Faecal contamination of a municipal drinking water distribution system in association with Campylobacter jejuni infections


ABSTRACT

After heavy rains Campylobacter jejuni together with high counts of Escherichia coli, other coliforms and intestinal enterococci were detected from drinking water of a municipal distribution system in eastern Finland in August 2004. Three patients with a positive C. jejuni finding, who had drunk the contaminated water, were identified and interviewed. The pulsed-field gel electrophoresis (PFGE) genotypes from the patient samples were identical to some of the genotypes isolated from the water of the suspected contamination source. In addition, repetitive DNA element analysis (rep-PCR) revealed identical patterns of E. coli and other coliform isolates along the distribution line. Further on-site technical investigations revealed that one of the two rainwater gutters on the roof of the water storage tower had been in an incorrect position and rainwater had flushed a large amount of faecal material from wild birds into the drinking water. The findings required close co-operation between civil authorities, and application of cultivation and genotyping techniques strongly suggested that the municipal drinking water was the source of the infections. The faecal contamination associated with failures in cleaning and technical management stress the importance of instructions for waterworks personnel to perform maintenance work properly.

Key words | Campylobacter, contamination, drinking water, microbiological method

INTRODUCTION

Campylobacter species have been described as common causative agents in waterborne gastrointestinal illness outbreaks all over the world and most of the illness cases have been associated with Campylobacter jejuni. For example, in England and Wales, Campylobacter was the major pathogen in outbreaks traced to private water supplies (Said et al. 2003).
and in Canada, Campylobacter was the second most common pathogen in 24 waterborne disease outbreaks during 1974–2001 (Schuster et al. 2005). In Finland, Campylobacter has been the most common bacterial pathogen identified in waterborne disease outbreaks, being implicated in 11 incidents between 1998 and 2004 (Miettinen et al. 2005). Drinking of undisinfected water was the leading factor increasing the risk of Campylobacter infection in a prospective case-control study conducted in Norway (Kapperud et al. 2005). The faeces of livestock, domestic and wild animals, wild birds and poultry and also sewage effluents are the sources of Campylobacter in water environments (Jones 2001). The high internal temperature of birds seems to make them especially good reservoirs for thermophilic campylobacters including C. jejuni (Waldenström et al. 2002).

In waterborne disease outbreaks, Campylobacter has rarely been detected directly from a suspected source even though there is convincing epidemiological data for a strong association with drinking water (Hänninen et al. 2003; Martin et al. 2006). This is probably due to the long lag between exposure and the appearance of symptomatic patients and the decision to initiate microbiological studies to search for a source (Hänninen et al. 2005). Another reason may be that Campylobacter cells are still present in the water at the time of sampling but they have entered the viable but nonculturable state prior to laboratory analysis (Tholozan et al. 1999). The international standard cultivation method for thermotolerant Campylobacter species from water is composed of concentration by membrane filtration and enrichment followed by cultivation on a selective medium in a microaerobic atmosphere (ISO 17995 2005). The conventional cultivation procedure requires several days and faster methods using a detection based on PCR with and without the enrichment step and fluorescent in situ hybridization (FISH) have been developed for environmental samples including water (Waage et al. 1999; Moore et al. 2001; Moreno et al. 2003; Yang et al. 2003; Lehtola et al. 2006). These advanced molecular detection tools have made it possible to partly overcome the loss of culturability and also to avoid the problems caused by interference of background growth.

The prevention of future waterborne outbreaks requires that the causes of past waterborne outbreaks are identified and eliminated. Various methods are available for microbial tracking of the faecal contamination source in water (Scott et al. 2002; Meays et al. 2004). Different genotyping methods such as pulsed-field gel electrophoresis (PFGE) have been used to determine whether the Campylobacter isolates from patients and suspected water source possess identical DNA profiles indicative of a common source (Hänninen et al. 2003). Also methods of examining repetitive DNA sequences (rep-PCR) used for faecal indicator bacteria that are isolated from water can help to track the sources of microbial contamination (Dombek et al. 2000). There are three groups of repetitive elements that are used in the applications of rep-PCR: repetitive extragenic palindromic (REP) sequence, enterobacterial repetitive intergenic consensus (ERIC) sequence, and BOX element (Rademaker et al. 1998). In the microbial source tracking studies conducted, the use of the rep-PCR method with Escherichia coli isolates has been library dependent with the goal of differentiation between human and non-human sources when the analysis of a large collection of micro-organisms from different sources is required (Simpson et al. 2002; McLellan et al. 2003). In addition to the PFGE and rep-PCR techniques, other molecular methods, such as amplified fragment length polymorphism (AFLP), and phenotypic methods including antibiotic resistance analysis are applied in microbial source tracking (Simpson et al. 2002; Leung et al. 2004).

The present study outlines how a combination of an enrichment method (ISO 17995 2005) and PCR for detection of Campylobacter was applied to water samples in a case of municipal drinking water contamination. In addition, the use of different genotyping methods for establishing the association between drinking water contamination and Campylobacter infection in three patients as well as in source tracking are described.

**METHODS**

**Drinking water contamination**

Contamination of drinking water occurred in a rural municipality with a population of 5,100 in eastern Finland in August 2004. The drinking water used by the community was non-disinfected ground water distributed by the local municipal waterworks. Before distribution, the pH was adjusted and, if necessary, the water was stored in a large storage tower. Faecal contamination was detected on
3 August 2004 in routine water quality monitoring samples taken on the previous day, and chlorination of water was initiated immediately, just before midnight. Extended water sampling was started and a boil-water notice was issued to consumers early on 4 August.

Microbiological analysis of water samples

*Escherichia coli*, coliform bacteria and heterotrophic bacteria counts were analysed in the local laboratory, Savolab, Mikkeli, Finland, from several sampling points including the raw water at the waterworks, water in the storage tower and four tap water points along the distribution line using their routine methods. After faecal contamination was detected at the local laboratory, the testing for thermophilic campylobacters, *Salmonella*, noroviruses, coliform bacteria, *E. coli*, intestinal enterococci and heterotrophic bacteria from an additional four tap water samples taken on 4 August 2004 were performed at the Laboratory of Environmental Microbiology, National Public Health Institute (KTL), Kuopio, Finland. The sampling was continued on 10 and 12 August 2004 when the presence of thermophilic campylobacters and the counts of coliform bacteria, *E. coli*, intestinal enterococci and heterotrophic bacteria were analysed from three tap water samples and from a raw water sample at the waterworks. In addition, these analyses were performed from a sample taken on 11 August 2004 from the suspected contamination source: the well which collected rainwater from the roof of the water storage tower.

Thermophilic campylobacters were analysed in 2,000–4,000 ml water samples following the principles of the draft standard ISO/DIS 17995:2003 which was finalized and published later (ISO 17995 2005). Briefly, the samples were filtered through membranes with 0.45 μm pore size (Millipore, Bedford, USA). Half of a sample was concentrated according to the draft standard on a filter which was enriched for 48 h in 50 ml of complete Bolton broth (LabM, Lancashire, UK) and the other half was concentrated on a filter which was initially enriched in 50 ml of Bolton broth for 4–12 h before the addition of the selective supplement. In the case of typical *Campylobacter* growth on mCCDA medium (Oxoid, Hampshire, UK), the cultures were confirmed to be campylobacters by gram-staining and testing for motility, aerobic growth, oxidase, catalase and hippurate hydrolysis. The *Campylobacter* isolates were stored at −70 °C in nutrient broth containing 15% glycerol.

Noroviruses were analysed after concentration of a 1,000 ml water sample and extraction of the RNA by reverse transcriptase (RT)–PCR method as described elsewhere, except that the prefiltration step was not used (Kukkula et al. 1999). *Salmonella* analyses were made from 1,000 ml sample volumes at Evira, Finnish Food Safety Authority, Kuopio Research Unit, Finland, using method NMKL 71 (Nordic Committee on Food Analysis 1999).

*E. coli* and coliform bacteria were analysed using membrane filtration with LES Endo and mFC media (SFS 3016 200a; SFS 4088 200b) and Chromocult® COLIFORM Agar medium (CC, Merck, Darmstadt, Germany). The incubation time was doubled to 48 h in cases when no coliform colonies were detected on CC in the first reading after the 24 h incubation. The presumptive *E. coli* and coliform colonies on CC were confirmed using the oxidase test and gram staining and all oxidase and gram negative cultures were tested for production of indole and gas formation from lactose as previously described (Pitkänen et al. 2007). The counts of intestinal enterococci were analysed according to the standard ISO 7899-2 (2000). Heterotrophic plate counts were determined at +22 °C according to the standard ISO 6222 (1999).

Patient interviews and their faecal *Campylobacter* isolates

Patients with microbiologically confirmed *Campylobacter* infection who lived in the community where the drinking water was contaminated and who had experienced symptoms between 31 July and 20 August 2004 were contacted by phone and interviewed. The onset date, description and duration of symptoms of *Campylobacter* infection were determined. The exposure was assessed for a 2-week period before the infection by asking if the patients had been travelling during that time and estimating the daily intake of unboiled tap water from the waterworks. In addition, other potential sources of *Campylobacter* infection were assessed. For example, the use of other water sources, other drinks, consumption of poultry or red meat or unpasteurized milk products was investigated. Stool specimens from the
patients were analysed for bacterial pathogens (Salmonella, Campylobacter, Yersinia, Shigella) in the local hospital laboratory. The patient isolates were stored at −70 °C at the Enteric Bacteria Laboratory, KTL, Helsinki, Finland.

Analyses of Campylobacter isolates

The species identification of Campylobacter isolates from water and patient samples was done by polymerase chain reaction – restriction enzyme analysis, PCR–REA (Fermer & Engvall 1999). The method differentiates between C. jejuni, C. coli, C. upsaliensis and C. lari with the primers THERM1 (Eyers et al. 1993) and THERM4 (Fermer & Engvall 1999) targeting the 23S rRNA gene of thermophilic campylobacters using the restriction enzyme, AluI (New England Biolabs Inc., Beverly, Massachusetts). In addition, PCR–REA was conducted with the DNA extracts from enrichment broth cultures of the water samples (Lehtola et al. 2006). Bacterial cell suspensions, enrichment broth cultures and DNA extracts from broths were stored at −20 °C prior to the analysis.

The similarity of the cultivated C. jejuni isolates from patients, drinking water samples and from the suspected contamination source was analysed from the pulsed-field gel electrophoresis (PFGE) patterns using two restriction enzymes, SmaI (Roche Diagnostics, Basel, Switzerland) and KpnI (Fermentas, Hanover, New Hampshire). The preparation of genomic DNA and running conditions were according to the PulseNet standardized protocol for molecular subtyping of C. jejuni (www.cdc.gov/pulsenet/protocols.htm).

DNA fingerprints of bacterial indicators

The similarity of a total of 47 E. coli isolates and nine other coliform bacteria isolates from tap water sampling points sampled during the drinking water contamination and isolated at the KTL were analysed by rep-PCR genomic fingerprinting (Rademaker et al. 1998). Repetitive BOX and ERIC elements were amplified employing two single primers, BOXA1R (Versalovic et al. 1994) and ERIC2 (Versalovic et al. 1991), respectively. The cell suspensions in sterile distilled water from colonies grown on tryptone soya agar (Oxoid, Basingstoke, UK) were used as PCR templates. The produced templates were stored at −20 °C prior to the analysis; 1 μl of template suspension was used in BOX-PCR and ERIC-PCR; the reaction mixture contained 0.5 U Dynazyme I enzyme (Finnzymes, Espoo, Finland) with optimized Dynazyme buffer (10 mM Tris-HCl, 1.5 mM MgCl₂, 50 mM KCl and 0.1% Triton X-100), 200 μM of each dNTP and 1 μM of primer (BOXA1R or ERIC2). The total reaction volume of 25 μl was prepared with nuclease free water (Promega, Madison, Wisconsin). The amplification consisted of 5 minutes’ denaturation at 95 °C followed by 30 cycles of 1 minute at 94 °C, 2 minutes at 56 °C and 3 minutes at 72 °C, and then final extension for 7 minutes at 72 °C (Tgradient or T1, Biometra®, Goettingen, Germany). The amplification products were electrophoresed (10 V/cm) in 2% agarose gels containing ethidiumbromide, then visualized and preserved digitally. Each run included Escherichia coli (ATCC 8739) and Enterobacter cloacae (ATCC 13047) as control strains. The DNA fingerprint patterns were compared using Bionumerics 4.1 software (Applied Maths, Sint-Martens-Latem, Belgium). The clustering of patterns was evaluated by the band based method of Jaccard creating an advanced consensus tree using both BOX and ERIC trees.

RESULTS

Faecal contamination of drinking water

E. coli and coliform bacteria were found in routine water quality monitoring from a municipal drinking water distribution system in samples taken on 2 and 3 August 2004 from sampling points 1 and 4 (Table 1). In the earlier monitoring samplings of the distribution system, microbial contamination had not been detected. Raw water samples at the waterworks before the distribution taken on 3, 4 and 12 August were negative for the analysed faecal microbes. A sample taken from the water tower on 3 August contained coliform bacteria 7 cfu 100 ml⁻¹, but in the resampling on 5 and 9 August coliforms were no longer detected.

During the week of 25 July to 1 August 2004, a considerable amount of precipitation occurred in the area (Figure 1). Further on-site technical investigations revealed that during the previous cleaning and maintenance of the
Water storage tower, one of the two rainwater gutters had been left in an incorrect position with the consequence that the gutter channelled the rainwater from the roof directly into the drinking water in the water tower instead of into the rainwater drain. The roof of the water storage tower was almost fully carpeted with bird faeces that were leached by rainwater directly into the stored drinking water which was then distributed to the consumers.

In a subsequent analysis of samples taken on 4 August, *C. jejuni* together with *E. coli*, other coliform bacteria and intestinal enterococci were found in the distribution sampling points 2, 3 and 4 (Table 1) as well as in a sample taken on 11 August from the well collecting rainwater flushed from the roof of the water storage tower. The counts of *E. coli*, coliform bacteria and intestinal enterococci in the rainwater sample were all above 400 cfu 100 ml\(^{-1}\). No culturable *C. jejuni* were found from sampling point 1. This sampling point was located at the beginning of the distribution network and the chlorine had already reached it at the time of sampling. Noroviruses or *Salmonella* were not detected in the analysed water samples.

Approximately one week after the first detected signs of faecal contamination, on 10 and 12 August, there were still a few sporadic coliform bacteria and *E. coli* present in

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**Table 1 | Microbiological indicator analysis of water in a municipality of 5,100 inhabitants in August 2004**

<table>
<thead>
<tr>
<th>Microbiological parameter</th>
<th>Date</th>
<th>Tap water, point 1</th>
<th>Tap water, point 2</th>
<th>Tap water, point 3</th>
<th>Tap water, point 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em> (cfu 100 ml(^{-1}))</td>
<td>2 Aug</td>
<td>5</td>
<td>nd</td>
<td>nd</td>
<td>115</td>
</tr>
<tr>
<td></td>
<td>3 Aug</td>
<td>43</td>
<td>nd</td>
<td>nd</td>
<td>103</td>
</tr>
<tr>
<td></td>
<td>4 Aug</td>
<td>–</td>
<td>18</td>
<td>30</td>
<td>1</td>
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<tr>
<td></td>
<td>5 Aug</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>2</td>
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<tr>
<td></td>
<td>6 Aug</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>4</td>
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<tr>
<td></td>
<td>9 Aug</td>
<td>nd</td>
<td>–</td>
<td>–</td>
<td>nd</td>
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<tr>
<td></td>
<td>10 Aug</td>
<td>nd</td>
<td>–</td>
<td>–</td>
<td>1(^a)</td>
</tr>
<tr>
<td></td>
<td>12 Aug</td>
<td>nd</td>
<td>–</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Coliform bacteria (cfu 100 ml(^{-1}))</td>
<td>2 Aug</td>
<td>15</td>
<td>nd</td>
<td>nd</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td>3 Aug</td>
<td>290</td>
<td>nd</td>
<td>nd</td>
<td>230</td>
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<tr>
<td></td>
<td>4 Aug</td>
<td>–</td>
<td>68</td>
<td>100</td>
<td>16</td>
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<tr>
<td></td>
<td>5 Aug</td>
<td>–</td>
<td>–</td>
<td>2</td>
<td>120</td>
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<tr>
<td></td>
<td>6 Aug</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>4</td>
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<td></td>
<td>9 Aug</td>
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<td>–</td>
<td>nd</td>
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<tr>
<td></td>
<td>10 Aug</td>
<td>nd</td>
<td>–</td>
<td>–</td>
<td>2(^a)</td>
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<tr>
<td></td>
<td>12 Aug</td>
<td>nd</td>
<td>–</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Intestinal enterococci (cfu 100 ml(^{-1}))</td>
<td>4 Aug</td>
<td>–</td>
<td>86</td>
<td>200</td>
<td>26</td>
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<td></td>
<td>10 Aug</td>
<td>nd</td>
<td>nd</td>
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<td>–</td>
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<tr>
<td></td>
<td>12 Aug</td>
<td>nd</td>
<td>–</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Heterotrophic plate count (cfu 100 ml(^{-1}))</td>
<td>2 Aug</td>
<td>2</td>
<td>nd</td>
<td>nd</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>3 Aug</td>
<td>28</td>
<td>nd</td>
<td>nd</td>
<td>21</td>
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<tr>
<td></td>
<td>4 Aug</td>
<td>–</td>
<td>11</td>
<td>10</td>
<td>1</td>
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<td></td>
<td>5 Aug</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>16</td>
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<td>6 Aug</td>
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<td>3</td>
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<td>nd</td>
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<td></td>
<td>10 Aug</td>
<td>nd</td>
<td>–</td>
<td>nd</td>
<td>–</td>
</tr>
</tbody>
</table>

nd, not done; –, not detected; \(^a\) cfu 250 ml\(^{-1}\); \(^b\) after 48 hours' incubation. All other coliform bacteria as well as *E. coli* results presented in this table were obtained after 24 hours' incubation.
distant parts of the distribution line where there were low chlorine concentrations (below 0.5 mg l\(^{-1}\)), but culturable *Campylobacter* were no longer detected.

**Patients**

According to local reports, there was no local increase in number of patients with gastrointestinal symptoms. In studies by the National Public Health Institute, three patients (A, B, C, Figure 1) who had characteristics matching both spatially and temporally with the drinking water contamination were identified and subsequently interviewed by telephone. The patients were young adults (two males and one female) aged between 20 and 33 years. All three had drunk water from the contaminated water network at the time when the incident occurred. The patients' symptoms started between 6 and 9 August 2004 and positive *C. jejuni* findings were detected from the faecal samples taken between 10 and 13 August. All three had abdominal pain as a symptom of the illness and, in addition, one patient had bloody diarrhoea and another had fever, diarrhoea and nausea. During the 2-week period before the onset of the symptoms, none of the patients had travelled abroad, and two out of three had travelled nearby but had not drunk unboiled tap water during those trips.

**Comparison of *Campylobacter* isolates**

23S rRNA PCR-REA pattern analysis of *Campylobacter* isolates and DNA extracts from the enrichment broth cultures showed two types of restriction pattern in tap water and rainwater well samples (Figure 2). The tested patient isolates, one isolate and one enrichment broth culture DNA extract both from tap point 2, one rainwater well isolate and one broth culture DNA extract exhibited similar patterns to *C. jejuni* ATCC 33291 used as a positive control. The other isolates and broth culture DNA extracts from tap points 3 and 4 as well as three isolates from tap point 2 and one from the rainwater well had slightly differing patterns from those of *C. jejuni* ATCC 33291.

Identical *Sma* I and *Kpn* I PFGE patterns were found among the three *C. jejuni* isolates from patients A, B and C and one isolate from the rainwater which had leached down from the roof of the water storage tower (Figure 3). Furthermore, one isolate from tap point 3 and two isolates from tap point 4 exhibited an identical pattern to an isolate
from the rainwater well (except for a faint approx. 320kb-size band in rainwater well isolate) and all these were different from those of the patient isolates.

**DNA fingerprints of indicator bacteria**

Seven clusters in a total of 56 coliform isolates from the water samples were identified in the BOX and ERIC consensus tree (Figure 4). *E. coli* isolates were in clusters A, B and G and the other coliform isolates were in clusters C, D, E and F. The *E. coli* isolates in cluster A (Figure 4) were identified in all three distribution sampling points (2, 3 and 4). Also the three coliform isolates in cluster C originated from the sampling points 2, 3 and 4. The *E. coli* isolates in cluster G had identical ERIC patterns, but had a variable set of BOX patterns.

**DISCUSSION**

In our studies a combination of several microbiological methods revealed an association between the faecal contamination of drinking water and *C. jejuni* infections among three inhabitants of the municipality. The battery of microbiological methods used included conventional cultivation methods and genotyping of both the pathogen involved, *C. jejuni*, as well as the bacterial indicators of faecal contamination, *E. coli* and other coliforms. The utilization of these methods as well as the multidisciplinary cooperation between the experts from public health, drinking water and research laboratory sectors were shown to be of crucial importance in identifying the association. This experience can be applied to other cases of faecal contamination of drinking water that might occur in the future.

In Finland, there is an obligatory notification and reporting system in place for suspected food- and waterborne disease outbreaks (Finnish Government 1994). Normally, an alert arises when patients with gastrointestinal symptoms contact health care personnel and these contacts lead to investigations of sources of the infection and potential
Figure 4 | ERIC and BOX fingerprint patterns from E. coli (E⁺) and other coliform bacteria (K⁺) isolates from three tap water sampling points (2, 3 and 4). The keys A–G represent the clusters identified from the consensus tree created by combining separate BOX and ERIC trees.
problems in drinking water treatment or distribution (Kuusi et al. 2004). However, in this incident, the starting point was the opposite: the faecal contamination of tap water was detected first, during a conventional monitoring programme for drinking water quality. Only subsequently was it appreciated that patients had been infected via contaminated drinking water. In the waterworks of the municipality, the regular monitoring of the hygienic quality of drinking water is normally performed six times per year and the quality of the system had been in compliance with regulations.

*C. jejuni*, *E. coli* and intestinal enterococci were found from three different points of the distribution network and it is evident that the whole distribution system had been microbiologically contaminated. However, according to the local health care authorities, the number of patients with gastroenteric symptoms requiring medical care was not higher than normal. Our results suggest that, despite an existing notification system, a proportion of waterborne disease outbreaks is probably never detected. It is common that people do not always contact the medical health care system if the gastrointestinal symptoms are mild. In fact, most patients with *C. jejuni* infections do not require medical treatment other than maintenance of hydration and electrolyte balance (Allos 2001). This might be the reason for the low number of sick people detected in the community in association with this abnormal drinking water contamination. A low number of patients may also indicate that successful corrective actions immediately after the contamination was detected may have prevented further infections. One explanation may also be that the number of viable *C. jejuni* cells in drinking water was low. Our results revealed that faecal material from wild birds on the roof of the water tower was the source of the contamination. However, it is likely that the number of *C. jejuni* in the faecal material had decreased when it was exposed to sunlight and desiccation in summer conditions before the rainy days, 25 July – 2 August 2004.

In summer 2004, there were exceptionally many rainy days and local rainstorms and thundershowers in Finland (Venäläinen et al. 2004). In the studies from the United States and Canada, respectively, statistically significant associations between intense precipitation and waterborne disease outbreaks have been found (Curriero et al. 2001; Thomas et al. 2006). In the municipality of our study the regular monitoring of drinking water quality was done exactly at the right time: shortly after a heavy rainfall. It is important that personnel of waterworks and public health authorities clearly recognize in their risk assessments that there may be an increased risk of microbiological contamination of drinking water after heavy rainfall and that the monitoring of drinking water quality in association with heavy downpours may detect faecal contamination and prevent waterborne disease outbreaks.

Several methods have been published for direct detection of *Campylobacter* species from water samples using PCR technology (Moore et al. 2001; Moreno et al. 2003; Yang et al. 2003). In waterborne disease outbreaks, the number of *Campylobacter* cells present in water at the time of sampling can be very low, and their detection requires concentration of a large quantity of water (Hänninen et al. 2003). The enrichment cultivation used prior to the PCR detection removes the inhibitory factors possibly present due to concentration (Hernandez et al. 1995; Waage et al. 1999; Josefsen et al. 2004). In our work, the combination of an enrichment method and PCR detection of thermotolerant *Campylobacter* species in water was used, as previously described in a Swedish outbreak investigation (Andersson et al. 1997). PCR detection substantially shortened the detection time for *Campylobacter* compared with the conventional cultivation method. Interestingly, some of the *C. jejuni* isolates had slightly different patterns from our *C. jejuni* positive control (Figure 2). It was found that the isolates yielding the unfamiliar pattern in *Alu* I digests had a one base mutation (G → C) in position 4074 on their 23S rRNA gene (position based on the gene for ribosomal RNA operon of *C. jejuni* ATCC 43431, accession no. Z29326). This mutation caused a new restriction site for *Alu* I and therefore a different pattern (results not shown). However, our results revealed that the use of restriction enzyme analysis of a 23S rRNA gene fragment as recommended by Engvall et al. (2002) represented a rapid and suitable tool for the identification of thermotolerant *Campylobacter* species.

The genotyping methods used proved to be advantageous for the identification of the association between drinking water contamination and the symptoms of the infected patients as well for tracking the contamination source of drinking water. *C. jejuni* isolates from all three patients and one isolate from the well of a rainwater drain
had identical SmaI/KpnI PFGE genotypes. Some C. jejuni isolates from tap water and from the rainwater well also had identical PFGE patterns. The identity of SmaI/KpnI PFGE genotypes combined with spatial and temporal analysis of rainfall, the timing of the water contamination event and patient drinking water habits indicated that the source of the infection was most probably the drinking water. In addition, rep-PCR fingerprinting data indicated that some E. coli and other coliform bacterial isolates isolated from different points of the distribution may have same origin. The rep-PCR findings combined with the local precipitation data provided additional evidence to support the hypothesis that the contamination source of the tap water had been the drinking water storage tower. Additional information about the failures in the maintenance of rainwater gutters in the water storage tower explained how faecal material from the roof had contaminated drinking water inside the tower. The faecal material originated from wild birds which are commonly known to excrete Campylobacter in their stools. It has been reported that over 20% of migrating birds might host Campylobacter species (Waldenström et al. 2002). Crows and gulls, which are common birds in eastern Finland where the municipality reported in this paper is located, seem to have high Campylobacter incidence rates (Kapperud & Rosef 1983; Broman et al. 2002).

Our results confirm the conclusions of Rademaker et al. (1998) that rep-PCR fingerprinting is well suited for the rapid analysis of large numbers of E. coli and coliform isolates. In our study, the E. coli isolates in cluster G (Figure 4) had identical ERIC patterns, but had a variable set of BOX patterns, suggesting that BOX fingerprinting is more discriminatory than ERIC fingerprinting. However, our work outlined the use of rep-PCR within one contamination incident and consequently our dataset consisted of only 56 coliform isolates all originating from the same system. In previously reported analyses using rep-PCR, a large pattern library is used, as the goal of studies has been to discriminate between different possible host sources (Dombek et al. 2000; Carson et al. 2003; McLellan et al. 2003; Leung et al. 2004). In our work, we did not aim to distinguish between possible hosts, since direct faecal samples from the suspected wild birds were not available. Instead, we found that rep-PCR analysis was useful in evaluating whether isolates from different parts of the drinking water distribution network and from different sampling times may have one common origin. The advantage of this approach is that rep-PCR can be applied without requiring a large library of analysed isolates, even though an existing library would allow further interpretation of the results.

In conclusion, rainfall had leached faecal material from wild birds into municipal drinking water as the consequence of a maintenance failure on the roof of the water storage tower. This led to a massive faecal contamination of the stored drinking water and subsequently the municipal drinking water distribution system. Our results suggest that it is important to undertake microbiological sampling after heavy rains since this may prevent major waterborne outbreaks. Molecular techniques applied in this study for revealing the contamination route and association of patient isolates with water contamination appeared to be very useful tools in the epidemiological analysis.

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