Epidemiological study of an outbreak of Norovirus *in a rest home in Italy*

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Keywords
Nursing home (hospice), Gastroenteritis, Italy, *Norovirus*.

Summary
A *Norovirus* GII.4 (variant 2006) epidemic occurred in a rest home in the Abruzzo region of Italy between January and March 2009. Rest homes are typologically exposed to *Norovirus* infections as often reported in the literature. The investigation proposed in this article focused on identifying the source of infection and the route of propagation of the virus in the infected rest home. Microbiological, chemical and hygiene/health investigations were conducted on residents and employees of the home and on its water supply. A questionnaire was designed and distributed to identify the habits of the subjects who promoted the spread of the virus, in order to obtain a retrospective view of the event.

Veterinaria Italiana 2013, **49** (2), 175-180. doi: 10.12834/VetIt.2013.492.181.186

Introduction

Noroviruses are micro-organisms of the *Caliciviridae* family which might be responsible for human gastroenteritis. They feature marked genetic variability, comprising 5 genogroups (GI-GV) and 31 gene clusters. Genogroups I, II and IV are pathogenic for humans (4, 11). Transmission may occur through person-to-person contact, but also following the ingestion of contaminated water or food or by direct contact with contaminated surfaces (2, 3, 10, 12, 17). The most common symptoms are nausea, vomiting and diarrhoea and, sometimes, fever, headache, and joint pains. The symptoms generally appear 12-48 hours after infection, and persist for up to 60 hours. There is a high rate of infection in both children and adults (25), due to the low infecting dose and the inability of common cleaning practices to eliminate the viral load (8).

The incidence of *Norovirus* is so high that specific mathematical models have been created for its control, especially in hospitals (30). There is no national *Norovirus* surveillance system in Italy, but the few available reports indicate that the most widespread genogroup in Italy as well as in other European countries is GII, with the related genotypes GII.4, variants GII.4 2006a and GII.4 2006b (1, 9, 10, 13, 15, 24). The main sources of contagion are human-to-human contact and occasional food contamination. The most commonly affected facilities are those providing long-term accommodation such as hospitals and care homes for the elderly, especially in winter (14, 17, 26, 27).

A *Norovirus* outbreak was recorded in a care home in the Abruzzo region of Italy from January to March 2009. Microbiological and chemical tests were conducted on stool and water samples to investigate the source of infection. A questionnaire was designed to identify the habits of the subjects who promoted the spread of the virus in order to obtain a retrospective view of the event.

Materials and methods

Structure of the care home

The care home consisted of two separate buildings (A and B) with diversified functions and their own medical and paramedical personnel. The epidemic affected only building B.

Building A housed elderly people with serious health problems and geriatric disorders, some of whom had impaired movement ability and required continuous care. The two-storey building consisted of 42 double and triple rooms with en suite bathroom. Each storey was provided with a dining room, infirmary, lounge with television and telephones in the corridor.

Building B mainly housed partly and wholly

self-sufficient elderly people, often with minor geriatric problems, some of whom could go out of the home autonomously. The two-storey building, each floor having a lounge with television, consisted of 76 single and 12 double rooms, all with en suite bathroom and telephone. The first of the 2 storeys, equipped with a dining room, was reserved for partly self-sufficient residents, and the second for wholly self-sufficient residents. On the ground floor were the dining room, recreation room, clinic, chapel, and the offices of the voluntary association (with an independent entrance) responsible for free transport of residents to hospital. Medical, paramedical, catering and cleaning services were provided by 29 people. In particular, common laundry was washed in the internal laundry, while personal clothing was washed by the residents themselves and external laundries. The building was cleaned by an external service cooperative, which cleaned the premises daily. Catering for both buildings was outsourced to a catering service, which delivered meals three times a day. Sixty-two residents were housed in building B during the period of the epidemic.

Epidemiological investigation

The epidemiological investigation was conducted by means of a cohort study involving all the residents of building B and the personnel working there. A questionnaire was devised to gather information relating to personal data, duties, time spent in the home, symptoms, food eaten and visits received.

Each person who had suffered at least three episodes of diarrhoea or vomiting in the last 24 h was classified as a 'case'.

For the epidemiological investigation, the subjects were divided into two groups: one consisting of 62 residents and one of 29 employees. By the end of the study, there were 54 respondents, comprising 46 residents and 8 employees.

Microbiological analysis of stool samples

A total of 13 stool samples from 13 people that had exhibited recent symptoms of gastroenteritis or gastroenteritis ongoing at the time of the interview were examined. The samples were analysed for *Campylobacter* spp., *Salmonella* spp., *Escherichia coli* O157, *Shigella* spp., *Listeria monocytogenes*, *Yersinia enterocolitica*, *Norovirus,* and *Rotavirus*.

The *Campylobacter* test was conducted by direct seeding on m-CCDA agar (Biolife, Milan) and Karmali agar (Biolife), incubated at 42°C in microaerophilia. The *Salmonella* spp. test was conducted in accordance with standard ISO 6872:2002, and the *Escherichia coli* O157, *Shigella* spp., *Yersinia enterocolitica* and *Listeria monocytogenes* tests were conducted by direct seeding on MacConkey Mug-sorbitol agar (Oxoid, Cambridge), *Salmonella-Shigella* agar (Biolife), CIN agar (Biolife), and blood agar (BioMérieux, Marcy L'Étoile), respectively. All the cultures were incubated at 37°C for 24 h.

Table I. *Oligonucleotide sequences and TaqMan probe for the ORF1-ORF2 junction region of* Norovirus *GI and GII.*

***Norovirus* GI**
NV1LCpr-probe: 6-FAM–5'-TGG ACA GGA GAY CGC RAT CT–3'-TAMRA
ON1F4: 5'-CGC TGG ATG CGN TTC CAT-3'
NV1LCR: 5'-CCT TAG ACG CCA TCA TCA TTT CC -3'
***Norovirus* GII**
QN1FS- probe: VIC-5'-AGC ACG TGG GAG GGC GAT CG -3'-TAMRA
QN1F2d: 5'- ATG TTC AGR TGG ATG GAG RTT CTC WGA -3'
COG2R: 5'- TCG ACG CCA TCT TCA TTC ACA -3'

The *Rotavirus* test was conducted by ELISA method using the commercial ProSpect kit (Oxoid). The *Norovirus* test was conducted in RT-PCR with the commercial IDEIA kit *Norovirus* (IDEIA, Dallas, USA). The RNA for RT-PCR was extracted with the commercial High Pure Viral Nucleic Acid Kit (Roche, Milano, Italia). The amplification was conducted with the SuperScript™ III Platinum® One-Step Quantitative RT-PCR system kit (Invitrogen, Carlsband, California). The manufacturer's instructions were followed in all cases.

The oligonucleotides used and the TaqMan probe for the ORF1-ORF2 junction region are listed in Table 1.

The thermal profile for the RT-PCR reaction was as follows: 50°C × 15 min and 94°C × 2 min (reverse transcription); 45 cycles consisting of 15 min at 95°C (amplification) and 60°C for 30 sec (annealing).

The analysis was conducted with the PCR Real Time instrument ABI Prism® 7900 HT Fast RT-PCR. An aliquot of the samples was sent to the Istituto Superiore della Sanità, Roma, for confirmation of the result.

Microbiological and chemical tests on water

Five water samples were taken. Three samples of drinking water were taken at different points of the supply: the water storage tank, the kitchen tap used by the voluntary association, and the automatic distributor in the recreation room. The 2 samples of non-drinking water were taken from the fire-fighting and refrigerating installations.

The samples were analysed for *Escherichia coli* and Coliform bacteria (ISO 308-1:2000), *Pseudomonas aeruginosa* (ISO 16266:2006), Enterococchi (ISO 7899-2:2000), total bacteria count at 37°C and 21°C (ISO 8199:2005) and *Clostridium perfringens* (6).

For virus analyses, the water samples were concentrated with PrepScale™ TFF cartridge filters (Millipore, Milan). The samples were then decontaminated and further concentrated by ultrafiltration with Centricon plus 20 (Millipore). Finally, the same virological methods used for testing the presence of the virus in the stool samples were applied to the concentrated sample of water.

The residual chlorine was determined with the chromogenic method using the Aquaquant® Chloride kit (Merck, Darmstadt, GE) in accordance with the instructions of the manufacturer. Solids in suspension were detected by filtering one litre of water with a 0.45 µm membrane system, and determined by gravimetry after drying at 103°C-105°C.

Health measures

All the common and private areas of the building were thoroughly disinfected, including furnishing.

Surfaces were disinfected by contact with a 1% Virkon® solution (DuPont, Subdory,UK) and utensils and objects were disinfected by immersion in the same solution for at least 10 min. Kitchenware and laundry were washed with hypochlorite at 1,000 ppm, at temperatures exceeding 60°C, as indicated in the international guidelines for prevention of viral gastroenteritis in hospitals (2,3). A meeting was held with the employees of the home, during which the rules of hygiene were reiterated and the correct use of individual protection systems was urged. Those involved in the epidemic were asked to perform more thorough personal hygiene, to stay in their rooms and, in the case of employees, not to go to work for 48 h after the symptoms had disappeared.

Results

A total of 62 residents and 8 employees answered the questionnaire. Useful information, however, was only obtained from 54 (59%) respondents, comprising 46 residents and 8 employees. The survey identified the respondents who said they had had symptoms of gastroenteritis from 1 January to 17 March 2009 (Figure 1). Eight respondents were classified as 'cases': 6 residents and 2 members of the care home's staff. In particular, 4 residents and 2 employees also reported episodes of fever. Seven respondents tested positive for *Norovirus*, regardless of whether they were classified as 'cases'. Four of them reported that they had had episodes of vomiting in the dining room.

The bacteriological test for enteric bacteria was negative for all 13 samples examined. The virological research included 12 stool samples; one sample was not examined because the quantity was insufficient. Five samples (41.7%) tested positive for *Norovirus* by ELISA and 8 (66.6%) by RT-PCR. The RT-PCR positive samples were genogroup GII4 variant 2006. One sample tested positive for *Rotavirus*.

As regards water analysis, the samples taken from the refrigerating installation, the tank and the distributor in the recreation room showed a high bacteria count. *Norovirus* contamination was not found.

Solids in suspension were found in the water of the refrigerating installation and residual chlorine in

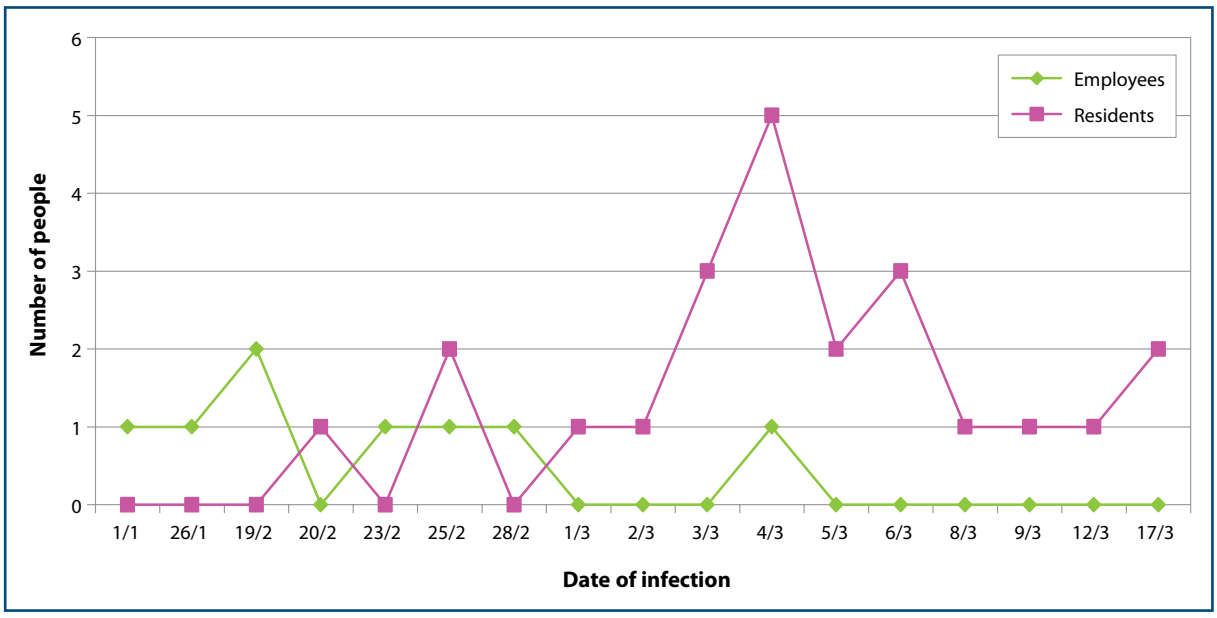

Figure 1. *Number of respondents presenting symptoms of gastroenteritis from 1 January to 17 March 2009.*

Table II. *Results of bacteriological and chemical tests on water.*

Sampling point	Type of water	Bacteria count 30°C (CFU/ml)	Bacteria count 21°C (CFU/ml)	Total coliform bacteria (CFU/100ml)	Faecal coliform bacteria (CFU/100 ml)	Streptococci (CFU/100 ml)	*P. aeruginosa* (CFU/100 ml)	*C. perfringens* (CFU/100 ml)	*Salmonella*	*Campylobacter*	Solids in suspension (mg/l)	Residual chlorine (mg/l)
Refrigerating installation	NP	160	160	20	0	3	28	6	A	A	1.1	NR (<0.1)
Fire-fighting installation	NP	0	0	0	0	0	0	0	A	A	NE	0.1
Reserve water tank	P	>300	>300	20	0	2	50	8	A	A	25.1	NR (<0.1)
Kitchen	P	0	0	0	0	0	0	0	A	A	NE	0.1
Water distributor	P	120	>300	0	0	0	40	0	A	A	NE	NE

NP = non-drinking water; P = drinking water; NR = not detectable; NE = not performed; A = absent.

that of the fire-fighting installation and the kitchen.

The results of the microbiological and chemical tests on the water are shown in Table II.

Discussion

On the basis of the initial investigations, the viral nature of the outbreak of gastroenteritis was suspected even before being confirmed by the microbiological tests, because the symptoms were consistent with Kaplan's description of *Norovirus* outbreaks: incubation period 24-48 h, episodes of vomiting in over 50% of cases, duration of illness between 12 h and 60 h (8).

The epidemiological investigation was conducted on the basis of the source of infection and its spread, and the structural conditions of the home were studied, together with the personal relations between residents and persons from outside the home, as human-to-human contact has a very high incidence in this type of infection (7).

The amount of data obtained with the questionnaire was less than expected. Simultaneously with the distribution of the questionnaire, it was decided to check on the state of the water supply and its possible correlation with the infection. The negative result of the bacteriological, virological and chemical tests demonstrated that the epidemic was unrelated to the water supply. Drinking water in the distributor and reserve water showed high bacteria count but enteric bacteria or virus were never isolated. The same catering service for both buildings, and only the involvement of people in building B, showed the non-involment of food epidemic. Unfortunately, the small number of stool samples on which microbiological tests could be conducted limited the objective data available for the study. Compared with the number of persons apparently involved, the samples were numerically small due to the incapacity of the patients and poor cooperation by care personnel in obtaining the samples. The relative non-comparability of the results obtained with ELISA and RT-PCR method is associated with the different sensitivities of the two methods (5, 28, 29).

The menu drawn up fortnightly by the catering service allowed us to evaluate, and rule out, the possible involvement of food in the epidemic, supporting the hypothesis that the virus spread only by human-to-human contact. This hypothesis was supported by the questionnaire completed by the first resident to suffer from symptoms, who reported that he never left the home. Sharing of common areas by self-sufficient residents consequently acquired crucial importance in the epidemic spread of gastroenteritis.

Following the cases of gastroenteritis recorded in the home, more stringent hygiene measures were introduced for preventive purposes, and this interrupted the transmission of the virus. This event suggests that isolation of the patient for up to 48 h after the disappearance of the symptoms, and simultaneous use of suitable disinfectants, is the only solution enabling this kind of epidemic to be terminated (3, 4).

Conclusions

Norovirus represents a major public health problem, because of its ability to produce clinically significant human infections in all age groups due to the high level of infectivity associated with the different transmission routes, and the inability of humans to develop lasting immunity.

In Italy, due to the lack of a suitable surveillance system and diagnostic protocols on a routine basis, together with the short duration of the symptoms and the immunity acquired, the actual incidence of *Norovirus* infection among the population is undoubtedly underestimated.

According to the psychiatrist and psychologist working at the home, the presence and persistence of gastroenteritis led to a state of reactive depression and anxiety in residents, which deteriorated their perceived quality of life. The study demonstrates the need to strengthen the differential diagnosis of forms of gastroenteritis so that specific preventive measures can be taken against the etiological agents involved. It is very helpful to implement an information plan for the population, to make them aware of the situation and encourage them to participate actively in limiting the spread of the infection. In our care home, where the average age of residents is about 80, providing information and modifying behaviour is difficult to implement, except for employees working in the facility.

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