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Publications of the National Public Health Institute A12/2004

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Application of modern epidemiological and microbiological methods

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PUBLICATIONS OF THE NATIONAL PUBLIC HEALTH INSTITUTE

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COVER PHOTO
Juho Kuusi

KTL A12/2004
ISBN 951–740–452–2
ISSN 0359–3584
ISBN 951–740–453–0 (pdf)
ISSN 1458-6290 (pdf)
Yliopistopaino, Helsinki 2004

ACADEMIC DISSERTATION

To be publicly discussed, with permission of the Medical Faculty, University of Helsinki. Biomedicum Helsinki, Haartmaninkatu 8, August 20, 2004 at 12 o’clock noon.

To my family

ABSTRACT

Since the implementation of a new notification system for suspected food- and waterborne outbreaks in Finland in 1997, the National Public Health Institute (KTL) has conducted several outbreak investigations per year. In this thesis, investigations of four suspected waterborne outbreaks are presented, and evaluated for strength of evidence of waterborne etiology. The outbreaks were caused by *Campylobacter jejuni* and norovirus; the two predominant microbes in waterborne outbreaks in Finland during last years.

The overall aim of this study was to describe and evaluate the application of different epidemiological approaches and new microbiological methods in the investigation of four suspected waterborne outbreaks in Finland. In addition, this study describes the public health interventions to control and prevent waterborne outbreaks and contributes to development of evidence-based public health policy and practice guidelines for waterborne outbreak investigations.

In waterborne outbreaks, the strength of evidence implicating water as the cause of an outbreak is determined on the basis of findings from epidemiological and microbiological investigations. Classifications for the assessment of the strength of evidence have been developed in the USA and England and Wales. Both classifications emphasise the importance of epidemiological investigation, because water microbiology often does not confirm that the water source was contaminated. However, if the same microbe is detected from the water and patients, comparing subtypes of the recovered microbes can provide further evidence about the water as the source of the outbreak.

In study (I), we conducted a matched case-control study, which showed a significant association between consumption of unboiled drinking water from the municipal supply and gastrointestinal illness. *Campylobacter jejuni* strains isolated from patients were indistinguishable by pulsed-field gel electrophoresis (PFGE), suggesting a common source of infection. In addition, *C. jejuni* was also isolated from a sample from the water system, and this strain also was indistinguishable by PFGE from the patient strains. The outbreak was categorized as strongly associated with drinking water based on epidemiological and microbiological findings.

In study (II), we conducted a cross-sectional survey among residents of the municipality. A 10% random sample of the population aged 15 years or more was included in the survey, which showed a significant association between consumption of unboiled drinking water from the municipal supply and gastrointestinal illness. The estimated total number of ill in the municipality was 2,700. *Campylobacter jejuni* was isolated from 45 out of 74 stool samples. Five strains were subtyped by PFGE, and the strains were indistinguishable. In this outbreak, *C. jejuni* was not detected from water samples. The outbreak was also categorized as strongly associated with drinking water from the community supply.

In the investigation of a norovirus outbreak in a rehabilitation centre (III), water was initially suspected as the source of the outbreak. However, in two retrospective cohort studies no association was found between drinking water or recreational water use and gastrointestinal illness. Samples from tap water and swimming pool water were negative for noroviruses. However, norovirus was detected from several environmental samples. Although sequencing of the environmental strains was not successful, clinical and environmental strains belonged to the same genogroup (GII) suggesting the same source of infection. It was concluded that the outbreak was not spread by water. The virus was likely transmitted from person-to-person, but environmental contamination may have contributed to the prolonged course of the outbreak.

In study (IV), a population survey conducted through the Internet showed a significant association between drinking water from the municipal supply and gastroenteritis. The estimated total number of ill was 5,500. Norovirus was detected from stool samples of ill persons but not from the water supply. Based on this evidence, the outbreak was categorized as probably associated with drinking water.

Isolation of campylobacter from water supply systems is challenging, and therefore has rarely been reported in waterborne outbreaks. In study (I) we recovered *C. jejuni* from the water supply as a result of intensive sample collection and investigation of large volumes of water. In addition, the water system was probably contaminated for several days. To increase the chances of detecting campylobacter from the water source, it is very important that water samples are collected promptly after the outbreak is recognized, sufficiently large water samples are collected, and the samples are investigated in a laboratory, which has good expertise in the field.

Investigation of a large waterborne outbreak may require substantial resources. Sending and collecting postal questionnaires or phoning to study participants, as well as entering data from a large study to a database is time-consuming. In studies (I–III) we used postal questionnaires to collect the data, but in study (IV), the regional computer network provided an opportunity to use the Internet for data collection. About 19% of households with access to the network participated in the study that showed an association between drinking water and illness. However, the demographics of our study participants differed considerably from the population demographics in the municipality. In an outbreak investigation, data collection through the Internet probably is best suited in communities with high Internet coverage, and to study associations that are not strongly related to socio-economic factors.

Investigation of waterborne outbreaks is teamwork where expertise from several different fields is needed. In Finland, municipal authorities have the main responsibility of outbreak investigations. However, in waterborne outbreaks, the investigation requires close collaboration between municipal authorities, epidemiologists, microbiologists, water system specialists and clinicians. Thorough investigation is important to identify and repair the deficiencies of the water supply, but also to learn from that particular outbreak and use the lessons learnt in other outbreak investigations, and to develop practice guidelines for investigation of waterborne outbreaks.

CONTENTS

DEFINITION OF ABBREVIATIONS

AR	Attack rate
CDC	Centers for Disease Control and Prevention, USA
CDSC	Communicable disease surveillance centre, England and Wales
CI	Confidence interval
DNA	Deoxyribonucleic acid
EELA	Food and Veterinary Research Institute, Finland
EHEC	Enterohemorrhagic Escherichia coli
EPA	Environmental Protection Agency, USA
EVI	National Food Agency, Finland
GBS	Guillain-Barré syndrome
GI	Genogroup I
GII	Genogroup II
KTL	National Public Health Institute, Finland
MOR	Matched odds ratio
NV	Norovirus
OR	Odds ratio
PCR	Polymerase chain reaction
PFGE	Pulsed-field gel electrophoresis
Ppm	Parts per million
RDD	Random digit dialling
RNA	Ribonucleic acid
RR	Relative risk
RT	Reverse-transcriptase
UV	Ultraviolet

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text by their Roman numerals (I–IV).

I Kuusi M, Klemets P, Miettinen I, Laaksonen I, Sarkkinen H, Hänninen ML, Rautelin H, Kela E, Nuorti JP. An outbreak of gastroenteritis from a nonchlorinated community water supply. *J Epidemiol Comm Health* 2004; 58: 273–277.

II Kuusi M, Nuorti JP, Hänninen ML, Koskela M, Jussila V, Kela E, Miettinen I, Ruutu P. A large outbreak of campylobacteriosis associated with a municipal water supply. Submitted for publication.

III Kuusi M, Nuorti JP, Maunula L, Minh NN, Ratia M, Karlsson J, von Bonsdorff CH. A prolonged outbreak of Norwalk-like calicivirus (NLV) gastroenteritis in a rehabilitation centre due to environmental contamination. *Epidemiol Infect* 2002;129:133–138.

IV Kuusi M, Nuorti JP, Maunula L, Miettinen I, Pesonen H, von Bonsdorff CH. Internet use and epidemiologic investigation of gastroenteritis outbreak. *Emerg Infect Dis* 2004; 10: 447–450.

INTRODUCTION

Provision of safe drinking water to all people living on this planet is one of the major challenges facing humanity. Microbiologically safe drinking water is considered a fundamental human right in many countries. However, waterborne pathogens represent a serious health hazard, and infectious diseases affect populations throughout the world. Ageing water treatment infrastructures are also a problem, as well as increasing recognition of pathogenic organisms resistant to conventional disinfection treatments. Diarrhoeal illness is the sixth leading cause of mortality worldwide, responsible for more than 2 million deaths in 1998.

A substantial burden of diarrhoeal illness is often attributed to contaminated water consumption, although the exact proportion due to waterborne pathogens is still unknown. Even in countries with effective surveillance systems, the source of infection often remains unknown. Considerable uncertainty remains about the number of waterborne disease outbreaks and the burden of such disease not associated with outbreaks. It is a major challenge to collect useful data on water quality and to improve data sharing between water suppliers and the public health community.

In Finland, water resources are abundant, and drinking water is even exported to other countries. Most people receive drinking water from a public water supply. More than half of the population receive water from a groundwater supply, and about 40% from a surface water supply [1]. Groundwater is rarely disinfected, because it is considered microbiologically safe while surface water is always disinfected. Groundwater supplies are regularly monitored for water quality, but the interval between samples is long and this practice may not necessarily help to prevent outbreaks.

Recently, a working group appointed by the Ministry of Social Affairs and Health proposed four actions that should be addressed to prevent waterborne outbreaks in Finland. Training of staff working in the municipal water utilities should be improved. Risk assessment should be carried out in all municipal water supplies. The microbiological quality of drinking water should be monitored more frequently, especially during seasons with increased runoff, e.g. in spring when the snow melts. Preparedness for disinfection should be improved; all water supply plants should be able to start disinfection within six hours after the suspicion of contamination has been aroused.

In 1997, a new outbreak early-warning system was implemented in Finland. The aim of the system is to distribute information about suspected food- or waterborne outbreaks to all relevant authorities locally, regionally and nationally [2]. During the past years more sensitive microbiological methods have also been developed enabling detection of certain microbes from the water. The source of infection can be more reliably confirmed by using molecular methods for typing of bacterial, viral or parasitic strains detected from patients and environmental samples. During last five years, epidemiologists trained in European and American field epidemiology programmes have increasingly lead outbreak investigations. All these factors have contributed to better investigation of waterborne outbreaks, and increasing understanding of potential problems in water systems in Finland [3].

Well-performed outbreak investigations have had a central role in the research and prevention of infectious diseases. Outbreak investigations have provided new knowledge about microbiology, clinical picture, risk factors, and mode of transmission of many diseases. Results from outbreak investigations have often lead to changes in practises, guidelines, or even laws, which aim to prevent similar outbreaks. Information about new, emerging microbes has mostly resulted from outbreak investigations where analytical epidemiological methods have been combined with microbiological and environmental studies [3].

The purpose of this study was to describe and evaluate the application of different epidemiological approaches and new microbiological methods in the investigation of four suspected waterborne outbreaks in Finland, and to evaluate whether the outbreaks were waterborne. These investigations identified risk points in all water supply systems associated with outbreaks. The subsequent public health interventions lead to substantial improvements in the safety of these water systems. This study also describes the public health interventions to control and prevent waterborne outbreaks and contributes to development of evidence-based public health policy and practice guidelines to improve the quality of waterborne outbreak investigations in Finland.

REVIEW OF LITERATURE

Water is one of the most important natural resources of life, since without it life cannot exist. Historically, communities developed by rivers, frequently at convenient crossing points. The rivers then provided a ready source of water and an even more convenient route for waste disposal. It was not until 1854 that a cholera outbreak in London, which caused more than 10,000 deaths, linked enteric disease to bacterial contamination of drinking water with sewage pollution [4]. The investigations of the cholera outbreaks in London by John Snow are one of the landmarks in the history of epidemiology and outbreak investigations. The dramatic decline in the incidence of waterborne disease in the early 1900s after the introduction of water treatment and disinfection has been clearly documented. For example, in 1900, the incidence of typhoid fever in the United States was approximately 100 per 100,000 population while by 1950 it had decreased to 1.7 per 100,000 [5].

Several viruses, including hepatitis A, hepatitis E and norovirus (NV) can be spread by water. Many enteric bacteria, e.g. Enterohemorrhagic *E. coli*, *Salmonella* Typhi, and *Campylobacter sp.* can survive in water, and have caused widespread waterborne outbreaks [6]. Enteric parasites, like giardia and cryptosporidium are highly resistant to disinfectants used in water treatment, and they have been linked to waterborne outbreaks. In fact, the largest waterborne outbreak ever reported was caused by cryptosporidium. This outbreak occurred in Milwaukee, Wisconsin, in 1993, affecting an estimated 403,000 persons [7].

In a number of countries, information on gastrointestinal illness associated with waterborne outbreaks has been collected for many years. However, the role that drinking water plays in endemic gastrointestinal illness is not known. Past outbreaks, together with recent studies [8, 9] suggest that a substantial proportion of endemic gastroenteritis cases may be caused by drinking water. Empirical evidence from a variety of water systems meeting federal drinking water standards suggests that 6–40% of gastrointestinal illness in the US may be water related [8, 10].

During the last decade there have been major developments in the detection and typing of microbial agents causing waterborne outbreaks. Polymerase chain reaction (PCR) was first described in 1985 and 1987 [11, 12]. Before the development of PCR techniques, detection of noroviruses was based on electron microscopy, a method that is far too insensitive for the demonstration of the virus in water samples [13]. Reverse-transcription polymerase chain reaction (RT-PCR) is much more sensitive, and enables detection of the virus also in water samples. The viral strain can be further characterized by sequencing. Finding identical sequences from clinical and environmental strains can be used to confirm the source of the outbreak. For campylobacter, development of new typing methods, including pulsed-field gel electrophoresis (PFGE) in the 1980s [14] has made it possible to compare strains from different sources. If indistinguishable strains of campylobacter are isolated from the water system and patient samples, the result strongly supports the water system as the source of the outbreak.

1. Waterborne-disease outbreaks

1.1 Surveillance of waterborne outbreaks in Finland

In Finland, municipal authorities are responsible for the investigation of outbreaks occurring in their area. If needed, they can consult provincial veterinary authorities, infectious disease consultants in the Health Care District or the National Public Health Institute (KTL) to receive epidemiological or laboratory assistance in the investigation. Each municipality should have an outbreak group which consists at least of the physician responsible for communicable diseases, head of the environmental health unit, infection control nurse and a health inspector. The group should have regular meetings to improve collaboration between authorities, and to define responsibilities in an outbreak situation. The group should also decide beforehand, who is responsible of notification to national authorities if a food- or waterborne outbreak is suspected.

Data on food- and waterborne outbreaks have been collected systematically since 1975. From 1975 to 1996 the municipal authorities sent outbreak reports to Food and Veterinary Research Institute (EELA). EELA then analysed these reports, and published summaries of outbreak surveillance data.

A major change in the surveillance of food- and waterborne outbreaks took place in 1997, when a new notification system for suspected food- and waterborne outbreaks was implemented. In the new system municipal authorities are encouraged to send a notification by telefax to National Public Health Institute (KTL) already at the stage when an outbreak is suspected. KTL relays the notification immediately to other national authorities, including EELA and National Food Agency (EVI), the Health Care District and province involved. The aim of the system is to distribute rapidly information about the outbreak to all relevant regional and national authorities so that the investigation of the outbreak and control measures can be carried out at the right level [2]. After the investigation, an outbreak report is sent to EVI. Data from outbreak notifications and outbreak reports are analysed annually, and an annual report on food- and waterborne outbreaks is written as a collaborative work between national authorities. After the new system was implemented, national authorities have been involved in several outbreak investigations annually.

About 100 notifications of suspected food- and waterborne outbreaks per year have been sent to KTL. In 75% of outbreaks, KTL has contacted the notifying person to confirm the outbreak and to obtain additional information about it. In one third of the outbreaks, KTL has provided wider assistance in the investigation, e.g. has sent examples of questionnaires, has given advice for collecting microbiological samples or has analysed questionnaire data collected by local authorities. Annually, KTL has taken a leading role in 5–6 outbreak investigations. These outbreaks have typically been large, involved several municipalities or have required international collaboration [15].

1.2 Outbreaks in Finland

From 1975 to 1983, about 80 food-and waterborne outbreaks were annually reported in Finland. In the 1990s the number of outbreaks decreased to about 30 per year. The majority of these outbreaks were foodborne; between 1980 and 1995, 30 waterborne outbreaks were reported to EELA. The number of waterborne outbreaks per year varied from 0 to 7. Fourteen of these outbreaks occurred in a community water system (5 surface water and 9 ground water). Eight outbreaks were linked to water from a private well, and 8 outbreaks to a non-community water system. The etiologic agent was unknown in 58%, virus in 17%, campylobacter in 13%, salmonella in 8%, and chemical substance in 4% of these outbreaks [16, 17].

After implementation of the new notification system for food- and waterborne outbreaks in 1997, the number of outbreak reports increased substantially to about 100 per year. Altogether 33 waterborne outbreaks were reported between 1998 and 2002; the number of outbreaks varied from 4 to 8 per year [18].

Fourteen outbreaks were associated with community water supply systems, and 19 with private water supplies. One large outbreak was due to water from a community surface water system, all other outbreaks were due to groundwater systems. Although less than half of the outbreaks were associated with community supplies, the total number of ill (14,000) in these outbreaks was much higher than in outbreaks associated with private water supplies (550). In four outbreaks, the estimated number of ill was more than 2,000 [18]. Norovirus was the etiologic agent in 15 (46%), campylobacter in 9 (27%), chemical substance in 2 (6%), and the agent remained unknown in 7 (21%) outbreaks [18,19].

Most outbreaks associated with community water supplies occurred in spring or late summer, and coincided with increased water runoff either due to melt water or heavy rainfall. Campylobacter outbreaks typically occurred in August. Outbreaks in private water supplies mostly occurred in June or July. The obvious reason for this was that these outbreaks occurred at camping sites or holiday resorts that only were open in summer [19].

1.3 Outbreaks in other Nordic countries

From 1988 to 2002, 72 waterborne outbreaks were reported in Norway. The total number of ill in these outbreaks was 10,616. The number of outbreaks varied from 1 to 12 per year. Campylobacter was the etiologic agent in 26% of outbreaks, and norovirus in 18% of outbreaks. In 46% of outbreaks, the etiologic agent was not identified. The three largest outbreaks were caused by norovirus, with 800–2000 persons affected. In 32 outbreaks the water was from a community water supply, in 22 outbreaks from a private water supply, and the type of the water supply was not reported in 18 outbreaks. Thirty-five outbreaks were associated with a surface water supply and 24 with a groundwater supply. All campylobacter outbreaks with more than 10 persons affected were linked to surface water supplies, while of the 13 norovirus outbreaks 7 were linked to groundwater supplies and 6 to surface water supplies. From 1988 to 1997, there were more outbreaks caused by community water supplies than by private water supplies. In contrast, from 1998 to 2002 the majority of outbreaks were caused by private water supplies [20].

In Sweden, 90 waterborne outbreaks were reported from 1980 to 1995. About 50,000 people became ill in these outbreaks, and two died. About 80% of these outbreaks were due to unknown agents. Campylobacter was the most commonly reported etiologic agent with 11 outbreaks, followed by *Giardia lamblia*. Other reported etiologic agents were enterotoxin-producing *E. coli*, norovirus, *Entamoeba histolytica* and cryptosporidium. Of the campylobacter outbreaks, three were large with more than 1,000 persons affected. Two of these outbreaks were linked to unchlorinated groundwater and one to chlorinated surface water [21].

1.4 Outbreaks in England and Wales

In England and Wales, between 1 January, 1992, and 31 December, 1995, 26 outbreaks with evidence for waterborne transmission were reported to the National Surveillance Centre [22]. In these outbreaks, 1756 laboratory confirmed cases were identified of whom 69 (4%) were admitted to hospital. In 19 outbreaks the illness was associated with consumption of drinking water; 10 of these were public supplies and 9 private supplies. Four outbreaks were associated with exposure to swimming pool water.

Cryptosporidium was the probable causative organism in all 10 outbreaks associated with public water supplies, and in the four outbreaks associated with swimming pool water. Eight of the public water systems were surface water supplies, and two were groundwater supplies. All public water supplies were chlorinated.

Campylobacter was the etiologic agent in six outbreaks associated with private water supplies, cryptosporidium in two outbreaks and *Giardia lamblia* in one outbreak. Of the private water systems, five were groundwater supplies and four were surface water supplies. Three of the private groundwater supplies were untreated, one had irregular chlorination and one had ultraviolet disinfection devices, which were not adequately maintained. Of the private surface water supplies, two were chlorinated, one had ultraviolet devices and one had no treatment [22].

1.5 Outbreaks in the United States of America

From 1991 to 1998, 230 waterborne outbreaks were reported in the United States. More than 443,000 cases of illness occurred, and an estimated 764 hospitalisations and 60 deaths occurred. Of the 126 drinking-water outbreaks, 109 were reported in public water systems, and 17 in individual water systems. An additional 104 outbreaks were associated with recreational water activities.

Forty-seven outbreaks were reported in community water systems, and 62 outbreaks in non-community systems. Twenty-two (47%) outbreaks in community systems occurred in systems using groundwater, and 22 outbreaks in community systems using surface water. Of the outbreaks in non-community systems, 52 (84%) were linked to groundwater systems, and only two to surface water systems, probably reflecting the few non-community systems that use surface water sources.

Seventeen outbreaks were classified as acute chemical poisoning, while a bacterial, viral or protozoan etiology was identified in 43 (39%) outbreaks. In 49 (45%) outbreaks an infectious agent was suspected but not identified. Of 31 outbreaks in community systems with a known or suspected infectious etiology, 13 were associated with groundwater sources, 15 with surface water sources and 3 with unknown water sources. Giardia and cryptosporidium were the most common infectious agents identified both in community groundwater and surface water outbreaks.

In non-community systems using groundwater, most (69%) outbreaks were of undetermined etiology. Bacterial agents caused 21%, giardia and cryptosporidium 8%, and viral agents 2% of outbreaks.

An infectious agent was identified in 8 outbreaks associated with individual water systems. Giardia and cryptosporidium accounted for 4 outbreaks, shigella for 2 outbreaks, and hepatitis A and *E. coli* for one outbreak each [23].

1.6 Summary

The number of reported waterborne outbreaks per year was 28.7 in USA, 6.5 in England and Wales, 6.6 in Finland, 4.8 in Norway and 5.6 in Sweden. However, the annual number of outbreaks per 100,000 population was about 10 times higher in the Nordic countries than in USA or in England and Wales. These differences most likely are due to different surveillance systems for outbreaks.

Also the etiologic agents in outbreaks linked to community waterborne outbreaks were different in Nordic countries compared with USA and England and Wales. In the Nordic countries, campylobacter and norovirus were the predominant pathogens, while in USA and England and Wales, cryptosporidium and giardia accounted for the majority of outbreaks.

In Finland, nearly all community outbreaks were linked to groundwater supplies, while in USA and England and Wales, about half of outbreaks were associated with surface water supplies and half with groundwater supplies.

2. Methods in the investigation of waterborne outbreaks

Although most public health practitioners working in communicable disease control have experience in outbreak management, few have managed an outbreak of waterborne disease. In the investigation of outbreaks of waterborne disease, the water extraction, treatment and distribution system, the population affected, and the health care system, which is responsible for the diagnosis, treatment and prevention of infectious diseases all have to be taken into account. The outbreak team must be aware of each of the areas in the system and consider how these may contribute to the outbreak or its investigation [24].

In principle, investigation of waterborne outbreaks follows the same steps as of any other outbreak. However, the team is likely to be different from teams investigating other types of outbreaks. In addition to representatives from the municipal health centre and the environmental health unit, the team should include an epidemiologist, a water supply expert, a microbiologist, and an expert from the environmental agency. It is important to define who is leading the team and who is responsible for communication to the press [24].

2.1 Detection and confirmation of an outbreak

Most waterborne disease outbreaks are detected because of an increase in the number of cases of a particular infection being reported through the local or national surveillance system.

Once the outbreak is detected, it is important to assess whether the increase of cases is real or an artefact. Pseudo-outbreaks as a result of laboratory error have been detected in more than one occasion [25].

2.2 Outbreak description

After the outbreak is confirmed, it should be described by collecting and summarizing certain key information about the people affected and their illness [2]. This is the most important stage in the investigation of any waterborne outbreak and may even be sufficient epidemiology [26]. The key information in descriptive epidemiology is often compressed to time, person and place, or in other words who, when and where. Demographic data on cases, the time when the illness developed, and the place where the person lives should be collected. These simple sets of data will often provide most of the information required to judge whether or not an outbreak is likely to be waterborne [24].

Case definition is needed to know whether an ill person should be included as a case in the study. Case definitions are usually phrased in terms of the illness, geographical location, and date of onset. Illness may be defined by clinical symptoms or by laboratory results.

Based on these data, the outbreak can be described in tabular and graphical form. The epidemic curve shows how the outbreak is progressing and whether control measures have been effective. It often provides information whether there is a continuous or a point source of infection. Geographical mapping showing how the cases are distributed is also useful to see whether the cases are clustered in one particular area.

2.3 Hypothesis formulation

As the initial data on the outbreak are analysed, it is important to evaluate what these data suggest about the possible cause of the outbreak. This is known as hypothesis generation. Descriptive epidemiology, initial environmental investigations, knowledge of the epidemiology or microbiology of the causative agent, clinical picture of the illness, and previous experience of similar outbreaks have to be taken into account when formulating the hypotheses about the cause of the outbreak.

It should be remembered that water is only one of several potential routes of transmission, persons may have multiple sources of exposure, persons may consume bottled water or water from several sources, and in some situations almost everyone may have some exposure to the water in question [27].

2.4 Hypothesis testing

The hypothesis generated by the investigators can be tested in an analytical epidemiological study i.e. retrospective cohort study or case-control study.

Cohort study is a good choice in small communities, where all persons in the community can be interviewed. In larger communities, when data on all residents cannot be collected, a cross-sectional study is an alternative. In the cross-sectional study, a random sample of the population is selected assuming that they are representative of the whole population. In principle, a cohort study follows up two or more groups from exposure to outcome. So, in a cohort study the experience of a group exposed to some factor is compared with another group not exposed to a factor. The advantage of a cohort study is that it allows calculation of incidence, relative risk and confidence intervals. In waterborne outbreaks, it often is of interest to calculate the estimated number of ill, which is possible in a cohort study or in a cross-sectional study. However, cohort studies may be very cumbersome to conduct. When interpreting the findings, sources of bias should be carefully considered.

In a case-control study, the incidence of a preceding event is compared among persons with the disease (cases) with a group of individuals who do not appear to have the disease (controls). These numbers allow to calculate odds ratios for different exposures. Case-control studies are relatively quick to conduct, and they can be used to examine several hypotheses simultaneously. Case-control studies cannot provide incidence rates, and therefore in waterborne outbreaks, the total number of ill in the community cannot be estimated. Case-control studies are more susceptible to bias than other comparative studies. Five main things should be taken into account when conducting case-controls studies. First, the case definition should be clearly stated, as well as eligibility criteria used for selection. Second, controls should come from the same population as the cases and their selection should be independent of the exposures of interest. Third, investigators should blind data gatherers to the case or control status of the participants. Fourth, data gatherers should be thoroughly trained to elicit exposure in a similar manner from cases and controls. Finally, investigators should address confounding either in the design stage or with analytical techniques [28].

The investigators often obtain habitual water usage from the multiple water sources persons consume during their daily routine (home, work, school, restaurants, etc). When focusing on exposures during the outbreak period, investigators should consider variations within each of these categories and the use of bottled water, the use of tap water to reconstitute beverages or foods, and exposure during bathing or other hygienic practices. Even with the most careful, detailed, retrospective exposure assessment, information may be insufficient to detect an association between drinking water exposure and illness, especially when there is little variability in drinking water exposures or there are few study participants [27].

2.5 Bias and misclassification

The key issue when conducting case-control studies is the selection of controls, which should be representative for the whole population. In waterborne outbreaks, overmatching may be a problem leading to underestimate of the true effect of exposure to drinking water. For example, if controls are matched to cases according to postal code, controls have been likely to use drinking water from the same source as cases, and a true association may remain undetected [24].

Incomplete ascertainment of cases may lead to selection bias if selected cases have exposures different from those of cases not included in the study. Although a case definition that includes laboratory confirmation may decrease the number of cases available in the study, it will increase study precision [27]. Selection bias may be a problem in case-control studies, because cases may be more active respondents [24, 29].

Objectivity in obtaining information helps to minimize but does not prevent information bias. Interviewer bias in an outbreak investigation is almost impossible to eliminate since the interviewer is part of the investigation team. Interviewer bias often leads to an exaggeration of the difference in exposure between cases and controls rather than an underestimated effect (differential misclassification). Recall bias is difficult to control, because cases are likely to think about exposure history more than controls. This is more likely to lead to an overestimate than an underestimate of the true odds ratio or relative risk [24].

Publicity is often impossible to avoid in waterborne outbreaks. It may affect both cases and controls (non-differential misclassification). In a community-based survey in North West England, following a large outbreak of cryptosporidiosis that generated a lot of media publicity, the investigators found that the prevalence of self-reported diarrhoea was higher in the control towns than in the outbreak towns [30].

Sometimes a modest degree of differential exposure misclassification can dramatically effect (over or underestimate) an association [31, 32]. Differential misclassification should be considered especially when evaluating suspected waterborne associations in outbreaks where few cases occur, attack rates are low, the observed association is small or illness occurs over a long time period [27].

3. Methodology of data collection

Traditionally, three methods have been used for collection of data in epidemiological studies: personal interviews, telephone interviews, and mailed self-administered questionnaires. Personal interviews are costly, and collecting data using mail and telephone interviews has been advocated as a cost-effective alternative for personal interviews [33].

Few comparisons have been made of factors like consent characteristics and data quality that are related to these two modes of data collection [34]. In most studies, telephone response rates have been higher than rates in a mail-administered mode [34-37]. However, in some studies response rates in mail mode have been higher compared to the telephone mode [38]. In one study, data were collected on 321 mail surveys conducted in the US and published in 1991. The mean response rate was 60%, and in some studies the response rate was less than 20% [39]. In northern and central Europe higher response rates have been reported than in southern Europe [37]. The response rates in mailed studies can be increased by using reminding letters, or by telephoning the study subjects before sending the questionnaire [40].

In addition to differential findings in response rates based on mode of administration, bias in terms of non-responders has been noted. Those who have been younger have been more likely to be non-consenters in mail-in studies, while older people have been less likely to participate in studies conducted by telephone interviews [41].

When looking at data quality, most studies have found higher missing data rates in the mail mode than in the telephone mode [38, 42, 43]. In a study on chronic bronchitis with 35 questions [43], the mean number of incomplete questions was 2.8 by using a postal questionnaire, and 0.6 by telephone interview. In the postal mode, 17 questions had more than 5% missing answers. In the telephone mode, few questions had any appreciable missing answers. This missing information may lead to differential non-response bias between studies. However, as a whole the results from studies conducted by postal questionnaires or by telephone interviews are comparable [43].

When large studies are conducted, each of these two modes of data collection require substantial resources either for mailing questionnaires and reminders or telephoning to study participants. In addition, workload from entering data into a database in a large study may be huge. Electronic data collection can reduce this workload and related errors and is therefore a tempting alternative to traditional data collection methods.

4. Internet and epidemiology

The Internet originated in the early 1970s in the USA as part of an Advanced Research Projects Agency research project on "internetworking". After developments made in the 1980s, the Internet became widely accessible in the 1990s, and currently more than 60% of the population in many developed countries can access the Internet from home [44].

Internet has had a major impact in epidemiology since 1995 [45]. It has been used for disseminating information [46], access to journal contents, access to confidential patient records [47], and management of multicentre randomised controlled trials [45, 48]. Internet has been used for partner notification and increasing community awareness during the investigation of a syphilis outbreak among users of Internet chat room [49]. In Denver, use of Internet was evaluated as a risk factor for sexually transmitted diseases [50].

Using the Internet for collection of data in epidemiological investigations was proposed already in 1997 [51]. The authors suggested that the main advantages of conducting a "webcohort" study would be fast recruiting of participants – hundreds by the hour – and fast access to the data for the researchers. Also follow-up of participants would be inexpensive and efficient. Selection bias in this kind of study would be inevitable, but the authors strongly recommended this approach to epidemiological investigation.

Several reports on the use of the Internet to conduct survey research have been published [52]. Advantages of a Web-survey include easy access, instant distribution and reduced costs. Examples of health-related surveys include: A web-based survey on the effects of ulcerative colitis on quality of life [53], collection of clinical data from atopy patients [54], a web-based survey looking at complementary and alternative medicine use by patients with inflammatory bowel disease [55], and a survey of dentists regarding the usefulness of the Internet in supporting patient care [56].

In open surveys conducted via the Internet where Web users, newsgroup readers, or mailing list subscribers are invited to participate by completing a questionnaire, selection bias is a major factor limiting the generalizability of results. Selection bias occurs due to the non-representative nature of the Internet population and the non-representative nature of respondents, also called the volunteer effect [57]. In a study comparing cigarette smokers recruited through the Internet or by mail there were numerous differences between these two groups in descriptive statistics. However, in the groups, the strength of associations between smoking-related variables was similar [58]. This suggests that the Internet may be a cost-effective method for data collection for analytical studies that assess associations between variables.

Electronic data collection has been used in few outbreak investigations. In a norovirus outbreak among employees of one company in Alaska, questionnaires were distributed and returned by e-mail with a response rate of 91% [59]. In a campylobacter outbreak in Australia among conference delegates, questionnaires were also distributed by e-mail with a response rate of 93% [60]. In a university outbreak of conjunctivitis, data were collected by using the Internet. An e-mail was sent out, encouraging students and staff members to complete the questionnaire. Responses were forwarded electronically to a database. The response rate was 50% [61]. However, there are no reports of community-based outbreak studies where data would have been collected through the Internet.

5. Etiologic agents in waterborne outbreaks

5.1 Campylobacter

Campylobacter sp. is the most common known bacterial pathogen causing gastroenteritis in the developed countries [62]. The ecology of *Campylobacter jejuni* involves wildlife reservoirs, particularly wild birds [63]. Species that carry *C. jejuni* include migratory birds – cranes, ducks, geese and seagulls [64]. The organism is also found in other wild and domestic bird species, as well as rodents. The intestines of poultry are easily colonized with *C. jejuni* and *C. jejuni* is a commensal organism of the intestinal tract of cattle [65].

Clinical features. The incubation period is usually from 2 to 5 days, but can vary from 1 to 10 days. Most typically, campylobacter infection results in an acute, self-limited gastrointestinal illness characterized by diarrhoea, fever and abdominal cramps [66]. In most patients the diarrhoea is either loose or watery or grossly bloody; 8-10 bowel movements occur at the peak of illness. In some patients, the diarrhoea is minimal and abdominal cramps and pain are the predominant features. Fever is reported by more than 90% of patients, and can be low-grade or over 40°C and persist for up to one week [67].

The most severe complication of *C. jejuni* infection is Guillain-Barré syndrome (GBS), an acute demyelinating disease of the peripheral nervous system that affects 1-2 persons per 100,000 population in the US each year. Although *C. jejuni* infection is a common trigger of GBS (30% of cases), the risk of GBS after *C. jejuni* infection is small (less than 1 per 1000 infections) [67, 68]. Reactive arthritis is another important complication of *C. jejuni* infection. The risk of reactive arthritis has varied from 0.7% to 8% in different studies [69, 70]. The clinical course of reactive arthritis is usually mild [71].

Transmission of campylobacter. Most campylobacter cases are sporadic [72]. Risk factors identified in case-control studies for sporadic campylobacteriosis include consumption of poultry, drinking unpasteurized milk, living or working on a farm, travel to foreign countries, consumption of milk from bottles attacked by birds, handling and cooking of food in relation to barbecuing, contact with food-producing animals and pets, and drinking untreated water [73-82]. Person-to-person transmission of campylobacter appears to be rare [83]. It has been estimated that 800-10^6 ingested organisms are needed to produce illness in 10%–50% of persons [84].

Campylobacter in water. Campylobacters are found in natural water sources throughout the year [65]. The numbers of campylobacter in surface waters is higher in the winter than in the summer [85, 86]. In England, campylobacters are absent in streams running through upland moors, but present in the same streams running through lowland, grazed pasture [87]. The presence of campylobacters correlates with upstream agricultural locations, such as farmyards, small-holdings and a slaughterhouse, and agricultural events such as emptying of slurry tanks and the spraying of farm slurry onto land [88]. In rivers, the same pattern has been observed: the upper, cleaner reaches of the river have been free of campylobacter but as the water flowed through grazed farmland, it became progressively more contaminated with campylobacters, particularly *C. jejuni* [87, 89]. A study of rivers and lakes in the Warsaw region of Poland showed that 70% of water samples were positive for thermotolerant campylobacter with *C. jejuni* making up to 65% of the isolates, *C. coli* 22% and *C. lari* 13% [89]. In a recent study in Finland, *Campylobacter sp.* was detected in 24 (17.3%) of 139 surface water samples. Campylobacter findings showed a clear seasonality, and in contrast with findings from other countries, during the winter the microbe was detected less frequently than during other seasons [90].

Groundwater is considered to be microbiologically clean. Contaminated groundwater sources have been implicated in the introduction of campylobacter into poultry flocks [91] and broiler chickens [92]. There is also bacteriological evidence that campylobacter can occur in groundwater [93]. The environmental conditions found within subsurface aquifers, i.e. low redox potentials, the absence of molecular oxygen with increasing depth, all year round low temperatures and protection from the effects of UV light and desiccation, favour the survival of campylobacter [85].

The presence of campylobacter is not always clearly correlated with indicator organisms for faecal contamination [94]. In central Washington, campylobacters were isolated from a number of natural water sources, including ponds, lakes, and small mountain streams, and although campylobacters were recovered throughout the year, especially in autumn and winter months, their densities did not show significant correlation with the faecal indicators [95]. In another study, however, faecal coliforms, but not faecal streptococci were strong predictors of *C. jejuni/coli* in a water source in southern Norway [96].

There have not been many systematic studies on the comparative survival of the different *Campylobacter* species in water. It has been reported that *C. coli* survives less well in surface waters than *C. jejuni* [86, 97]. However, new studies have shown that the survival times of *C. jejuni* and *C. coli* do not differ significantly from each other [98]. Survival is clearly dependent on temperature; the bacteria survive longest at 4°C. The mean survival of *C. jejuni* in distilled water at 4°C was 168 hours, and in artificial seawater 312 hours. These findings suggest that without a continuous source of contamination, after an incident the water may remain infective with campylobacter for several days.

Waterborne outbreaks of campylobacter infection. Waterborne outbreaks caused by campylobacter have been reported since 1970s, soon after campylobacter was identified as an important human pathogen. Outbreaks associated with mains water systems involving both surface water [99-103] and groundwater [104-111] have been reported, as well as outbreaks associated with individual water systems. In the largest waterborne campylobacter outbreaks, several thousand people have fallen ill [99, 104, 109] (Table 1).

The mechanisms for the contamination of the water systems include direct contamination of an open-top storage tank [105, 107], contamination with melt water [108], increased runoff due to heavy rains [99, 100, 111], and cross-connection of drinking water pipes with sewage water pipes [106, 109]. In some outbreaks, chlorinator failure has enabled spread of bacteria through the water supply [103, 107].

In waterborne outbreaks caused by *Campylobacter jejuni*, epidemiological studies often indicate that there is an association between consumption of drinking water and human illness, but neither faecal indicators nor campylobacters have been detected in the water samples studied, or coliforms but no campylobacters have been detected [112]. There are several reasons for this. It usually takes from several days to two weeks from the exposure, before the waterborne transmission of campylobacter in a community is recognized [113]. If the water source has been contaminated only for a short period, samples have been collected too late. The size of water sample may also be critical [113]. If the water volume is too small, contamination of the water supply may not be detected. It appears that water sample volumes of 100 to 1,000 ml proposed in the

Table 1. Waterborne outbreaks of campylobacter infection linked to community and non-community water supplies.

Date	Country	Number of persons affected	Type of water system	Water source	Mechanism of contamination	Epidemiological evidence (type of study)	Water microbiology
June 1978[99]	USA	3000	Community	Surface water	Increased runoff (heavy rainfall)	Survey	Negative
October 1980[104]	Sweden	2000	Community	Groundwater	Cross-connection	Descriptive	Coliforms
May 1981[105]	UK	257	Non-community	Groundwater (bore hole)	Contamination of open-top reservoir	Cohort	*C. jejuni*
May 1983[107]	USA	865	Community	Groundwater	Chlorinator failure	Survey	Coliforms
June 1984[102]	Norway	680	Community	Surface water	Increased runoff (heavy rainfall)	Cohort	*C. jejuni*
June 1985[216]	Finland	35	Non-community	Groundwater	Cross-connection	Descriptive	Coliforms
April 1985[108]	Canada	241	Community	Groundwater	Increased runoff (melt water)	Case-control	Coliforms
March 1986[100]	New Zealand	19	Community	Surface and ground water	Increased runoff (heavy rainfall)	Descriptive	Coliforms
November 1986 [106]	Finland	96	Non-community	Groundwater	Cross-connection	Descriptive	*C. jejuni*
August 1988[103]	Norway	330	Community	Surface water	Break of chlorination	Survey	Campylobacter negative
May 1993[217]	UK	43	Non-community	Spring water	Increased runoff (heavy rainfall)	Descriptive	*E. coli*
November 1995[109]	Denmark	2400	Community	Groundwater	Cross-connection	Descriptive	*C. jejuni*
June 1997[218]	Australia	23	Non-community	Rainwater	Animal faeces	Case-control	Coliforms
August 1998[110]	Austria	2200	Community	Groundwater	Overflow of sewage	Cohort	*E. coli*, SRSV, enterovirus
May 2000[111]	Canada	2300	Community	Groundwater	Increased runoff (heavy rainfall)	Cross-sectional survey	EHEC, coliforms

International Standardisation Organisation draft for detection of campylobacters from drinking water, are too small for detection of campylobacters in waterborne outbreaks [113]. In addition, the campylobacter strains, although remaining viable, may lose their culturability with time [112].

Subtyping of campylobacter strains. Campylobacters can be subtyped with several methods. Serotyping is a practical and valid phenotypic method for epidemiological typing of campylobacter and has been useful in both clinical and outbreak investigations, but it can produce ambiguous results [114]. This can be due to the occurrence of nontypable strains, transient antigen expression, and cross-reactivity between certain antigens. Pulsed-field gel electrophoresis (PFGE) is a somewhat labour-intensive method, but it has a highly discriminatory power for the typing of campylobacter [114]. PFGE has been successfully used for typing of campylobacter from food and clinical samples in several food-borne outbreaks [115-117], and to establish a link between the consumption of a contaminated food and illness or water and illness [109, 113] as human and environmental strains have been indistinguishable. PFGE is now used widely as PulseNet, a computer network in the United States, has added campylobacter among the bacteria included in the network [118]. Thus, a standardized protocol for typing campylobacter by PFGE will be widely available for routine use. Together with traditional epidemiological methods, the genotypic database will enable to make more accurate and relevant epidemiological conclusions [119].

5.2 Norovirus

Noroviruses, previously Norwalk-like caliciviruses, also referred to as small round structured viruses (SRSVs), appear to be the second most common agent of severe diarrhoea in children after rotavirus and the most common cause of outbreaks of acute gastroenteritis, including those that are foodborne. Their role in adults hospitalised for diarrhoea remains to be established, but outbreaks with fatal outcomes in the elderly are common [120]. In the Netherlands and England, noroviruses have been reported to account for 5–17% of cases of diarrhoea in the community and 5–7% of cases requiring treatment by physicians [121-123].

Noroviruses constitute one of the four genera of Caliciviridae [124, 125]. Human noroviruses are divided into two genogroups (G1 and G2) based on capsid sequence and polymerase sequence data [126-129]. To date, 15 distinct genotypes have been recognized, but as more strains are characterized, this number is likely to increase [124, 130].

Clinical features. Following a 1–3 day incubation period, infected persons may develop fever and vomiting, diarrhoea and headache as prominent symptoms. In adults, projectile vomiting occurs frequently. The clinical illness is considered to be self-limiting and mild, with symptoms lasting 2–3 days [131]. Up to 20% of infected persons, however, reported symptoms for more than 2 weeks, suggesting that norovirus infections may be less innocuous [132]. Sometimes parenteral fluid therapy is required, with up to 12% of cases hospitalised in a recent outbreak among military recruits [133, 134]. Deaths associated with norovirus outbreaks have been reported, but the etiologic association needs to be confirmed [130]. Virus is shed via stools and vomit, starting during the incubation period, and lasting up to 10 days, possibly longer [135].

Transmission of norovirus. The infectious dose is low: 10–100 viral particles may initiate infection [136-138]. Transmission from person-to-person is by far the most common route of infection [130]. The virus spreads through faecal-oral route [139], but also vomiting may create an aerosol with high number of viruses, and aerosolised vomit has been recognized as an important vehicle for transmission [136, 140, 141]. Seafood, especially oysters may be contaminated with noroviruses, and have been the source of infection in large outbreaks [142-144]. Contaminated berries have caused several outbreaks in Finland and other countries [145, 146]. Several foodborne outbreaks have resulted from an infected foodhandler who has contaminated the foods during preparation [147-150]. Outbreaks have also been associated with recreational water [151-155]. Environmental contamination may also transmit the disease, and outbreaks due to environmental contamination have been reported [156-159].

Detection of norovirus. The original Norwalk virus was identified from clinical specimens obtained in 1968 during the investigation of an outbreak of gastroenteritis among schoolchildren in Norwalk, Ohio [160]. The prototype strain was discovered by Kapikian in 1972 and was the first virus identified that specifically caused gastroenteritis in humans [161]. This discovery was followed by 20 years of failed attempts to cultivate the virus or develop simple and sensitive diagnostic tests. Epidemiological studies during this period were limited to small surveys conducted in reference centres, using electron microscopy (EM) for virus detection or immunoassays for serodiagnosis [120]. EM is a rather insensitive technique, requiring the presence of a minimum of 10^5–10^6 particles per ml of stool [130].

The cloning and sequencing of NV opened the way to new and more sensitive molecular diagnostics [162, 163]. At present, genome-based detection methods are available, in which fragments of the viral RNA are amplified directly from stool samples by reverse-transcriptase polymerase chain reaction (RT-PCR) [164-166]. RT-PCR is much more sensitive than EM, requiring only 10^1-10^3 viral particles per ml of stool [167]. Following detection of noroviruses by RT-PCR, the PCR products can be characterized further by sequencing. Many different genotypes of NV circulate in the general population, causing sporadic cases and outbreaks [122, 168-171]. Within outbreaks, strain sequences are usually identical. Thus, when identical sequences are found in different patients or clusters of illness, a common source of illness should be suspected. Also sequences from foods, water or environment can be compared with sequences from clinical samples, and finding identical sequences may confirm the source of infection [172]. An important question that cannot be answered with certainty is the extent to which a positive PCR result represents RNA not associated with viable virus. While it is possible that some of the positive PCR results represent non-infectious virus, NLVs have an ssRNA genome which is susceptible to RNAses found widely in the environment and it is likely therefore that positive signals are associated with virus particles [156].

Microplate hybridisation is also a useful confirmation method for diagnostic PCR of noroviruses. In outbreaks, the different hybridisation patterns obtained with different noroviruses may help tracking the infection route and exclude unrelated infections [173].

Norovirus in water. Noroviruses may survive in cold water up to one year [13]. It is very unlikely that EM would detect noroviruses from water samples, but RT-PCR has proven to be sufficiently sensitive also for detection from water samples. A sufficiently large volume of water is needed. A sample volume of less than one litre, possibly even as small as a few millilitres, may be sufficient for recovery of viruses from raw or primary-treated sewage. For drinking water, the virus levels are likely to be so low that hundreds or thousands of litres must be sampled to increase the probability of detecting virus [174]. In practice, the recommended sample volumes in outbreaks vary from 1 to 50 litres. If necessary, samples can be kept refrigerated for weeks prior to testing [13]. Viruses can be concentrated from water samples in different ways: flocculation, immunological methods or magnetic beads. Currently, insufficient data exist to recommend a standard method for concentration of noroviruses [174].

In a one-year study in the United States, monthly groundwater samples were collected in 29 sites and analysed for human enteric viruses. In 3% of the samples, noroviruses were detected [175]. In another study in Wisconsin, 50 household wells were sampled four times over a year, once each season. One of the 194 samples tested positive for norovirus G2 by RT-PCR [176]. In a study conducted in southwestern Finland in 2000–2001, 13 (9.4%) of 139 of surface water samples collected from rivers and lakes were positive for noroviruses by RT-PCR [90]. The prevalence of noroviruses did not show clear seasonal variation in this study.

Traditionally, indicator organisms have been used to estimate the waterborne occurrence of various pathogens, including noroviruses. However, because the indicators have different sizes, structures, and susceptibilities to physical treatment and disinfection, they may have deficiencies for describing the occurrence of noroviruses in water [137].

Table 2. Waterborne outbreaks of norovirus (SSRV) infection linked to community and non-community water supplies.

Date	Country	Number of persons affected	Type of water system	Water source	Mechanism of contamination	Epidemiological evidence (type of study)	Water microbiology
December 1976[189]	USA	418	Non-community	Groundwater (spring)	Cross-contamination	Cohort	Not reported
May 1978[178]	USA	400	Non-community	Groundwater	Cross-connection	Cohort	Coliforms
July 1978[177]	USA	~200	Non-community	Groundwater	Not reported	Cohort	Coliforms
August 1980[179]	USA	1,500	Community	Groundwater (spring)	Cross-connection	Cohort	Coliforms
September 1987[41]	USA	~100	Non-community	Groundwater	Increased runoff (heavy rainfall)	Cohort	Coliforms
April 1989[180]	USA	900	Non-community	Groundwater	Sewage infiltration	Cohort	Coliforms
April 1994[181]	Finland	2500	Community	Groundwater	Increased runoff (melt water)	Descriptive	*E. coli*, coliforms
June-July 1995[182]	USA	108	Non-community	Groundwater	Cross-contamination	Cohort	Norovirus, coliforms
March 1998[190]	Finland	1700-3000	Community	Surface water	Contamination of water source	Cohort	Norovirus, coliforms
August 1998[183]	Switzerland	1700	Community	Groundwater	Cross-contamination	Cohort	Norovirus etc.
July 2000[184]	Italy	344	Community	Not reported	Cross-connection	Cohort	Coliforms
February 2001[185]	USA	35	Non-community	Groundwater	Cross-contamination	Cohort	Norovirus, coliforms
May-June 2001[186]	Sweden	200	Non-community	Groundwater	Cross-contamination	Descriptive	Norovirus, coliforms
October 2001[187]	USA	84	Non-community	Groundwater	Cross-contamination	Cohort	Norovirus, coliforms
February-March 2002[188]	Sweden	500	Community	Groundwater	Cross-contamination	Cohort	Negative

Waterborne outbreaks of norovirus infection. The first waterborne outbreaks caused by norovirus were reported in the 1970s. Since then, several outbreaks associated with drinking water from community and non-community water supplies have been reported, including both ground water [41, 177-189] and surface water [190] systems. The most common mechanisms of contamination have been cross-connection between sewage and drinking water [41, 178, 179, 184] or increased runoff due to melt water [181] or heavy rains [41] resulting into infiltration of viruses to groundwater (Table 2).

Norovirus outbreaks have also been associated with recreational water. A group of visitors fell ill with norovirus gastroenteritis after swimming in a recreational artificial lake in Michigan in 1979 [152,153], but the mechanism of contamination remained uncertain. A school outbreak in Ohio in 1977 was associated with swimming in a pool. In this outbreak, the pool chlorinator had been disconnected, but also here the exact mechanism of contamination remained uncertain [154]. In 1994, a group of canoeists fell ill with norovirus gastroenteritis after exposure to contaminated recreational water in England [151]. In this outbreak, noroviruses of two genogroups were identified from stool samples of ill persons. In an outbreak in the Netherlands, about 100 children fell ill with norovirus gastroenteritis after playing in a recreational water fountain. Norovirus was detected in 88% of samples from ill children. The water sample from the fountain contained a norovirus strain that was identical to the RNA sequence found in stools [191].

Norovirus outbreaks associated with environmental contamination. Environmental contamination with norovirus may be a contributing factor for a prolonged course of an outbreak. In England in 1996, a prolonged outbreak occurred in a hotel. Environmental swabs were collected from 144 sites in the ninth week of the outbreak, and 61 (42%) were positive for norovirus by nested RT-PCR. The frequency of positive findings diminished with decreasing likelihood of direct contamination [156]. The highest frequency was detected in toilet rims and seats (73%) and carpets (64%). In UK in 1999, vomiting in a concert hall resulted in contamination of the facility with norovirus, and more than 300 people were affected. The reported cases were infected during five days after the incident. Environmental samples were not collected, but epidemiological data showed that the people were almost certainly infected as a consequence of environmental contamination [157]. In a hospital outbreak lasting for 17 days, environmental contamination was also documented by RT-PCR. In a large hotel outbreak, transmission may have occurred through environmental contamination, although person-to-person transmission was probably more important [158]. Environmental contamination is a factor that supports the rationale of cohorting sick patients during an outbreak in hospitals or nursing homes [159].

A common problem in the investigation of norovirus outbreaks is that the mode of transmission may be difficult to determine. As the virus spreads easily from person-to-person, an association with a food or water may be missed in the investigation due to different modes of transmission in the outbreak [130, 192]. In fact, outbreaks are often, if not always, the result of more than one mode of transmission [158, 193]. Therefore, it may be problematic in an outbreak investigation to differentiate between primary food- or waterborne and secondary contact cases, which has an impact on the calculation of incubation time and attack rates [194]. For example, incubation times of up to 7 days have been reported, which may have been due to misclassification of secondary cases.

5.3 Cryptosporidium and Giardia

Cryptosporidium has been the most common pathogen in outbreaks associated with mains water in the UK and US [22, 23]. Giardia is also an important agent causing waterborne outbreaks in US [23]. However, in Finland no outbreaks caused by cryptosporidium or giardia have been reported. It may be that samples have not been actively investigated for parasites, but it also appears that we do not have as much cryptosporidiosis or giardiasis as many countries in Europe and North America. However, in a recent study, *Cryptosporidi-*

um spp. was found in 14 (10,1%), and *Giardia spp.* in 19 (13.7%) of 139 surface water samples collected in south-western Finland [90].

5.4 Other etiologic agents

Other important microbes frequently linked to consumption of drinking water are Enterohemorrhagic *Escherichia coli* (EHEC) [195], *Shigella spp.* [196], *Salmonella spp.* [197], *Vibrio cholerae* [198], and hepatitis A [199]. However, in Finland, during the last five years, we have not had waterborne outbreaks caused by these microbes.

6. Community water supplies in Finland

6.1 Groundwater and artificial groundwater

In Finland, the use of groundwater and artificial groundwater for the public water supply has continuously increased over the period for which comprehensive statistics are available (1970–2001). At the beginning of 1970s the share of groundwater was only 30% but in 2001, approximately 61% of total water abstraction by public water works was groundwater or artificial groundwater. Of the 1560 separate water sources in the country, nearly 1500 are groundwater sources. The percentage of groundwater or artificial groundwater as raw water in public water utilities is foreseen to rise to about 70% by the year 2010. Water supply systems using artificial groundwater are located mainly in coastal areas, and account presently for about 8% of the total abstraction by public water utilities. The intention of artificial recharge is to produce groundwater that is similar to natural groundwater. This is done by using as few chemicals and in as small amounts as possible, and by infiltrating surface water taken from water bodies into soil, either by means of basin infiltration or by sprinkling infiltration.

Groundwater is typically slightly acidic, the average pH being 6.5. Most waterworks, especially the small ones representing 35% of the abstracted groundwater, deliver it untreated. If groundwater is treated, typical processes consist of pH adjustment with lime or sodium hydroxide addition or with limestone filtration. Regionally, many water utilities have to remove iron and manganese.

Groundwater is rarely disinfected. An increasing number of water utilities are introducing UV-disinfection in the plants where seasonal variation of water quality occurs. Only few waterworks using groundwater or artificial groundwater chlorinate the water.

6.2 Surface water

Surface water is mainly used in larger cities. The number of surface water sources is only about 70, but they produce 42% of the distributed water. Treatment of surface water includes removal of humic substances, adjustment of pH, and disinfection. Primary disinfection is mainly carried out at the final stage of surface water treatment with chlorine or at the intermediate stage with ozone. Helsinki water has additionally UV-disinfection. The doses of chloramine or chlorine are rather low (0.3–1.0 mg/l) resulting in low disinfectant residual (0–0.2 mg/l) in the distribution system [1].

7. Classification of waterborne outbreaks – strength of evidence

Waterborne outbreaks are classified according to the strength of evidence implicating water. The classification is based on the epidemiological, microbiological and water quality data provided in the outbreak report.

In the United States, epidemiological data are weighted more heavily than water quality data, and reports without supporting epidemiological data are excluded from the surveillance system. Weighting of epidemiological data does not preclude the relative importance of both types of data. A class I indicates that adequate epidemiological and water-quality data were reported (Table 3); however the classification does not necessarily imply whether an investigation was optimally conducted. Likewise, classes II, III, or IV should not be interpreted to mean that the investigations were inadequate or incomplete [23].

In England and Wales, the classification is slightly different. Based on epidemiological, microbiological and water quality data, the outbreaks are classified as strongly, probably or possibly associated with water (Table 4). This classification in England and Wales was introduced in 1996, and these definitions emphasize the importance of epidemiological investigation, while the previous classifications were mainly based on microbiological and water quality data [200, 201].

The reason for this is that water microbiology often does not confirm that the water source was contaminated. The water source may have been contaminated transiently, and samples therefore are negative. Routine monitoring is unlikely to help, because samples are collected infrequently and from few sites. Routine samples also are investigated only for indicator organisms and seldom for pathogens. In addition, some pathogens may be difficult to detect even if the contamination is not over.

Epidemiological evidence may come from descriptive or analytical epidemiological studies. In descriptive studies, if they suggest that the outbreak was waterborne, other possible modes of transmission should be ruled out.

Table 3. Classification of investigations of waterborne-disease outbreaks – United States.

Class	Epidemiological data	Water-quality data
I	*Adequate* Data were provided regarding exposed and unexposed persons, and the relative risk or odds ratio was ≥2 or the p-value was <0.05	*Provided and adequate* Historical information or laboratory data (e.g. the history that a chlorinator malfunctioned or a water main broke, no detectable free-chlorine residual, or the presence of coliforms in the water)
II	*Adequate*	*Not provided or inadequate* (e.g. stating that a lake was crowded)
III	*Provided, but limited* Data were provided that did not meet the criteria for Class I, or the claim was made that ill persons had no exposures in common besides water, but no data were provided	*Provided and adequate*
IV	*Provided, but limited*	*Not provided or inadequate*

Table 4. Classification of waterborne outbreaks in England and Wales – criteria for the strength of association between water and human infectious disease.

A Pathogen identified in clinical cases is also found in water	**B** Water quality failure and/or water treatment problem of relevance but outbreak pathogen is not detected in water
C Evidence from an analytical (case-control or cohort) study demonstrates association between water and illness	**D** Descriptive epidemiology suggests that the outbreak is water related and excludes obvious alternative explanations

Strongly associated if (A+C) *or* (A+D) *or* (B+C)
Probably associated if (B+D) *or* C *or* A only
Possibly associated if B only *or* D only

In analytical studies, the purpose is to find statistical evidence that exposure to water has caused the illness.

The definitions were tested in England and Wales retrospectively on two sets of outbreaks reported to Communicable Disease Surveillance Centre (CDSC). The first set were six outbreaks reported in peer-reviewed journal publications, and four of them were classified as strongly and two as probably associated with water. A further testing of evidence from 26 outbreaks showed that all were classifiable. The association was strong in 11, probable in 9, and possible in 6 outbreaks [201].

OBJECTIVES OF THE STUDY

The overall aim of this study was to describe and evaluate the application of different epidemiological approaches and new microbiological methods in the investigation of four suspected waterborne outbreaks in Finland. In addition, this study describes the public health interventions to control and prevent waterborne outbreaks and contributes to development of evidence-based public health policy and practice guidelines for waterborne outbreak investigations.

The specific objectives were:

1. To describe and evaluate the utility of different epidemiological study designs and data collection methods for investigation of waterborne outbreaks
    - Application of cross-sectional, case-control and retrospective cohort study designs to identify the source and factors associated with waterborne campylobacter and norovirus infections
    - Using the Internet to distribute and collect questionnaire data in addition to traditional postal surveys and evaluating potential biases.

2. To describe and evaluate the application of new microbiological methods in the investigation of suspected waterborne outbreaks.
    - Improving detection of microbes from water and environmental samples
    - Using molecular methods such as pulsed-field gel electrophoresis and sequencing for characterisation of campylobacter and norovirus strains.
    - Comparison of campylobacter and norovirus strains from environmental and clinical samples by molecular methods.

3. To contribute to development of evidence-based public health policy and practice guidelines to improve the scientific quality of waterborne outbreak investigations in Finland.
    - Evaluating the strength of evidence that the etiology of the outbreak was waterborne
    - Describing and assessing the public health interventions to control and prevent waterborne outbreaks.

MATERIALS AND METHODS

1. Community outbreak of campylobacteriosis associated with a municipal water supply – a case-control study (I)

1.1 Background

On August 3, 2000, six patients with diarrhoea, abdominal pain and fever contacted the health centre of Asikkala municipality. During the next days, additional persons with gastrointestinal symptoms were identified. On August 9, *C. jejuni* was isolated from three patients, and on August 10, KTL was notified of the outbreak. Interviews of initial patients suggested that the outbreak might be waterborne. Therefore, on August 11, a boil-water notice was issued and chlorination of the water supply system began on August 12. About 65% of the population received drinking water from the municipal supply; the rest living outside the centre had private wells.

1.2 Epidemiological investigation

The municipal health centre collected basic information on all persons who contacted them by phone or visited the clinic because of gastrointestinal symptoms and who had illness onset between July 25 and August 25.

We conducted a case-control study to determine the source of the outbreak. A case was defined as an illness with acute gastroenteritis, defined as diarrhoea with ≥3 loose stools per 24 hours and/or vomiting and/or abdominal pain, in a resident of Asikkala between July 31 and August 20. Of 250 eligible cases, a 50% sample was randomly selected to participate in the study. Twelve additional case-patients with positive stool cultures treated in the health centre were included in the study. For each case, three community controls were randomly chosen from the population registry. Controls were matched to cases according to sex, year of birth, and postal code of residency. In the postal questionnaire, participants were asked about symptoms, treatments, consumption of water from various sources (tap water, well water, bottled water), and consumption of poultry, eggs, and unpasteurized milk products. The questions referred to the two-week period before the onset of symptoms in the case.

1.3 Analysis of data

Mantel-Haenszel matched Odds ratios (MORs) with 95% confidence intervals for different water consumption categories and foods and chi-square for trend were calculated. To identify independent risk factors for campylobacteriosis, we used conditional logistic-regression analysis. The full model included all variables associated with the illness at $p<0.05$ in the univariate analysis. To determine the best model, we used backward elimination. The likelihood-ratio test was used to assess the statistical significance of each variable. All reported p-values are two-tailed.

1.4 Microbiological investigation

Stool samples were obtained from 74 (16%) of 463 patients who had gastrointestinal symptoms. The samples were analysed for the presence of *Salmonella*, *Shigella*, *Yersinia*, *Campylobacter*, *Aeromonas* and *Plesiomonas* species by routine bacteriological methods. Stool samples were cultured for campylobacters by using selective media and confirmed as *C. jejuni* by gram stain, and positive catalase, oxidase and hippurate tests.

For microbiological investigation of the water supply, 13 fresh water samples were collected from various sites of the water supply system. Coliforms and campylobacters were identified from the water samples as described elsewhere [113].

Sixteen *C. jejuni* isolates from case-patients and one isolate recovered from tap water were serotyped with commercially available antisera as described previously [202]. Eight patient isolates and the water isolate were genotyped by PFGE using SmaI, SacII and KpnI enzymes for digestion of DNA [203].

2. Community outbreak of campylobacteriosis associated with a municipal water supply – a cross-sectional study (II)

2.1 Background

Haukipudas (population approximately 15,000) is a municipality in northern Finland. Practically all (99%) residents receive drinking water from the municipal water supply. On Monday August 10, 1998, KTL was notified that during the weekend of August 7–8, about 50 persons had sought care at the municipal health centre because of gastrointestinal symptoms. On August 11, *Campylobacter jejuni* was isolated from stool samples of 15 patients with gastrointestinal symptoms. The wide geographical distribution of cases and preliminary interviews with patients suggested drinking water as the source of the outbreak. A boil-water notice was issued on August 11, and chlorination of water began on the same day.

2.2 Epidemiological investigation

For rapid characterisation of the outbreak, the municipal health centre collected basic information on all persons who contacted them by phone or visited the clinic because of gastrointestinal symptoms and who had illness onset between August 1 and August 20.

To determine the source and the extent of the outbreak, we conducted a population survey. For the survey, we selected a 10% random sample of all residents of Haukipudas aged $\geq$15 years from the population registry. A case was defined as a resident of Haukipudas with diarrhoea (three or more stools during 24 hours) or at least two of the following symptoms (fever, abdominal pain, vomiting, nausea), and illness onset from August 1 to 20. The questionnaire was mailed to participants on August 26. They were asked about onset and symptoms of gastrointestinal illness and use of health services. Information about consumption of tap water, private well water and bottled water, as well as exposure to mass catering, restaurant foods and poultry between July 25 and August 7 was also collected.

2.3 Analysis of data

Attack rates (AR) and relative risks (RR) with 95% confidence intervals (CI) for categorical variables, and Chi-square for trend were calculated. To identify independent risk factors for campylobacteriosis and control for confounding, we performed exact logistic regression analysis. The model included all variables associated with the illness at $p<0.05$ in the univariate analysis.

2.4 Microbiological investigation

Stool specimens were collected from 74 patients who had gastrointestinal symptoms. Twenty-six (35%) specimens were analysed for *Salmonella, Shigella, Yersinia, Campylobacter, Aeromonas* and *Plesiomonas* species as well as *Staphylococcus aureus*, *Bacillus cereus* and *Clostridium perfringens* by routine bacteriological methods. The remaining 48 (65%) specimens were cultured for *Campylobacter* sp. only. Ten specimens were investigated for *Cryptosporidium* and *Cyclospora* species and 20 specimens for noroviruses by RT-PCR.

Five of the *C. jejuni* isolates recovered from patients were genotyped by PFGE using SmaI and KpnI enzymes for digestion of DNA as described previously [204].

3. Outbreak in a rehabilitation centre due to environmental contamination (III)

3.1 Background

On 30 December 1999, KTL was notified that many guests at a rehabilitation centre had become ill with vomiting, diarrhoea and fever. During the next days, the number of cases decreased without control measures. However, on 25 January 2000, KTL was informed about a sudden increase of new cases with gastroenteritis with onset within the last 24 hours among both guests and staff of the centre.

3.2 Epidemiological investigation

The occupational health nurses of the rehabilitation centre collected information on the number of guests and staff who contacted them because of gastrointestinal symptoms.

Two retrospective cohort studies were conducted. A case was defined as a visitor to the centre between 24 and 26 December or between 22 and 23 January who had acute gastroenteritis (diarrhoea and/or vomiting and/or nausea) with onset between 25 and 29 December, or between 23 and 27 January, respectively. Data were collected on all guests who stayed in the centre from 24 to 26 December and on 20 employees of a pharmacy who stayed in the centre from 22 to 23 January by using a standard questionnaire. In both studies participants were asked about activities and treatments in the centre, meals and food items eaten and drinking water consumed during the stay, and time and symptoms of subsequent gastroenteritis.

3.3 Analysis of data

Food-specific and activity-specific attack rates and relative risks with 95% confidence intervals were calculated.

3.4 Microbiological investigation

Eighty-three stool samples from 67 persons were provided for virological investigation. Twenty-eight specimens were from symptomatic guests and 55 samples from kitchen staff. Twenty-eight stool samples were also cultured for *Salmonella, Shigella, Yersinia, Campylobacter, Aeromonas* and *Plesiomonas* species.

Water samples were collected from the water supply system and all four swimming pools. Thirty environmental swab samples were collected from surfaces of accommodation rooms, saunas, gym rooms, the main entrance and the restaurant.

Water filtration was performed as previously described [205]. Norovirus-RT-PCR detection for genogroups I and II and confirmation with microplate hybridisation was performed and nucleotide sequences of three norovirus amplicons were determined as previously described [173]. The first five patient samples were also examined by electron microscopy and astrovirus-RT-PCR with primers of Mitchell et al. [206].

3.5 Environmental investigation

All treatment areas, saunas, swimming pools and kitchen facilities as well as sections where guests were accommodated were inspected on 27 January 2000. The local environmental health unit in collaboration with the Department of Environmental Health of KTL assessed the water supply system.

3.6 Interventions

Accommodation rooms at the rehabilitation centre were cleaned and disinfected one section at a time, and new guests were accommodated in the cleaned sections. All surfaces in bathrooms and environmental surfaces in the rooms were disinfected with 500 ppm hypochlorite solution. Physiotherapy rooms and instruments were disinfected daily with 500 ppm hypochlorite. The chlorine concentration of swimming pools was increased from 1.0 to 2.5 mg/l each night. Alcoholic hand rub was made available in common toilets, treatment rooms and at the cafeteria. Guests were advised to pay special attention to hand hygiene. They were also instructed to refrain from treatments and to contact the nurses if gastrointestinal symptoms developed.

4. Internet use and investigation of a community outbreak of norovirus gastroenteritis (IV)

4.1 Background

On March 10, 2000, KTL was notified of an outbreak of gastroenteritis in Nurmes municipality in eastern Finland. During the following 2 weeks, an increasing number of patients with acute gastroenteritis were identified throughout the municipality. A boil-water notice was issued on March 24, and water was chlorinated. Drinking water for 75% of the municipality's population is provided from a community ground water supply, the rest receive water from private wells. The neighbouring two municipalities (Juuka, Valtimo) have their own water supply systems, and did not report increase of gastrointestinal illness.

In these three municipalities, a community computer network provides free access to Internet, local discussion groups, local information areas, and e-mail for private citizens, state and municipal authorities, private companies and other organizations. About 3,500 households (42% of households in the municipalities) with 4,100 persons regularly use the regional network.

4.2 Epidemiological investigation and use of the Internet

To determine the source and the extent of the outbreak, we conducted a survey among users of the community computer network in the three municipalities. A case was defined as member of a household with at least one registered user of the community computer network who had an episode of diarrhoea ($\geq$3 loose stools/day) and/or vomiting during March 2000. Data were collected by using a standard online questionnaire posted on the network. Only persons who completed the questionnaire from a home computer were included in the study. Data from the completed questionnaire was transferred directly to a database. From April 10 to 24, when a user logged on to the network, a notice appeared on their screen requesting them to complete the online questionnaire concerning the outbreak of gastroenteritis. To increase the participation rate, the survey was advertised on two different days in the local newspaper.

4.3 Analysis of data

We calculated attack rates (AR) by municipality and household as well as the relative risks (RR) and 95% confidence intervals (CI) for any drinking water from the municipal water supply, unboiled water from the supply and well water.

4.4 Microbiological and Environmental investigation

Stool specimens from 23 patients who were treated for acute gastroenteritis at the community health centre of Nurmes municipality between March 21 and 31 were examined for viruses by electron microscopy (EM) and by the reverse transcription polymerase chain reaction (RT-PCR) test for norovirus, sapovirus and astrovirus [206, 207]. Routine bacterial cultures and microscopy for parasites were also performed for the specimens.

Nine water samples from the municipal water supply of Nurmes were investigated for coliforms and noroviruses as described previously [205].

RESULTS

1. Community outbreak of campylobacteriosis associated with a municipal water supply – a case-control study (I)

1.1 Epidemiological investigation

Four hundred sixty-three persons who had gastroenteritis with illness onset between July 25 and August 25 contacted the health centre. One hundred thirteen cases (response rate, 84%) and 287 controls (response rate, 74%) completed the postal questionnaire. The epidemic curve (Figure 1) shows that on August 4, the number of cases increased suddenly with peak incidence on August 7. After August 10 cases began to decrease and few were recorded after August 20.

Of 113 case-patients, 106 (94%) reported having drunk unboiled tap water at home or outside the home during the two weeks before onset of illness compared with 140 (58%) of 241 control subjects (MOR 20.9, 95% CI 2.8 to 159.8). The odds of illness increased with increasing number of daily glasses of unboiled water consumed during the 2-week period suggesting a dose-response (p for trend <0.001). In a conditional logistic regression model, drinking unboiled tap water remained significantly associated with illness after adjustment for private well water, bottled water, boiled water and eggs (OR 11.1, 95% CI 1.4 to 90.2, p=0.03).

1.2 Microbiological investigation

C. jejuni was cultured from 24 (32%) of 74 stool samples submitted for examination from patients with gastroenteritis. *C. jejuni* was recovered from one tap water sample collected on August 10 at a day care centre.

Sixteen *Campylobacter jejuni* isolates from patients were serotyped and eight subtyped by PFGE. All sixteen were serotype Penner 12, and seven had indistinguishable SacII and KpnI PFGE patterns (Figure 2). The isolate

Figure 1. Cases of gastroenteritis among residents of Asikkala municipality by onset of illness, from July 25 to August 25, 2000.

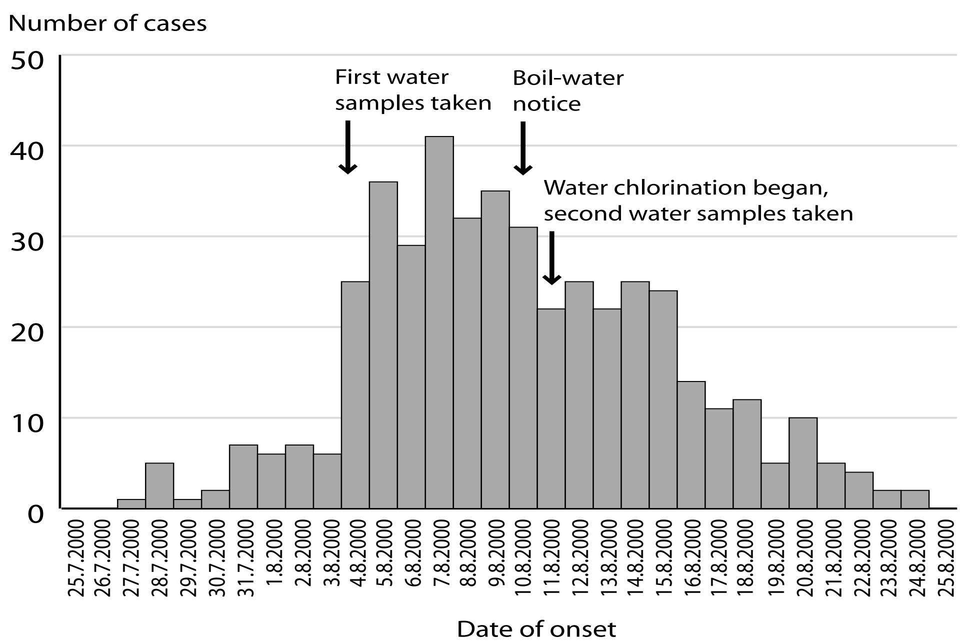
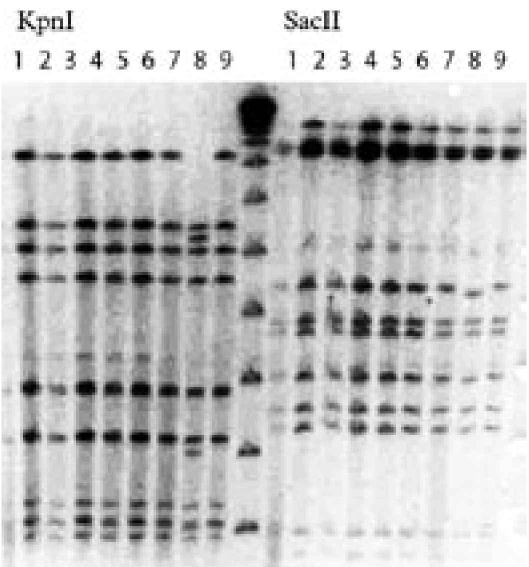


Figure 2. SacII and KpnI pulsed-field gel electrophoresis patterns of *Campylobacter jejuni* isolates from patients and water. Patients: lanes 1 to 6 and 8 to 9, water from the municipal system: lane 7. Asikkala municipality, August, 2000.



from water was also serotype Penner 12 and the PFGE pattern was indistinguishable from the seven patient strains.

1.3 Environmental investigation

The municipal water system had two ground water wells (well A and B). Water from the wells was pumped to two water reservoirs, both of which received water from both wells. From the reservoirs, the water was distributed to households. Water temperature measured at well B was 7.6°C. The water was not routinely chlorinated. Monthly water quality tests had not yielded positive findings before the outbreak. No construction or cleaning works of the water system had been carried out during the year 2000.

Both ground water wells were located about 30 meters away from a major lake. The vacation houses close to well B were not connected to the municipal sewage system. A dry toilet, and a compost heap for household wastes were located about 15 meters from well B. The wells were not fenced, allowing people and animals access to the area. Water reservoirs were accessible to anyone, and the lock in the door of one reservoir was broken. According to the National Weather Service, the total rainfall in this area in July 2000 was 104 mm compared with 58 mm in July 1999. The heaviest rains occurred between July 22 and July 25.

2. Community outbreak of campylobacteriosis associated with a municipal water supply – a cross-sectional study (II)

2.1 Epidemiological investigation

A total of 442 persons with gastroenteritis with illness onset from August 1 to 20, contacted the health centre. All age groups were affected with a median age of 39 years (range 1–89 years); 52% were men.

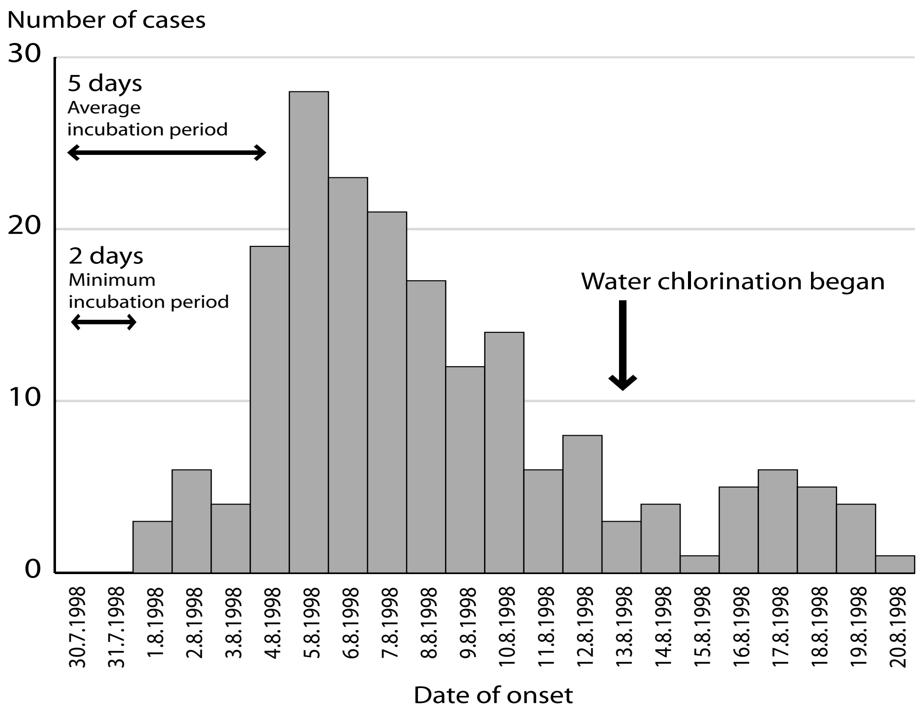
One thousand one hundred seventy-eight persons participated in the survey (response rate 78%). Two hundred eighteen (18.7%) persons met the case definition. Median age of cases was 41 years (range 15–88); 57% were women. The epidemic curve (Figure 3) is consistent with a point source outbreak. The attack rate was highest at the centre of municipality close to the water tower (postal code C) and lowest in the eastern part of municipality (postal code F) (Table 5, Figure 4).

Drinking unboiled tap water was significantly associated with illness (RR not calculable, $p<0.001$). The risk of illness increased significantly with increasing daily consumption of unboiled tap water (Table 6). In exact logistic regression, drinking unboiled tap water (OR 34.4; 95% CI 6.2–infinite), living in an apartment building (OR 2.8; 95% CI 1.4–5.6), age $\geq$65 years (OR 2.2; 95% CI 1.1–4.9), and living in postal code area C (OR 1.8; 95% CI 1.3–2.6) were all independently associated with illness.

2.2 Microbiological investigation

Campylobacter jejuni was isolated from 45 (61%) of the 74 stool samples tested. All five strains further analysed by PFGE showed an

Figure 3. Cases of gastroenteritis among residents of Haukipudas municipality by onset of illness, from August 1 to 20, 1998.



indistinguishable PFGE pattern (Figure 5). All water samples from various points of the water system collected on August 11, 12 and 13 were negative for campylobacter, indicator bacteria, noroviruses and parasites.

2.3 Environmental investigation

The water was pumped from ground water wells located in three different sites (Figure 4). At well A, 1,800 m^3/day was pumped from six abstraction wells. In the vicinity of well A excavation of gravel had reduced the thickness of earth layers protecting ground water. Well B produced 700 m^3/day from one abstraction well. Well C produced only 20 m^3/day. The water tower (capacity 1700 m^3) in the centre of the municipality was filled at night from wells A and B. Small holes were found on the roof of the water tower with connection to the water reservoir.

The eastern part of municipality (postal code F) received drinking water directly from well A, while other parts received their water through the water tower. The water was not chlorinated and water quality was monitored regularly for coliforms.

During a mains repair work close to the centre of municipality on July 29, sewage water had flooded and likely mixed with mains water. After the incident, the pipes were thoroughly flushed in order to prevent contamination of the main water system.

Table 6. Dose-response relation between the amount of daily consumption of unboiled tap water and attack rate of gastroenteritis, Haukipudas municipaility, August 1998.

Glasses/day	Cases	Total N=879	AR %
0	0	63	0
1-3	33	251	13.1
4-6	83	347	23.9
7 or more	86	278	30.9

Chi-square for linear trend = 43.48

p-value <0.0001

Figure 4. Map of Haukipudas municipality presenting the water supply system and postal code areas (A-F) in the municipality. Arrows indicate direction of water flow in the municipal supply.

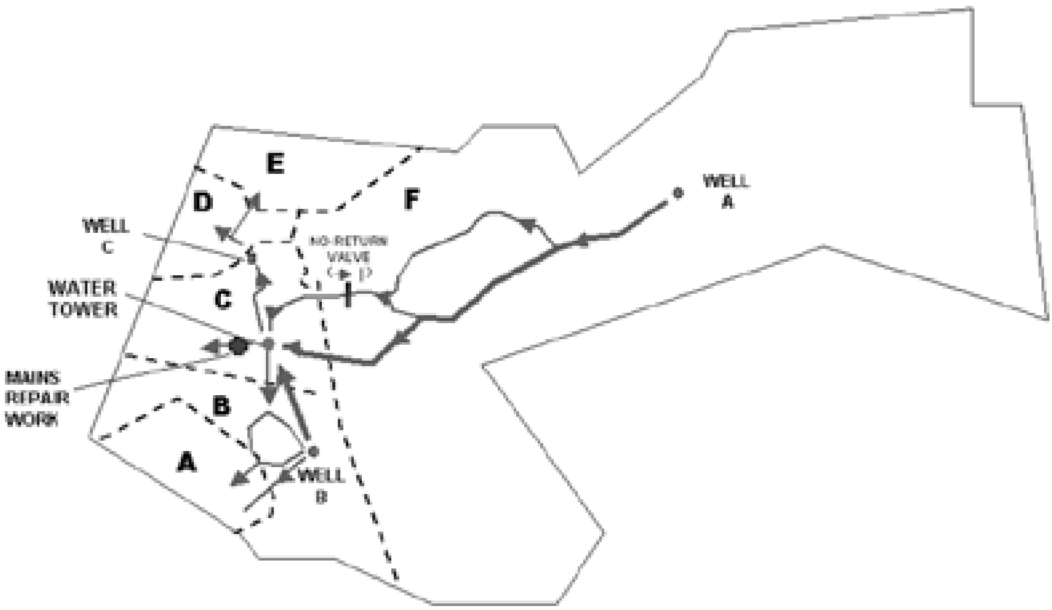
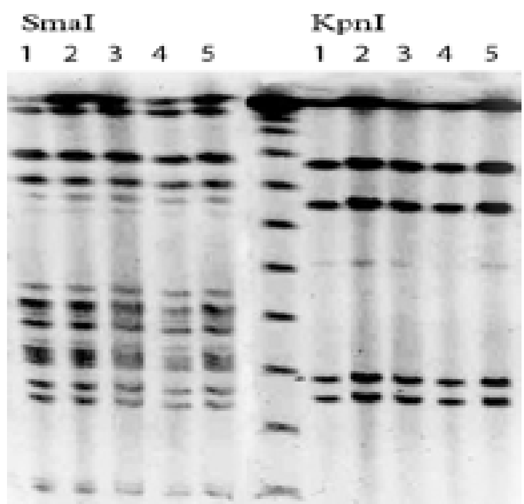


Table 5. Attack rates by postal code area according to the population-based survey, Haukipudas municipality, August 1998.

Postal code	Cases	Participants	Attack rate %
A	24	155	15.5
B	39	206	18.9
C	115	467	24.6
D	22	145	15.2
E	7	53	13.2
F	8	111	7.2*
Other	3	30	10.0
Total	218	1167	18.7

* p<0.0001 compared with highest AR

Figure 5. SmaI and KpnI pulsed-field electrophoresis patterns of five *Campylobacter jejuni* isolates from patients. Haukipudas municipality, August 1998.



3. Outbreak in a rehabilitation centre due to environmental contamination (III)

3.1 Epidemiological investigation

The epidemic curve (Figure 6) shows the first persons had gastrointestinal symptoms on December 21, and thereafter the outbreak occurred in three waves. Altogether 331 persons with symptomatic infection were recorded; 118 of them were employees and 213 were guests. The first illness among kitchen staff was recorded on December 27.

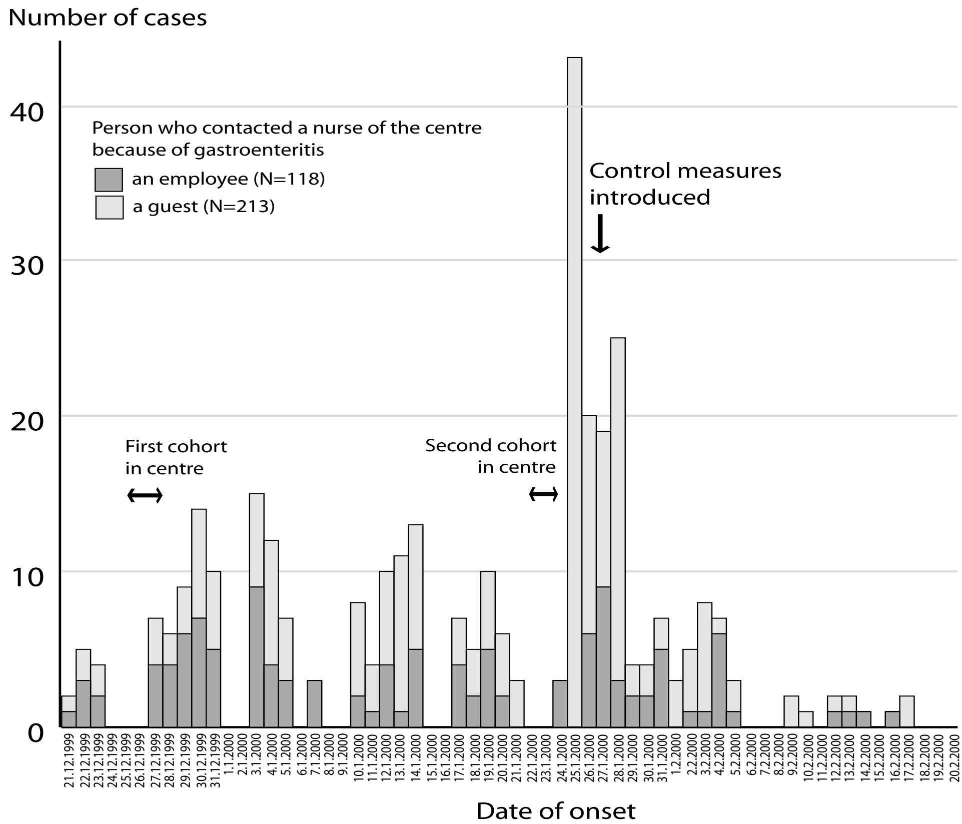
In the first retrospective cohort study, 208 persons completed the postal questionnaire (response rate 74%). One hundred and twenty-five (60%) met the case definition. None of the 29 food items eaten at meals between December 24 and December 26 was significantly associated with illness.

Among members of pharmacy staff, eleven respondents met the case definition (AR 55%). Symptoms began the next day after leaving the centre for most cases (7/11). None of the 39 food items or of the various activities conducted in the centre was significantly associated with illness.

3.2 Microbiological investigation

Bacterial cultures of the stool samples taken from guests and from staff did not yield any enteric pathogens. Norovirus genogroup II (GII) was the only virus detected in the virological investigations. In total, 20 (71%) of 28 samples from guests and 15 (38%) of 39 samples from staff were positive for NV GII.

Figure 6. Gastroenteritis by recording date among guests and employees at a rehabilitation centre in southern Finland, from December 21, 1999 to February 21, 2000.



All NV positive PCR products reacted similarly with the microplate hybridisation probe panel suggesting that they represented the same NV virus strain. The identity was further confirmed by nucleotide sequence determination of three of the NV amplicons.

Norovirus-PCR was positive in four environmental swab samples. Based on the reactivity to the used microplate hybridisation probes the environmental NV strain was identical to the strain detected from patient samples. Sequencing of the environmental strain was not successful.

Tap water samples as well as water samples from swimming pools were all negative for NV and indicator bacteria.

3.3 Environmental investigation

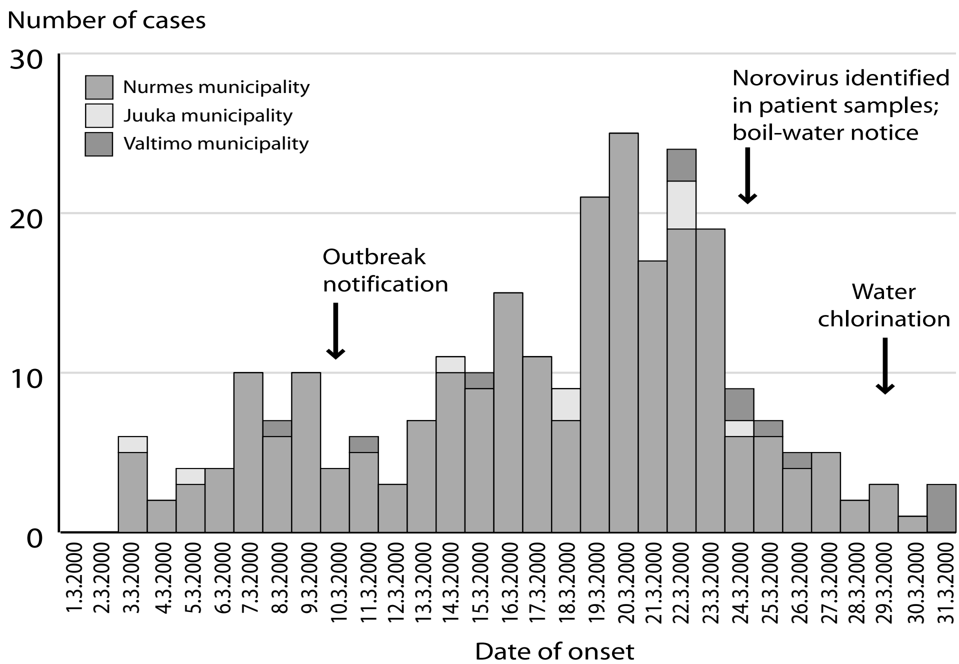
The treatment facilities, saunas, swimming pools and kitchen were all clean and well maintained. No disinfectant was used for cleaning the equipment in the gym room. In physiotherapy and massage departments, the treatment tables were wiped with disinfectant after each client. All treatment rooms were daily cleaned, but disinfectant was not used systematically. Investigation of the water supply system did not reveal any sites with risk of contamination.

4. Internet use and investigation of a community outbreak of norovirus gastroenteritis (IV)

4.1 Epidemiological investigation

Six hundred seventy-two persons in the three municipalities completed the online questionnaire. These persons represented an estimated 19% of registered households with access to the network. Of all respondents, 508 (76%) were from Nurmes; 59% were women. The median age was 27 years (range, 6–74). In Nurmes, the demographic characteristics of respondents were different from the population of the municipality: the proportion of

Figure 7. Cases of gastroenteritis by date of illness onset in a norovirus outbreak, eastern Finland, March 2000. Based on first episode of illness occurring in the household.



young adults aged 15–28 years was higher, and the proportion of persons aged ≥65 years much lower than in the general population (Table 7).

Of respondents, 368 (55%) met the case definition; 60% were females. According to the epidemic curve (Figure 7) cases began to increase in early March with peak incidence during March 19–23. Of the municipalities, AR by household was highest (63%) in Nurmes compared with the AR in Juuka (17%) and Valtimo (29%) [RR (Nurmes vs. Juuka and Valtimo combined) 2.8; 95% CI=2.0–3.9]. By direct standardization of the age-specific attack rates to the population, the total number of ill in Nurmes was 5,500. Among residents of Nurmes, drinking any unboiled tap water was significantly associated with illness (AR 65% vs. 42%; RR 1.6; 95% CI=1.1–2.2) while drinking water from a private well only was associated with decreased likelihood of illness (AR 24% vs. 71%; RR 0.3; 95% CI= 0.1–0.8).

4.2 Microbiological and environmental investigation

Nine stool specimens (one from a survey participant) were positive for norovirus by RT-PCR test. In two additional patients, electron microscopy for SRSV was positive. All stool samples were negative for bacterial pathogens and parasites. All samples from the municipal water supply were negative for NV by RT-PCR; the samples also were negative for indicator bacteria. Inspection of the water supply did not reveal the site of contamination.

Table 7. Attack rates of gastroenteritis by age group in Nurmes municipality, March 2000.

Age (y)	**Population** (%)	**Respondents** (%)	**Cases**	**AR%***
0-14	1,743 (17)	72 (14)	43	60
15-28	1,479 (15)	183 (36)	123	67
29-64	4,975 (49)	249 (49)	152	61
65-	1,911 (19)	4 (1)	1	25
Total	10,108 (100)	508 (100)	319	63

*AR, attack rate, based on first episode of illness occurring in the household

DISCUSSION

All these four outbreaks were notified to KTL through the national outbreak notification system implemented in 1997. The delay from the onset of the outbreak to the first notification about the outbreak was short in all cases. In investigations (I) and (II), according to the epidemic curve the delay from the sudden increase of gastroenteritis cases to the notification to KTL was approximately 6 days. In study (III), the number of cases increased during Christmas 1999, and KTL was notified on December 30. Also in this outbreak the delay was less than one week. In study (IV), KTL received three notifications on the outbreak. The first one was sent on March 10, 2000, about one week after the number of gastroenteritis cases began to increase in the municipality. These outbreaks would probably have been detected after a considerably longer time period through the national surveillance system for infectious diseases. From 1998 to 2001 KTL received altogether 425 notifications of suspected food- or waterborne outbreaks. The median delay from the onset of the outbreak to the notification was 4 days. However, in some outbreaks the interval was very long, the longest being 280 days. Thus, the notification system appears to be rather sensitive, although the sensitivity has not been systematically evaluated.

In both campylobacteriosis outbreaks, the investigation was initiated rapidly after the notification, but in the norovirus outbreaks the delay was much longer. The campylobacter outbreaks were explosive, many patients had fever and several were hospitalised. Therefore, it was obvious that rapid outbreak investigation and implementation of control measures were needed. In both norovirus outbreaks, the number of cases increased more slowly. In the investigation of the outbreak in the rehabilitation centre (III), it seemed that the outbreak was waning before the sudden increase of cases in late January 2000, which initiated new investigations and implementation of control measures. It may be rather common for norovirus outbreaks that the investigation is started late, because the disease usually is mild, and the majority of patients do not seek care at health services [190].

Between 1998 and 2002, 33 outbreaks associated with drinking water were reported in Finland. However, based on notifications of suspected outbreaks, 64 outbreaks in the same time period were suspected to be waterborne and resulted in consultation with KTL. The number of outbreaks leading to consultations varied from 11 to 16 per year. In Finland, the Department of Infectious Disease Epidemiology and the Department of Environmental Health of KTL have jointly assisted in these investigations. Many municipalities in Finland are small, and municipal authorities may have little experience of outbreak investigations; many of them have no experience of the investigation of waterborne outbreaks.

1. Strength of evidence

Public health lessons may be learned from every investigated waterborne outbreak, even with limited evidence of waterborne transmission. The outbreak investigation may result into findings in the water supply system that require repair or change in the disinfection procedures of the supply. The more convincing is the evidence from the outbreak investigation, the more likely are the waterworks and the population to accept these changes.

In study (I), the case-control study provided strong association (OR 20.9; 95% CI 2.8–154.8) between consumption of unboiled tap water from the municipal supply and gastroenteritis. *C. jejuni* was isolated from both the water supply and the patients, and the strains were indistinguishable by PFGE. In environmental investigation, several risk points were identified in the water supply, although the exact site of contamination remained unclear. Together the findings provided strong evidence that the outbreak was waterborne [201].

In study (II), we conducted a population-based survey in the municipality, including a 10% random sample of the population aged ≥15 years. In multivariable model, a strong association (OR 34.4; 95% CI 6.2–infinite) was found between consumption of unboiled municipal tap water and gastroenteritis. *C. jejuni* was isolated from patient samples and all strains tested were indistinguishable by PFGE. However, no bacteria were detected in samples from the water supply. In the environmental investigation, we detected several risk points in the water supply system, and the mains repair work close to the water tower was temporally compatible with the epidemic curve of the outbreak. Taken together, these findings provided strong evidence that the outbreak was waterborne.

In study (III), we did not find association between consumption of tap water and illness in the two retrospective cohort studies. There was no association between recreational water and illness either. Norovirus was detected in patient samples, but not in tap water or pool water samples. In contrast, noroviruses were detected in swab samples collected from environmental surfaces. We concluded that the outbreak was not waterborne, but was transmitted from person-to-person and through contaminated surfaces.

In study (IV), the survey conducted through the Internet found a relatively weak association (RR 1.6; 95% CI 1.1–2.2) between consumption of unboiled tap water from the municipal supply and gastroenteritis. Norovirus was detected in samples from patients, but not from the municipal water supply. Environmental investigation revealed that the main ground water pumping station was located on a large esker formation with high hydraulic conductivity allowing all surface water runoffs infiltrate easily into esker. Leakages in wastewater systems (wastewater wells or pipelines) could easily contaminate ground water. However, no leakages were detected. Based on epidemiological, microbiological and environmental findings, the outbreak was categorized as probably waterborne.

2. Epidemiological methods and sources of bias

2.1 Study design

We used cross-sectional, case-control and retrospective cohort design in our epidemiological investigations. In study (II), we had easy access to the population data of the municipality, and the cross-sectional study enabled us to estimate the total number of ill in the municipality and assess the differences of attack rates in postal code areas of the municipality. In study (I), the aim of the investigation was to gain experience of another type of study in a similar campylobacter outbreak situation. Therefore, we conducted a case-control study. In investigation (IV), the regional computer network provided an opportunity to conduct a survey by using the Internet. In study (III), two retrospective cohort studies were conducted.

In both campylobacter outbreaks, we collected data by using postal questionnaires. In study (I), the response rate for cases was 84% and for controls 74%. In study (II), 78% of participants completed the questionnaire. These response rates were high compared with studies conducted by postal questionnaires in other countries [39]. Also the response rate in the first cohort in the norovirus outbreak in the rehabilitation centre (III) conducted by postal questionnaires was high (74%). However, in the Internet survey (IV), the response rate was only 19% among the households with access to the network. Thus, selection bias because of non-respondents probably was not substantial in the studies conducted by postal questionnaires. However, in the survey conducted by using the Internet, non-response bias may have substantially affected the results.

Missing values were common in the studies conducted by postal questionnaires. This is a common problem when postal questionnaires are used[42,43]. Missing values were problematic in both campylobacter studies, and because of them in study (I), matched analysis for only bacteriologically confirmed campylobacter cases did not have sufficient power to show statistical significance. Missing values were also problematic in multivariable analyses, because they limited the number of variables that could be included in the model.

Our case definitions did not include microbiological confirmation in any of these outbreaks. Therefore, the case definition may have included persons with gastroenteritis caused by other infectious agents. This non-differential misclassification may have biased our results towards the null. However, the clinical features of illness were typical for campylobacteriosis in studies (I) and (II), and for norovirus infection in studies (III) and (IV). Therefore, this type of misclassification was unlikely to substantially affect our results.

The time interval between the outbreak and distribution of the questionnaire was short in all investigations conducted by using postal questionnaires (I–III) reducing the likelihood of recall bias. In the investigation conducted by the Internet, the interval was longer and as a result, the recall bias may have been more substantial.

In the three outbreaks associated with the municipal water supply (I, II, IV), a boil-water notice was issued before the epidemiological studies were conducted, and there was much media publicity about the outbreak. This may have biased the results, because ill persons may have been sensitised to the suspected risk factor while controls may have underestimated it resulting into overestimation of the odds ratio or risk ratio.

In addition, in study (I), cases and matched controls were from the same postal code area, and thus likely to have had the same source of household water. This overmatching may have lead to underestimation of the association between illness and drinking water.

2.2 Utility of the Internet in outbreak investigation

In study (IV), electronic collection of data had two major advantages. First, when questionnaires were completed online instead of a mail-in or telephone survey, several days were saved. Second, appropriate planning of the electronic questionnaire and direct transfer of data into a database saved much time and allowed a large sample size without the need for additional resources because manual data entry was not required.

However, only 42% of the households in our study area had access to the computer network. In telephone surveys, noncoverage is usually not a major concern, because in developed countries most households have at least one telephone [208]. In community-based Internet surveys, noncoverage may be a substantial problem.

Although detailed demographics of registered users of the community network were not available, among internet users, young age groups, persons with higher education, and persons with higher income are overrepresented[45]. Also in our online survey, young people were overrepresented among respondents. Only four respondents were aged 65 years or older, while this group represents nearly 20% of the population. Because the attack rate in elderly people may have been different from the rest of population, selection bias may have influenced the results. However, it is unlikely that the proportion of persons exposed to drinking water would have been substantially different from other age groups to confound the detected association between drinking water and illness. Representativeness of participants may also be a problem in investigations using traditional methods for enrolling subjects such as random digit dialling (RDD) [209].

In our survey, 19% of households with access participated but defining the exact sampling frame was not possible. This problem also is similar to telephone surveys using RDD [210]. In recent studies using RDD, estimated response rates from 28% to 35% have been reported [209, 211]. In an e-mail study of a defined group of employees in Alaska 91% of questionnaires were returned [59]. However, the higher response rates in e-mail studies are not directly comparable with our survey. The total number of respondents represented only 3% of the population in Nurmes, Juuka and Valtimo. Therefore, assessing whether the sample was population-based or not was difficult. Our study aimed to enrol enough subjects to have sufficient statistical power to detect a difference in drinking water exposures, even if the participant demographics differed considerably from the whole population.

In industrialised countries access to Internet and e-mail provides opportunities to increasingly conduct studies with online data collection. Defining the sampling frame, and appropriate design of questionnaire and database are essential. Response rates and demographics of respondents should be monitored to minimize selection bias. The method of choice for data collection in an outbreak investigation depends on the population and topic studied. Currently, online data collection seems best suited for investigations conducted in well-defined populations with high Internet coverage and where the exposures studied are unlikely to be strongly related to demographic and socio-economic factors.

3. Microbiological investigations

3.1 Isolation of campylobacter from water supply system

Isolation of campylobacter from water has rarely been reported in outbreak investigations [102]. The concentration of bacteria in the water may be low, and the strains may lose their culturability over time [212], making isolation difficult. The water supply may also be contaminated only for a short period or intermittently. The epidemic curve in study (II) suggests that exposure to contaminated water lasted one to three days. Samples from the supply were obviously taken after the contamination was already over.

In study (I) isolation of campylobacter from the water supply system was successful. The epidemic curve suggests that the water system was contaminated with campylobacter for several days. As the incubation period of campylobacter ranges from two to seven days, all cases were probably not infected simultaneously. However, it is possible that there was no continuous source of campylobacter, but after incidental contamination, campylobacter persisted in the water system for several days. The temperature of water in the municipal system was favourable for survival of campylobacter, which can survive in cold water for several weeks [213]. As only one campylobacter strain was detected among patients, repeated contamination was unlikely. This would have been expected to lead to contamination with several bacterial strains [108]. In this outbreak, samples from the water system were taken intensively, and large volumes of water were investigated. This may have been essential for the successful isolation of campylobacter.

3.2 Pulsed-field gel electrophoresis of campylobacter strains

The investigation of these campylobacter outbreaks illustrates the power of combining epidemiological data with molecular subtyping methods. PFGE has previously been used for tracing back the source in outbreaks associated with food handler contamination [115], and consumption of unpasteurized milk [117]. In these outbreaks, PFGE was an effective tool for subtyping campylobacter.

In study (I), the strains from both the water and patients were indistinguishable by PFGE confirming that cases in the community were related and linked to water [109]. The outbreak strain, Penner serotype 12 is one of the most common serotypes causing domestically acquired infections in Finland, and it has frequently been found in chickens [203]. One isolate from patients showed slightly different SacII and KpnI PFGE patterns suggesting either a genomic recombination or point mutations. These results demonstrate how the genotype of human *C. jejuni* strains may change in natural infections, a phenomenon that has been demonstrated in strains isolated from chickens [214].

In study (II), all subtyped human strains were indistinguishable by PFGE. Although we could not isolate the microbe from water samples, this suggests that these persons had the same source of infection, and combined with the epidemiological evidence strongly suggest that the outbreak was waterborne.

3.3 Norovirus and environmental contamination

In study (III), noroviruses were detected in environmental swab samples. Since only a few noroviruses are needed to cause infection, contaminated surfaces may be an important source of infection. Norovirus detected from environmental swabs have been reported in a hospital outbreak [159], and in a prolonged hotel outbreak [156]. In the latter study, toilet rims and seats, carpets, horizontal surfaces, toilet handles and taps, frequently handled objects and soft furnishings were positive for norovirus by RT-PCR-assays. On the other hand, in a prolonged outbreak in a long-term facility, environmental samples were RT-PCR negative [215]. Sampling methods may be important for detection of noroviruses; in the long-term facility outbreak [215], dry cotton swabs were used. In our study, norovirus was detected from both moistened and dry cottons swabs. Positive PCR results may also be obtained from non-viable virus. However, the norovirus RNA-genome is susceptible to RNAses found widely in the environment, and the findings probably represent viable noroviruses. Our results suggest that environmental contamination may have been important for the transmission of norovirus and the prolonged course of the outbreak.

Sequences of patient strains in this outbreak were identical. Based on the reactivity to the used microplate hybridisation panel, the environmental norovirus strains were identical to the strains detected from clinical samples. Sequencing of the environmental norovirus strains was not successful in this outbreak. It is not unusual that the small number of amplicons could not be sequenced and subsequently matched with patient isolates.

RECOMMENDATIONS

On the basis of the four studies we make the following recommendations:

- In the described investigations, the delay between the onset of outbreak and notification about the suspected waterborne outbreak to KTL through the early warning reporting system was relatively short. However, surveillance and reporting of illness should still be further improved to reduce time delays in recognizing outbreaks and initiating investigations.
- In epidemiological investigations of suspected waterborne outbreaks, particular attention should be paid to minimise recall bias and misclassification of exposure and disease outcomes. The study and the questionnaire should be designed in consultation with persons with epidemiological expertise. In addition, lessons learned from previous investigations should be utilized and investigators should have access to questionnaires and other materials used in previous investigations.
- If the setting is suitable and appropriate expertise in data management is available, electronic data collection by using the Internet could be considered during investigations of waterborne outbreaks. However, potential sources of bias should be identified and evaluated.
- To improve the quality of outbreak investigations, collaboration between epidemiologists, microbiologists, environmental engineers and drinking water specialists should be enhanced.
- Adequate laboratory facilities and expertise in microbiological methods should be available to optimise chances of identifying etiological agents from clinical specimens and the epidemiologically implicated water source.
- If the same microbe is detected from both the implicated water system and patients, subtypes of the recovered microbes should be compared to provide further evidence about the water as the source of the outbreak.
- The strength of evidence implicating water as the cause of an outbreak should be determined on the basis of combined findings from epidemiological and microbiological investigations in all waterborne outbreaks.
- Risk points identified during outbreak investigations should be eliminated to prevent similar outbreaks in the future. Experience from public health interventions should be used in developing national practice guidelines.

ACKNOWLEDGEMENTS

All but one of these outbreak investigations were carried out while I was working at the Department of Infectious Disease Epidemiology of KTL. Study (II) was conducted while I was an EPIET (European Programme for Intervention Epidemiology Training) fellow in Oslo. I am grateful that KTL invited me to come to Finland and to begin my career in the exciting field of outbreak investigations.

I would like to thank the former and the present heads of our institute, professor Jussi Huttunen and professor Pekka Puska, and the head of our department professor Pauli Leinikki for providing me excellent working facilities during the past years.

I am grateful to my supervisor docent Petri Ruutu for his encouragement and support during this work. For Petri it was crystal-clear from the beginning of my career at KTL that I would do a doctoral thesis – something that was not always so clear to me. My special thanks to docent Pekka Nuorti who has been my principal collaborator and supervisor in these outbreak investigations. It was originally Pekka's idea to write a thesis about outbreak investigations. Pekka has always thoughtfully commented my manuscripts and given valuable advice for scientific writing.

I also want to thank docent Outi Lyytikäinen for collaboration during this work, and for suggesting that I would concentrate on waterborne outbreaks. Outi has always been ready to give constructive comments on my manuscripts. Peter Klemets did most of the practical epidemiology work in study (I), my warmest thanks to him. I also want to acknowledge the work of all people in the Department of Infectious Disease Epidemiology of KTL. Without your work these investigations would never have been conducted. I am also grateful to Jukka Ollgren for statistical advice and to Pekka Holmström for technical assistance.

I thank professor Carl-Henrik von Bonsdorff and Leena Maunula, Ph.D., who were stimulating collaborators in the two norovirus outbreaks. I also thank professor Marja-Liisa Hänninen who did excellent work with campylobacters, and docent Ilkka Miettinen who assessed the water supply systems in all these investigations. This kind of collaboration is crucial for successful investigation of waterborne outbreaks. I also want to thank the numerous collaborators in all municipalities where these outbreaks occurred.

My sincere thanks to Aino Nevalainen and Johan Giesecke for sparing their time to review the thesis, and for giving constructive comments on the manuscript.

I also want to thank my mother, my brothers and sisters and their families, and my mother-in-law for their support and understanding during the past years.

Finally, I want to express my warmest gratitude to my dear family, my wife Ulla and my children Juho, Onerva and Matilda. You have brought joy and happiness to my life outside of work and epidemiology.

Järvenpää, August 2004

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