischer *et al.* (2007)
Ruminant marker	Concentration in faecal matter (log ME[d]/g)	Triangular (9.48; 9.57; 9.63)	Reischer *et al.* (2006)
E. coli human	Concentration in faecal matter (log CFU/g)	Triangular (5.30; 6.80; 8.88)	Reischer *et al.* (2007)
E. coli ruminant	Concentration in faecal matter (log CFU/g)	Triangular (6.60; 7.30; 7.89)	Reischer *et al.* (2006)

[a]Endemic conditions.
[b]Parameters for distributions: Lognormal 1 (5; 50; 95%), Lognormal 2 (location; 5; 95%), Beta (min; max; 5; 50%), Normal (mean; std. dev.), Triangular (min; likeliest; max).
[c]Samples of faecal matter from a herd represented at site 17.
[d]Marker equivalent.

sources (sites 3, 7, 18 and P) were estimated using human *Bacteroidales* genetic markers (BacH), while in the ruminant faecal contamination source (site 17) the pathogen concentrations were estimated using ruminant *Bacteroidales* genetic markers (BacR) as faecal indicators. For comparison, the pathogen concentrations in all faecal contamination sources were estimated using *E. coli* as a faecal indicator.

Coupled hydrodynamic and microbiological modelling

The coupled hydrodynamic and microbiological modelling was used to estimate the contributions from the above-mentioned contamination sources (Figure 1) to the norovirus, *Cryptosporidium* and *E. coli* O157/H7 concentrations at the water intake of the city of Mölndal under endemic and epidemic conditions. For this purpose the pathogen concentrations in the contamination sources estimated using BacH and BacR markers were used. For every pathogen the simulations were performed using 5, 50 and 95% values of estimated pathogen concentrations in the contamination sources around the lake for endemic and epidemic conditions as input data. In addition, the simulations were performed to estimate the contributions to the pathogen

concentrations at the water intake from the emergency sewer overflow (site P), the untreated stormwater source (site 18) and the cattle grazing area (site 17) for a hypothetical scenario in which these sources were located in the vicinity of the water intake on the north-western shore of the lake.

Hydrodynamic model of Lake Rådasjön

To simulate the water flows in Lake Rådasjön, the three-dimensional time-dependent hydrodynamic model MIKE 3 FM (DHI 2009) was used. The hydrodynamic model is based on the numerical solution of three-dimensional incompressible Reynolds-averaged Navier–Stokes equations invoking the assumptions of Boussinesq and of hydrostatic pressure (DHI 2009). The model consists of continuity, momentum, temperature, salinity and density equations and is closed by a turbulent closure scheme (DHI 2009).

The modelling domain was approximated with prisms (triangles in horizontal plane) using a flexible mesh approach (Figure 2). The length of the sides of the triangles varies from approximately 40 to 80 m, and was adjusted to describe the shoreline and bathymetry. The mesh resolution is finer in the narrow parts of the lake and where large
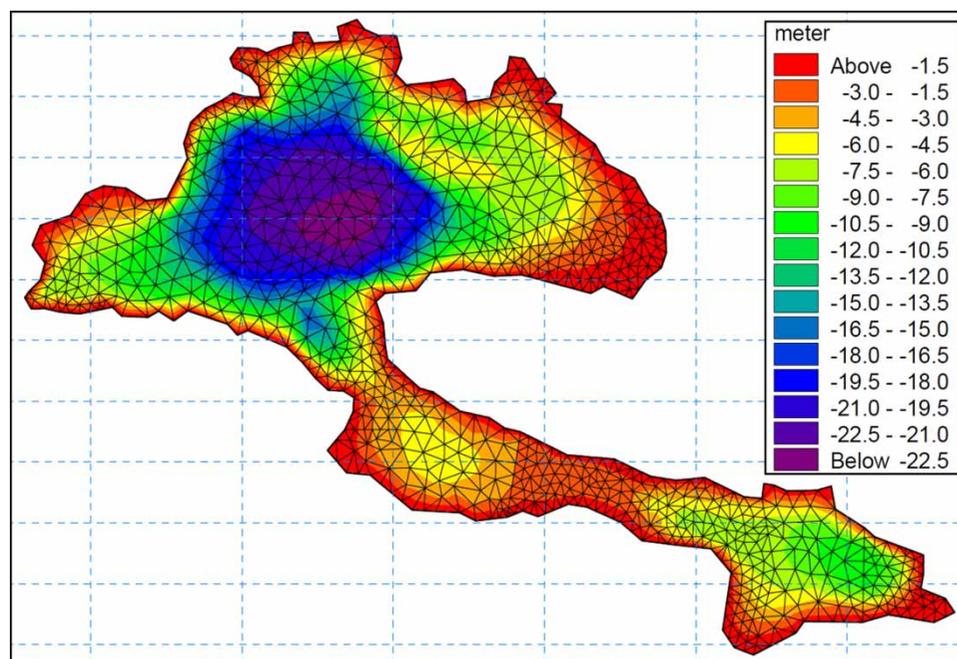

Figure 2 │ Lake Rådasjön: computational mesh and bathymetry (Sokolova *et al.* 2011). The colours represent water depth.

velocity gradients are expected, as well as in the vicinity of the emission points, to provide a better description of pollution spread. Vertically, the lake was divided into 27 layers. The thickness of the two uppermost layers can vary depending on the water level in the lake (sigma-layers), while the other layers have a fixed thickness (z-layers). In an undisturbed state the thickness of the two uppermost layers is 0.5 m each; these layers are followed by eight layers each of 1 m thickness down to a depth of 9 m. The range of depth from 9 to 16 m, where the thermocline is usually located, is divided into 14 layers each of 0.5 m thickness; below there are three layers with thicknesses of 1, 2 and 5 m.

The model was set up to simulate the hydrodynamic situation in Lake Rådasjön in the year 2008. The simulation was performed for the period January–December 2008. The output of the hydrodynamic model was then utilised in a microbiological model in order to simulate the transport of microbial contamination in the lake and estimate the pathogen concentrations at the water intake.

The initial rest conditions in the lake were defined by the constant surface elevation and the flow velocity was set to zero. The initial temperature field was described as homogeneous in the domain (2.1 °C) based on the assumption that in January the lake is well mixed and horizontal and vertical gradients of temperature at this time of year can be neglected.

The open boundary conditions (Figure 1, arrows) were defined by the discharge into the lake from the river Mölndalsån and by the water level in the downstream Lake Stensjön (Table 2). The land boundary was defined by zero normal velocity. The temperature on the open boundaries was described as zero gradients.

The model was set up to account for the hydrometeorological conditions (wind and precipitation on the lake surface), the withdrawal of water from the lake by WTPs, the inflow to the lake from small streams and to simulate the heat exchange between the atmosphere and the lake (Table 2). The water density was formulated as a function of temperature. The horizontal and vertical eddy viscosities were simulated using Smagorinsky and k-epsilon formulations, respectively. The bed resistance was described by a constant roughness height of 0.05 m.

Table 2 │ Input data for the hydrodynamic model

Type of data	Time resolution	Source
Wind speed	3 hours	SMHI[a]
Wind direction	3 hours	SMHI[a]
Precipitation	1 day	Härryda municipality
Air temperature	3 hours	SMHI[a]
Relative air humidity	3 hours	SMHI[a]
Clearness coefficient	3 hours	SMHI[a]
Withdrawal of water by WTPs	1 hour	Gothenburg Water, City of Mölndal
Discharge in the river Mölndalsån	1 day	City of Mölndal
Water level in Lake Stensjön	1 day	City of Mölndal
Inflow from small streams	1 day	Estimated[b]

[a]Swedish Meteorological and Hydrological Institute.
[b]The inflow to the lake from the small creeks was estimated using precipitation data for the catchment area of the respective creek and surface runoff coefficients, which were assigned based on the rate of exploitation and slope.

Microbiological model for norovirus, *E. coli* O157/H7 and *Cryptosporidium*

The fate and transport of pathogens in Lake Rådasjön were simulated using a microbiological water quality model ECO Lab (DHI 2004) which was coupled to the hydrodynamic model of the lake. ECO Lab uses flow fields from the hydrodynamic model to calculate the concentrations of pathogens or faecal indicators in the lake.

Inactivation of pathogens was described in the microbiological model according to Equation (3):

$$\frac{dC}{dt} = -kC \tag{3}$$

where k is the decay rate of pathogens in the water, t is the time and C is the pathogen concentration.

The decay coefficient for inactivation of norovirus and *E. coli* O157/H7 in the lake was described by Equation (4) (Mancini 1978; Sokolova *et al.* 2012):

$$k = k_0 \theta_I^{\mathrm{Int}} \theta_T^{(\mathrm{Temp}-20)} \tag{4}$$

where k_0 (day^{-1}) is the decay rate at 20 °C for a salinity of 0‰ and darkness; θ_I is the light coefficient; Int (kW m^{-2})

is the light intensity integrated over depth; θ_T is the temperature coefficient; and Temp (°C) is the water temperature.

It was assumed that the inactivation of norovirus and *E. coli* O157/H7 is well represented by the inactivation of faecal indicators somatic coliphages and *E. coli*, respectively (WHO 2008). The coefficients in Equation (4) for somatic coliphages and *E. coli* were experimentally determined in the microcosm trials performed during different seasons for the conditions of Lake Rådasjön (Sokolova *et al.* 2012).

The decay coefficient for the inactivation of *Cryptosporidium* in the lake is described by Equation (5) (Walker Jr & Stedinger 1999):

$$k = 10^{[(0.058 \times \text{Temp}) - 2.68]} \tag{5}$$

Sedimentation of *Cryptosporidium* was simulated according to Equation (6):

$$\frac{dC}{dt} = -\frac{v_s}{dz}C \tag{6}$$

where dz is the thickness of the layer and v_s is the settling velocity. The settling velocity was specified as 0.03 m day^{-1}, which is the settling velocity previously suggested for free oocysts (Medema *et al.* 1998). It was conservatively assumed that *Cryptosporidium* oocysts released into the lake were not attached to particles (Brookes *et al.* 2006).

RESULTS

Estimated concentrations of norovirus, *Cryptosporidium* and *E. coli* O157/H7 in the contamination sources

The concentrations of norovirus, *Cryptosporidium* and *E. coli* O157/H7 in the contamination sources around the lake were estimated for endemic and epidemic conditions (Figure 3). The estimated pathogen concentrations in the contamination sources were higher under epidemic conditions: the difference between epidemic and endemic conditions (comparing 50% values calculated using *Bacteroidales* markers) was approximately 2 log units for
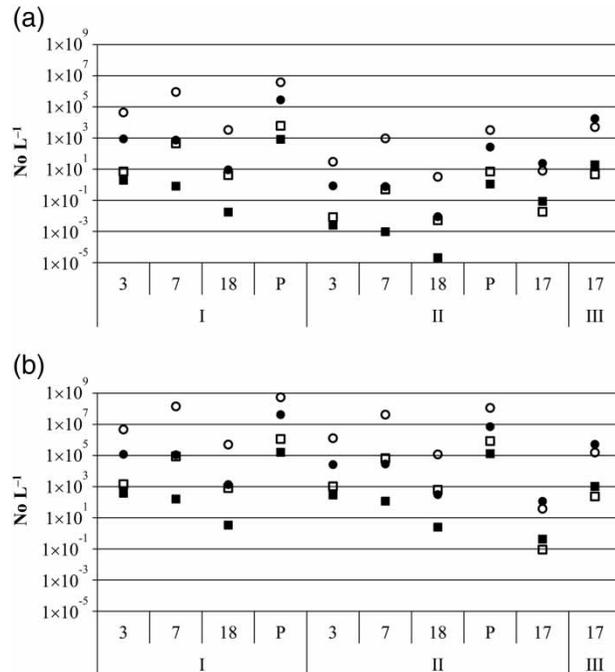

Figure 3 | Estimated concentrations of norovirus (I), *Cryptosporidium* (II) and *E. coli* O157/H7 (III) under (a) endemic and (b) epidemic conditions in the contamination sources: on-site sewers (3 and 7), untreated stormwater source (18), emergency sewer overflow (P) and cattle grazing area (17). BacH and BacR markers were used to estimate pathogen concentrations in human (sites 3, 7, 18 and P) and ruminant (site 17) contamination sources, respectively (50% (■) and 95% (•) values). The pathogen concentrations in both human and ruminant contamination sources were also estimated using *E. coli* (50% (□) and 95% (○) values).

norovirus, 5 log units for *Cryptosporidium*, and 2 log units for *E. coli* O157/H7 (Figure 3). The difference between the 50 and 95% values of the estimated (using *Bacteroidales* markers as faecal indicators) pathogen concentrations in the contamination sources was between 2 and 3 log units for all sources and pathogens under both endemic and epidemic conditions (Figure 3).

Comparing the 50% values calculated using *Bacteroidales* markers as faecal indicators, the highest norovirus concentrations in the contamination sources under both endemic and epidemic conditions were estimated for the emergency sewer overflow (site P), followed by the on-site sewers (sites 3 and 7) and the untreated stormwater source (site 18; Figure 3). In the same way, the highest *Cryptosporidium* concentrations in the contamination sources under endemic conditions were estimated for the emergency sewer overflow (site P) followed by the cattle grazing area

(site 17), the on-site sewers (3 and 7) and the untreated stormwater source (site 18); under epidemic conditions the order was the same with the exception of the cattle grazing area (site 17), for which the lowest concentrations were estimated (Figure 3).

The comparison of the 50% values of pathogen concentrations showed that the differences of more than 1 log unit between the estimations using *E. coli* and *Bacteroidales* markers were observed for on-site sewers (site 7) and untreated stormwater (site 18; Figure 3).

Simulated concentrations of norovirus, *Cryptosporidium* and *E. coli* O157/H7 at the water intake

The output results from the coupled hydrodynamic and microbiological modelling were the time series of pathogen concentrations at the water intake of the city of Mölndal during the year 2008 (e.g. Figure 4). These time series demonstrated that the contributions from different contamination sources to the pathogen concentrations at the water intake can vary strongly over time, up to 4 log units in case of norovirus (Figure 4). To simplify the analysis, the results were also presented using the arithmetic average values of the time series (Figure 5).

The modelling results illustrated that, under both endemic and epidemic conditions, on-site sewers (site 3) contributed the most to the norovirus concentration at the water intake followed by the emergency sewer overflow (site P), the on-site sewers (site 7) and the untreated stormwater source (site 18; Figure 5). In the case of *Cryptosporidium*, under endemic conditions the source that contributed most to the concentration at the water intake was the cattle grazing area (site 17) followed by the on-site sewers (site 3), the emergency sewer overflow (site P), the on-site sewers (site 7) and the untreated stormwater source (site 18). Under epidemic conditions the order was the same with the exception of the cattle grazing area (site 17), which contributed the least (Figure 5). According to the simulations, under endemic conditions the contributions from the studied contamination sources to the norovirus, *Cryptosporidium* and *E. coli* O157/H7 concentrations at
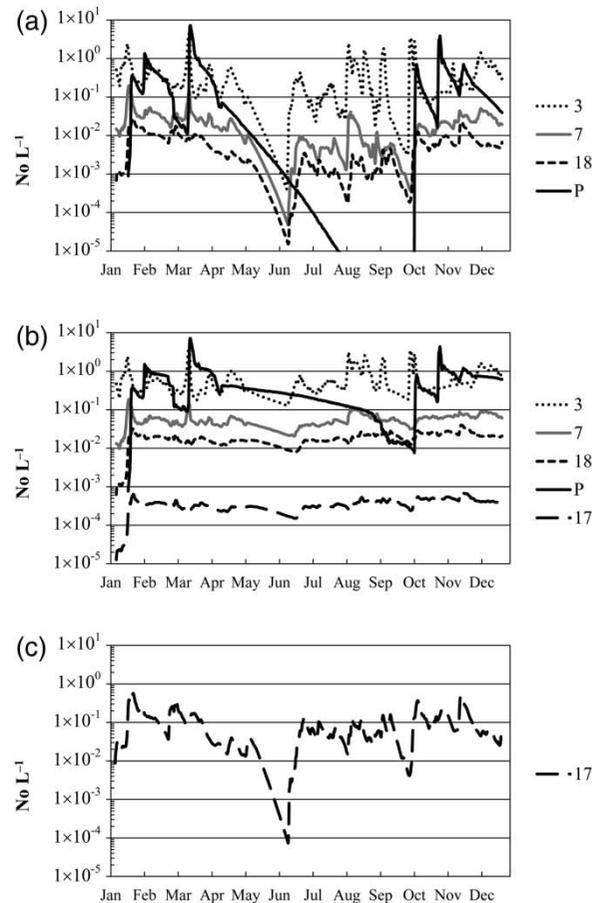

Figure 4 | Simulated contributions to the concentrations of (a) norovirus, (b) *Cryptosporidium* and (c) *E. coli* O157/H7 at the water intake under epidemic conditions from the contamination sources: on-site sewers (3 and 7), untreated stormwater source (18), emergency sewer overflow (P) and cattle grazing area (17). In the simulations the 50% values of pathogen concentrations in the contamination sources estimated for epidemic conditions using BacH and BacR markers were used.

the water intake were below 1 No. L^{-1}, while under epidemic conditions the pathogen concentrations at the water intake were expected to be much higher (Figure 5).

The simulations demonstrated that if the emergency sewer overflow (site P), the untreated stormwater source (site 18) and the cattle grazing area (site 17) were located in the vicinity of the water intake on the north-western shore of the lake, the contributions from these sources to the pathogen concentrations at the intake would be up to 1 log unit greater (Figure 5) than the contributions from their actual locations (Figure 1).
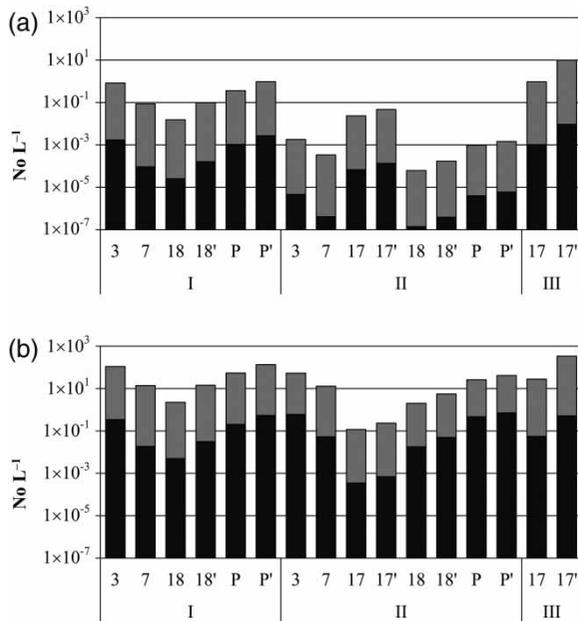
Figure 5 │ Simulated contributions to the concentrations of norovirus (I), *Cryptosporidium* (II) and *E. coli* O157/H7 (III) at the water intake under (a) endemic and (b) epidemic conditions from the contamination sources: on-site sewers (3 and 7), untreated stormwater source (18), emergency sewer overflow (P) and cattle grazing area (17). Numbers 18′, P′ and 17′ illustrate the contribution from the sources 18, P and 17, respectively, if these sources were located in the vicinity of the water intake. Black and grey colours represent 50 and 95% values of pathogen concentrations respectively. In the simulations the pathogen concentrations in the contamination sources estimated using BacH and BacR markers were used.

DISCUSSION

In this paper, we have suggested the combination of microbial source tracking with coupled hydrodynamic and microbiological modelling in order to obtain data on pathogen concentrations in a drinking water source. To the best of our knowledge, this is the first study where the microbial source tracking data were used to calculate pathogen concentrations in the contamination sources to provide input data for coupled hydrodynamic and microbiological modelling of pathogen fate and transport within a drinking water source.

The estimations of the pathogen concentrations in the microbial contamination sources were performed using BacH and BacR genetic markers as well as *E. coli* as faecal indicators. For the on-site sewers (site 7) and untreated stormwater (site 18) sources, the pathogen concentrations estimated using *E. coli* were higher than the estimations using BacH. A probable explanation for this is

that the stream which transports discharges from the on-site sewers (site 7, Figure 1) also drains a pasture area where horses graze, and untreated stormwater (site 18, Figure 1) can possibly transport faecal contamination from cats and dogs present in this urban area. Faecal contamination from horses, cats and dogs may have contributed to the total *E. coli* concentrations measured in these contamination sources. This might have resulted in an overestimation of pathogen concentrations since the only pathogen hosts of interest in case of these contamination sources were humans. Since BacH concentrations represent contamination from humans only, the estimations based on this indicator were considered to be more accurate. This example illustrates the advantages of using microbial source tracking data to estimate pathogen concentrations in contamination sources.

The *Cryptosporidium* concentrations were measured in the discharges from on-site sewers (site 3) on eight different occasions during 2010–2011 and varied from 0 to 3 oocysts/10 L (data from Gothenburg Water). These values can be compared to the *Cryptosporidium* concentrations in discharges from on-site sewers (site 3) estimated for endemic conditions using BacH markers: 0.02 and 8.5 oocysts/10 L as 50 and 95% values, respectively (Figure 3). The *Cryptosporidium* concentrations were measured at the WTP water intake on 26 different occasions during 2006–2011 and varied from 0 to 14 oocysts/10 L (data from Gothenburg Water). The concentration 14 oocysts/10 L, which was measured during the winter period, corresponds to the simulated *Cryptosporidium* concentrations for epidemic conditions (Figure 5).

The modelling results have demonstrated that the source with the highest estimated pathogen concentrations is not necessarily the main contributor to the pathogen concentrations at the water intake (Figures 3 and 5). Furthermore, the pathogen concentrations at the water intake can vary strongly over time (Figure 4). This illustrates that the risks for the drinking water supply are dependent on factors such as the magnitude of discharge from the contamination source and the pathogen fate and transport within the water source (Ashbolt *et al.* 2010). This emphasises the value of performing hydrodynamic and microbiological modelling in order to estimate the impact of various contamination sources on the water quality in a water source (e.g. Liu

et al. 2006; Hipsey *et al.* 2008; Thupaki *et al.* 2010; Schijven & De Roda Husman 2011).

In this study, we have combined microbial source tracking with fate and transport modelling in order to determine the dominant faecal sources and to provide input data for QMRA. In a study by Reischer *et al.* (2011), the dominant faecal sources were identified by linking microbial source tracking to catchment information. A faecal pollution source profile was used to formulate a hypothesis about the dominant sources of pollution in the catchment; this hypothesis was then tested using multi-parametric microbial source tracking. Schoen *et al.* (2011) estimated the contribution made by each source to the total infection risk in a water body, which was assumed to be impacted by a mixture of secondary-treated disinfected municipal wastewater and untreated sewage, by applying QMRA using norovirus as the reference pathogen.

The method suggested in this article provides the data needed to evaluate the current and the future safety of the drinking water supply in terms of infection risks. The greatest risks for waterborne disease outbreaks can be expected under epidemic conditions due to the increased load of pathogens to the water source. We have therefore suggested a method to investigate the contributions from the microbial contamination sources to the pathogen concentrations at the water intake under both endemic and epidemic conditions. Data from actual epidemiological studies can be utilised as input data for this method. Low pathogen concentrations and complexity of the microbial analyses often limit monitoring of pathogen concentrations at the water intake, and different surrogates have been suggested to overcome these limitations (e.g. Brookes *et al.* 2005; Field & Samadpour 2007). However, monitoring of pathogens or surrogates cannot provide information on the possible future risks (Ashbolt *et al.* 2010). The suggested method can therefore be used to address the limitations of monitoring. It has been demonstrated that this method can be used to identify, select and prioritise between sources of contamination and, consequently, to provide decision-support data on the development of the water source catchment area as well as to facilitate the prioritisation of mitigation measures (Bambic *et al.* 2011; Schijven & De Roda Husman 2011).

During the winter/spring period of 2010–2011, the presence of *Cryptosporidium* oocysts in the drinking water sources of Lake Storsjön and the river Skellefteälven (Sweden) in combination with insufficient treatment at the WTPs resulted in large waterborne disease outbreaks in the cities Östersund and Skellefteå, respectively. Most likely, *Cryptosporidium* oocysts entered the water sources with wastewater discharges and were transported to the intakes of the WTPs. Hydrodynamic and microbiological modelling integrated with microbial source tracking data would be appropriate to estimate the possible pathogen concentrations at the intakes to the WTPs in these two areas. Furthermore, the estimated pathogen concentrations at the intakes to the WTPs could be used as input data for QMRA to indicate the health risks for the consumers. In the case of the outbreak in Skellefteå, preliminary modelling results confirmed that wastewater discharges could have reached the raw water intake in the river Skellefteälven (Skellefteå Municipality 2011). The outbreak in Östersund occurred shortly after autumn destratification of Lake Storsjön, i.e. during the period when there were mixed conditions in the lake and the faecal contamination could reach the raw water intake located deep in the lake. These examples illustrate the importance of understanding the hydrodynamic processes in drinking water sources and the need for fate and transport modelling.

CONCLUSIONS

By combining microbial source tracking data with coupled hydrodynamic and microbiological modelling, we have estimated the pathogen concentrations in different contamination sources around a drinking water source and evaluated the contributions from these contamination sources to the pathogen concentrations at the WTP water intake. The suggested approach provides input data for QMRA and decision support data for risk management and the prioritisation of mitigation measures.

The concentrations of norovirus, *Cryptosporidium* and *E. coli O157/H7* in different contamination sources around Lake Rådasjön were estimated for endemic and epidemic conditions based on measurements of *E. coli* and *Bacteroidales* markers in these contamination sources. The estimated pathogen concentrations in the contamination sources under epidemic conditions were up to 5 log units

higher than under endemic conditions. The estimations based on *Bacteroidales* markers were considered to be source specific and thus more accurate than the estimations based on *E. coli*. From the results of coupled hydrodynamic and microbiological modelling, we can conclude that under endemic conditions the on-site sewers (site 3) were the source that contributed the most to the norovirus concentrations at the WTP water intake in Lake Rådasjön; the cattle grazing area (site 17) was the main contributor to the *Cryptosporidium* concentrations. Under epidemic conditions the on-site sewers (site 3) contributed the most to both norovirus and *Cryptosporidium* concentrations at the water intake. This modelling approach has proved to be a useful tool for simulating the pathogen fate and transport in a water source.

It can be concluded that integrating microbial source tracking with hydrodynamic and microbiological modelling yields more information than can be gained from applying these methods separately. The approach reported in this article can be used to prevent waterborne disease outbreaks caused by the faecal contamination of surface drinking water sources.

ACKNOWLEDGEMENTS

REFERENCES

Ashbolt, N., Grabow, W. & Snozzi, M. 2001 Indicators of microbial water quality. In: *Water Quality: Guidelines, Standards and Health* (L. Fewtrell & J. Bartram, eds). Published on behalf of WHO by IWA Publishing, London, pp. 290–316.

Ashbolt, N. J., Schoen, M. E., Soller, J. A. & Roser, D. J. 2010 Predicting pathogen risks to aid beach management: The real value of quantitative microbial risk assessment (QMRA). *Water Res.* **44** (16), 4692–4703.

Atmar, R. L., Opekun, A. R., Gilger, M. A., Estes, M. K., Crawford, S. E., Neill, F. H. & Graham, D. Y. 2008 Norwalk virus shedding after experimental human infection. *Emerg. Infect. Dis.* **14** (10), 1553–1557.

Bambic, D., McBride, G., Miller, W., Stott, R. & Wuertz, S. 2011 Quantification of pathogens and sources of microbial indicators for QMRA in recreational waters. *Water Intelligence Online* **10**, 384 pp. Available from: http://www.iwaponline.com/wio/2011/wio2011RF9781843395430.htm.

Bernhard, A. E. & Field, K. G. 2000 A PCR assay to discriminate human and ruminate feces on the basis of host differences in Bacteroides-Prevotella genes encoding 16S rRNA. *Appl. Environ. Microbiol.* **66**, 4571–4574.

Brookes, J. D., Hipsey, M. R., Burch, M. D., Linden, L. G., Ferguson, C. M. & Antenucci, J. P. 2005 Relative value of surrogate indicators for detecting pathogens in lakes and reservoirs. *Environ. Sci. Technol.* **39** (22), 8614–8621.

Brookes, J. D., Davies, C. M., Hipsey, M. R. & Antenucci, J. P. 2006 Association of Cryptosporidium with bovine faecal particles and implications for risk reduction by settling within water supply reservoirs. *J. Water Health* **4** (1), 87–98.

Converse, R. R., Blackwood, A. D., Kirs, M., Griffith, J. F. & Noble, R. T. 2009 Rapid QPCR-based assay for fecal Bacteroides spp. as a tool for assessing fecal contamination in recreational waters. *Water Res.* **43** (19), 4828–4837.

De Roda Husman, A. M., Lodder, W. J., Rutjes, S. A., Schijven, J. F. & Teunis, P. F. M. 2009 Long-term inactivation study of three enteroviruses in artificial surface and groundwaters, using PCR and cell culture. *Appl. Environ. Microbiol.* **75** (4), 1050–1057.

DHI 2004 *MIKE 21/3 Ecological Modelling: MIKE21/3 ECO Lab FM Short Description*. DHI Water & Environment, Horsholm. Available from: http://www.dhigroup.com/upload/dhisoftwarearchive/shortdescriptions/marine/MIKE213_FM_EL_Short_Description.pdf.

DHI 2009 *MIKE 3 Estuarine and Coastal Hydraulics and Oceanography, Short Description*. DHI Water & Environment, Horsholm. Available from: http://mikebydhi.com/~/media/Microsite_MIKEbyDHI/Publications/PDF/M3_ShortDescr.ashx.

Enemark, H. L., Ahrens, P., Lowery, C. J., Thamsborg, S. M., Enemark, J. M. D., Bille-Hansen, V. & Lind, P. 2002 Cryptosporidium andersoni from a Danish cattle herd: Identification and preliminary characterisation. *Vet. Parasitol.* **107** (1–2), 37–49.

Field, K. G. & Samadpour, M. 2007 Fecal source tracking, the indicator paradigm, and managing water quality. *Water Res.* **41** (16), 3517–3538.

Girdwood, R. W. A. & Smith, H. V. 1999 *Cryptosporidium*. In: *Encyclopaedia of Food Microbiology* (R. Robinson, C. Batt & P. Pattel, eds). Academic Press, London and New York, pp. 487–497.

Haas, C. N., Rose, J. B. & Gerba, C. P. 1999 *Quantitative Microbial Risk Assessment*. John Wiley & Sons, Inc., New York, USA.

Harwood, V. J., Levine, A. D., Scott, T. M., Chivukula, V., Lukasik, J., Farrah, S. R. & Rose, J. B. 2005 Validity of the indicator organism paradigm for pathogen reduction in reclaimed water and public health protection. *Appl. Environ. Microbiol.* **71** (6), 3163–3170.

Hipsey, M. R., Antenucci, J. P., Brookes, J. D., Burch, M. D., Regel, R. H. & Linden, L. 2004 A three dimensional model of Cryptosporidium dynamics in lakes and reservoirs: A new tool for risk management. *Int. J. River Basin Mgmt* **2** (3), 181–197.

Hipsey, M. R., Antenucci, J. P. & Brookes, J. D. 2008 A generic, process-based model of microbial pollution in aquatic systems. *Water Resour. Res.* **44** (7), pW07408.

Hrudey, S. E. & Hrudey, E. J. 2004 *Safe Drinking Water: Lessons from Recent Outbreaks in Affluent Nations*. IWA Publishing, London.

Hutchison, M. L., Walters, L. D., Avery, S. M., Synge, B. A. & Moore, A. 2004 Levels of zoonotic agents in British livestock manures. *Lett. Appl. Microbiol.* **39** (2), 207–214.

Kashefipour, S. M., Lin, B. & Falconer, R. A. 2006 Modelling the fate of faecal indicators in a coastal basin. *Water Res.* **40** (7), 1413–1425.

Kildare, B. J., Leutenegger, C. M., McSwain, B. S., Bambic, D. G., Rajal, V. B. & Wuertz, S. 2007 16S rRNA-based assays for quantitative detection of universal, human-, cow-, and dog-specific fecal Bacteroidales: A Bayesian approach. *Water Res.* **41** (16), 3701–3715.

Kistemann, T., Zimmer, S., Vågsholm, I. & Andersson, Y. 2004 GIS-supported investigation of human EHEC and cattle VTEC O157 infections in Sweden: Geographical distribution, spatial variation and possible risk factors. *Epidemiol. Infect.* **132** (3), 495–505.

Layton, A., McKay, L., Williams, D., Garrett, V., Gentry, R. & Sayler, G. 2006 Development of Bacteroides 16S rRNA gene TaqMan-based real-time PCR assays for estimation of total, human, and bovine fecal pollution in water. *Appl. Environ. Microbiol.* **72** (6), 4214–4224.

Liu, L., Phanikumar, M. S., Molloy, S. L., Whitman, R. L., Shively, D. A., Nevers, M. B., Schwab, D. J. & Rose, J. B. 2006 Modeling the transport and inactivation of E. coli and enterococci in the near-shore region of Lake Michigan. *Environ. Sci. Technol.* **40** (16), 5022–5028.

MacKenzie, W. R., Hoxie, N. J., Proctor, M. E., Gradus, M. S., Blair, K. A., Peterson, D. E., Kazmierczak, J. J., Addiss, D. G., Fox, K. R., Rose, J. B. & Davis, J. P. 1994 A massive outbreak in Milwaukee of Cryptosporidium infection transmitted through the public water supply. *N. Engl. J. Med.* **331** (3), 161–167.

Mancini, J. L. 1978 Numerical estimates of coliform mortality rates under various conditions. *J. Water Pollut. Control Fed.* **50** (11), 2477–2484.

Medema, G. J., Schets, F. M., Teunis, P. F. M. & Havelaar, A. H. 1998 Sedimentation of free and attached Cryptosporidium oocysts and Giardia cysts in water. *Appl. Environ. Microbiol.* **64** (11), 4460–4466.

Nordgren, J., Bucardo, F., Dienus, O., Svensson, L. & Lindgren, P. E. 2009 Novel light-upon-extension real-time pcr assays for detection and quantification of genogroup i and ii noroviruses in clinical specimens. *J. Clin. Microbiol.* **46** (1), 164–170.

Petterson, S. R. & Stenström, T. A. 2007 Generic QMRA tool for drinking water systems modelling. WaterMicro 2007, 14th International Symposium on Health-Related Water Microbiology, Tokyo, 10–15 September, 2007.

Reischer, G. H., Kasper, D. C., Steinborn, R., Mach, R. L. & Farnleitner, A. H. 2006 Quantitative PCR method for sensitive detection of ruminant fecal pollution in freshwater and evaluation of this method in alpine karstic regions. *Appl. Environ. Microbiol.* **72** (8), 5610–5614.

Reischer, G. H., Kasper, D. C., Steinborn, R., Farnleitner, A. H. & Mach, R. L. 2007 A quantitative real-time PCR assay for the highly sensitive and specific detection of human faecal influence in spring water from a large alpine catchment area. *Lett. Appl. Microbiol.* **44** (4), 351–356.

Reischer, G. H., Kollanur, D., Vierheilig, J., Wehrspaun, C., MacH, R. L., Sommer, R., Stadler, H. & Farnleitner, A. H. 2011 Hypothesis-driven approach for the identification of fecal pollution sources in water resources. *Environ. Sci. Technol.* **45** (9), 4038–4045.

Riou, P., Le Saux, J. C., Dumas, F., Caprais, M. P., Le Guyader, S. F. & Pommepuy, M. 2007 Microbial impact of small tributaries on water and shellfish quality in shallow coastal areas. *Water Res.* **41** (12), 2774–2786.

Sanders, B. F., Arega, F. & Sutula, M. 2005 Modeling the dry-weather tidal cycling of fecal indicator bacteria in surface waters of an intertidal wetland. *Water Res.* **39** (14), 3394–3408.

Schijven, J. F. & De Roda Husman, A. M. 2011 Applications of Quantitative Microbial Source Tracking (QMST) and Quantitative Microbial Risk Assessment (QMRA). In: *Microbial Source Tracking: Methods, Applications, and Case Studies* (C. Hagedorn, A. R. Blanch & V. J. Harwood, eds). Springer Science + Business Media, LLC, pp. 559–583.

Schijven, J. F., Teunis, P. F. M., Rutjes, S. A., Bouwknegt, M. & De Roda Husman, A. M. 2011 QMRAspot: A tool for Quantitative Microbial Risk Assessment from surface water to potable water. *Water Res.* **45** (17), 5564–5576.

Schoen, M. E., Soller, J. A. & Ashbolt, N. J. 2011 Evaluating the importance of faecal sources in human-impacted waters. *Water Res.* **45** (8), 2670–2680.

Silverlås, C., De Verdier, K., Emanuelson, U., Mattsson, J. G. & Björkman, C. 2010 Cryptosporidium infection in herds with

and without calf diarrhoeal problems. *Parasitol. Res.* **107** (6), 1435–1444.

Skellefteå Municipality 2011 *Discharges of wastewater caused Cryptosporidium infections: Report from the Department of Construction and Environment, Skellefteå Municipality on their work and conclusions (In Swedish: 'Utsläpp av avloppsvatten orsakade cryptosporidiesmittan – redovisning av bygg – och miljökontorets arbete och slutsatser').* Department of Construction and Environment, Skellefteå Municipality. Available from: http://www.skelleftea.se/Bygg %20och%20miljokontoret/Innehallssidor/Bifogat/ Slutrapport%20bygg-%20och%20milj%C3%B6kontoret.pdf.

SMI 2011a *Cryptosporidium in Östersund: The work of the Swedish Institute for Communicable Disease Control with the waterborne disease outbreak in Östersund in 2010–2011 (In Swedish: 'Cryptosporidium i Östersund: Smittskyddsinstitutets arbete med det dricksvattenburna utbrottet i Östersund 2010–2011').* Swedish Institute for Communicable Disease Control, Solna. Available from: http://www.smittskyddsinstitutet.se/upload/Publikationer/ Cryptosporidium-i-Ostersund-2011-15-4.pdf.

SMI 2011b *Statistics for Infectious Diseases (In Swedish: 'Statistik över smittsamma sjukdomar').* Swedish Institute for Communicable Disease Control, Solna. Available from: http://www.smittskyddsinstitutet.se/statistik/.

Sokolova, E., Borell Lövstedt, C. & Pettersson, T. J. R. 2011 Fate and transport modelling of microbial pollution in a lake used as a drinking water source. In *Proceedings of the*

34th IAHR World Congress, Brisbane, Australia, 26 June–1 July.

Sokolova, E., Åström, J., Pettersson, T. J. R., Bergstedt, O. & Hermansson, M. 2012 Decay of Bacteroidales genetic markers in relation to traditional fecal indicators for water quality modeling of drinking water sources. *Environ. Sci. Technol.* **46** (2), 892–900.

Stehr-Green, J. K., McCaig, L. & Remsen, H. M. 1987 Shedding of oocysts in immunocompetent individuals infected with cryptosporidium. *Am. J. Trop. Med. Hyg.* **36** (2), 338–342.

Stricker, A. R., Wilhartitz, I., Farnleitner, A. H. & Mach, R. L. 2008 Development of a Scorpion probe-based real-time PCR for the sensitive quantification of Bacteroides sp. ribosomal DNA from human and cattle origin and evaluation in spring water matrices. *Microbiol. Res.* **163** (2), 140–147.

Thupaki, P., Phanikumar, M. S., Beletsky, D., Schwab, D. J., Nevers, M. B. & Whitman, R. L. 2010 Budget analysis of Escherichia coli at a southern Lake Michigan Beach. *Environ. Sci. Technol.* **44** (3), 1010–1016.

Walker Jr, F. R. & Stedinger, J. R. 1999 Fate and transport model of Cryptosporidium. *J. Environ. Eng.* **125** (4), 325–333.

Westrell, T., Bergstedt, O., Stenström, T. A. & Ashbolt, N. J. 2003 A theoretical approach to assess microbial risks due to failures in drinking water systems. *Int. J. Environ. Heal. R.* **13** (2), 181–197.

WHO 2008 *Guidelines for Drinking-water Quality, 3rd edition, Incorporating First and Second Addenda.* World Health Organization, Geneva.