

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

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INTRODUCTION

Contaminated drinking water causes extensive outbreaks of illness because of the large number of people served by water supply facilities. Detection of such outbreaks requires the identification of an increase in illness (usually the rate of gastrointestinal disease) in the exposed population and confirmation that water was the route of transmission [1]. Many countries have routine reporting systems for detecting foodborne and waterborne outbreaks of infection. However, in

routine practice it is not easy to detect an increase in clinical cases and to link this increase to waterborne transmission; thus waterborne outbreaks are often unrecognised and underestimated [2]. Microbiological examination of water is more complex than examination of stools, and there is often a failure to detect pathogens in water. Norovirus, *Cryptosporidium parvum*, *Giardia intestinalis* and *Campylobacter jejuni* are the pathogens identified most frequently [3]. The present study describes a large waterborne community outbreak of infection with multiple pathogens, including *Campylobacter coli*.

METHODS AND MATERIALS

Background

On 23 August 2000, a general practitioner (GP) informed the local district health department that 16 cases of acute gastro-

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enteritis (AGE) had occurred among residents of a holiday camp in the Gourdon community since 21 August. Gourdon, which is located in the south-west of France, has a population of 4888 inhabitants, which increases by 50% in the July–August tourist season. Investigations at the holiday camp concluded that foodborne transmission was very unlikely, but found 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 serving 3800 water consumers in Gourdon; and facility B serving 1200 water consumers in Gourdon and the surrounding 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 (1100 m³ and 200 m³, respectively) before distribution. Facility B water is mixed with a fraction of facility A water in another 500 m³ 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 the origin of the outbreak. Therefore, the population of Gourdon was informed on 25 August to avoid the consumption of tap water or to boil it for 5 min.

Survey of gastroenteritis-related medical consultations

To confirm the outbreak, the 11 GPs in Gourdon and the local hospital emergency department were requested to provide the number of total daily visits and the number of cases of gastroenteritis cases occurring between 1 August and 11 September 2000.

Retrospective cohort study

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

Under the hypothesis that 60% of the population consumed tap water, an estimated 550 people were required for inclusion in the study to identify a relative risk (RR) of ≥ 3 , with a 5% α risk and a power of 80%. Based on an average of two individuals per household (INSERM 1999 population census), 300 households were required for inclusion in the survey.

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

The gender and age distribution of the cohort population were compared with that of the general population of Gourdon (INSERM 1999 population census) to assess whether it was representative. Under the hypothesis of representation, the total number of cases of gastroenteritis 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 for contamination of the water distribution system was based on the epidemic curve and the minimal incubation period of the pathogens involved.

Microbiological analyses of stools

Stool samples were examined for the presence of *Salmonella* spp., *Shigella* spp., *Staphylococcus aureus*, *Campylobacter* spp., *Yersinia enterocolitica*, *Escherichia coli*, enterococci, rotavirus groups A and C, astrovirus, calicivirus, adenovirus types 40 and 41, enterovirus, hepatitis A virus and *Cryptosporidium*. Viruses were detected using an enzyme immunoassay for group A rotavirus, astrovirus and adenovirus 40/41, and by 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 by means of phenotypic tests (API Campy; bioMérieux, Lyon, France), with use of PCR to differentiate *Campylobacter jejuni* from *Campylobacter coli* [7]. Random amplified polymorphic DNA fingerprinting was performed with primer 3881, 5'-AACGCGCAAC.

Environmental investigation

An environmental and microbiological investigation of the water distribution system was performed. 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 23 August, the water chlorine concentration and the presence of coliform bacteria were monitored daily at several sites of the water system before and after the resort treatment. Examinations for viruses (group A rotavirus, astrovirus, norovirus, enterovirus, hepatitis A virus) and *Campylobacter* spp. were undertaken on two 10-mL tap water samples collected in-house on 23 August, and on two 10-mL samples from groundwater sources A and B on 25 August. Viruses were detected by RT-PCR, followed by hybridisation with a specific primer to improve sensitivity, while *C. jejuni* and *C. coli* were detected by PCR with specific primers. Group A rotavirus isolates detected in stools and in water were sequenced using Beg 9 and End 9 primers [8] and compared using the Infobiogene GCG software (<http://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 for an 8-week period between 21 December 2000 and 7 February 2001.

RESULTS

Epidemiology

Between 1 August and 11 September 2000, 7104 Gourdon residents consulted a GP or the local hospital emergency department. Between 24 and 28 August, gastroenteritis accounted for 44% (479/1097) of all medical visits, compared with 6% (126/2185) during the first 15 days of August (Fig. 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 three for unknown reasons). The questionnaire was completed for 300 households (709 individuals). Data were missing concerning gender for one individual and age for 11 individuals. Overall, 331

(46.7%; 95% CI 44.5–49.0) of respondents were male and 377 (53.2%; 95% CI 50.9–55.5) were female. The median age was 44 years (range <1–94 years). The age and gender 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 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 females (31.8%) than in males (24.8%), and was highest in children aged <6 years (42.1%, Table 1). The epidemic curve (Fig. 2) suggests that the outbreak started between 14 and 19 August. The shape of the epidemic curve in the retrospective cohort study was very similar to that based on the number of visits to GPs for gastroenteritis.

Patients complained mainly of diarrhoea, abdominal pain and nausea (Table 2). No cases of bloody diarrhoea were notified. The mean duration of symptoms was 4.5 days (median 3, range 1–30 days). Among definite cases, 52% had visited a GP, 42% had taken sick leave for ≥ 1 day,

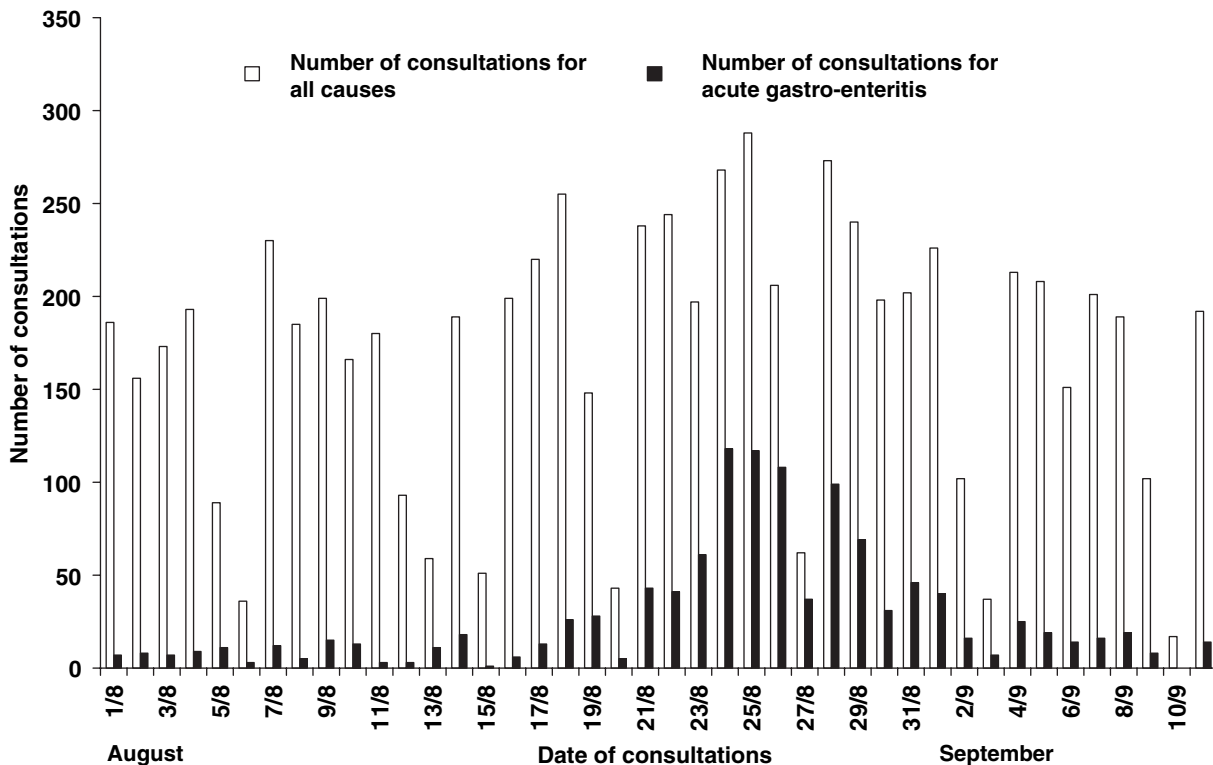


Fig. 1. Total number of medical visits per day, for all causes and for acute gastroenteritis, to general practitioners and the hospital emergency department, Gourdon, France, August–September 2000.

Table 1. Attack rate for definite cases of acute gastroenteritis according to age group (Gourdon, France, August–September 2000)

Age group ^a	Definite cases	Total	Attack rate (%)	95% CI
< 6 years	16	38	42.1	26.9–57.3
6–16 years	33	83	39.7	28.1–51.4
17–65 years	116	422	27.5	23.1–31.9
> 65 years	32	155	20.6	13.2–28.0

^aAge was unknown for 11 individuals.

32% had required bed rest, and 2.9% (six patients) had been hospitalised.

In total, 336 (47.4%) respondents had drunk tap water before 25 August, whereas 646 (91.1%) did not drink tap water after 25 August. The risk for illness, taking into account the sample cluster design effect of 1.4, was three-fold greater (95% CI 2.2–4.1) for those who had drunk tap water before 25 August, and increased with the amount of water consumed (chi-square trend: $p < 0.0001$; Table 3). The risk of gastroenteritis 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 illness.

Applying the AR of 37% to the total population of Gourdon exposed to the water supply (4888 residents + 2200 tourists), the number of individuals affected by this outbreak was estimated to be 2600 (95% CI 2400–2900), assuming an exposure

to tap water and a risk for gastroenteritis among the tourists similar to that among the inhabitants.

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 five (21.0%) samples, while nine (37.5%) samples were co-infected (four *C. coli* + rotavirus; one *C. coli* + norovirus; three rotavirus + norovirus; and one *C. coli* + rotavirus + norovirus). Of the 11 *C. coli* isolates, six were characterised as biotype 2, and were identical following RAPD analysis, while the remaining five were of biotype 1. Rotavirus typing by RT-PCR identified a single genotype P-(8), G1. Molecular characterisation of the norovirus isolates identified two 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 leaving the water-

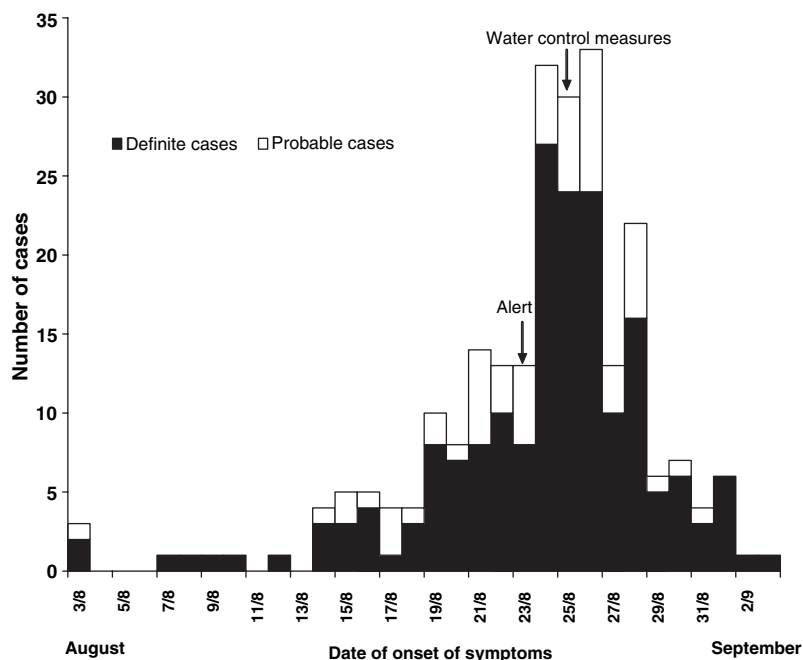


Fig. 2. Distribution of cases of acute gastroenteritis by date of onset of symptoms (retrospective cohort study, Gourdon, France, August–September 2000).

Table 2. Frequency of symptoms of gastroenteritis (Gourdon, France, August–September 2000)

Symptoms	Definite cases (<i>n</i> = 202)		Probable cases (<i>n</i> = 62)	
	<i>n</i>	%	<i>n</i>	%
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 ^a	27	13	7	11

^aasthenia, anorexia, headaches, shivers, dizziness.

Table 3. Attack rate of acute gastroenteritis among individuals who consumed tap water and those who did not, and the dose-effect relationship (Gourdon, France, August–September 2000)

	Definite cases (<i>n</i> = 202)	Total (<i>n</i> = 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

works on 23 and 25 August, revealed high concentrations of *E. coli* (2263/100 mL), enterococci (415/100 mL), total coliforms (90/100 mL), faecal streptococci (136/100 mL) and sulphite-reducing clostridia (10/100 mL), and an absence 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 containing a river, below a hamlet of 15 dwellings, 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 streams, with one originating close to a slaughterhouse, and was exposed to agricultural run-off and manure effluent; sheep and cattle grazed in meadows bordering the river, and a large amount of sewage from water treatment facilities was sprayed on to land close to the river. Cowsheds were close to the water catchment area. The immediate protected area around source A was too small and littered with building rubbish. Air vents without protection in the door made the source accessible to animals and insects.

Bacteriological analyses of the water samples from source A and the river revealed that the contamination was of both human and animal origin, with a higher concentration in the river. The levels and trends of the chemical parameters of source A were similar to those of the river, strongly suggesting that the river was connected to source A (Fig. 3). A retrospective review of routine data collected between 1991 and 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 15 households did not comply with sanitary rules. All but three ejected waste either into a cesspool or superficially without treatment.

Three regions of gene 9 of the rotavirus detected in stools and water were compared. Sequencing showed that the strains were closely related but not identical.

Assuming a likely onset of the outbreak between 14 and 19 August, and taking into account a minimum incubation period of 2 days for rotavirus A, norovirus and *C. coli*, the contamination of the water distribution system probably occurred between 12 and 17 August. Contamination probably persisted until 28 August, as intensive chlorination, beginning on 25 August, was effective only from 28 August onwards (Fig. 2).

DISCUSSION

The present descriptive and retrospective cohort studies confirmed that the outbreak of infection in the community of Gourdon was related to the consumption of tap water and affected 2600 individuals. The outbreak involved multiple pathogens, with group A rotavirus and *C. coli* being the most frequent, followed by norovirus. Results of environmental investigations suggested that water contamination was related to the contamination of the source, combined with a failure of the water chlorination system. The association with tap water consumption, the dose-effect relationship, 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 the implementation of control measures on

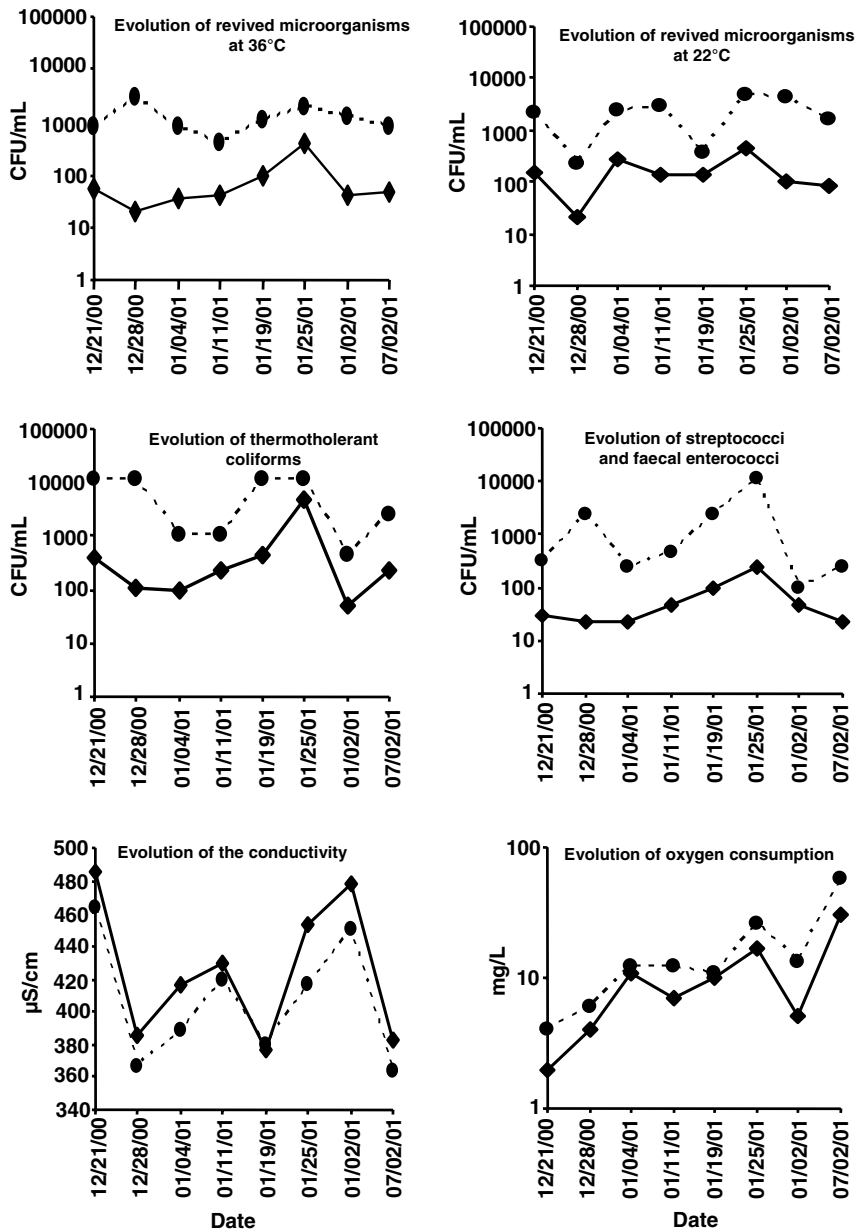


Fig. 3. Trends in bacterial, physical and chemical analyses of groundwater and river water (Gourdon, France, 21 December 2000 to 7 February 2001). For all recorded parameters, the data from the source parallel the data from the river, thus suggesting a link between the source and the river. Solid lines, data from the source; dotted lines, data from the river. All data are expressed in logarithms, except for the conductivity.

the water supply. No food products explored in the study were associated with illness.

An AR of 14.5% and 31% was observed among adults and children aged <6 years, respectively, who reported that they had not consumed tap water. This relatively high rate could be explained by several phenomena. Many individuals may have consumed tap water without being aware of

it, e.g., in drinks or ice cubes consumed outside of the home, via raw fruits and vegetables, dishes prepared with unboiled water, or while cleaning their teeth. It can be hypothesised 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), which is the most common route of transmission for rotavirus and calicivirus.

Waterborne contamination can affect large numbers of individuals [3,9–11]. In the present study, assuming a 50% increase in the population during the tourist season, the number of affected people was estimated to be between 2400 and 2900. The cohort study showed that almost half (46.5%) of the cases consulted a GP. Applying this proportion to the estimated number of cases yielded an estimated 1116–1349 ill individuals who consulted a GP for gastroenteritis, similar to the number of consultations for gastroenteritis actually reported by the GPs ($n = 1037$).

The stool sample analyses revealed the presence of multiple pathogens (rotavirus A, norovirus genogroups I and II and *C. coli*) as single or co-infections. Except for *C. coli*, these pathogens have been implicated in numerous waterborne outbreaks, suggesting an influx of sewage into the water supply system [10,12–18]. Rotaviruses are known to be responsible for large epidemics during winter seasons, affecting particularly children aged <3 years. These epidemics are caused by 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 massive contamination [15,16,19]. Different molecular sequences of rotavirus A genotype G1 found in stools and water indicated a human faecal source of infection. In the present study, contamination of water by sewage could be linked with the large amount of sewage from the water purification plant sprayed on to 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 previously as a causative agent in waterborne outbreaks, unlike *C. jejuni* [11,20–25], although *C. coli* has been isolated from 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 reported, although bloody diarrhoea is not constant in *Campylobacter* infections [27]. The high proportion of individuals who had to take sick leave, and the

fact that six individuals were hospitalised, suggested a relative severity of symptoms, which is much more compatible with *Campylobacter* infection than with virus infections. Among the hospitalised patients, 60% were infected with *C. coli*.

Among patients whose stools were analysed and whose age was known, cases infected only with rotavirus were younger (mean age 49 years; range, 5 months to 95 years) than cases infected only with *Campylobacter* (mean age 66 years; range 23–88 years) or co-infected with rotavirus plus *Campylobacter* (mean age 69 years; range 2–91 years). Moreover, the proportion (52%) of medical visits was unusually high compared with the proportion of visits observed during 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 [31]. Nevertheless, in the present study, the failure to detect *C. coli* in water, and the detection of noroviruses in stools, could be caused by the small (10 mL) water samples examined. Hänninen *et al.* [30] proposed that the volume used for detection of suspected pathogens in drinking water should be at least 8–10 L.

Groundwater is considered widely to be microbiologically clean, and is usually used for drinking without treatment. However, groundwater can be contaminated by the environment [26,32]. Stanley *et al.* [33] showed that *Campylobacter* can occur in groundwater, with hydrological evidence suggesting that the source of contamination in their study was a dairy farm situated within the hydrological catchment area of the groundwater, which was confirmed when identical strains of *C. jejuni* were isolated from groundwater and the dairy herd. In the present study, *Campylobacter* was not identified from groundwater, and dairy herds and other domestic animals in farms around the source were not investigated. However, results of bacterial, chemical and physical water analyses suggested strongly that a river contaminated by agricultural run-off from surrounding sheep and cattle grazing meadows was contaminating the source. In addition, the immediate area of the source was insufficiently protected and vulnerable to the environment. Wild birds, rodents, pigs and cows are all hosts for

C. coli and could contaminate surface water easily [26,27,34,35]. Campylobacters are sensitive to many environmental factors and to chlorine [33,36,37], but 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 that act together [18,20,23,38]. In some outbreaks, heavy rainfall has been responsible for increasing agricultural run-off into rivers, although this did not occur before the outbreak in Gourdon. This underlies the need for increased frequency of 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 in raising the alert. An outbreak can be recognised by: (i) the poor quality of water detected during regular controls; (ii) complaints about water quality; (iii) an incident in the supply and/or sewage water system during maintenance work; and (iv) an increased number of cases of gastroenteritis. In most instances, waterborne outbreaks are suspected when there are already a large number of declared cases [3,12,18,28,38,39]. In the present outbreak, the initial warning was given by a GP who notified officials 5–10 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 gastroenteritis 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 illness and complaints about the water quality should be used as an early warning source.

The retrospective cohort study of Gourdon residents allowed the scale and severity of the outbreak to be quantified precisely and the hypothesis that tap water was the vehicle of the outbreak to be tested. When provided with a cohort sample representative of the study population, the number of people affected can be estimated. However, as the exposure of the population was high, a relatively large sample size was needed to test the association between the disease and the exposure. Because of 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.* [42] proposed categorising levels of evidence for waterborne outbreaks. 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 an outbreak is associated strongly with water consumption. Faecal indicators of water quality are currently insufficiently sensitive for some pathogens, notably viruses and parasites (e.g., *Cryptosporidium*). Therefore, in the absence of evidence from an analytical study to demonstrate an association, the link between illness and water can only be classified as probable or possible. However, total coliform counts are good indicators of faecal contamination with other pathogens (e.g., rotaviruses, *E. coli*, *Campylobacter*) [43].

In conclusion, the waterborne outbreak described in the present study was most probably caused by faecal contamination of source A by groundwater, resulting 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* infection. In-depth environmental investigations to identify the origin of water contamination are important in order to better understand microbial transmission from the environment and to implement appropriate controls and preventive measures. In addition, surveys on vehicles and vectors that transmit *Campylobacter* between hosts are crucial in order to better understand the epidemiology of these organisms.

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