

Multidisciplinary management of a norovirus outbreak in Tuscany, Italy

Clara Girardi,^{1,2} Lucia Kundisova,³ Francesca Marconi,¹ Alessandra Guidi,¹ Johanna Alexandra Iamarino,⁴ Veronica Gallinoro,⁴ Silvia Mele,⁴ Giovanni Nardone,² Maurizio Grani,² Giovanni Munaò,⁵ Luca Cianti,⁵ Paola Picciolli,³ Ylenia Zizzo,⁶ Loria Bianchi,⁷ Martina Sartoni¹

¹Department of Veterinary Sciences, University of Pisa; ²Veterinary Public Health and Food Safety Functional Unit, Local Health Competent Authority Toscana Centro, Pistoia; ³Hygiene and Public Health Service Function Unit, Local Health Competent Authority Toscana Centro, Pistoia; ⁴Medical Specialization School of Hygiene and Preventive Medicine, University of Florence; ⁵Veterinary Public Health and Food Safety Functional Unit, Local Health Competent Authority Toscana Centro, Firenze, Calenzano; ⁶Preventive Healthcare Activities Simple Departmental Operative Structure, Local Health Competent Authority Toscana Centro, Empoli-Prato-Pistoia; ⁷Microbiology Unit, S. Jacopo Hospital, Pistoia, Italy

Correspondence: Martina Sartoni, Department of Veterinary Sciences, University of Pisa, viale delle Piagge 2, 56124 Pisa, Italy. Tel: +39-050-2216987. Fax number: +39.050.2210654. E-mail: martina.sartoni@vet.unipi.it

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Abstract

Norovirus (NoV) is one of the most frequent agents responsible for foodborne outbreaks. Transmission occurs through the consumption of contaminated food or water or *via* contact with contaminated surfaces. The low infectious dose and high environmental resistance of the virus facilitate its spread within communities and healthcare settings, complicating epidemiological investigations. This study aims to highlight the management strategies and key entities involved in outbreak response, emphasizing the importance of coordinated efforts to share best practices. The outbreak occurred in Tuscany in April 2024 and affected multiple school groups. The response team was activated in accordance with guidelines for managing foodborne diseases and comprised various public health authorities. The team undertook a series of coordinated actions, including epidemiological investigation, official controls, sampling, and analysis of relevant matrices to identify high-risk foods. Food and water samples collected during the official inspection at the accommodation facility involved in the first wave of the outbreak were tested for potential pathogenic bacteria. Additionally, fecal samples from ten hospitalized cases were analyzed for pathogenic bacteria and viruses. Among all these analyses, only the fecal samples from the examined cases tested positive for NoV genogroups GI and GII. In light of these results, during the second wave, food and water were tested again for the same potential pathogenic bacteria and for the presence of NoV. Furthermore, NoV was investigated in staff fecal samples and environmental surfaces. A total of four fecal samples from staff and two environmental swabs tested positive for NoV genogroups GI and GII. Our multidisciplinary investigation suggests that an initial foodborne transmission may have led to environmental contamination. This finding underscores the critical role of food safety culture in outbreak prevention and control. To reduce the risk of viral gastroenteritis, specific awareness initiatives and training programs should be offered to both food sector operators and the general public.

Introduction

Access to safe, adequate, and nutritious food is a fundamental human right and essential for good health (FAO, 2014). Foodborne diseases (FBD) adversely affect people's health and well-being, and still cause a substantial public health, economic, and social burden throughout the world (WHO, 2017). The full burden of FBD remains largely unknown, despite being considered substantial. This gap in accurate data is primarily attributed to underreporting, as

many affected individuals do not seek medical care, while others who do remain undiagnosed due to the absence of definitive laboratory testing. Consequently, FBD and outbreaks frequently go undetected and unrecorded (WHO, 2021). The majority of FBD, hospitalizations and fatalities are attributed to eight common pathogens: norovirus (NoV), nontyphoidal *Salmonella*, *Clostridium perfringens*, *Campylobacter*, *Staphylococcus aureus*, *Toxoplasma gondii*, *Listeria monocytogenes*, and *Shiga* toxin-producing *Escherichia coli* (Switaj *et al.*, 2015).

According to a recent report on zoonoses (EFSA-ECDC, 2024), NoV and other caliciviruses were the third most common agents responsible for foodborne outbreaks in several European countries in 2023, ranking second in the number of cases reported. The number of outbreaks caused by this pathogen increased by 8.1% compared to 2022 (EFSA-ECDC, 2024). NoV causes outbreaks of acute gastroenteritis with significant social and economic impacts. It is estimated that NoV results in an annual global economic burden of around 60 billion dollars, mainly due to healthcare costs and productivity losses (Carlson *et al.*, 2024). Although reported outbreaks of NoV are increasing, accurately estimating their incidence remains difficult due to diagnostic limitations and the typically mild, self-limiting nature of the disease (Randazzo *et al.*, 2018). Enhanced reporting of foodborne viruses is essential to reduce the incidence of these pathogens (Magalhães-Guedes *et al.*, 2019).

Currently, 10 genogroups of NoV, *i.e.*, GI to GX, have been identified (Cates *et al.*, 2020). NoV GI, GII, and GIV genogroups are known to infect humans (Chhabra *et al.*, 2020), with genogroup GII representing the predominant strain responsible for approximately 50-70% of epidemic outbreaks in recent decades (Cates *et al.*, 2020). The transmission of NoV can occur through the fecal-oral route, *via* the consumption of contaminated food or water, *e.g.*, consumption of raw shellfish, salads, berries, contaminated water, cold foods, sprouts, herbs, and spices (De Graaf *et al.*, 2017; Tan *et al.*, 2024), as well as through contact with contaminated surfaces (Aprea *et al.*, 2021). NoV on surfaces can persist for up to 2 weeks (FDA, 2023). Aerosol transmission, *via* vomit micro-pellets, is particularly significant: up to 3×10^7 viral particles can be released (Barker *et al.*, 2004). Viral excretion through feces occurs on average for 72 hours and can extend up to 8 weeks at low levels (Glass *et al.*, 2009). As it lacks an envelope (Karst *et al.*, 2014), it is presumed to be resistant to lipophilic disinfectants such as alcohols and quaternary ammonium salts (Lin *et al.*, 2020). Additionally, NoV shows resistance to temperatures above 60°C and to the chlorine levels commonly used to disinfect drinking water (Hall *et al.*, 2014). Therefore, to sanitize contaminated surfaces, it is necessary to implement several alternating steps of washing with detergents and disinfectants (*e.g.*, sodium hypochlorite) (Aprea *et al.*, 2021). The aforementioned characteristics, together with the NoV low infectious rate (from 10 to 100 viral particles) and that approximately until 21.8% of infected individuals are asymptomatic in outbreaks (Wang *et al.*, 2023), encourage the spread of the virus especially in communities and hospitals (Aprea *et al.*, 2021; Carlson *et al.*, 2024) and complicate the work of epidemiological investigation personnel.

This study aims to describe the management of a NoV outbreak that occurred in April 2024, involving four school groups accommodated at a facility in Tuscany. The outbreak management involved the collaboration of various public health authorities as the Department of Prevention of the Local Health Unit (LHU) *Toscana Centro*, the Hospital Trust, the *Istituto Zooprofilattico Sperimentale Lazio-Toscana* (IZSLT), and the National Reference Laboratory for Foodborne Viruses of the *Istituto Superiore di Sanità* (ISS). These

authorities undertook a series of coordinated actions, which proved crucial in deepening the understanding of the toxic-infectious phenomenon. The highlight of the study is on the management and key entities involved in outbreak response, emphasizing the importance of coordinated efforts to share best practices. By analyzing these processes, it seeks to propose a potential model to enhance outbreak investigation.

Materials and Methods

Clinical presentation and outbreak notification

On the 5th of April 2024, the LHU was notified about the infectious event. Hospital physicians reported that several individuals from different school groups staying at the same accommodation facility presented symptoms consistent with FBD, as both students and teachers experienced gastrointestinal symptoms and fever. The outbreak developed into two epidemic waves: the first on April 5th, involving 54 cases across three school groups, and the second on April 14th, involving 9 cases in a single school group. Notably, two staff members at the facility also experienced gastrointestinal symptoms—one during the first epidemic wave and the other between the two waves. Following the event notification, the foodborne outbreak Response Team was activated in line with the guidelines for managing FBD, established by the Regional Reference Center for Foodborne Diseases of Tuscany (Ce.R.R.T.A). The team consists of specialized healthcare professionals, including physicians, veterinarians, public health assistants, prevention technicians (PTs), and administrative staff. Based on the initial hospital reports, the team coordinator launched an epidemiological investigation, drafting a medical mandate that detailed the actions to be undertaken.

Epidemiological investigation and analysis

Physicians and health assistants of the Hygiene and Public Health Service (PHS) carried out the epidemiological investigation through interviews with the school groups and hotel staff. Due to the high number of exposed individuals during the first wave, interview data were obtained with the support of the teachers and recorded on Excel documents. The recorded data and the list of foods consumed during the stay were essential information for each student. Additionally, physicians held interviews with the hotel manager and dining/kitchen staff, questioning them about the served food preparation and storage methods. To track the possible subsequent onset of symptoms, follow-up phone interviews were conducted with students and teachers upon their return home to evaluate the health status of asymptomatic individuals exposed during the outbreak. During the first epidemic wave, the collected data enabled a descriptive (*i.e.*, interviews) and analytical [*i.e.*, calculation of attack rate (AR) and relative risk (RR)] epidemiological analysis of the outbreak aimed at identifying any potentially risky food product. As stated in the FBD outbreaks guidelines (WHO, 2008), AR – incidence of cases among the “exposed” (those who ate that specific food) and “non-exposed” (those who did not eat that specific food) – was calculated in order to identify the high-risk food item associated with ER visits and symptom presence. Values were calculated using the formula [Eq. 1]:

$$\begin{aligned} \text{AR exposed} &= \text{n. of cases among the exposed} / \text{n. exposed} \\ \text{AR non-exposed} &= \text{n. of cases among the non-exposed} / \text{n. non-exposed} \end{aligned} \quad [\text{Eq. 1}]$$

Then, RR, as the ratio between the two AR for each food item (exposed/non-exposed) (WHO, 2008), was determined using the formula [Eq. 2]:

$$RR = AR \text{ exposed} / AR \text{ non-exposed} \quad [\text{Eq. 2}]$$

The RR value indicates how much higher the risk of developing symptoms is in the group exposed to a specific risk factor (the food item under study) compared to the non-exposed group. An RR value greater than 1 indicates a higher risk in the exposed group (WHO, 2008). For the second epidemic wave, the information received indicated that all the students and teachers consumed the same foods. This condition did not make it possible to calculate AR and RR. New interviews were conducted with the dining and kitchen staff, covering the previously mentioned topics and, additionally, staff training and health, as well as cleaning/sanitation management of the facilities, with particular attention to textile furnishings (towels, tablecloths, sheets, *etc.*).

Official controls

Several official controls were conducted by an LHU team at the facility, which is registered under EC Regulation No. 852/2004 (European Parliament and Council of the European Union, 2004). During the first epidemic wave, the official control included the assessment of: i) official authorization; ii) prerequisite programs (*i.e.*, food storage, cleanliness of premises, equipment, and personnel, pest control); iii) Hazard Analysis and Critical Control Points system. Also, a sampling was arranged based on the preliminary information obtained through the epidemiological investigation. During the second epidemic wave, a new official control focused on water management in the accommodation facility, by reviewing the general documentation and verifying the management of bottled mineral water, potable water storage tanks, and the methods of water service to clients.

Sampling and analysis of matrices

Food samples

Sample collection was carried out by the PTs, and the subsequent analyses were conducted by competent laboratories. During the first epidemic wave, the food sampling was performed on the same day as the notification of the FBD event (5th of April). However, meals consumed by the school groups in the previous 2 days were no longer available. In agreement with the coordinator of the Response Team, the PTs proceeded to sample other food items found in the facility and the drinking water from a sink located near the kitchen. The food and water samples were analyzed by the Public Health Laboratory through microbiological tests for the main pathogens responsible for FBD (*e.g.*, *Salmonella spp.*, *Campylobacter spp.*, *Bacillus cereus*, *Escherichia coli* STEC, *Listeria monocytogenes*, *Staphylococcus spp.*). During the second epidemic wave, food items from the facility were resampled for further analysis (*i.e.*, *Enterobacteriaceae*). To ensure thorough testing, the samples were sent to two different local laboratories based on available resources. The Public Health Laboratory conducted microbiological analyses to detect major pathogens causing FBD. The IZSLT performed reverse transcription polymerase chain reaction (RT-PCR) testing to identify NoV GI/GII in the food samples.

Biological human samples

During the first epidemic wave, it was decided to carry out stool

cultures on the hospitalized patients (n=10). All the samples were analyzed using a multiplex PCR microarray for bacterial/viral/parasitic pathogens most frequently responsible for gastroenteritis. During the second epidemic wave, HPHS physicians proposed a screening for the presence of NoV in stool samples to the hotel manager and kitchen and dining staff; seven employees agreed to participate, providing their stool samples, which were collected and then analyzed by the ISS for the detection of NoV GI and GII using RT-PCR.

Water samples and environmental swabs

Personnel from ISS collected and analyzed water samples from a cistern and from a sink near the kitchen, used by a symptomatic worker during the first wave. The water samples were filtered and concentrated, then tested for NoV GI and GII using RT-PCR. During the second epidemic wave, PTs conducted 23 environmental swabs in the following areas: kitchen (major tools, including a teflon cutting board, stainless steel work surface, two water faucet handles, microwave handle, slicer handle, beverage dispenser keypad, refrigerator handle for water, refrigerator handle for fruit), staff restrooms (a toilet flush button and two toilet seats, shower handle, restroom door handle), staff locker room (two handles of two lockers), rooms occupied by patients from the first and the second waves (four handles of four access doors, and a spot on the carpet), and elevators (two keypads). All swabs were identified, recorded, and processed by the ISS to detect NoV GI and GII using RT-PCR.

Results

Investigation and epidemiological analysis

During the first epidemic wave, the investigation reported that on April 3rd and 4th, the school groups consumed self-service buffet-style breakfasts and dinners in the hotel dining room. Interviews identified a hotel staff member who experienced gastrointestinal symptoms during this period. Although this individual ate meals at the hotel, he consumed different foods from those served to the students. Investigations further revealed that some exposed students and teachers, initially asymptomatic, developed gastrointestinal symptoms after returning home.

For the second epidemic wave, records indicated that the school groups consumed meals in the same dining hall: dinner on April 13th and 14th, and self-service buffet-style breakfasts on April 14th and 15th. Additionally, discussions revealed that a kitchen and dining room staff member exhibited symptoms similar to those of the guests during the interval between the two epidemic waves.

The epidemiological analysis was conducted only for the first epidemic wave and was based on the results of food questionnaires completed by two of the three school groups involved, encompassing 94 individuals: 51/94 cases (54%) presented symptoms compatible with acute gastroenteritis and/or foodborne illness, and 37/94 cases (39%) visited the ER. The most common symptom was abdominal pain (50%), followed by vomiting (42%), diarrhea (32%), and fever (32%). As for the food consumed, the investigation focused on the meals served on April 3rd dinner (pasta with tomato sauce, roast meat with roasted potatoes, tiramisu, sealed bottled water, and pitchers of drinking water), and on April 4th dinner (pasta with cream and ham, veal escalope in tomato sauce with a side of peas, chocolate cake, sealed bottled water, and pitchers of drinking water). Almost all guests ate pasta with tomato sauce (95%) and roasted potatoes (94%), while less than half of the guests consumed chocolate cake (48%) and peas (41%).

Table 1 shows the calculation of the AR and the RR for the outcome of symptom presence.

The roast meat and the pasta with cream and ham were identified as high-risk foods, with an RR of 1.76 and 1.68, respectively. The RR indicates how many times more or less likely an event/illness is to occur in one group compared to another. In this case, those who ate the roast meat and the pasta with cream and ham had about twice the risk of developing symptoms compared to those who did not consume them. Table 2 shows the calculation of the AR and RR for the outcome of emergency room (ER) visits. In this case, the roast meat and the pasta with cream and ham are identified as high-risk foods, with an RR of 4.98 and 2.38, respectively. Those who ate the roast meat had nearly 5 times the risk compared to those who did not eat it. Similarly, those who ate the pasta with cream and ham had a 2.4 times higher risk.

Finally, the RRs for the two outcomes – ER visits and the presence of symptoms—were compared (Table 3). The analysis showed that the RR values were much higher for ER visits compared to the appearance of symptoms.

Official controls

During the first and second epidemic waves, minor non-compliances were identified during the on-site inspections conducted by the PTs. Deficiencies were noted in relation to: management and cleaning of the drinking water tanks, inadequate cleanliness of premises and equipment, lack of staff training, and absence of monthly pest monitoring.

Collection and analysis

Food samples

The analyses conducted by the Public Health Laboratory on food and drinking water did not reveal any non-compliances. The tests conducted on food by the IZSLT did not detect the presence of viral RNA for NoV.

Human biological samples

While the analyses conducted on the cases were positive for NoV GI/GII, out of the seven fecal samples from staff, four tested positive for NoV GII.

Water samples and environmental swabs

The analysis of two environmental swabs, related to one staff restroom and one spot on the floor carpet in a room occupied by some cases, detected the presence of NoV GI and GII. The analyses of the water were negative for the presence of NoV GI and GII.

Discussion

The transmission of NoV is influenced by multiple factors, including genetic variants, contamination pathways, excretion patterns, environmental persistence, and meteorological conditions (Marshall and Bruggink, 2011). Given its widespread presence and the recurring nature of outbreak epidemics, these factors play a

Table 1. Attack rate and risk ratio for the outcome “symptoms presence”.

Food product	n. of subjects who ate the food product				n. of subjects who did not eat the food product				RR
	Sick	Not sick	Total	AR (%)	Sick	Not sick	Total	AR (%)	
Tomato pasta	43	38	81	53.09	2	2	4	50.00	1.06
Roast meat	38	27	65	58.46	6	12	18	33.33	1.75*
Roasted potatoes	41	36	77	53.25	3	2	5	60.00	0.89
Tiramisu	32	25	57	56.14	11	13	24	45.83	1.22
Cream and ham pasta	41	32	73	56.16	4	8	12	33.33	1.68*
Veal escalope in tomato sauce	29	22	51	56.86	16	17	33	48.48	1.17
Peas	21	13	34	61.76	23	26	49	46.94	1.32
Chocolate cake	24	16	40	60.00	20	23	43	46.51	1.29

AR, attack rate: the value is expressed in percentage and calculated using the formula: $AR_{exposed} = n. \text{ of cases among the exposed} / n. \text{ exposed}$; $AR_{non-exposed} = n. \text{ of cases among the non-exposed} / n. \text{ non-exposed}$. RR, risk ratio: the value is expressed in percentage and calculated using the formula: $RR = AR_{exposed} / AR_{non-exposed}$. *Foods with the highest risk of symptom presence.

Table 2. Attack rate and risk ratio for the outcome “ER visits”.

Food product	n. of subjects who ate the food product				n. of subjects who did not eat the food product				RR
	Sick	Not sick	Total	AR (%)	Sick	Not sick	Total	AR (%)	
Tomato pasta	29	52	81	35.80	2	2	4	50.00	0.72
Roast meat	36	29	65	55.38	2	16	18	11.11	4.98*
Roasted potatoes	29	48	77	37.66	2	3	5	40.00	0.94
Tiramisu	24	33	57	42.11	6	18	24	25.00	1.68
Cream and ham pasta	29	44	73	39.73	2	10	12	16.67	2.38*
Veal escalope in tomato sauce	23	28	53	43.40	8	26	33	24.24	1.79
Peas	15	19	34	44.12	15	34	49	30.61	1.44
Chocolate cake	17	23	40	42.50	14	29	43	32.56	1.31

AR, attack rate: the value is expressed in percentage and calculated using the formula: $AR_{exposed} = n. \text{ of cases among the exposed} / n. \text{ exposed}$; $AR_{non-exposed} = n. \text{ of cases among the non-exposed} / n. \text{ non-exposed}$. RR, risk ratio, the value is expressed in percentage and calculated using the formula: $RR = AR_{exposed} / AR_{non-exposed}$. *Foods with the highest risk of emergency room visits.

critical role in its spread. In the investigated outbreak, food and water samples were collected and analyzed, as they represented common sources of exposure among cases and are frequently implicated in outbreaks occurring in communal settings. A systematic review of NoV outbreaks in China from 2000 to 2018 highlighted that person-to-person transmission was the most common (41.64%), followed by foodborne (22.80%) and waterborne transmission (21.88%) (Yu *et al.*, 2022). NoV can persist for long periods in other food products, such as cooked turkey (at least 10 days at 7°C), processed foods (at least 2 days at 6°C), apples (at least 7 days at 11°C), and water (for 60 to 728 days) (Cook *et al.*, 2016).

Water and its management were investigated because the first official control revealed suboptimal management of the tanks for drinking water storage. NoV was not tested in food and water during

Table 3. Comparison of the risk ratio for the two different outcomes.

Food product	Risk ratio (presence of symptom)	Risk ratio (ER visits)
Tomato pasta	1.06	0.72
Roast meat	1.75	4.98
Roasted potatoes	0.89	0.94
Tiramisu	1.22	1.68
Cream and ham pasta	1.68	2.38
Veal escalope in tomato sauce	1.17	1.79
Peas	1.32	1.44
Chocolate cake	1.29	1.31

ER, emergency room.

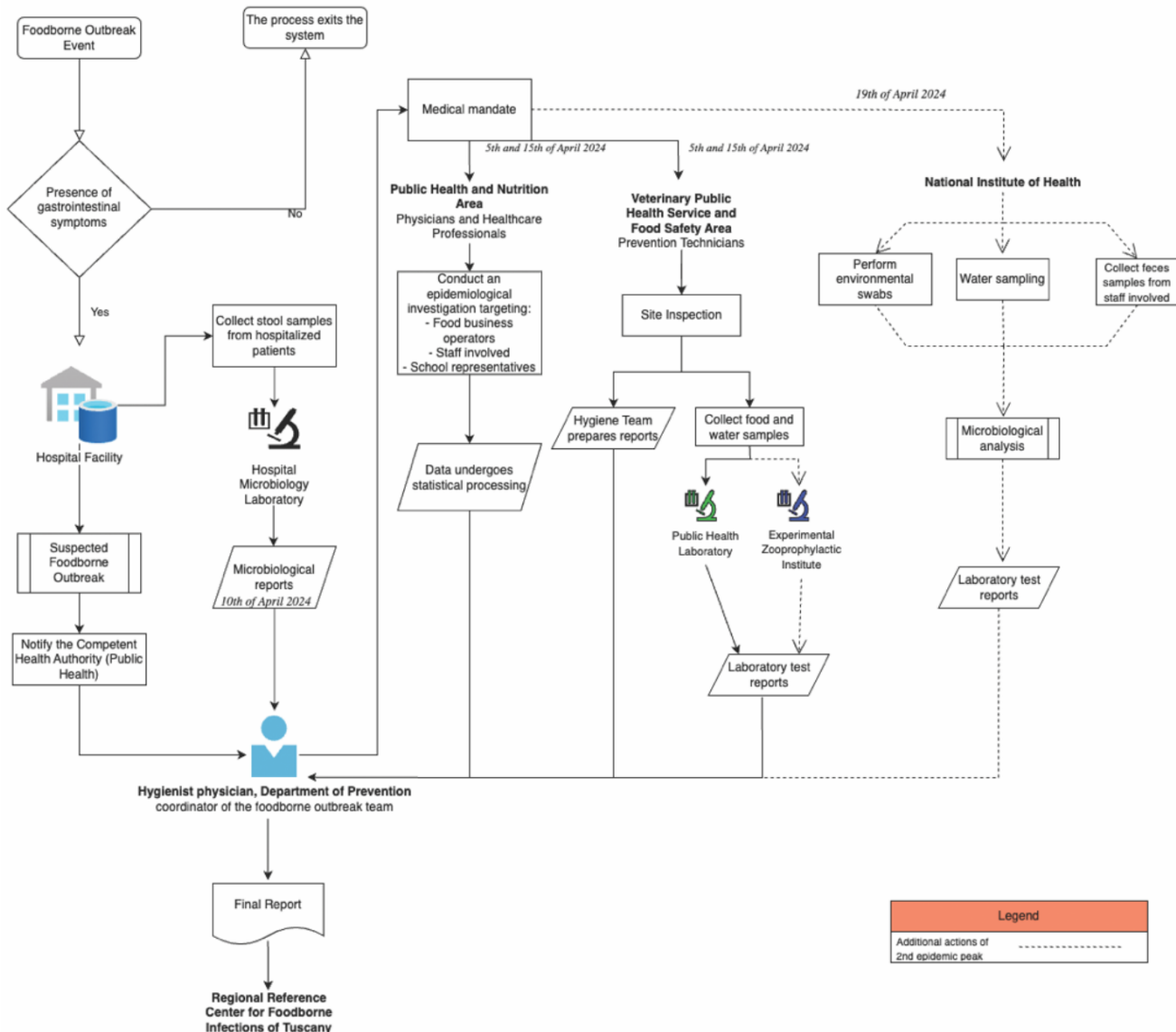


Figure 1. Mixed foodborne outbreak management flowchart.

the first epidemic wave because such analysis is not carried out by the Public Health Laboratory. Later, it was no longer possible to conduct tests on the same food products, as the samples were quantitatively insufficient to be sent to other laboratories. For the second epidemic wave, this difficulty was overcome by involving the IZSLT and the ISS. The tests on food and water did not detect the presence of NoV or other pathogens.

The results obtained from the epidemiological analysis could not exclude or confirm the involvement of contaminated food. For various foods under study, the results were inconsistent. The association of at-risk foods was much stronger for those who were hospitalized compared to those who only exhibited symptoms. Based on the calculations, the foods most at risk were roast meat and pasta with cream and ham, consumed on different days. The limitations of the epidemiological study were missing data for a school group and the inability to precisely determine the incubation period. Given the negative results for bacteria tested in food and water, the occurrence of gastrointestinal symptoms even among asymptomatic individuals exposed during the first wave after several days, and the conflicting epidemiological results, the hypothesis was made that the pathogen had a mixed transmission. Therefore, NoV was sought in the feces of the hospitalized cases from the first epidemic wave. The fecal tests were positive for NoV GI/GII.

Following the positive fecal results, it was considered appropriate to proceed with environmental swabs and to collect fecal samples from dining and kitchen staff. The feces of two staff members, who had experienced gastrointestinal symptoms, were positive for NoV GII. The feces remained positive for NoV GII even 7 and 15 days after the clinical symptoms. The feces of two other staff members, who reported not having any symptoms, were also positive for NoV GII.

Two environmental swabs carried out by the ISS tested positive for NoV GI and GII. This might indicate that the cleaning actions between the first and second waves were not sufficiently effective. For environmental and food processing surfaces, NoV is resilient on carpets, formica, stainless steel, PVC, and ceramic surfaces (Cook *et al.*, 2016). Further evidence of environmental transfer comes from a report of two carpet fitters who became ill after removing a carpet from a hospital ward 13 days after the last case in an NoV outbreak (Barker *et al.*, 2004).

All the reports from official controls, epidemiological investigations, and laboratory test results were forwarded to the coordinator of the Response Team for further analysis and the preparation of the Final Report, which was then sent to Ce.R.R.T.A, as required by regional guidelines.

Similar studies show that positivity is often found in environmental surfaces as well as staff working within collective settings. In 2016, a NoV outbreak in Spain affected 151 people in a hotel. The presence of NoV was detected in the patients' feces, as well as in surface samples from the rooms and common areas (Doménech-Sánchez *et al.*, 2020). Another example occurred in Oregon, USA: 75% of the employees of a car dealership contracted a NoV infection after consuming a communal meal consisting of sandwiches. Initially, foodborne transmission was suspected; however, subsequent investigations confirmed an environmental exposure attributed to an employee who, after changing the diaper of a child infected with NoV, left the restroom without taking adequate hygiene measures (Repp *et al.*, 2013). To provide greater clarity and understanding, we created a flowchart on Mixed Foodborne Outbreak Management, illustrating all the actions taken and all the stakeholders involved (Figure 1).

Conclusions

Based on our investigation and the reviewed studies, it is evident that the characteristics of NoV, as multiple transmission routes and prolonged persistence on surfaces and in the feces of cases, can make the investigation of the outbreak itself challenging. The multidisciplinary approach adopted helps to overcome the obstacles that may be encountered in the investigation of such events. The difficulties faced, along with the determination of all team members to understand the nature of the infectious outbreak and prevent its spread over time, encouraged the professionals involved to collaborate and seek support from other healthcare facilities. Our multidisciplinary investigation highlighted how, from a possible initial foodborne transmission, as indicated by statistical analysis, NoV can contaminate the surrounding environment. Additionally, the staff of the hospitality facility, both symptomatic and asymptomatic, can remain positive for several days after infection, further contributing to the spread of the pathogen.

This finding emphasizes the importance of a food safety culture, which should extend not only to food sector operators but also to hospitality staff handling food and to citizens. Regarding the prevention of viral gastroenteritis, the Centers for Disease Control and Prevention offers several brochures and training events aimed at both food sector operators and citizens (CDC, 2024). A similar approach could be applied in our region to limit such infectious events and simultaneously reduce their social and economic consequences.

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