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A large multi-pathogen waterborne community outbreak linked to faecal contamination of a groundwater system, France, 2000)

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Abstract: A large waterborne outbreak of infection that occurred during August 2000 in a local community in France was investigated initially via a rapid survey of visits to local physicians. A retrospective cohort study was then conducted on a random cluster sample of residents. Of 709 residents interviewed, 202 (28.5%) were definite cases (at least three liquid stools/day or vomiting) and 62 (8.7%) were probable cases (less than three liquid stools/day or abdominal pain). Those who had drunk tap water had a three-fold increased risk for illness (95% CI 2.4–4.0). The risk increased with the amount of water consumed (chi-square trend: $p < 0.0001$). Bacteriological analyses of stools were performed for 35 patients and virological analyses for 24 patients. Campylobacter coli, group A rotavirus and norovirus were detected in 31.5%, 71.0% and 21% of samples, respectively. An extensive environmental investigation concluded that a groundwater source to this community had probably been contaminated by agricultural run-off, and a failure in the chlorination system was identified. This is the first documented waterborne outbreak of infection involving human C. coli infections. A better understanding of the factors influencing campylobacter transmission between hosts is required.

Keywords: Campylobacter coli, epidemiology, gastroenteritis, norovirus, rotavirus, waterborne outbreak

INTRODUCTION

Contaminated drinking water causes extensive outbreaks of illnesses because of the large number of people served by water supply facilities. Their detection requires the identification of an increase in illness (usually gastrointestinal) rate in the exposed population and confirmation that water was the route of transmission (1). Many countries have routine reporting systems for foodborne and waterborne outbreaks. However, in routine practice it is not easy to detect an increase in clinical cases and to link it to waterborne transmission, thus waterborne outbreaks are often unrecognised and underestimated (2). Microbiological examination of water is much more complex than that of stools and thus detection of pathogens in water often fails. Norovirus, *Cryptosporidium parvum*, *Giardia intestinalis* and *Campylobacter jejuni* are most frequently identified (3). We describe a large waterborne outbreak with multiple pathogens including *Campylobacter coli.*

METHODS

Background

On 23rd August 2000, a general practitioner (GP) informed the district health department that 16 cases of acute gastro-enteritis (AGE) had occurred among residents of a holiday camp in the Gourdon community since 21st August. Gourdon, which is located in the south west of France, has a population of 4,888 inhabitants which increases by 50 percent in the July-August tourist season. Investigations at the holiday camp concluded that foodborne transmission was very unlikely and that tap water was highly contaminated with faecal coliforms. Preliminary information collected from physicians in Gourdon indicated an increase of the number of consultations for AGE. Gourdon tap water is supplied by two underground water facilities; facility A with 3,800 water consumers supplied 95 % of the inhabitants of Gourdon and facility B with 1,200 water consumers supplied 5 % of the inhabitants of Gourdon and neighboring communities. Water treatment in facility A consists of chlorination which involves two automatic pumps working in rotation. The water then passes through two semi in-ground reservoir tanks (1,100 m^3 and of 200 m^3) before distribution. Facility B water is mixed with a fraction of facility A water in another 500 m^3 semi in-ground reservoir tank before being distributed. This mixing with the treated water from facility A is intended to disinfect the water from facility B. Since the increase in AGE was noted only in Gourdon, contaminated tap water from facility A was suspected to be at the origin of the outbreak. Therefore, on 25th August, the population of Gourdon was informed to avoid the consumption of tap water or to boil it for five minutes.

Survey on gastro-enteritis related medical visits

To confirm the existence of the outbreak, the 11 Gourdon GPs and the local hospital emergency department were requested to provide the number of total daily visits and the number for gastro-enteritis occurring between 1st August and 11th September.

Retrospective cohort study

A retrospective cohort study was performed on a sample of Gourdon households between 5th and 15th September 2000. Households were randomly selected from the telephone directory, with the exclusion of companies. A definite case was defined as a Gourdon resident who had 3 or more liquid stools per day or vomiting between 1st August and 3rd September 2000 and who had been present in Gourdon at least for one day between 1st and 31st August; if less than three liquid stools per day or abdominal pain had occurred, the case was classified as probable. Exposure was defined as the consumption of tap water before 25th August 2000. A standardised questionnaire on demographic data (gender, birth-date), food consumption (commercially prepared dishes, sea-food, raw milk, pastry), tap water consumption (before and after 25th August, daily number of glasses, consumption of boiled tap water), symptoms of gastro-enteritis (date of onset, number of liquid stools per day, vomiting, nausea, abdominal pain, fever), medical care (in or out-patients) and consequences on daily activities (sick leave, bed confinement) was completed by telephone for each member of the selected household.

Under the hypothesis that 60% of the population consume tap water, an estimated 550 people should be included in the study to identify a relative risk (RR) of 3 or more, with a 5% alpha risk and a power of 80%. Based on an average of two persons per household (INSERM 1999 population census), an estimated 300 households should be included in the survey.

The association between AGE and the consumption of tap water was assessed by calculating the risk ratio (RR) and its 95% confidence interval (95% CI), taking into account a household cluster design effect. A dose-effect relationship was evaluated by analysing the trend of the RR for different categories of daily number of glasses of tap water consumed ($\chi 2$ trend). Proportions were compared using the corrected $\chi 2$ test, means were compared using the Student test or Mann and Witney test. Data were analysed with Epi-Info 6.04c.

The gender and age distribution of the cohort population were compared to that of the general population of Gourdon (INSERM 1999 population census) to assess its representativeness. Under the hypothesis of representativeness, the total number of gastro-enteritis cases was estimated by applying the attack rate to the total population of Gourdon including the tourist population (data provided by the Gourdon tourism office). The most likely period of contamination of the water distribution system was based on the epidemic curve and the minimal incubation period of the implicated pathogens.

Microbiological analyses of stools

Stool samples were examined for the presence of *Salmonella sp.*, *Shigella sp.*, *Staphylococcus aureus*, *Campylobacter sp.*, *Yersinia enterocolitica*, *Escherichia coli*, Enterococci, group A and C rotavirus, astrovirus, calicivirus, adenovirus types 40 and 41, enterovirus, hepatitis A virus and C*ryptosporidum*. Viruses were detected using an enzyme immunoassay for group A rotavirus, astrovirus and adenovirus 40/41 and by the reverse transcriptase polymerase chain reaction (RT-PCR) for calicivirus, group C rotavirus, enterovirus and hepatitis A virus (4-6). *Campylobacter* isolates were sent to the National Reference Centre for Campylobacter for identification and fingerprinting. Species identification was done by phenotypic tests (Api Campy) and PCR to differentiate *C. jejuni* from *C. coli* (7). Fingerprinting was performed using the Random Amplified Polymorphic DNA (RAPD) method with one primer: 5'-AACGCGCAAC-3' (No 3881).

Environmental investigation

An environmental and microbiological investigation of the water distribution system was carried out. The inspection concerned the water catchments, the water distribution and sewage system, agricultural practices and private households around the sources. Bacteriological (*Escherichia coli*, Enterococci, faecal Streptococci, thermotolerant coliforms, sulphite reducing *Clostridia*) and chemical (chlorine) analyses were performed on tap water and on the water sampled from the two groundwater sources. After 23rd August, the water chlorine concentration and the presence of coliform bacteria were daily monitored at several sites of the water system before and after the resort treatment. A search for viruses (group A rotavirus, astrovirus, norovirus, enterovirus, hepatitis A virus) and Campylobacter was undertaken on two 10 ml tap water samples collected in-house on 23rd August and on two 10 ml samples in both groundwater sources A and B on 25th August. Viruses were detected using the RT-PCR method and hybridization with a specific primer to improve sensitivity, and *C. jejuni* and *C. coli* by PCR with specific primers. Group A rotavirus strains detected in stools and in water were sequenced using Beg 9 and End 9 primers (8) and compared using the GCG software from infobiogen (www.infobiogen.fr).

Bacteriological (revived 36°C, revived 22°C, thermotolerant coliforms, Streptococci and faecal Enterococci), physical (temperature, conductivity) and chemical (oxygen consumption, ammonium, nitrates, phosphorus) parameters from source A and the river were analysed. Trends were compared over eight weeks between 21st December 2000 and 7th February 2001.

RESULTS

Epidemiology

Between 1st August and 11th September 2000, 7,104 Gourdon residents consulted a GP or the local hospital emergency department. Between 24th and 28th August, gastro-enteritis accounted for 44% (479/1,097) of all medical visits compared to 6% (126/2,185) during the first 15 days of August (Figure 1). Among the 498 households contacted for the household survey, 198 were excluded (103 did not respond, 49 did not fulfil the inclusion criteria, 43 refused to participate, and 3 for unknown reasons). The questionnaire was completed for 300 households including 709 persons. There was one missing data for gender and 11 for age. Three hundred and thirty one (46.7%; 95% CI 44.5 – 49.0) were male and 377 (53.2%; 95% CI 50.9 – 55.5) were female. The median age was 44 years (range < 1 to 94 years). The age and sex distribution of the sample was similar to that of the general population. During the outbreak period, 264 of the 709 respondents (attack rate [AR] 37.2%) had been ill with 202 being definite cases (AR = 28.5%; 95% CI 24.6 – 32.4) and 62 probable cases (AR = 8.7%; 95% CI 6.5 – 11.0). The AR was higher in women (31.8%) than in men (24.8%) and highest in children under six years old (42.1%, Table 1). The epidemic curve (figure 2) suggests that the outbreak started between 14th and 19th August. The shape of the epidemic curve in the retrospective cohort study is very similar to the one based on the number of visits to GPs for gastro-enteritis.

Patients complained mainly of diarrhoea, abdominal pain and nausea (Table 2). No bloody diarrhea was notified. The mean duration of symptoms was 4.5 days (median 3, range 1 to 30 days). Fifty two percent of definite cases included a visit to the GP, sick leave for at least one day (42%), bed rest for 32% and hospitalisation for six patients (2.9%).

Three hundred and thirty six (47.4%) respondents had drunk tap water before 25th August, whereas 646 (91.1%) did not afterwards. The risk for illness, taking into account the sample cluster design effect of 1.4, was 3 (95% CI 2.2 – 4.1) times greater among those who had drunk tap water before 25th August than among those who had not and increased with the amount of water consumed (χ^2 trend: $p< 0.0001$, Table 3). The risk of gastro-enteritis associated with drinking tap water did not change substantially after adjustment for age

(RR_{MH} = 3.3, 95% CI 2.5 – 4.4) and there was no modification effect according to age. Food consumption was not associated with the occurrence of the illness.

Applying the AR of 37% to the total Gourdon resident population exposed to the water supply (4,888 residents + 2,200 tourists), the number of people affected by this outbreak was estimated to be 2,600 (95% CI: 2,400 – 2,900) assuming an exposure to tap water and a risk of gastro-enteritis for the tourists similar to that for the inhabitants.

Figure 1: Total number of medical visits per day for all causes and for acute gastro-enteritis to general practitioners and the hospital emergency department, Gourdon, France, August-September 2000.

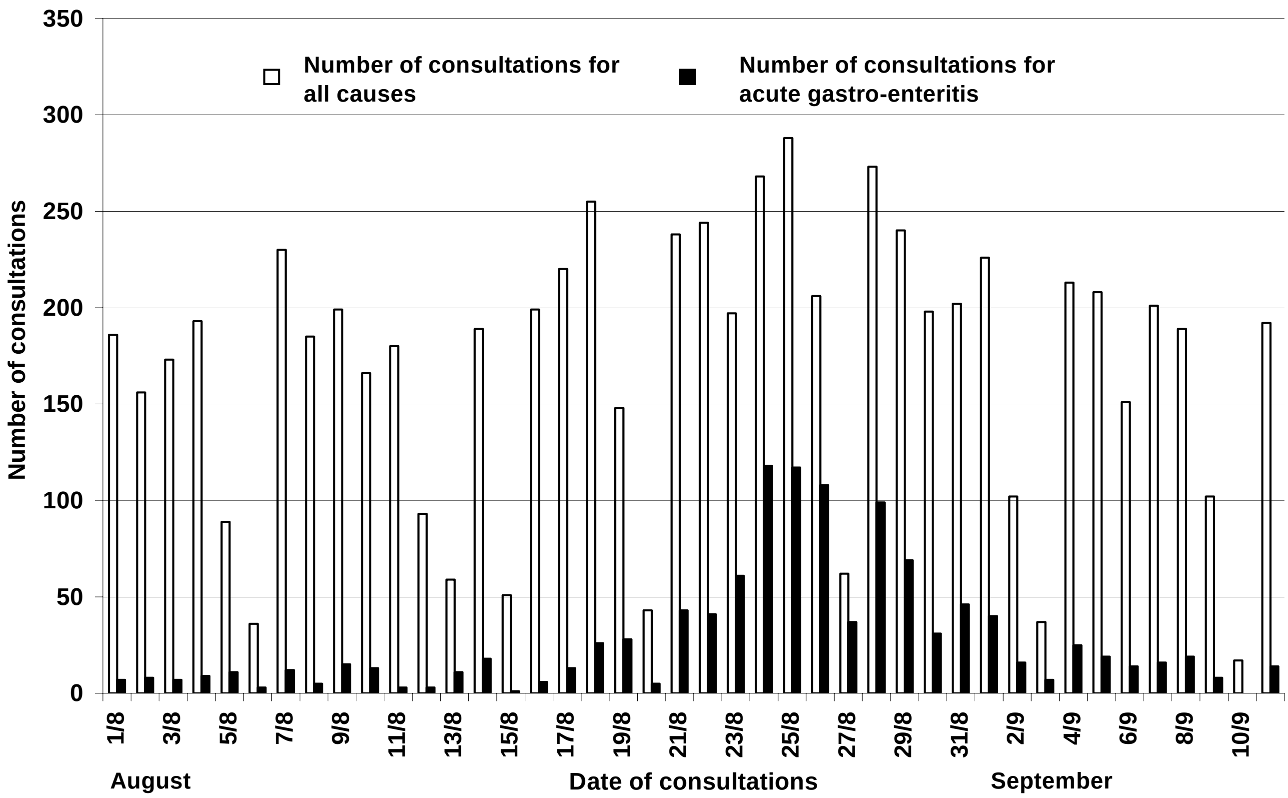


Table 1: Attack rate of definite cases of acute gastro-enteritis according to the age group, Gourdon, France, August-September 2000.

Age group*	Definite cases	Total	Attack Rate %	95% CI
<6 y	16	38	42.1	26.9 – 57.3
6-16 y	33	83	39.7	28.1 – 51.4
17-65 y	116	422	27.5	23.1 – 31.9
> 65 y	32	155	20.6	13.2 – 28.0

* Age was unknown for 11 persons.

Table 2: Frequency of symptoms, Gourdon, France, August-September 2000.
*asthenia, anorexia, headaches, shivers, dizziness.

Symptoms	Definite cases (N = 202)		Probable cases (N= 62)	
	n	%	n	%
Diarrhoea	189	94	44	71
Abdominal pain	169	84	0	0
Nausea	128	63	25	40
Vomiting	99	49	45	73
Fever	62	31	8	13
Others*	27	13	7	11

Table 3: Attack rate of acute gastro-enteritis among people who consumed tap water and those who did not, and dose-effect relationship, Gourdon, France, August-September 2000.

	Definite cases (N=202)	Total (N= 709)	Attack rate %	Relative risk	95% CI
Tap water consumption					
Yes	148	336	44.0	3.0	2.2 – 4.1
No	54	373	14.5	-	
Number of glasses of water consumed per day					
0	54	373	14.5	-	-
1-3	30	99	30.3	2.1	1.4 – 3.0
4-7	45	103	43.7	3.0	2.2 – 4.2
> 7	56	98	57.1	3.9	2.9 – 5.3

CI: confidence interval

Microbiological stool analyses

Among the 35 stool samples analysed for bacteria, 11 (31.5%) were positive for *C. coli*. Twenty-four of the 35 samples were also tested for viruses: rotavirus A was detected in 17 (71.0%) and norovirus in 5 (21.0%) samples, while nine (37.5%) samples were co-infected (4 *C. coli* + rotavirus; 1 *C. coli* + norovirus; 3 rotavirus + norovirus; and 1 *C. coli* + rotavirus + norovirus). Of the 11 *C. coli*, six were characterized as biotype 2 and were identical by RAPD while the remaining five were of biotype 1. Rotavirus typing by RT-PCR identified a single genotype P-[8]; G1. The molecular characterization of norovirus showed 2 different patterns with three isolates being identical and close to the Saragota strain (genogroup I) and two close to the new GGIIb variant (genogroup II).

Environmental investigation

All of the water samples from source B were negative regardless of the site of the distribution system tested. Analyses of the water originating from source A, sampled after the waterworks on 23rd and 25th August, revealed high concentrations of *E. coli* (2,263/100 ml), Enterococci (415/100 ml), total coliforms (90/100 ml), faecal Streptococci (136/100 ml) and sulphite reducing *Clostridia* (10/100 ml), and a lack of chlorination. Rotavirus A genotype G1 was detected in the crude water sampled from source A. The analyses for enterovirus, astrovirus, norovirus, hepatitis A virus and Campylobacter were negative.

Source A was situated in a valley, crossed by a river, below a hamlet of about ten habitations including farms breeding cattle, pork and sheep. The source was not supposed to be supplied by the river. The river resulted from the convergence of about ten different sources with one originating close to a slaughterhouse and was exposed to agricultural run-off and effluent of manure: Sheep and cattle grazed in meadows bordering the river, larges amount of sewage from water treatment facilities were sprayed onto land close to the river. Cowsheds were close to the water catchment area. The immediate area of protection of source A was too small and littered with building rubbish. Air vents without protection in the door made the resort accessible to animals and insects.

Bacteriological analyses of the water samples of source A and the river revealed that the contamination was from both human and animal origin with a higher concentration in the river. The levels and trends of chemical parameters of source A were similar to those of the river, strongly suggesting that the river supplied source A (Figure 3). The retrospective review of routine data collected from 1991 to 2000 showed irregularities in the maintenance of the water distribution system and the water treatment plant, particularly during the month of August. In the hamlet, the sewage system of the fifteen households did not comply with the sanitary rules. All but three ejected the waste into a cesspool or superficially without treatment.

Three regions of gene 9 of the rotavirus detected in stools and water were compared. The sequencing showed that the strains were close but not identical.

Assuming a likely onset of the outbreak between 14th and 19th August, and taking into account a minimum incubation period of two days for rotavirus A, norovirus and *C. coli*, the contamination of the water distribution system would have occurred between 12th and 17th August. The contamination persisted most likely until 28th August since intensive chlorination, beginning on 25th August, was effective only from that date onwards (Figure 2).

Figure 2: Distribution of cases of acute gastro-enteritis by date of onset of symptoms, retrospective cohort study, Gourdon, France, August-September 2000.

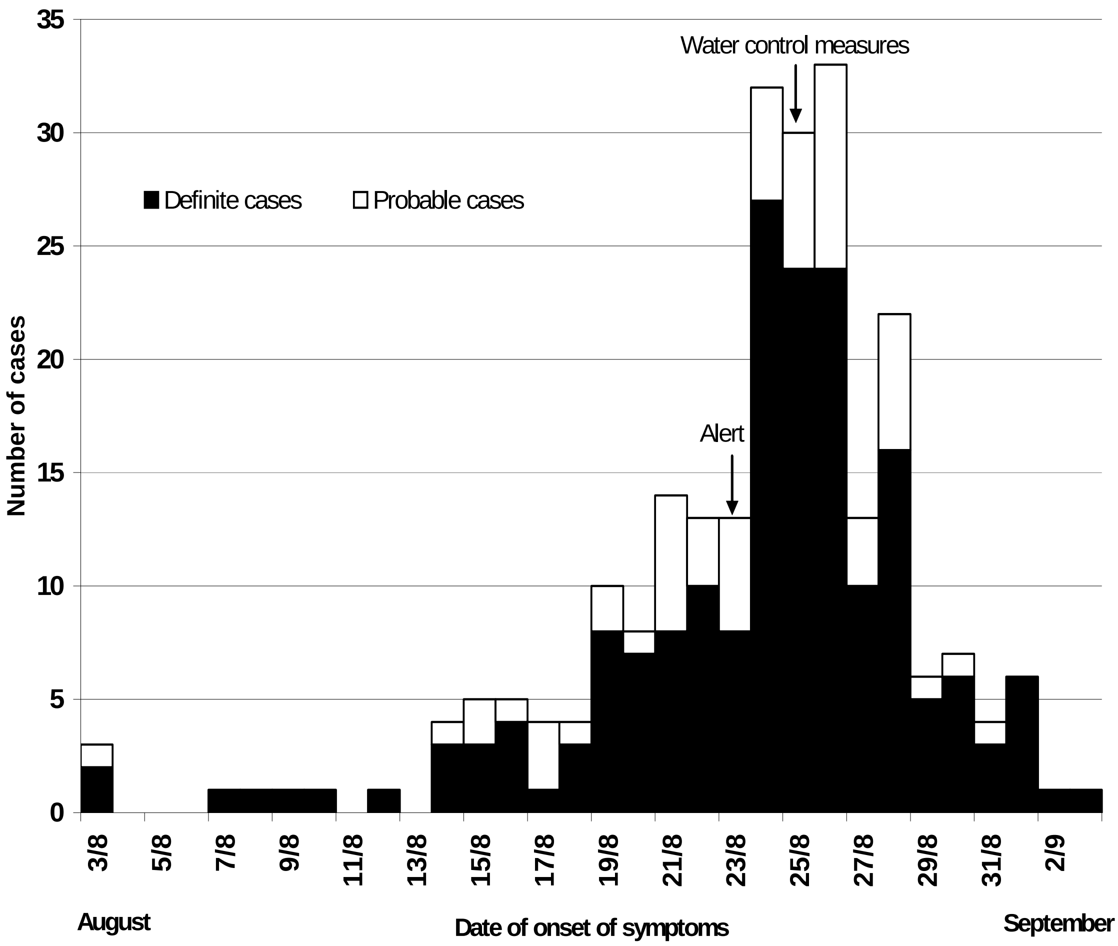
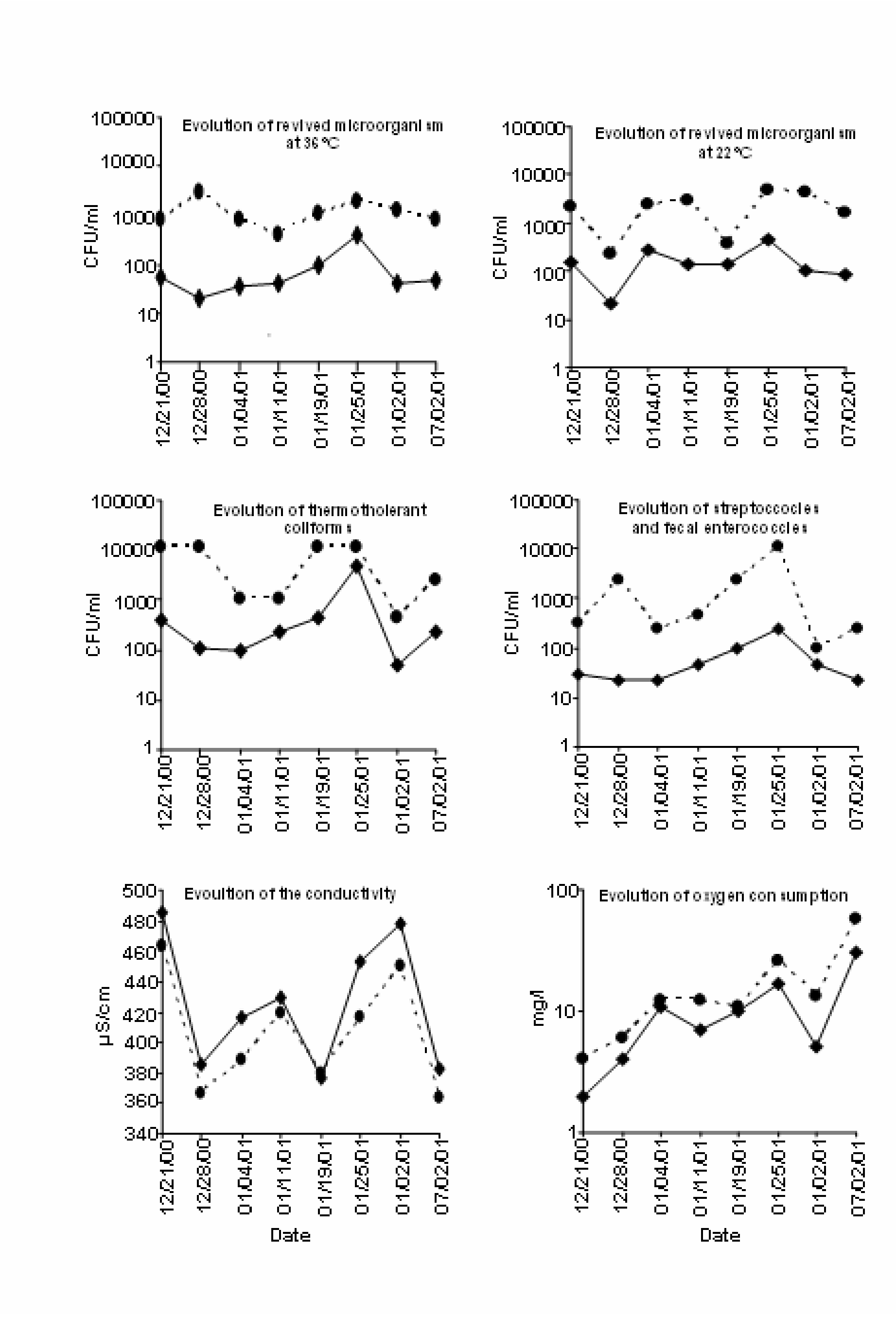


Figure 3 : Trends in bacterial, physical and chemical analyses of groundwater and river water (Gourdon, France, 21 December 2000 to 7 February 2001).



For all recorded parameters, the data from the source parallel the data from the river, thus suggesting a link between the source and the river.
Solid lines : data from the source. Dotted lines : data from the river.
All data are expressed in logarithms except for the conductivity.
CFU : Colony Forming Unit.

DISCUSSION

Our descriptive and retrospective cohort studies confirmed that the outbreak that occurred in the community of Gourdon was related to the consumption of tap water and affected an estimated 2,600 people. The outbreak involved multiple pathogens with group A rotavirus and *C. coli* being the most frequent followed by norovirus. Results of the environmental investigations suggested that water contamination was related to the environmental contamination of the source combined with a failure of the water chlorination system.

The association with tap water consumption, the dose-effect relation and the fact that multiple pathogens were involved were all strongly in favour of tap water as the origin of infection. Moreover, the number of cases decreased dramatically after the water restriction advice and after control measures on the water supply had been implemented. No food products explored in the study were associated with illness. We observed an attack rate (AR) of 14.5% and 31 % among people and children aged under six respectively who had reported not to have consumed tap water. This relatively high rate could be explained by several phenomena. Many persons may have consumed tap water without being aware of this, such as in drinks or ice cubes consumed out of the home, in raw fruits and vegetables or dishes prepared with unboiled water, or through teeth cleaning. We can hypothesize that people who became ill, without being aware of having consumed tap water, were exposed to small amounts of water and had been subjected to a small infective dose. In addition, children, more than adults, may have been contaminated through person to person transmission (e.g. family contact) that is the most common route of transmission for rotavirus and calicivirus.

Waterborne contamination can be responsible for a large number of ill persons (3, 9-11). In our study, assuming a 50 percent increase in the population during the tourist season, the number of affected people was estimated between 2,400 and 2,900. The cohort study showed that almost half of the cases (46.5%) consulted a GP. Applying this proportion to the estimated number of cases provided an estimated 1,116 to 1,349 ill persons who consulted a GP for gastro-enteritis, similar to the number of consultations for gastro-enteritis reported by the GPs (n = 1,037).

The stool sample analysis revealed the presence of multiple pathogens, rotavirus A, norovirus genogroups I and II and *C. coli* as a single infection or co-infection. Except for *C. coli*, these pathogens have been previously implicated in numerous waterborne outbreaks suggesting an influx of sewage into the water supply system as already described in other waterborne outbreaks (10, 12–18). Rotaviruses are well known to be responsible for large epidemic during cold seasons, affecting particularly young children under three years of age. These epidemics are due to the faecal route of contamination (person to person transmission). The high proportion of stools positive for rotavirus (71%) is uncommon in waterborne outbreaks and probably reflects a massive contamination (15, 16, 19). Different molecular sequences of rotavirus A genotype G1 found in stools and water associated with different norovirus strains in stools indicated a human faecal source of infection. In our study, the massive contamination by sewage water could be linked to the large amount of sewage from water purification resort sprayed onto land close to the river and the water catchments. In this outbreak, *C. coli*, which was identified in 32% of stools, was the second most frequent pathogen. To our knowledge, human *C. coli* contamination has not been described before as a causative agent in waterborne outbreaks, unlike *C. jejuni* (11, 20-25). However, *C. coli* has been identified in water (26). The main symptoms observed (diarrhoea, abdominal pain, nausea) and the mean duration of the disease (4.5 days) were compatible with a Campylobacter infection. No bloody diarrhoea was noticed. However, bloody diarrhoea is not constant in Campylobacter infections (27). The high proportion of persons who had to take sick leave and the fact that six persons were hospitalised suggested a relative severity of symptoms which is much more compatible with Campylobacter infection than with viral infections. Sixty percent of the hospitalized patients were infected with *Campylobacter coli*. Among patients who had stools analysis and whom age was known, cases infected only with rotavirus were younger (mean age 49 years [5 months to 95 years]) than cases infected only with campylobacter (mean age 66 years [23 to 88 years]) or co-infected with rotavirus + campylobacter (mean age 69 years [2 to 91 years old]) (Data not shown). Moreover, the proportion of medical visits (52%) was unusually high compared with the proportion of visits observed in other waterborne outbreaks (13, 16, 28).

Campylobacter needs specific atmospheric conditions for growth and survival and its detection can be very difficult (29, 30). Because of a rapid physiological transformation to a viable though non-cultivable state, it has been suggested that conventional culture methods often fail to detect Campylobacters (31). Nevertheless, in this study the lack of detection in water of *C. coli* and noroviruses identified in stools could be due to the insufficient quantity of water sampled. Indeed, only 10 ml of water were sampled for each analysis. Hänninen *et al.* proposed that the volume used for detection of the suspected pathogen in drinking water should be not less than 8 to 10 liters (30).

Groundwater is widely considered to be microbiologically clean and is usually used for drinking without treatment. However, groundwater can be contaminated by the environment (26, 32). The first cultivable evidence that Campylobacter can occur in groundwater was provided by Stanley et al. (33). In their study, hydrological evidence suggested that the source of contamination was a dairy farm situated within the hydrological catchment area of the groundwater, and this was confirmed when identical strains of *C. jejuni* were isolated from groundwater and the dairy herd. In our study we failed to identify Campylobacter in groundwater, and research in dairy herds and other domestic animals in farms around the source was not performed. However, results of bacterial, chemical and physical water analyses strongly suggested that a river contaminated by agricultural run-off from bordering sheep and cattle grazing meadows supplied the source. In addition, the immediate area of the source was insufficiently protected and vulnerable to the environment. Many of animal species (wild birds, rodents, pigs, cows) are hosts for *C. coli* and could easily contaminate surface water (26, 27, 34, 35). Campylobacter are sensitive to many environmental factors and to chlorine (33, 36, 37). A failure of the chlorination system could have resulted in the presence of a high concentration of *C. coli*. Waterborne outbreaks are often caused by several factors, which in this case acted together (18, 20, 23, 38). In some of outbreaks, heavy rainfalls have been responsible for increasing in agricultural run-off into the river. Nonetheless, the area of Gourdon did not experience such climatological event break prior the outbreak. This underlies the need for an increased frequency in routine water quality monitoring, chlorination of the water supply according to environmental conditions and implementation of preventive measures.

This outbreak raised the question of the delay of the alert. The origin of an alert can be 1) the poor quality of the water detected during regular controls, 2) complaints about water quality, 3) an incident in the supply and/or sewage water system during maintenance work, and 4) an increase in the number of cases of gastro-enteritis. In most instances waterborne outbreaks are suspected when there are already a large number of declared cases (3, 12, 18, 28, 38, 39). The initial warning was given by a GP who notified officials of a foodborne outbreak in a holiday camp 5 to 10 days after the assumed date of onset. This highlights the role of GPs in the detection of an unusual event, particularly during the summer when an increase in acute gastro-enteritis is less expected than during winter (40, 41). In addition, a routine water quality monitoring system able to detect contamination of drinking water before the occurrence of illnesses and complaints about the water quality should be used as an early warning source.

The retrospective cohort study of Gourdon residents allowed us to quantify precisely the scale and severity of the outbreak and to test the hypothesis that tap water was the vehicle of the outbreak. When provided with a cohort sample representative of the study population, the number of people affected can be estimated. As the exposure of the population was high, a relatively large sample size was however needed to test the association between the disease and the exposure. Due to misclassification of exposure (unrecognised consumption of tap water), the attributable risk is probably underestimated.

In the absence of obvious alternative explanations, water contamination is the most likely common cause of an explosive outbreak of AGE occurring in a large population. Tillett et al. proposed categorizing levels of evidence for waterborne outbreaks (42). A descriptive study suggesting a water-related outbreak, together with the identification of the same pathogen in stools and water, should be sufficient to conclude that the outbreak is strongly associated with water consumption. So far, faecal indicators of water quality remain insufficiently sensitive to some pathogens, notably viruses and parasites (i.e. *Cryptosporidium*). Therefore, without evidence obtained from an analytical study demonstrating an association, the link between illness and water could only be classified as probable or possible. However, total coliforms are good indicators of a faecal contamination with other pathogens (i.e. rotaviruses, *E. coli*, Campylobacter) (43).

The origin of the waterborne outbreak was most probably due to the faecal contamination of groundwater of source A arising from deficiencies in the maintenance of the water distribution system. Corrective measures were implemented based on the results of the investigation. To our knowledge, this is the first documented waterborne outbreak involving human *C. coli* infections. In depth, environmental investigations to identify the origin of water contamination are very important to better understand microbial transmission from the environment and to implement better controls and preventive measures. In addition, surveys on vehicles and vectors which transmit Campylobacter between hosts are critical points to enrich the knowledge of the epidemiology of this organism.

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