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BRIEF COMMUNICATION

Detection of Microbial Pathogens and Indicators in Sewage Effluent and River Water During the Temporary Interruption of a Wastewater Treatment Plant

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Abstract In this case study, the impact of a temporary (4 days) interruption of a sewage treatment works on the microbial quality of a drinking water source in SE Norway to which the sewage was discharged was investigated. Samples of wastewater and river water were taken before and during the interruption of the sewage treatment and analysed for various microbial indicators and pathogens: faecal indicator bacteria (*E. coli* and total coliforms); F-specific RNA bacteriophages; pathogenic viruses (norovirus GI and GII, adenovirus); protozoan parasites (*Giardia* and *Cryptosporidium*). Water at the inlet to the drinking water treatment plant, 20 km downstream from the sewage discharge, was not found to be significantly affected with respect to bacteria and parasites, with higher concentrations measured prior to the sewage treatment interruption, probably associated with a period of heavy rainfall. For viruses, however, the heavy rainfall appeared to be of lesser relevance to the contamination levels. These data demonstrate the importance of water providers having a clear overview not only of sewage discharge in water catchment areas but also of other potential sources of contamination with different pathogens and indicators, and the effects of weather conditions on contamination events.

Keywords Environmental microbiology · Indicator bacteria · Protozoa · River water · Virus · Wastewater

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Introduction

Discharge of untreated sewage has been associated with increases in pathogen concentrations in drinking water sources and, in some instances, outbreaks of waterborne infection (Svraka et al. 2007; Beaudeau et al. 2008; Baldursson and Karanis 2011).

The River Glomma is 604 km long and an important source of drinking water in SE Norway. A small part of the catchment is covered by agricultural area (5.8 %), while most of the catchment is by mountain and forest areas. The drinking water treatment plant (DWTP) associated with the river produces approximately 42,000 m^3 water per day and serves 150,000 PE. Although the water quality of the Glomma is considered satisfactory, there are eight cities in the river catchment and several potential sources of contamination with microbial pathogens, including: effluent from wastewater treatment plants (WWTP), storm water overflows/combined sewer overflows, runoff from riverbanks, and discharges from septic tanks (Grizzetti et al. 2007).

Fjellfoten WWTP treats up to 150 m^3 of sewage per hour, and treated effluent discharges into the Glomma. Typical sewage discharge is 2,800 m^3/day (0.032 m^3/s). The treatment at the start of this study consisted only of primary sedimentation, but in October 2012, renovation of the WWTP meant that it would be closed for 4 days, during which raw sewage would be discharged untreated into the river. Transport time in the Glomma from the sewage discharge point (SDP) until the intake to the DWTP is approximately ten hours. The purpose of this case study was to investigate contamination of the Glomma during the WWTP interruption in order to evaluate the impact of the event on the microbial quality of this drinking water source.

Table 1 Sampling plan

Sampling time relative to WWTP interruption	−6 days	−1 day	Interruption	+1 days	+2 days	+3 days	Total
Number of samples analysed							
Bacteria	2ww	2ww; 5Gl; 1rw	1ww; 5Gl; 1rw	1ww; 5Gl; 1rw	1ww; 5Gl; 1rw	1ww; 5Gl; 1rw	38
Parasites	2ww	2ww; 1rw	1ww; 1rw	1ww; 1rw	1ww; 1rw	1ww; 1rw	13
Virus	1ww	1ww; 1Gl; 1rw	1ww; 1Gl; 1rw	1ww; 1Gl; 1rw	1ww; 1Gl; 1rw	1ww; 1Gl; 1rw	16

ww wastewater, *Gl* river water, *rw* raw water at DWTP inlet

Table 2 Concentration of microbial pathogens and indicators in the wastewater at WWTP

Date	−6 days		−1 days		Interruption	+1 days	+2 days	+3 days
Type	Untreated	Treated	Untreated	Treated	Untreated	Untreated	Untreated	Untreated
E. coli (MPN/100 ml)	4.0×10^6	2.2×10^5	2.0×10^6	4.4×10^5	4.6×10^6	5.5×10^6	6.9×10^6	6.5×10^6
Total coliforms (MPN/100 ml)	1.2×10^7	6.7×10^5	4.9×10^6	1.0×10^6	7.7×10^6	1.4×10^7	1.9×10^7	1.7×10^7
Giardia (cysts/l)	4,600	<170	3170	830	1,500	1,000	2,700	3,700
Cryptosporidium (oocysts/l)	<170	<170	170	170	500	<170	300	<170
Bacteriophages (PFU/l)	1.7×10^5	2.5×10^4	5.9×10^4	1.5×10^3	3.7×10^5	4.3×10^5	3.0×10^5	7.5×10^5
Norovirus GI (gene copies/l)	ND	9.2×10^4	2.9×10^3	5.6×10^3	5.5×10^5	ND	8.1×10^5	1.4×10^6
Norovirus GII (gene copies/l)	1.1×10^6	2.0×10^5	6.0×10^4	ND	2.9×10^6	1.1×10^7	1.1×10^8	1.4×10^7
Adenovirus (gene copies/l)	5.8×10^5	1.7×10^5	3.3×10^5	9.8×10^5	5.3×10^4	2.2×10^4	4.8×10^3	1.2×10^5

ND not detected

Methods

Parameters Measured

In order to obtain an overview of microbial contamination, the following microbiological parameters were investigated in sewage and in river water samples: faecal indicator bacteria (*E. coli* and total coliforms); F-specific RNA bacteriophages; pathogenic viruses (norovirus GI and GII, adenovirus); protozoan parasites (*Giardia* and *Cryptosporidium*). Due to differences in expense and ease of analysing for different microbes, not all samples were analysed for all organisms (Table 1).

In addition, water flow data in the river during the study period were collected at the measuring station approximately 10 km downstream from the SDP, and precipitation information was obtained from the nearest meteorological station at Årnes, approximately 4 km from the WWTP.

Sampling for Microbiological Analyses

Five sampling points were selected in the Glomma, from 1 km upstream to 30 km downstream from the SDP (Table 2). At each sampling point, 3 sub-samples were taken: 1 from the centre of the Glomma, and one from each side, and these were combined to make a single mixed sample. In addition, river water samples were taken daily at the intake of the DWTP, approximately 20 km downstream from the SDP, from one day prior to the WWTP interruption until the end of the renovation period.

Samples of raw and treated sewage (mechanical- and chemical- treatment) were taken six days and one day prior to interruption of the WWTP, and samples of raw sewage were taken daily during the WWTP interruption (Table 1).

Analytical Methods

Bacteriology

Samples of sewage and river water were collected in sterile 500 ml bottles and analysed at the Norwegian Institute for Water Research for *E. coli* and total coliforms using ISO method 9308-2:2012, with Colilert®-18 / Quanti-Tray® (IDEXX, USA) following the manufacturer's instructions. Briefly, the reagent is mixed with 100 ml sample, then poured into a Quanti-Tray, sealed and incubated at 36 ± 2 °C for 18–22 h. Most probable number (MPN) is calculated from the number of positive wells (Boubetra et al. 2011).

Virology

River water samples (10 l) were filtered at the Norwegian School of Veterinary Science (NVH), using NanoCeram 147 mm nanoalumina disc filters, 2 μm pore size (Argonide, Sanford, FL) (Gibbons et al. 2010), then eluted in 40 ml

Tr alk buffer (Hamza et al. 2009) and reconcentrated using polyethylene glycol (PEG 8000) precipitation (Comelli et al. 2008). The pellet was resuspended in 3 ml lysis buffer and stored at −80 °C until nucleic acid extraction. For wastewater samples (50 ml), PEG precipitation was the only concentration method used. ISO method 10705-1:1995 Part 1 was used for enumeration of F-specific RNA bacteriophages.

Total nucleic acids were extracted in the NucliSens®easyMAG (Biomerieux, USA) and copyDNA (cDNA) was synthesized using superscript III (Invitrogen, USA), following manufacturer's instructions. All samples were amplified in a MX3005P qPCR system (Agilent Technologies Inc., USA) using TaqMan® Environmental Master Mix (Applied Biosystems, UK) and 2 µl cDNA/DNA in 20 µl total reaction volume. Primers AdvJTVXF, AdvJTVXR (0.25 µM), probe AdvJVXP (0.15 µM) were used for detection of human adenovirus (Jothikumar et al. 2005). For norovirus GI, primers QNIF4 and NVLC1R (0.3 µM), probe NVGGI (0.2 µM) were used (Silva et al. 2007), while for norovirus GII were primers COG2R and QNIF2d (0.3 µM), probe QNIFS (0.1 µM) (Kageyama et al. 2003; Loisy et al. 2005). The corresponding volume of water tested in the qPCR was 1 L for norovirus and 0.4 L for adenovirus. Absolute quantification of norovirus GI, GII and human adenoviruses was performed using serial tenfold dilutions of a DNA plasmid designed to include the respective PCR amplicons (Genscript, USA).

Parasitology

Water samples (10 l) were analysed at NVH by an accredited internal method based on ISO Method 15553 (2006) and US EPA Method 1623 (2005), commencing with filtration through a membrane filter of nominal porosity 2.0 µm, elution in a detergent buffer, concentration by centrifugation, purification by immunomagnetic separation (IMS; Invitrogen) according to the manufacturer's instructions. Final 50 µl concentrates were air-dried onto welled slides, before fixing with methanol and staining with a FITC-labelled monoclonal antibody (mAb) cocktail against *Cryptosporidium* oocysts and *Giardia* cysts (Waterborne Inc., New Orleans, USA) and 4,6-diamidino-2-phenyl indole. Samples were screened using a fluorescent microscope equipped with appropriate filter blocks and Nomarski optics. Putative parasites were scored as such and confirmed according to characteristic features.

For sewage samples, 2 ml samples were found to provide the best recovery efficiencies (compared with 50 µl and 200 ml samples). Thus, 2 ml samples were purified by IMS as previously described (Robertson et al. 2006) and examined by immunofluorescent antibody testing as described for water samples.

Results

Considerable rainfall (22mm) was measured between day −3 and −2 prior to interruption of the WWTP, but negligible precipitation between day −2 (pre-interruption) and day 2 (post-interruption). On day 2, 1.4 mm of precipitation was measured. Water flow in the Glomma during this period was, on average, 640 m^3/s (hourly values varied from 575 to 665 m^3/s).

Microbial Detection in Wastewater

All microbial pathogens and indicators were detected in both untreated and treated wastewater (Table 2), demonstrating that discharge from this WWTP contributes to microbial contamination of the river. Average concentration in untreated sewage before the interruption was 3.0×10^6 for *E. coli*; 8.5×10^6 for total coliforms; 3,885 for *Giardia*; <170 for *Cryptosporidium*; 1.2×10^5 for bacteriophages; 2.9×10^3 for norovirus GI; 5.8×10^5 for norovirus GI; 4.6×10^5 for adenovirus, and for treated sewage 3.3×10^5 for *E. coli*; 8.4×10^5 for total coliforms; 500 for *Giardia*; <170 for *Cryptosporidium*; 1.3×10^4 for bacteriophages; 4.9×10^4 for norovirus GI; 2.0×10^5 for norovirus GII; 5.8×10^5 for adenovirus. Data from the 2 occasions of sampling both untreated and treated wastewater indicates that the microbial removal efficiency of the plant was relatively low, ranging from under 1 log removal to around 2 log removal depending on organism (Table 2).

After the interruption, average concentrations were 5.9×10^6 for *E. coli*; 1.4×10^7 for total coliforms; 2225 for *Giardia*; 285 for *Cryptosporidium*; 1.9×10^6 for bacteriophages; 9.2×10^5 for norovirus GI; 3.5×10^7 for norovirus GI; 5.0×10^4 for adenovirus. While concentrations of indicator organisms and parasites in untreated wastewater remained approximately constant during the interruption period, concentrations of norovirus were approximately 2 logs higher, and of adenovirus 1 log lower.

Bacteria and Virus Detection at Different Sites in the River Glomma

River water collected upstream of the wastewater discharge point prior to its interruption contained both adenovirus (average 4.8×10^4) and faecal coliforms (average 192break *E. coli*, 1,252 total coliforms), demonstrating that the source of contamination was not just this WWTP (Table 3). Downstream, concentrations of *E. coli* were high on the day prior to interruption (358 MPN/ml on average), but subsequently decreased to an average of 269 MPN/ml. Concentration of adenovirus upstream from the WWTP was 2.4×10^5 GC/L 1 day after the interruption, 3 or 4 log higher than on any of the other sampling occasions (Table 3).

Table 3 Concentrations of microbial indicators at sampling sites before and after WWTP interruption

Sampling sites	−1 day	Interruption	+1 day	+2 days	+3 days
E. coli (MPN/100 ml)					
GL1, 1 km upstream	280	230	230	130	90
GL2, 10 km downstream	370	210	230	440	330
GL3, 11 km downstream	360	120	260	140	210
GL4, 21 km downstream	310	200	330	330	330
GL5, 31 km downstream	390	110	440	310	320
Total coliforms (MPN/100 ml)					
GL1, 1 km upstream	1,700	880	1,500	880	1300
GL2, 10 km downstream	1,500	1,900	2,100	2,100	1,700
GL3, 11 km downstream	2,100	1,100	2,000	720	700
GL4, 21 km downstream	1,700	100	2,100	1,700	1,200
GL5, 31 km downstream	1,800	1,200	2,600	1,100	1,500
Adenovirus (gene copies/l)					
GL1, 1 km upstream	445	32	2.4×10^5	31	168

Table 4 Microbial pathogens and indicators detected in the raw water at DWTP intake

Date	−1 days	Interruption	+1 day	+2 days	+3 days	Mean (2008–2012)	Maximum (2008–2012)
E. coli (MPN/100 ml)	230	190	110	120	190	64	240
Total coliforms (MPN/100 ml)	2,100	930	610	800	810	240	2400
Giardia (cysts/5L)	3	ND	ND	ND	ND	0.4	3
Cryptosporidium (oocysts/5L)	2	ND	ND	ND	ND	ND	ND
Norovirus GI (gene copies/L)	ND	ND	ND	205	ND	Not analysed	Not analysed
Norovirus GII (gene copies/L)	ND	ND	ND	66	70	Not analysed	Not analysed
Adenovirus (gene copies/L)	56	ND	22	439	2464	Not analysed	Not analysed

ND not detected

Microbial Concentrations in River Water at the DWTP Intake

Samples from the intake to the DWTP showed faecal contamination before the WWTP interruption, including contamination with *Giardia* (3 cysts), *Cryptosporidium* (2 cysts) and adenovirus (56 gene copies) (Table 4). However, although parasites were not detected in samples taken from the same point during the WWTP interruption, virus concentrations increased 2 days after the interruption (Table 4). Concentrations of *E. coli* and total coliforms during the interruption were higher than the 2008–2012 average (measured every 14 days at the DWTP), but higher values were obtained prior to the interruption (Table 4).

Discussion and Conclusions

Faecal indicator bacteria like *E. coli* and total coliforms, as well as F-specific RNA bacteriophages, are not pathogenic, and thus occur in wastewater irrespective of the health of the human population in the catchment area. They may also have zoonotic sources.

In contrast, the concentrations of parasites and pathogenic viruses in wastewater are related to the number of infected people in the catchment area and their occurrence may reflect the microbial health of the contributing population. In addition, zoonotic sources of *Cryptosporidium* and *Giardia* are possible.

This is the first time that the removal of microorganisms at this WWTP was evaluated and our data suggest it was low, albeit similar to previously reported in Sweden (Nordgren et al. 2009). Although the data obtained from this case study are limited, they demonstrate that the temporary interruption of the WWTP had no obvious effect on the concentrations of faecal bacteria or protozoa in the river water intake of the DWTP plant 20 km downstream. Previous studies have demonstrated that removal of protozoan parasites in WWTP is greatest during the secondary treatment stages and may be minimal if there is only primary treatment (Robertson

et al. 2000; Robertson et al. 2006). Virus removal is also minimal during primary treatment, and generally reduction is most studied and most significant in secondary treatment and disinfection stages (Nordgren et al. 2009; Carducci et al. 2009; Francy et al. 2012; Alcalde et al. 2012). However, our data are also affected by recovery efficiency of the methods for the different organisms in the different matrices, as well as time and other parameters. Indeed, highest concentrations of bacterial indicators and protozoa were measured before the interruption and were probably related to the period of heavy rainfall prior to sample collection. Other sources of contamination, such as run-off and sewer overflow, may also be speculated to have impact on contamination of this water source with parasites and bacteria.

For viruses, however, a different picture emerges. Although the lack of information on the recovery efficiency of the method for each virus requires that virus concentrations be indicative only, the results show that virus is present in the sewage and in the river water both upstream and downstream from the WWTP, similar to what has been described in other regions and treatment plants (Rodríguez-Lázaro et al. 2012). Given the increase in adenovirus concentrations measured at the DWTP during the study, while their concentrations in the wastewater remained stable or even decreased, it seems likely that, as previously suggested by Ottoson et al. (2006), the heavy rainfall was of less relevance to the viral load in the river than sewage discharges.

Overall, these data demonstrate that water providers should not only have a clear overview of discharges from WWTP in water catchment areas but also of other potential sources of contamination with different pathogens and indicators. The effects of weather conditions on contamination events should be further researched, as well as the different potential contamination sources and pathways in the Glomma catchment. As expected, microbial removal capacity of WWTPs is an important factor for drinking water source management, and it may be a significant contributor to water contamination if it is low.

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