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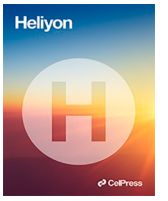

Research article

Contamination source identification for the prompt management of a gastroenteritis outbreak caused by norovirus in drinking water in Northern Italy

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A R T I C L E I N F O

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A B S T R A C T

In June 2022, a gastroenteritis outbreak occurred in a town in Northern Italy, possibly associated with the ingestion of norovirus from public drinking water. Noroviruses are highly infectious RNA viruses, with high stability in the environment. They are the primary cause of non-bacterial gastroenteritis worldwide, and despite the fact that the disease is mainly self-limiting, norovirus infection can lead to severe illness in the immunocompromised, the elderly and children. Immediately after the notification of the suspected norovirus outbreak, faecal specimens were collected from hospitalised patients, and water samples were collected from public drinking fountains in the affected area, to confirm the presence of norovirus. Norovirus was detected in 80 % (95 % CI 0.58–0.91) of the faecal specimens, and in 50 % (95 % CI 0.28–0.72) of the water samples using RT (reverse transcription) Real-time PCR. The identification of GII genotype in all samples confirmed public drinking water as the source of norovirus contamination. In addition, in one faeces and one water sample, the co-presence of genotypes GI and GII was detected. The strains were typed by sequencing, with most of them belonging to the genotype GII.3. Immediately after the confirmation of norovirus contamination in public drinking water, the local competent authorities applied safety measures, resulting in a decline in number of cases. Moreover, after the application of disinfection protocols in the water plant, the sampling was repeated with negative results for norovirus in the affected area. However, positive samples were found in the neighbouring area (prevalence 10.00 %, 95 % CI 0.02–0.40) and in the water spring (prevalence 50.00 %, 95 % CI 0.21–0.78), suggesting norovirus persistence and spread from the water source. The prompt identification of the source of contamination, and collaboration with the local authorities guided the implementation of proper procedures to control viral spread, resulting in the successful control of the outbreak.

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Abbreviations

ATS	Agenzia di Tutela della Salute; Bergamo, Italy
CA	Competent authority
G	Genogroup
IZSLER	Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Brescia, Italy
GI	Norovirus genogroup I
GII	Norovirus genogroup II
ORF	Open reading frame
95 % CI	95 % confidence interval

1. Introduction

Human noroviruses are small viruses highly infectious for humans, causing sporadic cases and outbreaks of acute gastroenteritis in people of all ages [1]. They represent the primary cause of non-bacterial gastroenteritis, causing almost 685 million cases per year, 210,000 deaths, and 15 million disability-adjusted life years annually [2–4]. Noroviruses are non-enveloped viruses classed into the *Norovirus* genus, *Calciviridae* family, with a single stranded RNA approximately 7.5 kb in length. Noroviruses are currently classified into ten genogroups (G) further divided in 48 genotypes, with GI, GII, and GIV infecting humans, and GI and GII being responsible for the greater part of clinical cases [2,5]. Norovirus infection is generally self-limiting, and healthy individuals recover in 1–2 days without complications, but it can lead to severe dehydration, hospitalisation, and potentially death in immunocompromised, the elderly, and children [6]. Norovirus infection causes almost 200 million cases in children, with 50,000 child deaths every year, mainly in developing countries [3]. Norovirus infection seems a problem also in developed countries, costing annually $60 billion in healthcare and productivity decrease [3].

Norovirus transmission occurs mainly via the faecal-oral route, through contact with infected people or contaminated surfaces, via infectious aerosols, or via the ingestion of contaminated water or food [7]. Concerning environmental transmission, noroviruses have been found in a broad variety of water sources: groundwater, surface water, drinking water, tap water, rivers, swimming pools, and wastewater, highlighting the importance of water monitoring to prevent norovirus outbreaks [8–11]. Moreover, the presence of norovirus in natural water supplies suggests a risk for human health, given noroviruses low infectious dose, and high resistance in the environment [12–14]. Indeed, the virus can persist for long periods in the springs used for irrigation, human or animal drinking water, and washing food, leading to viral spread and outbreaks [7,15–17]. In Italy, waterborne norovirus outbreaks have often been associated with exposure to surface or drinking water, reinforcing the need to collect data to study viral spread, epidemiology, and possible effective control measures and strategies to prevent outbreaks [18–24].

In this study, we describe the investigation and management of a gastroenteritis outbreak of suspected waterborne origin caused by norovirus, reported in June 2022 in a municipality in Lombardy (Northern Italy).

2. Materials and methods

2.1. Gastroenteritis outbreak setting and management

In June 2022, the local competent authorities (CAs, Agenzia di Tutela della Salute – ATS, Bergamo, Italy) were informed about a possible gastroenteritis outbreak affecting about 150 citizens and tourists, possibly associated with norovirus ingestion from public drinking water. Indeed, all the people involved used the public drinking water of the town for drinking or washing and preparing foods, and then reported norovirus-associated symptoms, such as nausea, vomiting, diarrhoea, abdominal cramps and slight fever; some of them were even hospitalised.

Following outbreak notification, 20 faecal samples were collected from hospitalised patients with norovirus symptoms, to confirm the presence of norovirus.

Information was collected on the design and management of the water supply, with collaboration of the local CAs, the supervisors of the water supply service and water-distribution plant, and the laboratory intended to perform the analysis (Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna – IZSLER, Brescia, Italy). Before the outbreak, the water intended for public consumption was monitored monthly, with negative results for both norovirus and coliforms (indicators of faecal contamination).

Five days after the outbreak notification, 16 water samples were immediately collected by CAs from public drinking fountains, to investigate the possible source of the outbreak. Three additional water samples were collected and analysed from public drinking fountains in a neighbouring village to check for the possible spread of the viral contamination; indeed, all consumption-points were supplied by the same spring and water-distribution plant.

Eight days after the outbreak notification, disinfection protocols were applied in the water-distribution plant to deal with possible contamination. Chlorine dosage was increased up to 0.30–0.45 mg/L in the tanks gathering water before distribution, to guarantee values between 0.15 and 0.18 mg/L in the water network of the critical area. A UV system was also installed at the entrance to the tanks, the plant was sanitized, and the water pump of the plant was replaced. At the same time, the local CAs promptly applied safety measures, closing public water sources, and recommending that people boil tap water before use for both drinking and washing or

preparing food.

Sixteen days after the outbreak notification, six water samples were collected from the same points in the town, and 10 water samples from the neighbouring village, to check for possible contamination persistence, and monitor possible viral spread. Finally, 15 water samples were collected along the water-distribution plant, and 8 from the spring suppling both the outbreak area and the neighbouring area.

Ninety days after the outbreak notification, and after further disinfection of the spring, another sample was collected to confirm the absence of norovirus in the water. Fig. 1 and Table 1 show the flow diagram describing the outbreak management, and the sampling plan, respectively.

2.2. Water sample preparation and RNA extraction

Each water sample (500 mL) was spiked with 10 µl of mengovirus (ATCC VR-1597™) as process control, as per the UNI EN ISO 15216–2:2019 [25], and the water was filtered. The membrane was washed with 3 mL of Beef extract (3 %, pH 9.5), and incubated for 5 min at room temperature. The eluate was collected for RNA extraction.

For faeces, 900 µL of water for molecular biology (Sigma-Aldrich – USA) were added to 100 mg of sample, and centrifuged at 10,000 g for 5 min. One mL of supernatant was used for RNA extraction.

The extraction and purification of viral RNA were conducted using the NucliSENS® MiniMag kit (bioMérieux – France), including a negative control in each extraction run.

2.3. Norovirus GI and GII RT real-time PCR

Norovirus GI and GII RNA was detected according to the UNI EN ISO 15216–2:2019 guideline by One-step RT (reverse transcription) Real-time PCR targeting a sequence on ORF1/ORF2 (open reading frame) junction regions of viral genome [25]. Norovirus GI and GII were detected using specific primers and TaqMan probes, as described in Table 2.

The samples were analysed in two separate reactions, each corresponding to a specific genogroup, using the RNA UltraSense™ One-Step Quantitative RT-PCR System (Invitrogen – USA) in a CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad – USA), and including negative and positive controls in each run. The reaction was performed in a total volume of 25 µL, including 5 µL of Ultrasense mix (5X), 1.25 µL of foward primer (10 µM), 2.25 µL of reverse primer (10 µM), 0.63 µL of probe (10 µM), 0.5 µL of Rox reference dye (50X), 1.25 µL of enzyme mix, 12.49 µL of Nuclease-Free Water (Promega Corporation – USA), and 5 µL of RNA. Both reactions were carried out performing reverse transcription for 60 min at 55 °C, denaturation for 15 min at 95 °C, and 45 amplification cycles at 95 °C for 15 s, 60 °C for 1 min and 65 °C for 1 min.

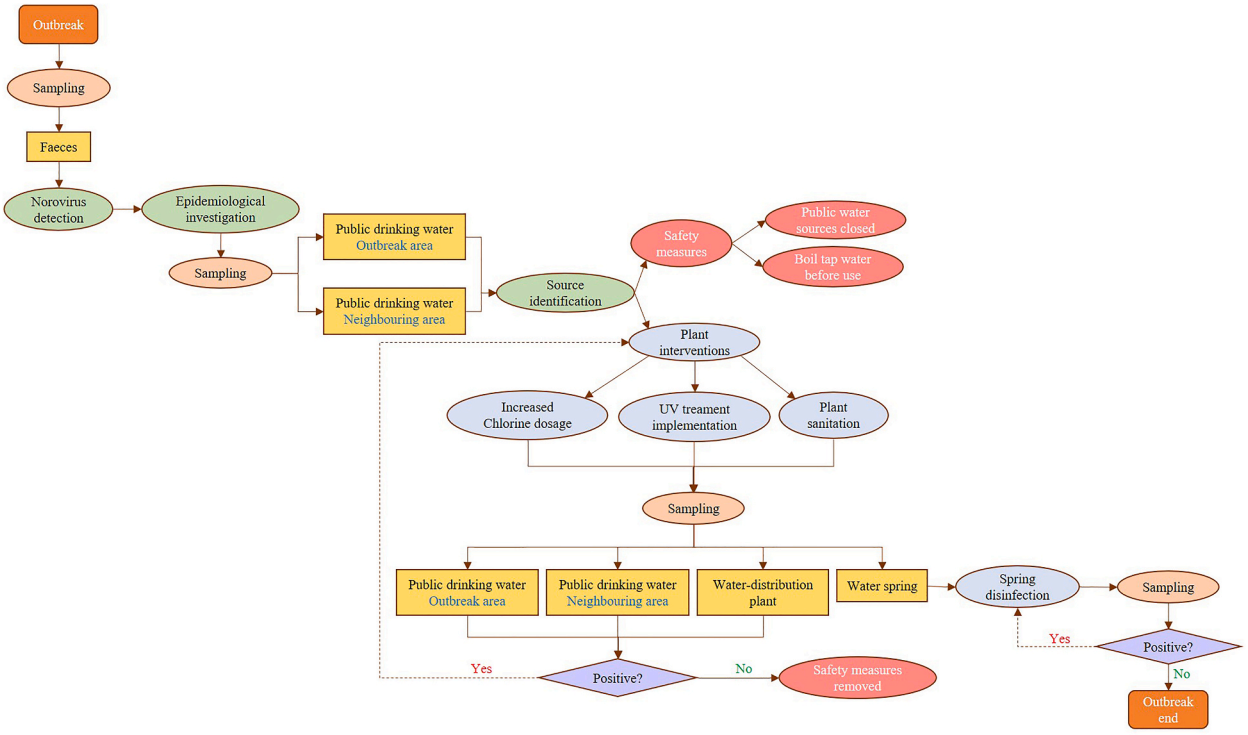

Fig. 1. Flow diagram representing the investigation and management of the norovirus outbreak.

Table 1
Sampling plan used to investigate and manage the norovirus outbreak. The samples are listed based on collection time (days after the outbreak notification) and area.

Time of sampling	Sample type	Area of sampling	Number of samples
Outbreak notification	Faeces	Hospital – patients with gastroenteritis symptoms	20
5 days	Water	Public drinking fountains – outbreak area	16
	Water	Public drinking fountains – neighbouring area	3
16 days	Water	Public drinking fountains – outbreak area	6
	Water	Public drinking fountains – neighbouring area	10
	Water	Water-distribution plant	15
	Water	Water spring	8
90 days	Water	Water spring	1

Table 2
Primers and probes used to detect norovirus GI and GII in reverse transcription Real-time PCR [25].

Target	Name	Type	Sequence
Norovirus GI	QNIF4	Forward	5′-CGCTGGATGCGNTTCCAT -3′
	NV1LCR	Reverse	5′-CCTTAGACGCCATCATCATTTAC-3′
	NVGG1p	Probe	5′-FAM-TGGACAGGAGAYCGCRATCT-TAMRA-3′
Norovirus GII	QNIF2	Forward	5′-ATGTTCAGRTGGATGAGRTTCTCWGA-3′
	CIG2R	Reverse	5′-TCGACGCCATCTTCATTCACA-3′
	QNIFS	Probe	5′-FAM-AGCACGTGGGAGGGCGATCG-TAMRA-3′

2.4. Mengovirus detection

Real-time PCR for mengovirus detection was carried out using primers and a TaqMan probe, as reported in Table 3.

Mengovirus detection was performed in the CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad – USA) using the RNA UltraSense™ One-Step Quantitative RT-PCR System (Invitrogen – USA), and including both negative and positive amplification controls. A total volume of 25 μL contained 5 μL of Ultrasense mix (5X), 1 μL of foward primer (12.5 μM), 1 μL of reverse primer (22.5 μM), 1 μL of probe (6.25 μM), 0.5 μL of Rox reference dye (50X), 1.25 μL of enzyme mix, 10.25 μL of Nuclease-Free Water (Promega Corporation – USA), and 5 μL of RNA; positivity was identified when Ct $\leq$ 40. After reverse transcription (1 h at 55 °C), an incubation for 5 min at 95 °C was performed, followed by an amplification for 45 cycles at 95 °C of 15 s, at 60 °C for 1 min and at 65 °C for 1 min.

2.5. Genotyping and phylogenetic analysis

Genotyping was carried out on positive samples, sequencing the ORF1/ORF2 junction regions of the norovirus genome. RT was performed using the specific reverse primers described by Kojima et al., 2002 (G1SKR primer for norovirus GI detection, and G2SKR primer for norovirus GII) [26]. The first end-point PCR was performed using the primers described by Kojima et al., 2002 and Kageyama et al., 2003 (COG1F and G1SKR primers for GI detection, and COG2F and G2SKR primers for GII) [26,27]. The semi-nested PCR was performed using the primers described by Kojima et al., 2002 (G1SKF and G1SKR primers for GI detection, and G2SKF and G2SKR for GII) [26].

Reverse transcription was conducted in a GeneAmp™ PCR System 9700 Thermal Cycler (Applied Biosystems – USA) at 50 °C for 60 min and 94 °C for 5 min, in a volume of 40 μL that included 16 μL of dNTPs pool (10 mM), 8 μL of $MgCl_2$ (25 mM), 8 μL of Transcriptase Buffer (5X, Invitrogen – USA), 2 μL of primer (50 μM, G1SKR and G2SKR), 1 μL of RNAse Inhibitor (40 U/μL, Invitrogen – USA), 0.5 μL of M-MLV Reverse Transcriptase (200 U/μL, Promega Corporation – USA), and 4.5 μL of extracted RNA.

For each genogroup, a first end-point PCR reaction was performed in the GeneAmp® PCR System 9700 Thermal Cycler (Applied Biosystems – USA) starting with 5 min at 95 °C, then 40 cycles of 30 s at 94 °C, 30 s at 50 °C and 1 min at 72 °C were performed, and finally an extension for 5 min at 72 °C. Each reaction contained 15 μL of QuantiNova® Probe PCR Master Mix (2X, Qiagen – Germany), 0.3 μL of each primer (50 μM, COG1F and G1SKR for GI, and COG2F and G2SKR for GII), and 11.4 μL of Nuclease-Free Water (Promega Corporation – USA), and 3 μL of cDNA.

A semi-nested PCR was then performed for each genogroup using 12.5 μL of QuantiNova® Probe PCR Master Mix (2X, Qiagen – Germany), 0.25 μL of each primer (50 μM, G1SKF and G1SKR for GI, and G2SKF and G2SKR for GII), and 9.5 μL of Nuclease-Free Water

Table 3
Primers and probe used for mengovirus detection in reverse transcription Real-time PCR [25].

Name	Type	Sequence
Mengo 110	Forward	5′-GCGGGTCCTGCCGAAAGT-3′
Mengo 209	Reverse	5′-GAAGTAACATATAGACAGACGCACAC-3′
Mengo 147	Probe	5′-FAM-ATCACATTACTGGCCGAAGC-MGB-3′

Table 4
Number of positive samples for norovirus GI and GII. The samples are listed based on collection time (days after the outbreak notification) and area. Prevalence and 95 % confidence intervals are indicated in brackets.

Target	Faeces (Outbreak notification)	Public drinking water–outbreak area (5 days)	Public drinking water–neighbouring area (5 days)	Public drinking water–outbreak area (16 days)	Public drinking water–neighbouring area (16 days)	Water-distribution plant (16 days)	Water spring (16 days)	Water spring (90 days)
Norovirus GII	16/20 (80.00 %, 0.58–0.91)	8/16 (50.00 %, 0.28–0.72)	0/3	0/6	1/10 (10.00 %, 0.02–0.40)	0/15	4/8 (50.00 %, 0.21–0.78)	0/1
Norovirus GI	1/20 (5.00 %, 0.01–0.24)	1/16 (6.25 %, 0.01–0.28)	0/3	0/6	0/10	0/15	0/8	0/1

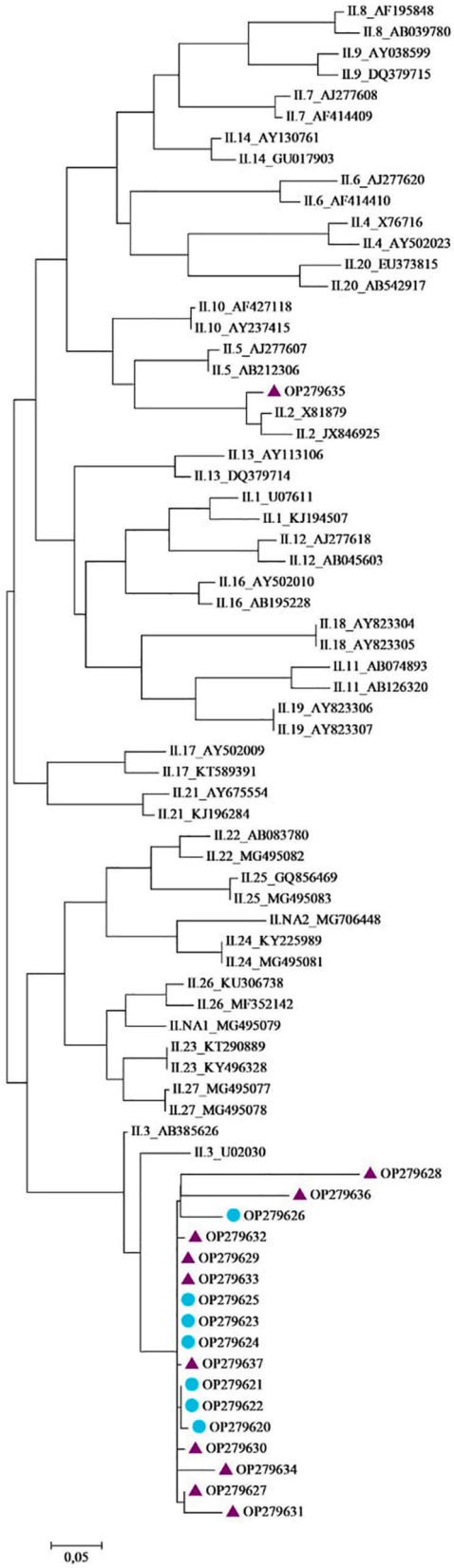

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Fig. 2. Maximum likelihood phylogenetic tree of norovirus GII sequences. The circles indicated the norovirus strains from this study detected from water samples, whereas the triangles from faeces samples. Norovirus GII reference sequences from Chhabra et al., 2019 are also considered in the dendrogram [5]. The tree was drawn to scale, with branch lengths measured in number of substitutions per site.

(Promega Corporation – USA). Semi-nested PCRs were performed adding 2.5 μL of DNA previously amplified to 22.5 μL of master mix, using the same thermal profile of the first end-point PCR. Each reaction included a negative and a positive control.

The electrophoretic run was performed in 3 % agarose gel loading the PCR products and a 100 bp marker (Invitrogen – USA). The expected bands (GI = 329 bp, GII = 343 bp) were detected using UV transillumination.

The enzymatic purification of the positive amplification products was performed with FastAP™ Thermosensitive Alkaline Phosphatase (1 U/μL) and ExonucleaseI (20 U/μL, Thermo Fisher Scientific – USA). The sequence reactions were prepared independently using the primers for semi-nested PCR (2 μL of primer, 1.6 μM), and adding 2 μL of Big Dye Terminator Reaction Mix, 1 μL of Big Dye Terminator 5X Sequencing Buffer, 3 μL of Nuclease-Free Water (Promega Corporation – USA), and 2 μL of purified amplification product. Purified products underwent incubation for 1 min at 96 °C, followed by amplification for 25 cycles at 96 °C for 10 s, 50 °C for 5 s and 60 °C for 4 min. The BigDye XTerminator® Purification Kit (Thermo Fisher Scientific – USA) was used for the purification of the sequence reaction products, that were then sequenced in a SeqStudio Genetic Analyser (Applied Biosystem Inc. – USA); the generated sequences were genotyped using the Norovirus Typing Tool (Version 2.0) [28]. The phylogenetic analysis was conducted in MEGA11 software, and the phylogenetic tree was deduced using the Maximum likelihood method (Tamura-Nei model), using norovirus GII reference sequences from Chhabra et al., 2019 [5,29,30].

2.6. Data analysis

Prevalence and the corresponding 95 % confidence intervals (95 % CI) were computed by Wilson confidence limits using the Epitools software [31].

3. Results

3.1. Norovirus GI and GII detection

Table 4 reported the results of norovirus GI and GII detection; mengovirus was detected in every sample, confirming the process effectiveness.

Norovirus GII prevalence in faeces was 80.00 % (95 % CI 0.58–0.91), including one sample with both genogroups detected (prevalence 5.00 %, 95 % CI 0.01–0.24, Table 4).

Five days after the outbreak notification, norovirus GII was detected in 50.00 % of the public drinking water collected from the outbreak area (95 % CI 0.28–0.72), including one sample containing both genogroups (prevalence 6.25 %, 95 % CI 0.01–0.28, Table 4). In the neighbouring area, no sample was tested positive for norovirus.

After the application of disinfection protocols, sampling was repeated (16 days after the outbreak notification), with all the water samples collected from the outbreak area negative, whereas one sample collected from the neighbouring village was positive for norovirus GII (prevalence 10.00 %, 95 % CI 0.02–0.40, Table 4). In the water-distribution plant, no sample tested positive for norovirus. In the water spring, 50.00 % (95 % CI 0.21–0.78) of the water was found to be positive for norovirus GII, whereas after the disinfection of the water spring (90 days after the outbreak notification), the water collected tested negative.

3.2. Genotyping and phylogenetic analysis

Genotyping was performed on sequences obtained from 11 faeces and 7 water samples, confirming norovirus GII.3 in all samples, apart from one faeces sample which contained norovirus GII.2. Thus, the co-presence of norovirus GI and GII detected using RT Real-time PCR was not confirmed by sequencing.

The phylogenetic tree in Fig. 2 shows that the majority of the water and faeces sequences belonged to the same clade correlated to norovirus GII.3. These sequences showed an intra-group nucleotide identity (nt. id.) ranging from 65.8 % to 100 %, with the highest nt. id. observed with a norovirus strain previously identified in the US in human cases (U02030, nt. id. 76.8 %).

Moreover, one faeces sample (OP279635) belonged to norovirus GII.2, even though it had the same geographical and temporal provenance of the other sequences. This sequence showed a nt. id. of 78.9 % with the sequence X81879, previously identified in humans in the UK.

The nucleotide sequences of the 18 norovirus GII strains were deposited in the GenBank database (Accession Numbers: from OP279620 to OP279626 for water samples, and from OP279627 to OP279637 for faeces samples).

4. Discussion

In our study, the timely and rapid identification of the outbreak was essential to its effective control, with molecular methods for investigating the causal role of norovirus in acute gastroenteritis outbreaks, and inspecting the possible viral source and reason for spread of the contamination. Indeed, many studies have already reported contaminated drinking water as the source of norovirus

epidemiologically linked to faecal specimens from human gastroenteritis cases [32–37]. Immediately after the notification, norovirus GII was detected in the faeces of patients with gastroenteritis, and in public drinking water collected from the outbreak area. The detection of the same genogroup both in faecal specimens and water samples suggested water was the likely source of norovirus causing the outbreak, facilitating the CAs in implementing disinfection measures to tackle the outbreak. Norovirus GII is recognized worldwide as the most common genogroup infecting humans, and has already been found several times in Italy; but in literature, its role in waterborne outbreaks is discordant [38–41]. Indeed, some studies reported that faecal samples containing norovirus GII presented a viral load that was 100 times greater than norovirus GI positive samples, implying a possible higher transmissibility of norovirus GII strains through the faecal-oral route, whereas, other studies associated mainly norovirus GI with waterborne outbreaks, suggesting a better stability in water than norovirus GII [7,32,36,42–44].

In our study, the co-presence of norovirus GI and GII was detected using RT Real-time PCR, but norovirus GI involvement in the outbreak was not confirmed by sequencing. However, the typing of only norovirus GII could not exclude the existence in the affected area of other different genogroups not associated with the outbreak, or the additional role of norovirus GI in the outbreak, with norovirus GII as the predominant strain. The literature has already reported norovirus outbreaks caused by water contaminated with strains of different genogroups, highlighting the importance of norovirus monitoring in human outbreaks and in the environment [33,34,42,45,46]. Therefore, viral typing was performed to investigate the strain responsible for the outbreak, and norovirus circulation in the affected area. The sequencing of the outbreak samples was effective in only some positive samples, probably caused by a low viral titer, or a scarce-quality sample. Previous studies have already reported some difficulties occurred in genotyping viruses from environmental samples caused by a possible lack in sensitivity of sequencing PCRs, which actually resulted less sensitive than the Real-time PCR used for norovirus initial detection [36,47]. The analysis of the typed strains confirmed an association between public water and the onset of gastroenteritis, with the same strain found in water and in patients' stools, strengthening the hypothesis of water as the vector for norovirus transmission. In fact, almost all water and faeces samples contained norovirus GII.3, and only one faeces sample contained norovirus GII.2, but without any associated water sample, suggesting a source of infection different from public water, or that the associated water sample was not typeable. Recent studies have already reported norovirus GII.3 and GII.2 as a common cause of sporadic gastroenteritis in children, but less transmissible to adults, even if they showed a high propensity for recombination [48–51].

In our study, the absence of positive samples in the neighbouring area at the beginning of the outbreak was reassuring, suggesting a viral spread limited and circumscribed to the drinking water sources in the outbreak area. However, the particular ability of norovirus to be stable in the environment was a concern for managing the outbreak. In particular, the persistence of norovirus genome for up to 2 months has already been demonstrated in groundwater, river water, mineral and tap water [8–10]. In groundwater, norovirus genome was found even after 2 years, and a similar persistence was supposed also for its infectivity [8,10,52]. Interestingly, a prototype of human norovirus (Norwalk virus) spiked in groundwater, was still able to infect human volunteers after storage at room temperature in the dark for 61 days [10]. Therefore, following the confirmation of norovirus contamination in public drinking water, the local CAs decided immediately to apply safety measures, reducing the number of cases. A UV system was also installed in the water-distribution plant, as a common disinfection method in wastewater treatment plants, effective for bacteria, coliphage, and enteric viruses [53,54]. Furthermore, the plant was sanitized, and the chlorine concentration in the water supply was increased to guarantee values of 0.15–0.18 mg/L in the water network. In Italy, water intended for human consumption cannot exceed a chlorine concentration of 0.2 mg/L, so the supervisors of the water supply had to challenge the norovirus contamination while maintaining water potability [55]. Moreover, the exact chlorine concentration needed to inactivate norovirus in water is not known [2]. One study reported that Norwalk virus was inactivated only in high concentrations of chlorine (>2 mg/L), whereas another study on murine norovirus described a 3-log reduction when chlorine concentration was comparable to our concentration [56]. Therefore, it was difficult to know whether the norovirus would be inactivated or still infectious at the concentration reached in the water network of this study. Nevertheless, after the disinfection procedures, all the water samples collected from the outbreak area tested negative, except for one sample from the neighbouring village. This was not surprising, given that some samples from the spring also tested positive, suggesting a possible delayed spread of the norovirus to the neighbouring area, or that in the first sampling, norovirus was not detected because of the small number of samples analysed. The strain persistence could be explained by a deficient state of current sewage systems, possibly contaminating the spring, but more probably by the continued presence of the source of viral infection which periodically contaminated the spring, despite the disinfection protocols applied. Finally, the results of this last environmental sampling should be interpreted with caution, as it is difficult to correlate viral genome detection in water with the likelihood of disease transmission [43,57,58]. For example, one study on a surrogate for human norovirus reported that the treatment with UV radiation or chlorine resulted in loss of infectivity, but kept the RNA detectable, as it damages only the viral capsid [59]. In any case, no further human cases were reported probably thanks to the safety measures taken by the CAs, preventing further viral spread. Accordingly, the CAs decided on the partial lifting of the safety measures in force, keeping closed only the public drinking fountains still contaminated in the neighbouring area, until the absence of norovirus in the water spring was also confirmed.

The successful management of this outbreak highlighted the importance of rapid investigation into cased of local epidemic by implementing viable and cost-effective methods to detect and trace the contamination in a timely manner. Indeed, the fast confirmation of norovirus in the water, and the detection of the same norovirus genotype in both faeces and water were decisive to recognising a waterborne infection, and guide the implementation of proper procedures to control viral spread, limiting the impact on the vulnerable population and health systems.

Finally, the occurrence of norovirus in drinking water highlighted the role of water as important vehicle for viral transmission, reinforcing the interest in improving water monitoring to gain insights into the transmission and circulation of norovirus, preventing possible outbreaks [60]. Currently, norovirus surveillance is performed on a voluntary basis, leading to differences in norovirus control

among countries, with even no surveillance at all in some states [61]. Thus, starting from a reporting system at local level, the ongoing efforts seek to integrate all the available data in a global and strong surveillance network, in order to monitor outbreaks, identify the emergence of potential new variants, and study their possible impact and severity on human health [1,61–63]. In fact, the death burden of norovirus-associated diseases over the years has remained high in developing countries, but has increased among the elderly in industrialized countries, even if the global trend has decreased in the past three decades, highlighting the fact that efforts should be directed towards a common norovirus control approach [64].

Ethics declaration

Review and/or approval by an ethics committee is not needed for this study because no experimental test was performed. Informed consent was not required for this study because the samples included in the study were collected by the competent authority in the context of official diagnostic activities carried out to investigate and control the outbreak. The study is limited to the characterization of pathogens and does not involve any data related to the patients.

Data availability statement

Data associated with this study have not been deposited into a publicly available repository because data are all included in the manuscript.

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CRediT authorship contribution statement

Sara Arnaboldi: Writing – original draft, Visualization, Formal analysis, Conceptualization. **Francesco Righi:** Writing – review & editing, Investigation, Formal analysis, Data curation, Conceptualization. **Lucia Mangeri:** Investigation, Data curation. **Elisa Galuppini:** Investigation, Data curation. **Barbara Bertasi:** Supervision. **Guido Finazzi:** Writing – review & editing, Supervision. **Giorgio Varisco:** Supervision. **Stefania Ongaro:** Supervision, Resources, Data curation. **Camillo Gandolfi:** Supervision, Conceptualization. **Rossella Lamera:** Supervision, Data curation. **Paolo Amboni:** Supervision, Data curation. **Elena Rota:** Supervision, Data curation. **Deborah Balbino:** Resources, Investigation. **Constanza Colombo:** Resources, Investigation. **Martina Gelmi:** Resources, Investigation. **Alessandra Boffelli:** Resources, Investigation. **Serena Gasparri:** Resources, Investigation. **Virginia Filipello:** Writing – review & editing, Supervision. **Marina-Nadia Losio:** Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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