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'Evidence for waterborne origin of an extended mixed gastroenteritis outbreak in a town in Northern Greece, 2019'

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Abstract

We investigated a large gastroenteritis outbreak that occurred in Northern Greece in 2019. A case was defined as anyone presenting with diarrhoea and/or vomiting from 24 January 2019 to 04 February 2019. We conducted a case-control study (CCS) using random selection of participants >16 years of age, residents of town X, who visited the health care centre between 25 and 28 January 2019. Moreover, we conducted a retrospective cohort study (CS) at the four elementary schools of the town. We collected clinical and water samples and the water supply system was inspected. In total, we recorded 638 cases (53% female; median age was 44 years (range 0–93)). Forty-eight cases and 52 controls participated in the CCS and 236 students in the CS. Both CCS and CS indicated tap water as the most likely source (OR 10, 95% CI 2.09–93.4, explaining 95.7% of cases; RR = 2.22, 95% CI 1.42–3.46, respectively). More than one pathogen was detected from stool samples of 6 of the 11 cases tested (norovirus, *Campylobacter jejuni,* Enterohemorrhagic *E. coli* (EHEC) and Enteropathogenic *E. coli* (EPEC)). Water samples, collected after *ad-hoc* chlorination, tested negative. Technical failures of the water tanks' status were identified. Our results suggested a waterborne outbreak. We recommended regular monitoring of the water supply system and immediate repair of technical failures.

Introduction

In recent years, there has been an increase in the incidence of waterborne infections internationally. This is mainly due to the ageing of water and sewage systems in some countries and possibly, also to improved laboratory methods used for the detection of waterborne pathogens [1–3]. This increase has also been attributed to factors, such as overpopulation and urbanisation, mass migration and an increase of immunocompromised people in the population [4, 5].

Waterborne infections are of great public health importance. They can appear as sporadic cases but can go on to affect many people in a short time [6–8].

Most of the identified waterborne outbreaks (WBOs) in developed countries are caused by *Campylobacter jejuni*, norovirus, *Cryptosporidium* and *Giardia lamblia* [7, 9–14].

Between 2005 and 2018 EU Member States and reporting EU non-Member States reported 286 WBOs affecting more than 54 609 people [15]. In Greece, 32 WBOs were recorded between 2004 and 2018. Seven of them had more than 200 recorded cases each. A large WBO challenges the health care system in different geographical regions of Greece every 2–3 years [10, 16–18].

On 26 January 2019, health care services in Northern Greece informed the National Public Health Organization (EODY) on the occurrence of a high number of gastroenteritis cases. The event attracted wide media and public attention.

This study aims to present the results of the outbreak investigation and summarise the particular characteristics and lessons learned from its management.

Methods

Setting

Town X has 8903 permanent residents (last census of 2011) and a dedicated water supply system that is distinct from those of neighbouring towns.

Epidemiological investigation

Descriptive epidemiology

We collected information from patients presenting at the local general hospital (GH) and the health care centre (HCC) with diarrhoea and/or vomiting with the onset of symptoms from 24 January 2019 to 04 February 2019. Demographic characteristics (age, sex), date of symptoms' onset, clinical manifestations (diarrhoea, vomiting, bloody diarrhoea and fever), hospitalisation and place of residence were collected using a structured form. Descriptive data were analysed to generate hypotheses on the possible source of the outbreak.

Case-control study

We performed an unmatched 1:1 case-control study (CCS) from the list of visitors at the HCC. As most recorded cases were residents of town X, the study population was restricted to the residents of the town. Moreover, the study population was restricted to the population aged over 16 years of age because of the lack of a paediatrician at the HCC, as minors aged between 16 and 18 years of age are treated by general practitioners like adults, in Greece.

We selected cases and controls randomly from the registry of the HCC since all visitors HCC – along with the reason for their visit – are recorded there.

We defined a case as any resident of the town, older than 16 years of age who visited the HCC between 25 and 28 January 2019 presenting diarrhoea and/or vomiting and control as any resident of the town, older than 16 years of age that visited the HCC between 25 and 28 January 2019 for reasons other than gastroenteritis symptoms, for example prescribing, scheduled appointments, diagnostic examinations.

A list of all cases with gastroenteritis symptoms and a list of controls were acquired, individuals were numbered and after creating random numbers with Microsoft EXCEL, cases and controls were randomly selected.

To develop the questionnaire, we conducted preliminary interviews with several cases on-site using generic questionnaires regarding their habits, recent activities, participation to events etc. Based on the collected information, we formed the hypothesis of the probable waterborne origin of the outbreak. Taking into consideration the information collected from these cases and local authorities and in accordance with the questionnaires used in the literature for the investigation of similar outbreaks, the questionnaire was finalised. Public health nurses of the HCC conducted face-to-face interviews with cases and controls after obtaining their verbal informed consent. Interviews took place from 31 January 2019 to 03 February 2019.

Information on demographic characteristics, date of symptoms' onset, duration of illness and absenteeism from work were included in the questionnaire. All participants were asked whether they had participated in a social event, whether they had consumed specific food items, tap or bottled water at home and at work, and the daily number of consumed water glasses. For each case and control, we recorded the number of household contacts and the number of contacts who developed gastroenteritis symptoms after the 24 January 2019.

Norovirus was detected in the first clinical specimens tested. As norovirus was suspected as the causative organism questions addressed to cases concerned a 2-day period prior to the onset of symptoms. Controls were questioned about possible exposures on 24–25 January 2019, as most cases had reported 26 January 2019 as the day of their symptoms' onset.

Cohort study

We conducted a retrospective cohort study (CS) among the students of the four elementary schools of the town.

We defined a case as any student aged 6–12 years who presented vomiting and/or diarrhoea from 25 to 28 January 2019.

A structured questionnaire, similar to the one used in the CCS, was distributed to the students on 30 January 2019, to be completed by their parents or guardians. We asked students to return questionnaires the following day. The questions concerned the 2-day period prior to the onset of the symptoms for cases and the period 24–25 January 2019 for non-cases.

Ethical issues

The study protocols for CCS and CS were reviewed and approved by the Scientific Board of EODY.

Interviews were conducted after informed consent was obtained.

The questionnaires used in both CCS and CS were developed by the investigation team consisting of epidemiologists from EODY that visited on-site and the local public health authorities and were approved by the Scientific Board of EODY.

All collected data were anonymised and entered in a database by the members of the investigation team at the EODY's premises. Data processing and analysis were managed in accordance with national and European Union laws.

Statistical analysis

We entered data in EpiData Manager statistical package and performed statistical analysis using Stata v12. We presented quantitative variables as means ± standard deviations or medians and interquartile ranges (IQR) and qualitative variables as absolute frequencies and percentages. Associations between the categorical variables and the occurrence of gastroenteritis symptoms were quantified using contingency tables with the calculation of chi-square test or Fisher's exact test. We calculated odds ratios (ORs) and 95% confidence intervals (CI) for the CCS and risk ratios (RR) for the CS. *P*-values <0.05 were considered as statistically significant. Stratification by consumption of tap water was conducted for variables statistically significant at univariate analysis.

Laboratory testing of clinical samples

We collected stool samples from patients with gastroenteritis symptoms that visited the health care services and sent to the Regional Public Health Laboratory (PEDY) of Thessaly. Samples were tested for bacteria (*Salmonella* spp., *Shigella* spp., *Campylobacter* spp., *Yersinia enterocolitica, Escherichia coli O157*) by standard culture methods.

All stool samples were also tested by polymerase chain reaction (PCR) for *Campylobacter* spp. Detection. Where non-fermenting sorbitol *E. coli* strains were isolated, H7:O157 serotype was confirmed by PCR, where fliCh7 and *rfbO157* genes were targeted for the detection of O antigen and flagellar antigen of *E. coli* O157:H7. All non-fermenting sorbitol *E. coli* strains were tested for the presence of eae, stx1 and stx2 toxin-producing genes. For the nucleic acid extraction, the iPrepTM Invitrogen Purification Instrument (Thermo Fisher Scientific) with the iPrep PureLink Invitrogen Virus Kit (Thermo Fisher Scientific)

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Table 1. Primers, their sequence and use for the laboratory investigation of the waterborne outbreak, town in Northern, Greece, January–February 2019

Code	Target	Sequence	Use
JV12Y	ORF1 region	5′-ATACCACTATGATGCAGAYTA-3′	NoV detection and ORF1 region (partial RdRp) sequencing
JV13I	ORF1 region	5′-TCATCATCACCATAGAA[I]GAG-3′	NoV detection and ORF1 region (partial RdRp) sequencing
GIG1SK F	ORF2 region	5′-CTGCCCGAATTYGTAAATGA-3′	NoV GI detection and ORF2 region (capsid) sequencing
GIG1SK R	ORF2 region	5′-CCAACCCARCCATTRTACA-3′	NoV GI detection and ORF2 region (capsid) sequencing
GIIG2SK F	ORF2 region	5′-CARGARBCNATGTTYAGRTGGATGAG-3′	NoV GII detection and ORF2 region (capsid) sequencing
GIIG2SK R	ORF2 region	5′-CCRCCNGCATRHCCRTTRTACAT-3′	NoV GII detection and ORF2 region (capsid) sequencing
*rfb*O157 F	O antigen in *E. coli* O157:H7	5′-GGATGACAATATCTGCGCTGC-3′	Detection of O antigen in *E. coli* O157:H7
*rfb*O157 R	O antigen in *E. coli* O157:H7	5′-GGTGATTCCTTAATTCCTCTCTTTCC-3′	Detection of O antigen in *E. coli* O157:H7
flicH7F	flagellar antigen in *E. coli* O157:H7	5′-GCG CTG TCG AGT TCT ATC GAG C-3′	Detection of flagellar antigen in *E. coli* O157:H7
flicH7R	flagellar antigen in *E. coli* O157:H7	5′-CAA CGG TGA CTT TAT CGC CAT TCC-3′	Detection of flagellar antigen in *E. coli* O157:H7
stx1F	A subunit region of *stx*1	5′-ATAAATCGCCATTCGTTGACTAC-3′	Detection of *stx*1 gene
stx1R	A subunit region of *stx*1	5′-AGAACGCCCACTGAGATCATC-3′	Detection of *stx*1 gene
stx2F	A subunit coding region of *stx*2	5′-GGCACTGTCTGAAACTGCTCC-3′	Detection of *stx*2 gene and variants
stx2R	A subunit coding region of *stx*2	5′-TCGCCAGTTATCTGACATTCTG-3′	Detection of *stx*2 gene and variants
eaeAF	*eaeA* region	5′-GACCCGGCACAAGCATAAGC-3′	Detection of region conserved in EPEC and STEC strains
eaeAR	*eaeA* region	5′-CCACCTGCAGCAACAAGAGG-3′	Detection of region conserved in EPEC and STEC strains
cadF	*cadF* gene of *Campylobacter* genus	5′-TTGAAGGTAATTTAGATATG-3′	Detection of *Campylobacter* spp.
cadR	*cadF* gene of *Campylobacter* genus	5′-CTAATACCTAAAGTTGAAAC-3′	Detection of *Campylobacter* spp.

was used and for the PCR assays the Phusion High-Fidelity DNA Polymerase (Thermo Fisher Scientific).

Reverse transcriptase PCR (RT-PCR) for norovirus detection was performed, using the Super Script III One-Step qRT-PCR System with Platinum Taq Polymerase kit (Invitrogen). All PCR reactions were performed in a validated Applied Biosystems Verity 96 well Thermal Cycler. Norovirus positive samples were sequenced and sequence identity was determined using RIVM Noronet, Norovirus Genotyping Tool version 2.0 [19]. The primers and the PCR conditions used for the performed molecular analyses are provided in detail in Tables 1 and 2, respectively [20–23].

Stool samples were also tested using a multiplexed molecular assay, the BioFire FilmArray Gastrointestinal (GI) Panel, targeting 22 different pathogens in a single reaction; Enterotoxigenic *E.coli* (ETEC), Enteropathogenic *E. coli* (EPEC), Enteroaggregative *E. coli* (EAEC) and Shiga-like toxin-producing *E. coli* (STEC) with specific identification of *E. coli* O157, Enteroinvasive *E.coli*/*Shigella* (EIEC), *Campylobacter* (jejuni/coli/upsaliensis), Vibrio (parahaemolyticus/vulnificus/cholerae), *Yersinia enterocolitica*, Plesiomonas shigelloides, *Clostridium difficile* (Toxin A/B), *Salmonella*, *Cryptosporidium*, Cyclospora cayetanensis, Entamoeba histolytica, Giardia lamblia, Adenovirus F40/41, Astrovirus, Norovirus GI/GII, Rotavirus A and Sapovirus (genogroups I,II,IV and V) [24].

Environmental investigation and sampling

We collected information on the town's water supply, water chlorination and drainage system, as well as the proximity of livestock holdings to water abstraction points and storage and obtained the municipality chlorination records and records of technical works carried out at the water distribution system since the beginning of December 2018.

The local authorities performed water sampling from the water supply system on 25 and 26 January 2019. Local public health authorities also performed water sampling on 26 January 2019 from the system and one of the town's springs. All water samples were tested for microbiological indicators at PEDY; total aerobic count at 22 °C and 37 °C (ISO 6222:1999), number of total coliforms colonies per 100 ml and number of *E. coli* colonies per 100 ml (ISO 9308-01:2000), number of *Enterococci* spp. colonies per 100 ml (ISO 7899-02: 2000) and the number of *Clostridium perfringens* colonies per 100 ml (ISO 14189:2013).

In addition, 10 l of water sampled from the water supply system and the spring by the local public health authority on 26 January 2019 and were molecularly tested for norovirus at PEDY. The procedure for the concentration of the viruses was based on the elution of the viruses with glycine-alkaline buffer followed by organic flocculation with skimmed-milk, as described

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Table 2. PCR conditions for all molecular analyses performed during the investigation of the waterborne outbreak, town in Northern, Greece, January–February 2019

Molecular analysis	PCR conditions
NoV detection ORF1 region (partial RdRp) definition	42 °C for 60 min 94 °C for 180 s 40 cycles at 94 °C for 60 s 37 °C for 90 s 68 °C for 60 s
Genogroup assignment and ORF2 region (capsid) definition	45 °C for 20 min 95 °C for 60 s 40 cycles at 95 °C for 10 s 60 °C for 15 s 72 °C for 30 s 72 °C for 10 min
Escherichia coli O157:H7 detection (*fliCh7* and *rfbO157* genes detection)	95 °C for 2.5 min 35 cycles at 94 °C for 30 s 58 °C for 1 min 72 °C for 1 min 72 °C for 5 min
Detection of *stx*1, *stx*2 gene and *eaeA* region	10 cycles at 95 °C for 1 min 65 °C for 2 min 72 °C for 1.5 min 14 cycles at 95 °C for 1 min 60 °C for 2 min 72 °C for 1.5 min 9 cycles at 95 °C for 1 min 60 °C for 2 min 72 °C for 2.5 min
Detection of *Campylobacter* spp. (*cadF* gene detection)	95 °C for 6 min 38 cycles at 95 °C for 45 s 50 °C for 1 min 72 °C for 1 min 72 °C for 10 min

by Calgua *et al.* [25]. After sample concentration, nucleic acid extraction followed using the iPrepTM Invitrogen Purification Instrument (Thermo Fisher Scientific) with the iPrep PureLink Invitrogen Virus Kit (Thermo Fisher Scientific) and subsequently, RT-PCR for norovirus detection was performed, using the Super Script III One-Step qRT-PCR System with Platinum Taq Polymerase kit (Invitrogen). Similar to the clinical samples, the PCR reactions were performed in a validated Applied Biosystems Verity 96 well Thermal Cycler. The primers used and the PCR conditions are included in Tables 1 and 2 [20–23].

On 5 February 2019, the local public health authority inspected the water supply system and measured the residual chlorine at the pumping station, the tanks and various points of the town's water distribution network. Local weather reports were reviewed for January 2019 [26].

Results

Descriptive analysis

In total, we recorded 638 gastroenteritis cases with symptoms onset from 25 January 2019 to 04 February 2019. Almost half of them (53%) were female and the median age was 44 years (range: 0–93). The majority (79%) were residents of town X. The first gastroenteritis cases appeared early on Friday, 25 January 2019, with increasing numbers of cases recorded between 25 January 2019 and 29 January 2019. Most cases had an onset of symptoms on 26 January 2019. From 27 January 2019, numbers of cases decreased, with the last case occurring on 4 February 2019 (Fig. 1).

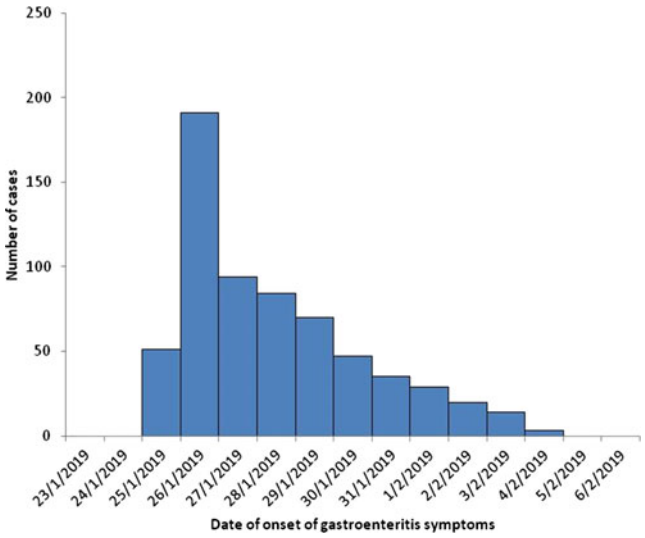

Fig. 1. Number of gastroenteritis cases by date of onset of symptoms ($n = 638$), health care centre and general hospital, January–February 2019.

The main reported symptoms were diarrhoea (89%) and vomiting (86%). Hospitalisation, mainly short stay, was required for 430 (80%) patients; 183 (43%) cases were hospitalised at the GH and 247 (57%) at the HCC to receive fluids intravenously usually for some hours. We did not record any death.

Case-control study

We selected 50 cases and 50 controls. We excluded two cases (4%) that did not meet the case definition. The median age of cases and controls was 51 (range: 23–78) and 56 (range:25–87) years old, respectively. Sex distribution among cases and controls was similar (45% and 55% of cases and controls, respectively, were females) (P-value = 0.3).

Cases reported diarrhoea (92%), fatigue (89%), vomiting (79%), arthralgia (77%), abdominal pain (68%), nausea (52%) and fever (>38 °C) (37%). The median duration of symptoms was 4 days (range: 3–5 days).

In total, 22 of the 31 (71%) cases, that the respective information was available, reported absence from work for at least 1 day (median: 2 days; range: 1–3 days).

According to the results of the univariate analysis, cases were more likely to consume tap water in the 2 days before symptoms onset (OR 10.6, 95% CI 2.20–99.0) and were more likely to use tap water to produce ice cubes (OR 3.7, 95% CI 1.24–12.0) (Table 3). A total of 45 of the 47 cases (96%) reported consuming tap water. Cases were less likely to consume bottled water (OR 0.16, 95% CI 0.02–0.80). No dose-response effect was observed and the likelihood of being a case did not increase with the number of glasses of tap water consumed. No food consumption or common activity had a statistically significant association with the occurrence of gastroenteritis symptoms.

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Table 3. Results of the univariate analysis for the consumption of tap water, various foods and drinking water consumption estimators (proxies), case-control study, town in Northern, Greece, January–February 2019

	Cases		Controls				
Risk factor	*n*	(%)	*n*	(%)	OR	95% CI[a]	*P*-value
Consumption of tap water	45/47	96	34/50	68	10.59	2.20–98.96	0.001
Consumption of bottled water	2/45	4	11/48	23	0.16	0.02–0.80	0.010
Use of water for fruit/vegetable washing	45/47	96	44/50	88	3.07	0.51–32.33	0.166
Use of water for ice cube preparation	17/43	40	7/47	15	3.74	1.24–12.04	0.008
Use of water for juice dilution	6/35	17	3/47	6	3.03	0.58–19.96	0.123
Use of water filter	11/47	23	11/50	22	1.08	0.37–3.13	0.869
Consumption of milk	19/44	43	12/46	26	2.15	0.81–5.79	0.088
Consumption of cold cuts	17/47	36	11/43	26	1.81	0.67–4.97	0.197
Consumption of seafood	0/47	0.0	2/46	4	0.00	0.00–1.87	0.148
Consumption of fresh vegetables	40/47	85	35/47	74	1.96	0.62–6.52	0.199
Consumption of pasta/rice	22/44	50	27/41	66	0.52	0.20–1.36	0.139
Consumption of fish	2/43	5	5/47	11	0.41	0.04–2.70	0.289
Consumption of dairy products	30/44	68	27/45	60	1.43	0.55–3.75	0.421
Consumption of eggs	16/45	36	18/42	43	0.74	0.28–1.90	0.486
Consumption of sweet	13/43	30	10/42	24	1.39	0.48–4.10	0.505
Consumption of canned food	1/44	2	2/46	4	0.51	0.01–10.24	0.584
Consumption of meat	16/45	36	16/43	37	0.93	0.36–2.43	0.872
Consumption of chicken	21/45	47	20/44	45	1.05	0.42–2.63	0.909
Consumption of snacks	5/43	12	5/42	12	0.97	0.21–4.61	0.968
Consumption of fruits	42/47	89	42/47	89	1.00	0.21–4.69	1.000
Consumption of shellfish	0/44	0	0/48	0	.		
Event participation	1/47	2	1/50	2	1.00	0.01–85.30	0.965

[a]OR, odds ratio; CI, confidence interval.

Stratification by consumption of tap water for bottled water and the use of tap water for making ice cubes did not show a confounding or effect modification.

Of the 137 persons who lived in the same house as a case, 79 (58%) also developed gastroenteritis symptoms at the time of the interview.

Cohort study

Of 452 students enrolled at the four schools studied, 236 participated in the study (response rate: 55%); 123 (53%) were girls and 123 met the case definition (attack rate: 53%). Age had a statistically significant association with being a case (P-value < 0.001).

Reported symptoms were vomiting (84%), abdominal pain (81%), fatigue (72%), diarrhoea (68%), nausea (54%), fever (>38 °C) (39%) and arthralgia (24%). The median duration of symptoms was 2 days (range: 2–3 days).

Of the total, 71% of the cases were absent from school for at least 1 day; the median duration of absence was 2 days (range: 1–3 days). The total number of lost school days among the 123 cases was 162.

In the univariate analysis, the tap water consumption within 2 days prior to the onset of the symptoms was associated with gastroenteritis (RR = 2.22, 95% CI 1.42–3.46) (Table 4). The consumption of bottled water had a negative effect (RR = 0.49, 95% CI 0.34–0.71). We were not able to detect an association between the daily number of consumed glasses of tap water and illness. No food consumption or activity was found to be associated with being a case.

After adjusting for age, the occurrence of gastroenteritis symptoms was statistically significantly associated with tap water consumption (OR 4.4, 95% CI 2.15–9.07).

Out of the 344 household contacts, 260 (76%) had also developed gastroenteritis symptoms by the time of responding to the questionnaire.

Laboratory results of clinical samples

More than one pathogen was detected in six of the 11 stool samples tested (Table 5); four were positive for two and two were positive for three pathogens each. *Campylobacter* spp. was the most commonly identified pathogen (found in eight clinical samples), followed by norovirus (found in five). *Campylobacter* spp. was detected together with various pathogens, mostly with diarrheagenic *E.coli*. Two norovirus strains were sequenced and belonged

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Table 4. Results of the univariate analysis for the consumption of tap water, various foodstuffs and tap water estimators (proxies), cohort study, town in Northern Greece, January–February 2019

	Cases		Non- cases				
Risk factor	*n*	(AR%)	*n*	(AR%)	RR	95% CI[a]	*P*-value
Consumption of tap water	104/169	62	15/54	28	2.22	1.42–3.46	<0.001
Consumption of bottled water	23/72	32	90/139	65	0.49	0.34–0.71	<0.001
Use of water for fruit/vegetable washing	110/196	56	4/10	40	1.40	0.65–3.03	0.317
Use of water for ice cube preparation	25/51	49	82/148	55	0.88	0.65–1.21	0.430
Use of water for juice dilution	4/9	44	103/190	54	0.82	0.39–1.72	0.566
Use of filter	31/50	62	84/157	54	1.16	0.89–1.51	0.292
Consumption of milk	90/157	57	23/41	56	1.02	0.76–1.38	0.888
Consumption of cold cuts	44/94	47	70/110	64	0.74	0.57–0.95	0.016
Consumption of seafood	6/9	67	108/193	56	1.19	0.74–1.92	0.527
Consumption of fresh vegetables	60/105	57	50/88	57	1.01	0.79–1.29	0.964
Consumption of pasta/rice	76/127	60	34/65	52	1.14	0.87–1.50	0.318
Consumption of fish	10/17	59	104/184	57	1.04	0.69–1.58	0.855
Consumption of dairy products	70/121	58	42/74	57	1.02	0.79–1.31	0.881
Consumption of eggs	38/71	54	79/134	59	0.91	0.70–1.18	0.455
Consumption of sweet	49/87	56	61/105	58	0.97	0.76–1.24	0.805
Consumption of canned food	9/16	56	99/175	57	0.99	0.63–1.56	0.980
Consumption of meat	68/116	59	47/85	55	1.06	0.83–1.35	0.638
Consumption of chicken	30/61	49	82/139	59	0.83	0.62–1.11	0.198
Consumption of snacks	28/50	56	79/137	58	0.97	0.73–1.29	0.839
Consumption of fruits	75/139	54	38/58	66	0.82	0.65–1.05	0.135
Consumption of shellfish	0/2	0	114/199	57	0,00	–	0.104

[a]RR, relative risk; CI: confidence interval.

to the GII.Pe-GII.4_Sydney_2012 genotype (GenBank accession No MN923214-MN923215).

Environmental investigation results

The springs supplying the town also provided water to other areas where gastroenteritis cases were not reported. From the springs, water is channelled to the autonomous water supply system of the town. This system consists of a pumping station where chlorination takes place. Four pumps fill two two-room tanks and a conduit, which is branched into two parallel conduits, spaced about 10 m apart.

Residual chlorine levels are monitored weekly by the municipality. From 01 December 2018 to 24 January 2019 residual chlorine ranged between 0.10 mg/l and 0.20 mg/l. On 25 January 2019, eight measurements of the residual chlorine were carried out, with values ranging from 0.10 mg/l to 0.50 mg/l. Four of these measurements were conducted before the *ad-hoc* chlorination that was ordered by the municipality authorities later on the same day. This response measure led to an increase of the residual chlorine content to 0.50 mg/l in the water reservoir for 24 h. However, when residual chlorine was measured at different points of the town's water supply system during the inspection by the public health inspectors, the values ranged from 0.07 mg/l (at a point of the water distribution network) to 0.31 mg/l (entrance of water in one of the tanks).

The low levels of residual chlorine at certain distribution points could not be supported by any record of missed chlorination events at the pumping station. Technical municipal records also did not report any malfunctions since early January 2019.

However, an inspection of the water supply system revealed technical failures; disintegration of the cement, especially on the tank roofs, a lack of a protective surrounding enclosure in one of the tanks and a lack of appropriate protective equipment on the windows of both the pumping station and the tanks.

Microbiological testing of water samples collected by the municipality were negative.

Six water samples were collected on 26 January 2019 by the public health inspectors after ad-hoc chlorination. The five samples collected from the water supply network were negative for all the indicators while the sample from the spring was positive for *E. coli* (<4 cfu/100 ml) and total coliforms (15 cfu/100 ml). Molecular testing of spring water for norovirus was negative.

Finally, there were no livestock holdings close to the springs or tanks' area and the wastewater pipeline was not in close proximity with the drinking-water pipeline. Almost all town residences were connected to the public sewage system. There was no recorded extreme weather event in the area in January 2019.

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Table 5. Summary of laboratory results of stool samples collected from patients with gastroenteritis symptoms ($n = 11$), town in Northern Greece, January–February 2019

	Symptoms' onset	Date of sampling	Health Care Service	Laboratory result
1	26 January 2019	26 January 2019	GH[a]	Norovirus
2	26 January 2019	26 January 2019	GH	Norovirus & *Campylobacter* spp.
3	28 January 2019	30 January 2019	GH	Negative
4	29 January 2019	30 January 2019	GH	*E. coli O157*
5	25 January 2019	30 January 2019	HCC[b]	Norovirus & *Campylobacter jejuni* & *E. coli O157* (stx1-positive)
6	27 January 2019	30 January 2019	HCC	Norovirus & *Campylobacter* spp.
7	29 January 2019	30 January 2019	HCC	*Campylobacter* spp.
8	28 January 2019	30 January 2019	GH	*Campylobacter jejuni* & *E. coli O157* (stx1-positive)
9	28 January 2019	30 January 2019	HCC	Norovirus & *Campylobacter jejuni* & *EPEC*
10	29 January 2019	30 January 2019	HCC	*Campylobacter jejuni*
11	29 January 2019	30 January 2019	HCC	*Campylobacter* spp. & *EPEC*

[a]GH, general hospital; [b]HCC, health care centre of the town.

Discussion

Between 25 and 29 January 2019, we identified a sudden increase in the occurrence of gastroenteritis cases, with a peak on 26 January 2019 in a town of Northern Greece.

The results of the outbreak investigation point to the possible waterborne source. As well as epidemiological evidence, a waterborne source is also supported by the outbreak's size, the absence of a common activity or food item consumption among cases and the fact that there was no recorded increase of gastroenteritis cases in nearby areas/villages with a different water supply system.

The significance of the epidemiological investigation of this outbreak arises from the fact that laboratory confirmation was not possible.

In this outbreak, we recorded 638 cases. However, the actual burden of the disease was much higher given the estimated attack rate among students in the CS and the high proportion of symptomatic household contacts of cases in the CCS and the CS. High attack rates were recorded in previous WBOs, however, the total cost for the local community in such outbreaks cannot be easily estimated [10, 16–18, 27, 28]. In this outbreak, the hospitalisation rate and the number of consultations depicted the burden for the local HCSs, while absenteeism from work and school indicated a high indirect cost [29, 30]. However, the overall duration of the outbreak was relatively short, possibly due to the implemented measures.

The identification of the mixed aetiology of the outbreak supported the hypothesis for possible faecal contamination of the water. Verification of the aetiological agent of WBOs is challenging as multi-pathogen outbreaks are common and adequate laboratory capacity for a variety of pathogens (bacteria, viruses, parasites) is required [6, 31, 32]. In this respect, the added value of molecular methods for the detection of multiple pathogens in a single test, with the significantly reduced turnaround time for accurate results should be emphasised. Moreover, the high probability of mixed aetiology of WBOs and the different incubation period of the pathogens potentially involved may indicate the need to continue testing of samples even after the initial detection of a pathogen from the first clinical specimens.

Mixed origin is suggestive in that water may have become contaminated with faeces from sewage, rather than from a single person. Most large WBOs occur after environmental contamination of the water distribution system combined with the failure of chlorination systems and the inadequate maintenance of the system [3, 13]. The present outbreak investigation did not lead to a definitive conclusion on how water might have become contaminated and whether the faecal contamination was of human or animal origin. Technical failures related to the status of the water tanks and their enclosure allow us to assume that contamination of the water in the tanks due to the access of animals or unauthorised persons might be possible. Moreover, low levels of residual chlorine found during the inspection could possibly imply deficiencies in water sanitation practices.

The main limitation of the outbreak investigation was that laboratory investigation was not optimal overall. Water samples were collected after *ad hoc* chlorination and the waterborne origin of the outbreak was not confirmed. Furthermore, water samples were not tested for parasites due to the lack of a dedicated laboratory for testing of parasites in water samples. However, the patients' clinical manifestations and duration of symptoms were not compatible with parasitic infections [33].

In conclusion, this possible WBO demonstrates the impact of the contamination of the public water supply system on the local community and the health care services, the added value of analytical epidemiology in the investigation, the need for testing for a variety of pathogens when a waterborne source is suspected and the importance of having standard operational procedures for water sampling [34].

This outbreak led to an increased awareness in the local authorities on the measures needed to ensure water safety including upgrading the water supply system, continuous monitoring for early detection of faults, systematic water quality control appropriate treatment processes and protection during storage and distribution of water.

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References

1. **Yang K *et al.*** (2012) Global distribution of outbreaks of water-associated infectious diseases. *PLoS Neglected Tropical Diseases* **6**, e1483.
2. **Pons W *et al.*** (2015) A systematic review of waterborne disease outbreaks associated with small non-community drinking water systems in Canada and the United States. *PLoS One* **10**, e0141646.
3. **Moreira NA and Bondelind M** (2017) Safe drinking water and waterborne outbreaks. *Journal of Water & Health* **15**, 83–96.
4. **Eisenberg JN *et al.*** (2007) Environmental determinants of infectious disease: a framework for tracking causal links and guiding public health research. *Environmental Health Perspectives* **115**, 1216–1223.
5. **Semenza JC and Menne B** (2009) Climate change and infectious diseases in Europe. *The Lancet Infectious Diseases* **9**, 365–375.
6. **Craun MF *et al.*** (2006) Waterborne outbreaks reported in the United States. *Journal of Water & Health* **4**(suppl. 2), 19–30.
7. **Gallay A *et al.*** (2006) A large multi-pathogen waterborne community outbreak linked to faecal contamination of a groundwater system, France. *Clinical Microbiology and Infection* **12**, 561–570.
8. **Robertson LJ *et al.*** (2004) *Cryptosporidium parvum* infections in Bergen, Norway, during an extensive outbreak of waterborne giardiasis in autumn and winter 2004. *Applied and Environmental Microbiology* **72**, 2218–2220.
9. **Jakopanec I *et al.*** (2008) A large waterborne outbreak of campylobacteriosis in Norway: the need to focus on distribution system safety. *BMC Infectious Diseases* **8**, 128.
10. **Karagiannis I *et al.*** (2010) A waterborne *Campylobacter jejuni* outbreak on a Greek island. *Epidemiology and Infection* **138**, 1726–1734.
11. **Hewitt J *et al.*** (2007) Gastroenteritis outbreak caused by waterborne norovirus at a New Zealand ski resort. *Applied and Environmental Microbiology* **73**, 7853–7857.
12. **Riera-Montes M *et al.*** (2011) Waterborne norovirus outbreak in a municipal drinking-water supply in Sweden. *Epidemiology and Infection* **139**, 1928–1935.
13. **Semenza JC and Nichols G** (2007) Cryptosporidiosis surveillance and water-borne outbreaks in Europe. *Eurosurveillance* **12**, E13–E14.
14. **Daly ER *et al.*** (2010) Outbreak of giardiasis associated with a community drinking-water source. *Epidemiology and Infection* **138**, 491–500.
15. **European Union Summary reports on trends and sources of zoonoses, zoonotic agents and foodborne outbreaks for the years 2005-2018**. Available at https://efsa.onlinelibrary.wiley.com/loi/18314732 (Accessed 24 February 2020).
16. **Mellou K *et al.*** (2013) Epidemiological investigation of two parallel gastroenteritis outbreaks in school settings. *BMC Public Health* **13**, 241.
17. **Mellou K *et al.*** (2014) A large waterborne gastroenteritis outbreak in central Greece, March 2012: challenges for the investigation and management. *Epidemiology and Infection* **142**, 40–50.
18. **Vantarakis A *et al.*** (2011) A gastroenteritis outbreak caused by noroviruses in Greece. *International Journal of Environmental Research and Public Health* **8**, 3468–3478.
19. **Norovirus Genotyping Tool- RIVM**. Available at https://www.rivm.nl/mpf/typingtool/norovirus/ (Accessed 24 February 2020).
20. **Sai L *et al.*** (2013) Clinical and molecular epidemiology of norovirus infection in adults with acute gastroenteritis in Ji'nan, China. *Archives of Virology* **158**, 2315–2322.
21. **Gordillo R *et al.*** (2011) Development of PCR assays for detection of *Escherichia coli* O157:H7 in meat products. *Meat Science Journal* **88**, 767–773.
22. **Paton AW and Paton JC** (1998) Detection and characterization of Shiga toxigenic *Escherichia coli* by using multiplex PCR assays for stx1, stx2, eaeA, enterohemorrhagic E. coli hlyA, rfbO111, and rfbO157. *Journal of Clinical Microbiology* **36**, 598–602.
23. **Harzandi N *et al.*** (2015) Molecular detection and speciation of Campylobacter species in children with gastroenteritis using polymerase chain reaction in Bahonar Hospital of Karaj City. *International Journal of Enteric Pathogens* **3**, 1–4.
24. **BioFire**. A BioMerieux company. E-document Center. Available at https://www.online-ifu.com/ITI0009 (Accessed 24 September 2020).
25. **Calgua B *et al.*** (2013) New methods for the concentration of viruses from urban sewage using quantitative PCR. *Journal of Virological Methods* **187**, 215–221.
26. **National Meteorological Service**. Significant Weather and Climatic Events in Greece. Available at http://www.hnms.gr/emy/el/climatology/climatology_extreme (Accessed 24 February2020).
27. **Belliot G *et al.*** (2014) The burden of norovirus gastroenteritis: an important foodborne and healthcare-related infection. *Clinical Microbiology and Infection* **20**, 724–730.
28. **Widerström M *et al.*** (2014) Large outbreak of *Cryptosporidium hominis* infection transmitted through the public water supply, Sweden. *Emerging Infectious Diseases* **20**, 581–589.
29. **Baker EL *et al.*** (1979) Economic impact of a communitywide waterborne outbreak of gastrointestinal illness. *American Journal of Public Health* **69**, 501–502.
30. **Chyzheuskaya A *et al.*** (2017) Economic assessment of waterborne outbreak of cryptosporidiosis. *Emerging Infectious Diseases* **23**, 1650–1656.
31. **O'Reilly CE *et al.*** (2007) Outbreak working group, a waterborne outbreak of gastroenteritis with multiple etiologies among resort island visitors and residents: Ohio, 2004. *Clinical Infectious Diseases* **44**, 506–512.
32. **Parasidis T *et al.*** (2008) Outbreak of gastroenteritis occurred in North-Eastern Greece associated with several waterborne strains of noroviruses. *International Journal of Infectious Diseases* **12**, E104–E105.
33. **Abeywardena H, Jex AR and Gasser RB** (2015) A perspective on Cryptosporidium and Giardia, with an emphasis on bovines and recent epidemiological findings. *Advances in Parasitology* **88**, 243–301.
34. **Geneva, Switzerland:World Health Organization & International Water Association,** 2009. *Water Safety Plan Manual: Step-by-Step Risk Management for Drinking-Water Suppliers*. World Health Organization. Available at https://apps.who.int/iris/handle/10665/75141 (Accessed 24 February 2020).

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