

An outbreak of gastroenteritis in a holiday resort in Italy: epidemiological survey, implementation and application of preventive measures

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Summary

A major gastroenteritis outbreak was reported in a vacation resort in Central Italy in 2003. A total of 183 cases were identified. The case-control study identified a statistically significant correlation between the disease and sea bathing, use of sanitary facilities in bungalows and of common showers. Stool samples taken from people affected were found positive for *Norovirus* (68%, 13 of 19 samples), *Rotavirus* (38%, 1 of 14 samples) and *Campylobacter* (7%, 3 of 8 samples). Environmental investigations revealed serious faecal contamination of the groundwater and the presence of *Norovirus* in the seawater near the resort. The mixing of groundwater and seawater with the non-drinking water system – which was also found to be connected to the drinking water system – had a primary role in the onset and spread of infection within the village. The complete absence of any gastroenteritis epidemics among the site guests since 2006 demonstrates the effectiveness of the environmental corrective measures taken.

Keywords

Campylobacter, Drinking water, Gastroenteritis, Groundwater, Hygiene, Italy, *Norovirus*, Resort, *Rotavirus*, *Salmonella*, Seaside, Water, Water-borne disease.

Focolaio di gastroenterite in un villaggio turistico: indagine epidemiologica, definizione e applicazione di misure di profilassi

Riassunto

In un villaggio turistico, nel 2003 si è verificato un importante focolaio di gastroenterite. Complessivamente sono stati identificati 183 casi. Lo studio caso-controllo ha identificato l'esistenza di una associazione statisticamente significativa tra la patologia osservata e bagni in mare, l'uso dei servizi igienici dei bungalow e delle docce comuni. I campioni di feci raccolti dagli individui colpiti sono risultati positivi per Norovirus (68%, 13 di 19 campioni), Rotavirus (38%, 1 di 14 campioni) e in Campylobacter (7%, 3 di 8 campioni). I risultati delle indagini ambientali hanno evidenziato nell'acqua di falda una intensa contaminazione fecale e nell'acqua di mare, antistante il villaggio, la presenza di Norovirus. La commistione dell'acqua di falda e di mare con la rete non potabile, risultata in connessione con la rete di acqua potabile, ha avuto un ruolo primario nella insorgenza e nella diffusione dell'infezione nella struttura turistica. Le misure preventive suggerite sono risultate tese a impedire l'inquinamento fecale

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dell'acqua potabile. L'assenza di segnalazioni di casi di gastroenteriti negli ospiti del campeggio a partire dal 2006, dimostra l'efficacia delle misure suggerite.

Parole chiave

Acqua di falda, Acqua potabile, *Campylobacter*, Gastroenterite infettiva, Italia, *Norovirus*, Patologie idrodifuse, *Rotavirus*, *Salmonella*, Spiaggia, Villaggio turistico.

Introduction

Gastrointestinal infections are the most common diseases recorded among holiday village guests (28). They are caused by numerous agents and are mainly transmitted by the faecal-oral route, often in contaminated water and food (7). In recent years, *Noroviruses* have increasingly been reported as the major cause of gastroenteritis in holiday resorts (8, 28). In United States, 12% of 348 *Norovirus* gastroenteritis outbreaks detected between 1996 and 2000 were found in holiday resorts. The role of drinking water as the source of contagion has been well documented in the literature for numerous countries. Major defects were identified in leaks from non drinking water or sewage system or cross connection between the water supply and waste water system (4, 13). It has also been reported that drinking water has been contaminated by polluted river water, infiltrating groundwater (30).

In Italy, water transmission has been reported only once, at a holiday resort in the south of the country (5).

In previous years, several episodes of acute gastroenteritis were observed in holiday resorts in coastal towns of central Italy. A study was planned to identify the source of infection and eliminate its causes. The investigation was conducted between June and September 2003 in a holiday village where the greatest number of cases had been observed previously. The village provided a daily medical service. This study describes the onset and progress of the epidemics, the activities conducted to trace the sources of infection, the measures proposed to prevent recurrence of outbreaks and the results obtained.

Materials and methods

Resort

The survey was conducted at a holiday village that guaranteed the presence of two doctors for an hour a day, in a place where numerous cases of gastroenteritis had been reported the previous year (2002).

The holiday village covers an area of 150 000 m² overlooking the seashore from a private beach. The last stretch of a river runs on one side. The structure is organised in two different areas, namely: the village (with 275 bungalows) and the campsite (with 602 sites for campers, roulettes or tents). The campsite is provided with four large structures with bathrooms, showers, sinks, etc. The entire holiday village can accommodate a maximum of 4 080 people.

The village also has a sports centre with four swimming pools (Fig. 1). The following activities were conducted in the holiday village:

- an epidemiological survey and tracing of the outbreak's sources of infection
- an environmental survey, based on the results of the epidemiological survey to verify the validity of the suggested origin of infection
- a proposal for the implementation of preventive measures.

Epidemiological survey

A paired samples case-control study was conducted. The choice of a case-control study was justified by the following:

- rapid turnover of the resort population, leading to the absence of a large number of subjects from the longitudinal study
- high incidence of gastroenteritis, making it impossible to study all subjects in the cohorts involved.

Case definition: an individual present in the holiday resort investigated (as a guest or worker) who had shown at least three episodes of diarrhoea or three episodes of vomiting in a 24 h period during his/her stay in the resort (12). Each case was paired with a randomised control, consisting of a symptom-free individual of the same age group who had

stayed in the resort at some stage during the week prior to the onset of symptoms in the paired case. The following age groups were selected to pair cases and controls: 0-11 months, 1-4 years, 5-14 years and >14 years.

Individuals who suffered from gastroenteritis (cases) were subsequently contacted for the epidemiological survey. At the peak of epidemic, not all cases had consulted a doctor for therapy.

The following epidemiological data were collected: personal details, type of accommodation in the resort, period of stay, date of onset and duration of symptoms, symptoms, any contact with other infected subjects, use of medical services, hospitalisation, food products eaten in the three days prior to onset of symptoms, type and mode of use of water within the resort, use of swimming pools and sea bathing.

When possible, stool samples were taken for laboratory testing for: *Campylobacter* (20),

Escherichia coli O157 (24), *Salmonella* (25), *Listeria monocytogenes* (21), *Shigella* (1), *Vibrio cholerae* (10), *Clostridium perfringens* toxin (*Perfringens* enterotoxin-reversed passive latex agglutination [PET-RPLA], Oxoid, Basingstoke) (15), *Bacillus cereus* toxin (*Bacillus cereus* enterotoxin (diarrhoeal type)-reversed passive latex agglutination [BCET-RPLA], Oxoid) (17, 29), *Norovirus* (IDEIA™ *Norovirus* Dako-Cytomation Ltd, Ely) (6, 14) and *Rotavirus* (IDEIA™ *Rotavirus*, DakoCytomation Ltd) (9, 16). For confirmation of *Norovirus* diagnosis and strain genotyping, samples were further assayed by reverse transcriptase-polymerase chain reaction (RT-PCR) using biotin-labelled JV12 and JV13 primers targeting the RNA-dependent RNA polymerase (RdRp) region of open reading frame 1 (ORF1) and reverse line blot hybridisation (RLBH).

To match the cases, 181 controls (consisting of 128 customers and 53 employees) were selected and interviewed.



Figure 1
Holiday resort map

The risk factors were analysed with univariate statistical techniques. Statistically significant results were analysed by multivariate logistic regression.

The number of cases and controls, exposed and not exposed to each risk factor, were used to calculate the odds ratio (and confidence limits) and the Chi-square test (31). To confirm the association of specific risk factors suggested by the preliminary univariate analysis, a stepwise multivariate logistic regression was performed (18).

Environmental survey

The environmental survey was based on the results of the epidemiological survey to verify the validity of the suggested origin of the infection. The survey examined the following two aspects:

- layout and condition of the water pipelines
- chemical, physical and microbiological quality, main flow direction and origin of any pollution of groundwater originating from the river near the resort.

Water distribution pipelines

The water for the resort was provided by two pipelines, one of which came from the public aqueduct and supplied drinking water and the other originated from two wells that collected groundwater from the river.

The drinking water was distributed to the village bungalows, public fountains and swimming pools, to some of the basins and to the sinks in the shared sanitary facilities provided for dishwashing.

The well water was filtered and chlorinated and then distributed to the showers, to some of the basins and to the sinks in the shared sanitary facilities provided for dishwashing. Part of the well water was not purified but was distributed directly to the public toilets and to the hoses used for watering gardens.

Seventy-six samples (49 samples of drinking water, 20 non-drinking water samples and 7 water samples from the swimming pools) were screened for the presence faecal or pathogen contamination (Table I) (10, 19, 22, 23, 26, 27). Sampling points were selected at the end of each main branch of the pipeline network in the holiday village.

To detect *Norovirus*, 101 water samples were subjected to an ultra-filtration process, and the final 1.5 ml suspension was examined as described for stool samples, namely: enzyme-linked immunosorbent assay (ELISA) detection (DakoCytomation Ltd), confirmation with RT-PCR and genotyping with RLBH (6, 14). Distilled water samples were used as negative controls.

The sodium fluorescein test (34) was used to check whether there was any connection between the drinking and non-drinking water systems in the resort. A total of 100 g of sodium fluorescein was added to the 100 m³ of non-drinking water in tanks (10⁻³ g/l) and the passage of any coloured water through the drinking water pipe system was monitored at seven supply points by on-the-spot spectrophotometric measurements and laboratory testing with the LS-50 spectrofluorimeter (Perkin-Elmer, Norwalk, Connecticut) with a detection limit of 10⁻⁹ g/l. Eight activated carbon fluorescence traps were used to absorb any sodium fluorescein in the four days following the test and were then analysed by spectrofluorimetry.

Ground and seawater

The chemical and microbiological quality of the groundwater that originated from the river near the resort was evaluated between 22 October and 3 November 2003 by selecting 19 wells in the area, from which 19 samples had been taken (one per well). Samples were tested for ammonia, nitrites (3) and chlorides (test Spectroquant 14755, Merck KgaA, Darmstadt).

Nitrates were measured in a 5 ml sample volume by direct spectrophotometry (Lambda 11, Perkin Elmer, Norwalk, Connecticut) at 220 nm. The detection limit was 0.1 mg/l.

Microbiological tests were conducted for *C. perfringens*, total coliforms, *E. coli* (23), *Enterococci* (22), *Campylobacter* (20), *E. coli* 0157 (24), *L. monocytogenes* (21) and *Salmonella* (19).

Between 25 August and 13 October 2003, three samples of seawater from the sea in front of the resort and three samples of water from the wells in the resort area were collected and tested for *Norovirus*.

Table I
Microbiological analysis of water used within the holiday village in central Italy, a case control study (June-September 2003)

Risk factor	Univariate statistical analysis				Multivariate logistic regression	
	Odds ratio	95% CI	χ^2	p value	Coefficients	95% CI
Swimming in the sea	6.62	2.92-15.00	24.50	<0.01	4.76	1.99-11.42
Use of toilets and showers in cabins and chalets	3.4	1.95-5.90	19.50	<0.01	3.44	1.89-6.24
Use of cabins and villa showers supplied with drinking water	3.11	1.78-5.42	16.30	<0.01	-	-
Use of shared shower facilities supplied with non-drinking water	2.96	1.68-5.20	14.80	<0.01	2.49	1.32-4.68
Use of non-drinking water for various purposes (e.g. laundry, washing dishes, oral hygiene)	2.63	1.46-4.72	10.80	<0.01	-	-
Use of drinking water distributed to cabins and villas	2.15	1.24-3.72	7.54	<0.01	-	-
Use of swimming pools	2.11	1.21-3.69	6.99	<0.01	-	-
Use of bottled drinking water	0.36	0.09-1.39	2.39	>0.05	-	-
Bathing in rivers	3.05	0.31-29.81	1.02	>0.05	-	-
Use of common toilets	1.38	0.81-2.33	1.44	>0.05	-	-
Use of drinking water collected in bathrooms, showers, sinks and fountains	1.55	0.77-3.11	1.51	>0.05	-	-
Use of mineral water for cooking	1.63	0.61-4.37	0.96	>0.05	-	-
Use of ice	0.79	0.44-1.44	0.57	>0.05	-	-
Use (for cooking) of drinking water collected in bathrooms, showers, sinks and fountains	1.22	0.70-2.11	0.49	>0.05	-	-
Use of drinking water collected at permanent facilities (cabins and villas)	1.19	0.52-2.69	0.17	>0.05	-	-
Water for massage at swimming pool	0.85	0.28-2.61	0.08	>0.05	-	-
Consumption of food items						
Sterile canned food	6.18	0.04-862.34	3.54	>0.05	-	-
Cooked vegetables	2.82	0.23-34.85	3.70	>0.05	-	-
Cooked eggs and egg preparations	1.97	0.04-106.07	0.61	>0.05	-	-
Pasta and cooked cereals	1.73	0.32-9.48	2.15	>0.05	-	-
Salami	1.18	0.14-9.68	0.13	>0.05	-	-
Milk and dairy products	1.00	0.2-5.04	0.00	>0.05	-	-
Cooked meat preparations	0.76	0.16-3.57	0.62	>0.05	-	-
Pizza, sandwiches, etc.	0.49	0.1-2.53	3.88	>0.05	-	-
Salads, fruits, raw vegetables	0.29	0.06-1.47	12.2	>0.05	-	-
Cooked fish preparations	0.25	0.03-1.93	10.2	>0.05	-	-
Croissants	0.18	0-26.86	2.97	>0.05	-	-

CI confidence interval

- not performed

Results

Epidemiological survey

The first gastroenteritis cases were identified in July 2003 by doctors at the holiday resort.

Infection spread rapidly and had a short incubation period. In total, 183 cases were identified (169 guests and 14 employees at the resort), showing acute gastroenteritis symptoms. Of the 169 cases among the resort

guests, 122 belonged to family clusters, ranging from 2 to 4 cases.

The most commonly reported symptoms were diarrhoea (77.0%), vomiting (68.9%), abdominal cramps (67.2%), nausea (62.8%), fever (42.1%) and headaches (38.3%). In 50% of cases the doctor was called; 12% of these cases sought assistance from the first aid station and 5% needed admission to hospital. The mean duration of symptoms was 3 days (range 1-30). Figure 2 shows the time distribution of cases.

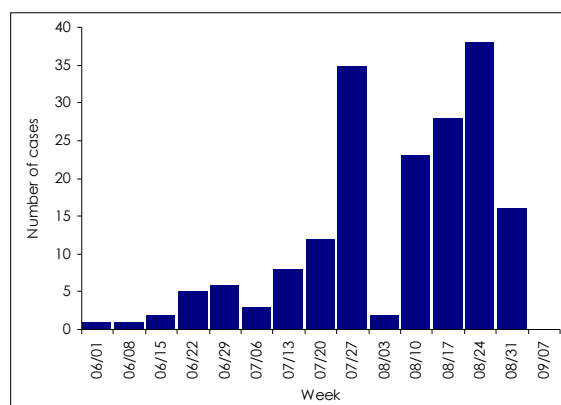


Figure 2
Epidemic curve of cases studied

The epidemics lasted three months, with the last case recorded on 4 September 2003. The epidemic curve shows two distinct peaks, each lasting approximately three weeks. To investigate the length of incubation, the cases that occurred during the epidemic peaks (since 10 July) were plotted as a cumulative percentage against the number of days since arrival at the resort (Fig. 3). Approximately two-thirds of cases presented symptoms within six days of their arrival.

Three risk factors (sea bathing, use of bungalow and chalet sanitary facilities, use of shared showers supplied with non-drinking water) were found to be significantly more frequent in the cases than in the controls and were therefore included in the final model generated by the logistic regression. The significant risk factors, their logistic regression coefficients and the 95% confidence intervals are presented in Table II.

Data on the consumption of various food items were compiled for 71 cases and 68 controls.

Some of these cases and controls were identified after interviews with other cases. On account of the delay, many were unable to remember what they ate before the onset of symptoms. The odds ratios and 95% confidence intervals for food items are given in Table II. The confidence intervals included value 1 in all cases; consequently, no significant association was found between food items and the disease.

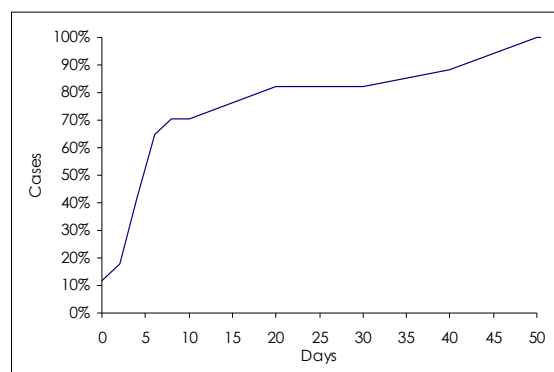


Figure 3
Cumulative percentage of cases against number of days since arrival in holiday village from 10 July 2003

Laboratory analyses on stool samples from patients

Stool samples were collected from 19 patients between 15 July and 4 September 2003, mainly at the beginning of the epidemic. Of these, 13 samples (68%) were found to be positive for *Norovirus*: 2 by PCR alone, 6 by ELISA and 5 using both techniques.

Most of the stool samples were collected at the beginning of the epidemic curve. Subsequently, both the identification of *Norovirus* as the likely agent and the consistency of clinical signs, and the discomfort of patients induced the physician involved to cease the active collection of samples.

Sequencing of these viruses was not possible due to the low amount of DNA amplified. RLBH hybridisation with genotype-specific probes was performed confirming 9 samples; 4 were genotyped as Birmingham, 1 as Lordsdale, 2 as Leeds and 2 samples were not identified.

Table II

Risk factors and food items associated with the presence of gastroenteritis in guests at a seaside resort in central Italy, a case-control study (June-September 2003)

Test	Results, positive/No. samples examined		
	Drinking water	Non-drinking water ^(a)	Swimming pool water
Microbial count at 36°C ^(a)	4/49 (8%)	8/20 (40%)	0/7
Microbial count at 22°C ^(b)	4/49 (8%)	9/20 (45%)	0/7
<i>Clostridium perfringens</i> ^(c)	0/49	5/20 (25%)	Not tested
Total coliforms ^(d)	0/49	8/19 (42%)	Not tested
<i>Escherichia coli</i> ^(e)	0/49	0/20	0/7
Enterococci ^(f)	2/49 (4%)	7/20 (35%)	0/7
<i>Pseudomonas aeruginosa</i> ^(g)	9/49 (18%)	5/20 (25%)	1/7 (14%)
<i>Salmonella</i> spp. ^(h)	0/13	1/17 (6%)	0/5
<i>Vibrio cholerae</i> ⁽ⁱ⁾	Not tested	0/9	Not tested
Coagulase-positive staphylococci (<i>Staphylococcus aureus</i> and other species) ^(j)	Not tested	Not tested	3/7 (43%)
Norovirus antigen ^(k)	Not tested	3/3 (100%)	Not tested

Limits of acceptability

- a) drinking water = 100 cfu/ml; swimming pool water = ≤100 cfu/ml
- b) drinking water = 100 cfu/ml; swimming pool water = ≤200 cfu/ml
- c) drinking water = 0 cfu/ml
- d) drinking water = 0 cfu/100 ml
- e) drinking water = 0 cfu/ml; swimming pool water = 0 cfu/100 ml
- f) drinking water = 0 cfu/100 ml; swimming pool water = 0 cfu/100 ml
- g) drinking water = 0 cfu/100 ml; swimming pool water = ≤1 cfu/100 ml
- h) drinking water = absent/100 ml; swimming pool water = absent/100 ml
- i) drinking water = absent/100 ml
- j) swimming pool water = ≤1 cfu/100 ml
- k) drinking water = sample volume examined

A *Campylobacter* strain was isolated in 1 of the 14 samples tested (7%) and 3 of 8 samples (38%) (all belonged to the 1-14 age group) tested positive for *Rotavirus*. All other microbiological tests gave negative results.

Environmental survey

Water distribution pipelines

Table I gives the results of microbiological tests conducted on the water samples that were evaluated according to the reference limits established for drinking water (2).

Noroviruses were present in all of the three non-drinking water samples examined, and

were identified as belonging to either genotype Lordsdale or Leeds.

The sodium fluorescein test immediately traced fluorescein to two bungalows. A subsequent analysis of the fluorescence traps set along the pipelines revealed the presence of fluorescein in a fountain supplied by the drinking water system and in two other bungalows, as shown in Table III.

As expected, the tracer was also found in all facilities that received the non-drinking water (Fig. 4).

Table III

Results of fluorescence trap analysis

Collection site	Units of fluorescence x 1 tracer	Quantity of sodium fluorescein in 10 g of fluorescence trap (µg)
Bungalow No. 420	26.5	0.149
Bungalow No. 626	2.9	0.016
Fountain	12.7	0.071



Figure 4
Fluorescein diffusion in water system

Ground and seawater

Microbiological testing of the groundwater revealed the presence of total coliforms in all samples examined, at concentrations varying from 100 to 2 000 cfu/100 ml; 78.9% of samples (15/19) were contaminated with *E. coli*, 57.9% (11/19 samples) with *Enterococci* (6-700 cfu/100 ml) and 15.8% (3/16) with *C. perfringens* (3-100 cfu/100 m) (Fig. 5). All well water samples and two seawater samples gave positive results for *Norovirus* (genotypes Lordsdale and Leeds).

Chemical tests of chloride, ammonia, nitrite and nitrate concentrations were also conducted on water sampled in different areas near the resort. The geographic distribution of these chemical residues is illustrated in Figure 6. Chloride ranged from 13 to 132 mg/l (mean 53.4 mg/l) and ammonia from 0 to 1.37 mg/l (mean 0.23 mg/l). Nitrites and nitrates were found at concentrations that ranged between 0 and 0.65 mg/l (mean 0.07 mg/l) and between 3 and 70 mg/l (mean 20.5 mg/l), respectively. Chloride, ammonia and nitrite levels were significantly higher in the wells near the sea shore, in particular near the river mouth, in

comparison with the wells that were situated further inland. A completely opposite trend was seen in the distribution of nitrates which revealed higher concentrations inland, decreasing towards the sea, in line with the change in landscape profile from agriculture to industry and tourism, the principal activities conducted on the territory (Fig. 6).

Measures proposed to prevent recurrence and results obtained

Since the direct involvement of water in the origin of outbreaks was strongly suggested by the data collected, a series of recommendations was made to the management of the holiday village. These included the following:

- eliminate connections and leaks between non-drinking and drinking water systems
- stop using well water and/or install efficient water purification systems
- clean the clear water pipeline, conduct routine maintenance and have a water quality surveillance plan
- prepare internal standard operating procedures for the management of *Norovirus* infections in holiday resorts (33, 35).

Additional recommendations were issued for the local health authority. The preventive measures suggested were implemented between 2004 and 2005. No epidemic of gastroenteritis was reported among holiday village guests during the 2006 summer holiday season.

Discussion

Laboratory testing of stool samples from the affected cases revealed that the following infectious agents were possibly involved: *Norovirus*, *Rotavirus* and *Campylobacter*.

The specific clinical signs, age of patients and occurrence of secondary cases support the conclusion that *Norovirus* was the most likely cause of the outbreak.

The rapid spread of the disease and the high rate of infection led to the hypothesis of a common source of infection, to which a large number of guests at the resort had been exposed simultaneously. The prolonged

duration of the epidemic was probably due to the persistence of the source of infection. The double peak observed in the epidemic curve was probably due to the change of guests at the resort between the end of July and

beginning of August, when the lowest number of new cases was recorded. Approximately two-thirds of patients presented symptoms within the first six days of their stay in the resort (Fig. 3).

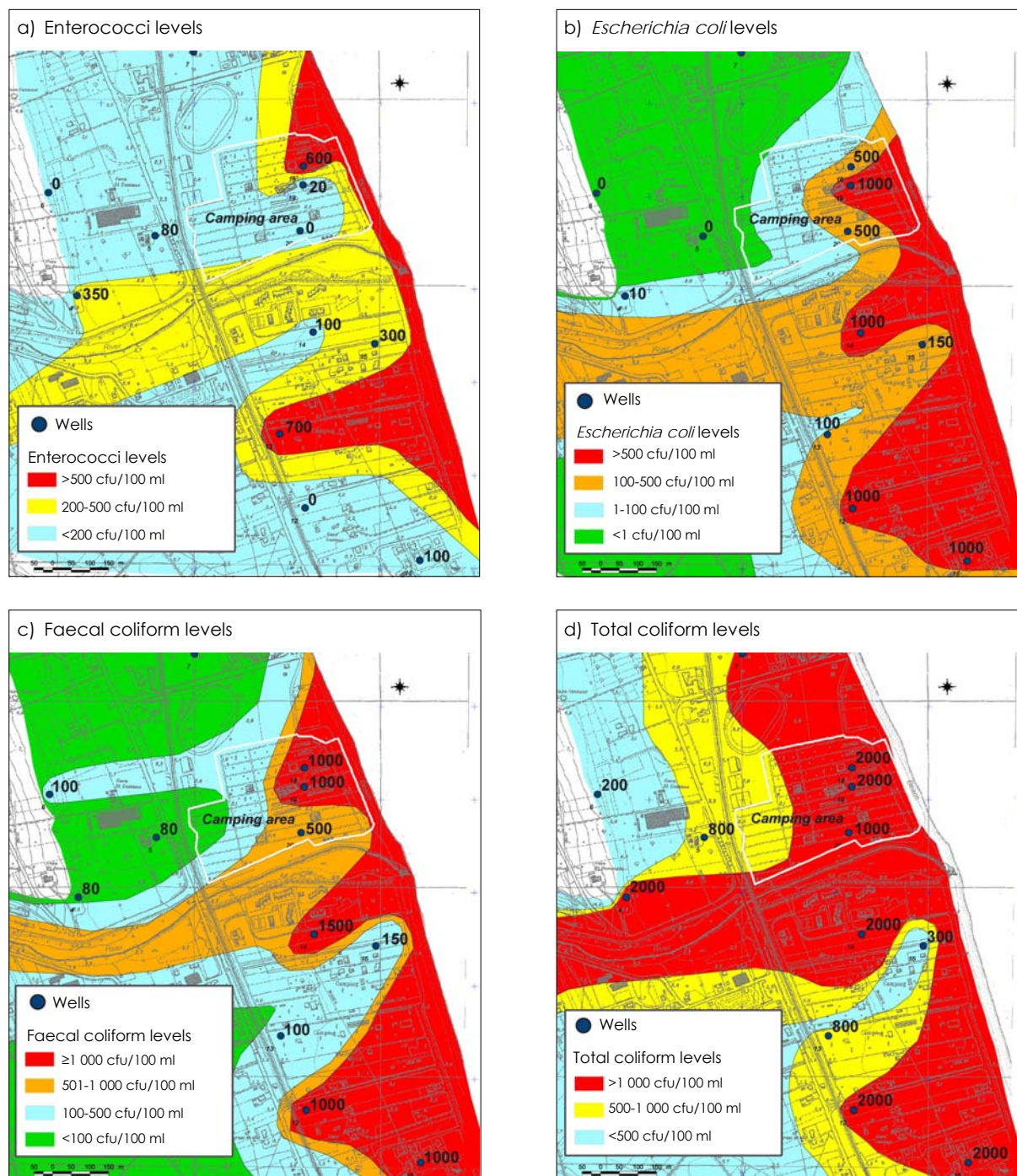


Figure 5 Geographic distribution of bacteriological contamination

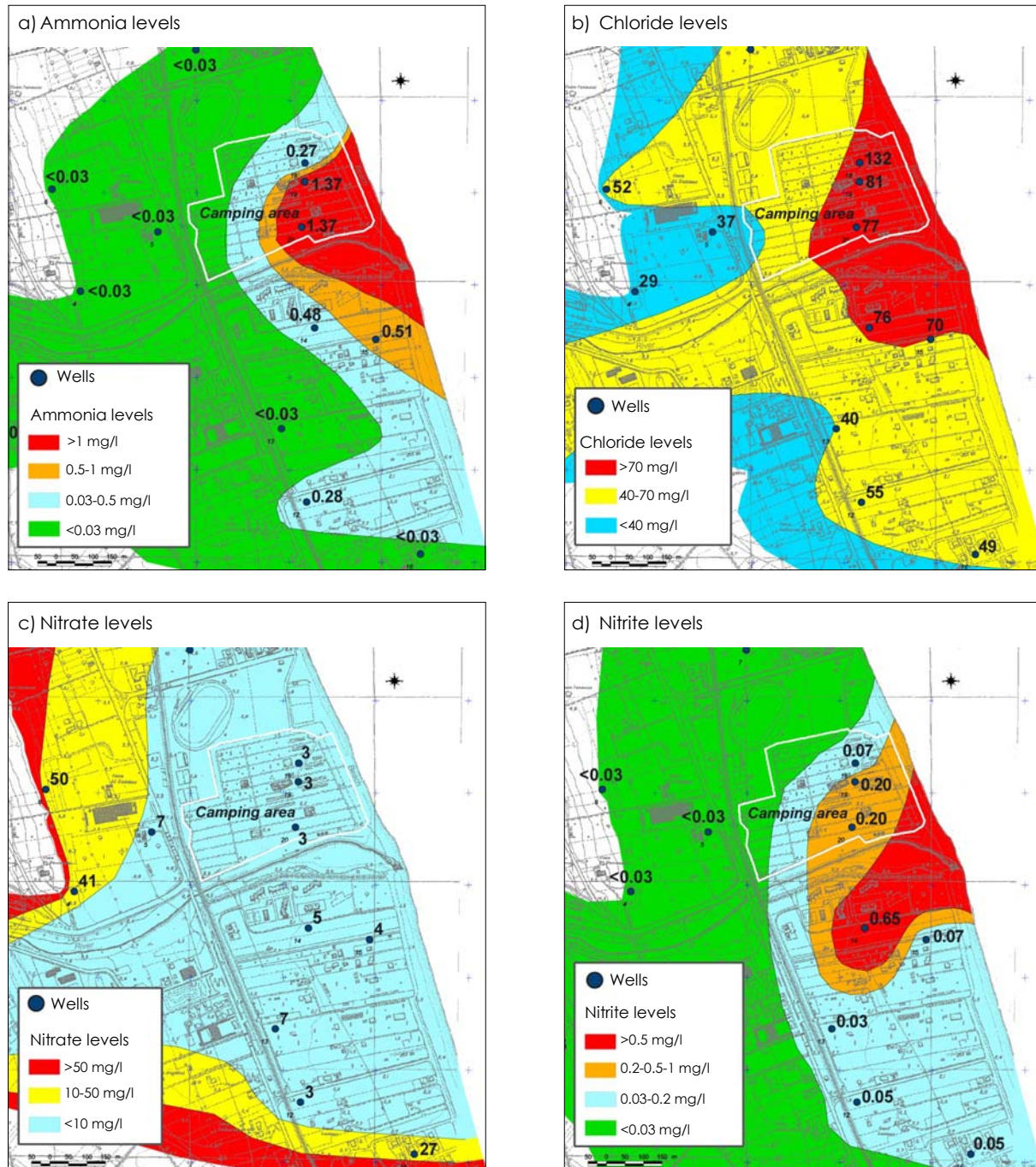


Figure 6
Geographic distribution of chemical residues

This is consistent with a rapid exposure to the source of infection of many guests, probably soon after their arrival.

The case-control study identified the existence of the following statistically significant risk factors: sea bathing, use of sanitary facilities in the bungalows and use of shared showers. Nygard *et al.* have reported that showering is

an efficient method of spreading infection (32). This may be due to the faecal contamination detected in the seawater and the well water that is used to supply the shared showers. Tests conducted on the well water samples revealed the presence of total coliforms, *Enterococci* and *Salmonella togba*, suggesting specific contamination with faecal material.

The correlation between infection and use of the sanitary facilities at the bungalows was probably a consequence of the subsequent contamination of these areas by infected individuals. This hypothesis is supported by the observation of a high frequency of family clusters of infection, as follows: of the 183 cases, 122 were grouped in family clusters. Faecal contamination was also found in the drinking water systems. This may have occurred through poor connections in the non-drinking water system in the resort or may be due to damaged pipelines. The discovery of the fluorescein tracer in water from the bungalow taps and in the fountains supplied with drinking water showed that the two systems were connected.

The presence of *Pseudomonas aeruginosa* confirmed the very deteriorated condition of the water distribution pipeline (11).

The groundwater that was responsible for contamination of both the drinking and non-drinking water systems, had probably been contaminated by illegal household spills and the infiltration of seawater. The latter is polluted by excrement from both coastal and inland houses, through the river that flows into the sea near the holiday village. The infiltration of seawater in the inland groundwater is demonstrated by the geographic distribution of chemical indicators of seawater (chlorides) and organic pollution (ammonia and nitrites), which follow the gradient of the coast towards the inland.

All well water samples and two seawater samples tested positive for *Norovirus*. Two of the three genotypes found in the patients were identified in these samples (Lordsdale and Leeds). It was not possible to establish why the Birmingham genotype – the most common in the samples from patients – was not found in the environmental samples.

The possibility that other agents transmitted by the faecal-oral route were responsible for some of the cases identified cannot be excluded, as demonstrated by the isolation of *Campylobacter* and the identification of *Rotavirus* in stool samples from patients.

In 2004, the holiday resort implemented the measures proposed to prevent the recurrence of gastroenteritis. Cases notified to the health services that were epidemiologically related to the holiday resort totalled 120 in 2003 and 1 in 2005. No cases were recorded in 2006.

The absence of any epidemic of gastroenteritis cases in campsite guests for 2006 demonstrates the efficacy of the activities suggested.

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