The role of staff and contaminated environmental surfaces in spreading of norovirus infection in a long-term health care facility in Italy

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Summary
Some residents and people from the staff in an Italian geriatric health care facility, developed acute gastroenteritis from March 8th to March 21st 2017, in Teramo province. A prompt epidemiological investigation was conducted to identify the etiological agent of the outbreak and the potential mode of transmission. The cases (N = 50) were investigated according to an epidemiological questionnaire. Samples from all the cases (faeces) and highly transmissible environmental surfaces (swabs) were collected for analysis. Among faecal samples, 34 out of 50 were positive for norovirus (NoV) with no other pathogen detected. In particular, 2 (2/34) were positive only to NoV genogroup I (GI), 31 (31/34) were positive only to NoV genogroup II (GII), and one sample (1/34) was positive to both NoV GI and GII. Moreover, people from the canteen were also tested and they resulted negative to NoV detection in faeces. Among the positive samples, 12 NoV strains were subtyped as NoV GII.4 Sydney_2012 variant. Person-to-person close contact and contaminated environmental surfaces were the probable transmission route among the people of the health care facility. The members of the staff were considered to play an important role in transmission of NoV. A proper disinfection procedure applied during the outbreak could have been critically important to limit the dissemination of the viral infection.

Introduction

Norovirus (NoV) is a single-stranded positive-RNA virus of the family *Caliciviridae*. Being resistant to several stressors (e.g. high temperatures and desiccation), including disinfectants (Atmar and Estes 2006), Noroviruses are able to persist in the environment for several days. However, its presence is strongly linked to human population density and inadequacy of wastewater treatments (Bonadonna *et al.* 2019, Fusco *et al.* 2019, Purpari *et al.* 2019). NoVs are classified into ten genogroups (G) based on the variation of the major capsid protein (VP1), and are further divided into 49 genotypes. Of the 5 identified genogroups (GI to GV), only 3 have been shown to be pathogenic to humans; amongst them, GII.4 Sydney_2012 is the most widespread genotype since the mid-1990s (Ahlfeld *et al.* 2015, De Graaf *et al.* 2015). NoV is the leading cause of acute gastroenteritis in people of all ages worldwide. It is estimated to cause 12-24% of community-based or clinic-based cases of acute gastroenteritis, 11-17% of emergency room or hospital cases and approximately 70,000-200,000 human deaths of all ages, annually (Bányai *et al.* 2018). NoVs are highly contagious, and 10-100 viral particles may be sufficient to infect an individual (Zarb *et al.* 2012). In immunocompetent people, the

disease is self-limited with recovery within 2-5 days while in those who are immunecompromised, it can cause severe dehydrating diarrhoea. The main clinical signs of viral gastroenteritis also include vomiting accompanied by nausea, abdominal cramps, and fever. There are a number of different routes through which NoV transmission could occur. The main transmission mode is person to person through faecal-oral route. Other possible way include contaminated food, water or surfaces (Lin *et al.* 2011, Parrón *et al.* 2020). Several NoV outbreaks have been recorded in cruise ships, hospitals and long term care facilities (LTCFs), where infections spread rapidly, have high attack rates and are difficult to control (Centers for Disease Control And Prevention 2003, Hofmann *et al.* 2020). In particular, in LTCFs most residents are bedridden and elderly, and the NoV spread is facilitated by enclosed living quarters and reduced levels of personal hygiene, because of faecal incontinence, immobility, dementia or need of assistance (Yang *et al.* 2010, Ali *et al.* 2014). In these settings, environmental surfaces and health care assistants can play a key role in the transmission of NoV.

From the 8th to the 21st of March 2017, a gastroenteritis outbreak occurred in a geriatric nursing facility in Teramo province (Abruzzo, Italy) and involved 38 elderly patients, 8 health care workers, 2 nurses, 1 animator and 1 maintenance technician. The outbreak was investigated, the causative pathogen identified, the main routes of transmission hypothesized and the risk factors analysed. In particular, our investigation highlighted the role of health care workers and environmental surfaces in the spread of NoV infection. Moreover, a new protocol for cleaning and disinfection of the healthcare environments that helped to control the viral infection in the LTCFs has been presented.

Methods

Case definition and data collection

The total number of residents in the LTCF was 177 people; 72 of these were health care assistants. In this study a norovirus case was defined as residents or staff in the geriatric nursing facility with at least 1 of the following symptoms: (1) diarrhoea (more than 3 times in a 24-hour period), (2) vomiting, (3) nausea, and (4) abdominal pain, occurring from the 8th to the 21st of March 2017. The investigation was based on an epidemiological questionnaire where information on the onset of symptoms, history of contact with infected persons and personal hygiene habits was collected.

Environmental investigation

An environmental investigation was carried out in order to collect information related to the layout of buildings, the disinfection procedures and staff organization within the premises.

The LTCF was organised in 2 different buildings: the building A and the building B. The building A had 4 floors, each with 1 living room. In this structure, there were 76 single rooms and 12 double rooms, each with a toilette. The building A was reserved to partially or completely self-sufficient elderly people, generally with minor health problems, and able to leave the residence, independently. The second building (B), was divided into 2 floors, each with a refectory, infirmary and living room. The building B consisted of 5 units with a total of 42 double and triple rooms, equipped with bathrooms. It housed elderly people with serious health problems and senile pathologies, some of them with movement difficulties and the need of continuous assistance.

Inside the 2 buildings, each nursing assistant was in charge of about 25 residents and there was no precise distinction between staff from the building A and the building B. The most part of nursing assistants did not have systematic professional nursing training.

The canteen was located at the first floor of the building A; a private company managed the cooking and distribution with its own staff.

Sample collection

Between the 8th and the 21st of March, 180 samples were collected. They include 58 faecal and 122 environmental surface samples. Of the 58 faecal samples, 50 were individually collected from all cases (N = 50), while 8 were from people of the canteen staff with no gastrointestinal symptoms. One-hundred-and-two samples were collected from highly touched environmental surfaces between the 10th and the 11th of March, to check the effectiveness of the ongoing cleaning/disinfection procedures. These surface samples were taken from all the different areas of buildings A and B.

The protocol for cleaning/disinfection described in Table I was applied from the end of day 16th of March 2017, which differed from the protocol commonly used before in the following parts: the usage of disposable cloth when changing surfaces; the application of sodium hypochlorite for 5 minutes at minimum. Soon after the application of the new protocol, 20 further samples were taken (on the 17th of March) from the same previously positive surfaces, in order to verify the success of this new cleaning/disinfection procedure.

All samples were transported to the laboratory

Table I. *Cleaning and disinfection procedure applied during the outbreak.*

Step	Action
1	Surface cleaning with a common detergent
2	Sanitization with sodium hypochlorite 50,000 ppm (5%), left on surfaces for 5 minutes
3	Removal with a disposable wet cloth
4	Cleaning with disposable cloth moistened with alcohol 90°

to detect *Escherichia coli* (ISO/TS 13136: 2012), *Salmonella* spp. (UNI EN ISO 6579-1: 2017), *Shigella* spp. (ISO 21567: 2004), *Yersinia enterocolitica* (ISO 10273:2017), *Vibrio* spp. (ISO 8914: 1990), Rotavirus and Adenovirus (Van Maarseveen *et al.* 2010), NoV and Hepatitis A virus (ISO 15216-2: 2013).

Sample processing and viral RNA extraction

For the viral concentration step, approximately 1 gram (gr) of each faecal sample was dissolved into 1 millilitre (ml) of phosphate buffered saline (PBS) at pH 7.2, added with gentamicin, penicillin, nystatin and streptomycin. In the case of environmental samples, each cotton swab from the surfaces was absorbed in 2 ml of PBS at pH 7.2, immediately after sampling. Ten ± 0.1 microliters (µl) of mengovirus (process control virus, National Reference Laboratory for Foodborne Viruses, Istituto Superiore di Sanità, Rome, Italy) were added to each sample at the final concentration as reported in ISO 15216-2: 2013. After vortexing for 1 minute (min), faecal samples were clarified by centrifugation at 10,000 gravity (g) for 10 min at room temperature. RNA was extracted from 500 µl suspension using the NucliSens MiniMAG platform with the NucliSens magnetic extraction Kit (BioMeırieux, Marcy-l'Étoile, France) according to the manufacturer's instruction.

Real time RT-PCR detection of NoV

RNA samples were amplified by real time reverse transcriptase polymerase chain reaction (RT-PCR) for NoV GI and GII, as described by the ISO 15216-2: 2013, using UltraSenseTM One-Step Quantitative RT-PCR System kit (Invitrogen, Germany).

Sequence analysis of NoV

Real time RT-PCR positive samples were also analysed by end-point RT-PCR, with primer sets G1SKF/G1SKR and G2SKF/G2SKR annealing to Open Reading Frame (ORF) 2 and specific for GI and GII, respectively (Kojima *et al.* 2002).

The RT-PCR was followed by a semi-nested PCR, using the primer sets COG1F/G1SKR for GI and COG2F/ G2SKR for GII.

DNA amplicons were purified by the QIAquick PCR Purification Kit (Qiagen, Milan, Italy) or by the ExoSAP (Affimetrix, USA) enzyme and sequences were elaborated by Eurofins (Milan, Italy). Sequencing data were edited and aligned using MEGA6. The genotypes were assigned using the public database NoroNet typing tool (http://www.rivm.nl/mpf/norovirus/typingtool).

Results

Detection of pathogens involved in the outbreak

Of the 180 samples tested in this survey, 62 (34.44%) [34 (54.83%) faecal samples and 28 (45.16%) surface swabs] were positive for NoV. No other pathogens (intestinal bacteria and enteric viruses) were detected.

Genotype determination of NoV

Viral RNA from 12 faecal and 5 surface samples was successfully characterized (BLASTn and NoroNet Typing Tool database) as NoV GII.4 Sydney_2012 variant (sequence submitted to GenBank database; accession numbers MN581063 - MN581079). For the other positive samples it was not possible to identify the genotype.

Epidemiological investigation

The epidemiological investigation started only from the 8th of March 2017, when it appeared clear to public authority that the spreading of the disease was worsening.

All residents in this LTCF were old (average 84.64 years old) and nursing assistants provided most of their daily living care.

From the 8th to the 21st of March, 34 out of 50 cases were confirmed to be infected with NoV (25 residents, 7 social health operators, 1 nurse and 1 maintenance technician) (Table II). In particular, 2 (2/34) were positive to NoV GI, 31 (31/34) to NoV GII and one sample (1/34) was positive to both NoV GI and GII. Twelve NoV GII positive samples were genotyped as NoV GII.4 Sydney_2012 strain (99% nucleotide identity with the reference GII.4 Sydney_2012 strain accession number JX459908). All obtained sequences showed 100% nucleotide identity. Faecal samples from 8 people of the canteen staff with no gastrointestinal symptoms were negative to NoV. As being the total number of

residents 177, and the total number of employees 72, the attack rate was respectively of 21.47% (38/177) and 13.89% (10/72).

In addition, 28/122 environmental samples resulted positive to NoV GII. The viral strains from the contaminated surfaces (5/28) were identified as NoV GII.4 Sydney_2012 (Table III).

Sequence alignment of the strains detected in human and environmental samples showed 100% nt identity.

Environmental investigation

At the beginning (from the 8th to the 11th of March), the outbreak involved only residents from building A (N = 12) and health care workers (N = 4) (Table II). The first cases among the residents in building B appeared on the 13th of March. Nevertheless, the evidence of the NoV presence in surface samples in building B was detected on March 10th and 11th (Figure 1), demonstrating that the virus was already present in those premises before the onset of the first cases.

In total, 13 residents from the building A and 12 from the building B were found infected with NoV between March 8th and 21st, 2017. In the remaining 13/38 residents, though falling into case definitions (gastrointestinal symptoms), NoV was not detected and so they were excluded from the NoV cases.

Table II. *Details of confirmed human cases showing clinical manifestations of NoV infection.*

ID number	Date	Age	Role	Gender	Symptoms	Setting
1	08.03.2017	56	health care worker	female	vomiting and diarrhea	-
2	08.03.2017	52	health care worker	female	vomiting and diarrhea	-
3	08.03.2017	75	resident	female	vomiting and diarrhea	building A, 2nd floor
4	08.03.2017	93	resident	female	vomiting, diarrhea, dehydration, sensory deprivation	building A, 2nd floor
5	08.03.2017	90	resident	male	vomiting, diarrhea, dehydration, sensory deprivation	building A, 1st floor
6	08.03.2017	78	resident	male	vomiting and diarrhea	building A, 3rd floor
7	08.03.2017	66	health care worker	female	vomiting and diarrhea	-
8	08.03.2017	93	resident	female	vomiting, diarrhea, dehydration, sensory deprivation	building A, 3rd floor
9	08.03.2017	89	resident	male	vomiting and diarrhea	building A, 4th floor
10	08.03.2017	92	resident	female	vomiting, diarrhea, dehydration, sensory deprivation	building A, 3rd floor
11	08.03.2017	94	resident	male	vomiting, diarrhea, dehydration, sensory deprivation	building A, 2nd floor
12	08.03.2017	95	resident	female	vomiting, diarrhea, dehydration, sensory deprivation	building A, 1st floor
13	08.03.2017	88	resident	female	vomiting, diarrhea, dehydration, sensory deprivation	building A, 1st floor
14	11.03.2017	54	health care worker	female	vomiting and diarrhea	-
15	11.03.2017	92	resident	male	vomiting, diarrhea, dehydration, sensory deprivation	building A, 4th floor
16	11.03.2017	85	resident	male	vomiting and diarrhea	building A, 4th floor
17	13.03.2017	36	nurse	female	vomiting and diarrhea	-
18	13.03.2017	80	resident	female	vomiting, diarrhea, dehydration, sensory deprivation	building B, Unit 2
19	13.03.2017	87	resident	female	vomiting, diarrhea, dehydration, sensory deprivation	building B, Unit 2
20	14.03.2017	85	resident	female	vomiting, diarrhea, dehydration, sensory deprivation	building B, Unit 5
21	14.03.2017	90	resident	female	vomiting, diarrhea, dehydration, sensory deprivation	building B, Unit 5
22	14.03.2017	91	resident	female	vomiting, diarrhea, dehydration, sensory deprivation	building B, Unit 5
23	15.03.2017	87	resident	female	vomiting, diarrhea, dehydration, sensory deprivation	building B, Unit 1
24	15.03.2017	81	resident	female	vomiting and diarrhea	building B, Unit 2
25	15.03.2017	75	resident	female	vomiting and diarrhea	building A, 1st floor
26	16.03.2017	43	health care worker	female	vomiting and diarrhea	-
27	16.03.2017	73	resident	female	vomiting and diarrhea	building B, Unit 1
28	20.03.2017	90	resident	female	vomiting, diarrhea, dehydration, sensory deprivation	building B, Unit 3
29	20.03.2017	63	health care worker	female	vomiting and diarrhea	-
30	20.03.2017	88	resident	female	vomiting and diarrhea	building B, Unit 4
31	20.03.2017	68	resident	male	vomiting and diarrhea	building B, Unit 4
32	20.03.2017	84	resident	female	vomiting, diarrhea, dehydration, sensory deprivation	building B, Unit 4
33	21.03.2017	30	health care worker	female	vomiting, diarrhea, dehydration, sensory deprivation	-
34	21.03.2017	24	maintenance technician	male	vomiting and diarrhea	-

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Table III. *List of environmental surfaces resulted positive to the detection of NoV GII during the outbreak.*

Environmental surfaces	Number of surfaces tested	Number of surfaces positive to NoV GII (%)	Building A	Building B
Bed rails	10/122	6/10 (60)	3	3 (Unit 5)
Elevator push-button panel	10/122	2/10 (20)	0	1 (Unit 3) 1 (Unit 2)
Cleaning trolley handles	12/122	2/12 (16,7)	1	1 (Unit 3)
Handrails	6/122	1/6 (16,7)	0	1 (Unit 4)
Laundry trolley clamps	6/122	1/6 (16,7)	0	1 (Unit 3)
Bathroom taps	31/122	8/31 (25,8)	3	1 (Unit 4) 1 (Unit 3) 1 (Unit 2) 1 (Unit 1) 1 (Unit 5)
Door handles	39/122	6/39 (15,4)	3	1 (Unit 3) 2 (Unit 5)
Sink pedals	5/122	1/5 (20)	0	1 (Unit 3)
Radio buttons (from a shared radio in one of the living rooms)	3/122	1/3 (33,3)	0	1 (Unit 5)

Control measures

After the application of the cleaning/disinfection procedure showed in Table I, only 1 out of the 20 surface samples taken was still positive for NoV GII to real time RT-PCR screening. Nevertheless, the genome sequence was not achieved and subtype was not possible to be identified, due probably to the low amount of the virus particles.

Moreover, it was also suggested to reduce the staff movements among different units of the buildings as much as possible. Other actions that were taken included cases isolation, health education on hand hygiene habits, more frequent cleaning and disinfection of bathrooms and toilets in the rooms.

Eight new cases were observed on March 20. Five of them were due to NoV GII. On the 21st of March, 2 further cases were confirmed. GII.4 subtype was identified as responsible for these cases. No more cases were observed from the 22nd of March and the outbreak was considered officially closed.

Discussion and conclusions

NoV is characterized by a low infectious dose and a strong stability in the environment (Atmar and Estes 2006). However, experimental studies estimated the 50% human infectious dose measured was similar to that of other RNA viruses (Atmar *et al.* 2014).

This virus can be transmitted to the residents of

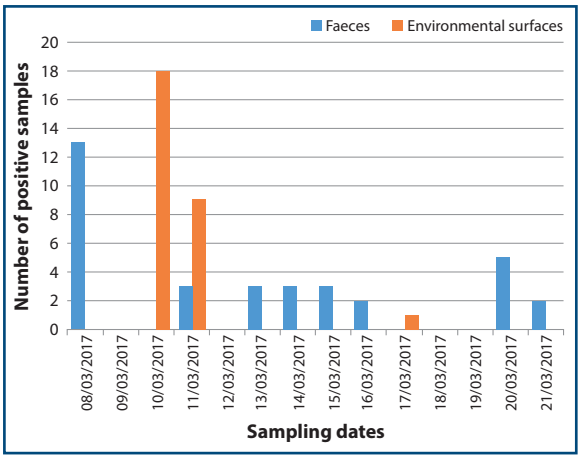

Figure 1. *Positive samples (faeces and environmental swabs) collected and analysed during the outbreak period.*

health care settings by visitors and staff who may be asymptomatic, pre-symptomatic or symptomatic.

Also, contaminated food can be the source of NoV introduction in these settings and could be responsible for the beginning of outbreaks (Rushton *et al.* 2019).

A previous NoV outbreak occurred in the same LTCF in 2009 (Di Giannatale *et al.* 2013). As at that time, also in the present study no role was supposed to be played by food and canteen staff, since the canteen itself was located in a separate area of building A. No members of the staff fell within case definitions and the infections showed a progressive involvement of different areas of the two buildings. If the outbreak originated from contaminated food, a simultaneous involvement of all areas of both the LTCF buildings since the food prepared in the canteen was the same distributed in the whole facility, every day.

In this outbreak, the source of NoV and the identification of the first case were not defined, but the epidemiological investigations suggested that health care staff assistants have played a crucial role in the secondary spread of the infection, as frequently reported in literature (Danzmann *et al.* 2013, Lai *et al.* 2013, Di Giannatale *et al.* 2013, Ho *et al.* 2015, Zheng *et al.* 2015, Chong and Atmar 2019).

The detection of the same, unique NoV strain, GII.4 Sydney_2012 variant, during the whole period of surveillance, indicated that person-to-person transmission has significantly contributed in the spreading of the virus infection. The same virus was also detected in the environmental swabs, implying the occurrence of cross-contaminations, which may have also contributed to the spread of the infection.

The outbreak started from building A and then progressively involved the building B, from March 13. In this scenario, the personnel of the LTCF provided continuous assistance to the residents, being in

constant contact with them. However, the finding of NoV on the surfaces of building B detected on the 10th and the 11th of March let us suggests that the virus was already present in these premises before the occurrence of the first cases. None of the residents were transferred from building A to B and no contacts among residents were recorded. Health care staff was promiscuous between the buildings, and the contact with residents was continuous.

From the results of this survey, it appears that health care assistants were responsible for spreading the infection not only by direct contact but alos by contaminating the surfaces of the health care facility (Lin *et al.* 2011). Environmental surfaces not well disinfected have been demonstrated to be source of infection for residents and nursing assistants (Wu *et al.* 2005).

In our study, 28 out of 122 (22.95 %) surface samples were positive for NoV GII (Table III). Highly touched surfaces from the 2 buildings were chosen for sampling (swabbed).

Our results corroborated what is already reported in the literature regarding the most common surfaces involved in spreading the virus in close and semi closed settings (Rico *et al.* 2020) (Table III). The sample from 1 bed rail continued to test positive for NoV GII also after accurate cleaning and disinfection (1 sample over 20).

This paper also reports for the first time the application of an ISO method (ISO 15216-2: 2013) for the detection of NoV from environmental surfaces, while the laboratory methods used in the previously cited papers were developed in-house. In particular, we successfully applied the same part of the standard procedure that describes the detection of NoV RNA from food contact surfaces. No validation activities were carried out to verify the quality parameters in relation to this modification to the standard method adopted; therefore, more in-depth studies should be carried out in the future at this regard.

Although the source of NoV was not clearly identified in this outbreak, NoV GII.4 can indeed be considered as the cause of infection. This variant has been reported as the most frequent cause (70-80%) of NoV associated gastroenteritis outbreaks worldwide since the mid-1990s (De Graaf *et al.* 2015).

Apart from 3 faecal samples positive to NoV GI, all the other samples collected in this outbreak were positive to NoV GII. NoV GII.4 was detected on all contaminated surfaces. The main control measures to be implemented during an outbreak include: quarantine of infected individuals, enhanced environmental decontamination and enhanced hand hygiene (Arias *et al.* 2013). However, the application of these procedures can be difficult for many reasons; in our case scenario, in particular, shortage of staff as well as the time-consuming activities for an effective disinfection, may have been the most critical factors that contributed to the spreading of NoV infection.

To date, no methods have been demonstrated efficacious for the inactivation of human NoV. The development of real time RT-PCR protocols for NoV RNA detection improved the possibility to identify viral fragments in different matrices, but the inability to assess the viability of viral particles is still an important limit. Murine NoV is often used as human NoV surrogate, but there are deep differences between the 2 viruses including the susceptibility to inactivation methods (Cromeans *et al.* 2014). There are some indications about the efficacy of environmental cleaning against NoV using sodium hypochlorite at concentrations of 1,000-5,000 ppm (Keswick *et al.* 1985, CDC 2011). Nevertheless, some kind of faecal and soil may render 5,000 ppm of sodium hypochlorite not effective against infective particles and longer exposure time could be needed (Barker *et al.* 2004). For these reasons, in our study, we decided to use a more concentrated sodium hypochlorite solution (50,000 ppm), that was applied in the protocol described in Table I, and that probably helped in reducing the number of new cases up to the 19th of March. However, 8 new cases were identified on day March 20th, with 62.5% of confirmed diagnostic positivities to NoV GII (5/8), and 2 more confirmed cases on day March 21st. Nevertheless, the outbreak was considered officially closed the 22nd of March, with no more cases.

In conclusion, in this work we reported a NoV associated acute gastroenteritis outbreak that occurred in a long-term care facility in Italy in 2017. NoV GII.4 was the genotype associated with this outbreak. Person-to-person close contact and contaminated environmental surfaces were the probable transmission routes, with health care assistants playing the key role in virus spreading. In particular, for NoV outbreaks, the implementation of infection control measures is fundamental. From our point of view, an effective environmental cleaning and disinfection, accurate and frequent hand washing and limited circulation of the staff among different areas of the premises, are the most important actions to prevent viral infections spreading when residents from LTCFs are involved. These measures could be important also as general principals, in order to mitigate the burden related to the environmental NoV contamination from infectious people (Bonadonna *et al.* 2019, Fusco *et al.* 2019, Purpari *et al.* 2019).

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References

Ahlfeld B., Li Y., Boulaaba A., Binder A., Schotte U., Zimmermann J.L., Morfill G. & Klein G.2015. Inactivation of a foodborne norovirus outbreak strain with nonthermal atmospheric pressure plasma. *MBio*, **6** (1), e02300-14.

Ali M.M., Aburowes A.H., Albakush A.M., Rzeg M.M., Alrtail A. & Ghenghesh KS.2014. Identification of multidrug-resistant bacteria and *Bacillus cereus* from healthcare workers and environmental surfaces in a hospital. *Libyan J Med*, **9** (1), 25794.

Arias A., Emmott E., Vashist S. & Goodfellow I. 2013. Progress towards the prevention and treatment of norovirus infections. *Future Microbiol*, **8** (11), 1475-1487.

Atmar R.L. & Estes M.K. 2006. The epidemiologic and clinical importance of norovirus infection. *Gastroenterol Clin*, **35** (2), 275-290.

Atmar R.L., Opekun A.R., Gilger M.A., Estes M.K., Crawford S.E., Neill F.H., Ramani S., Hill H., Ferreira J. & Graham D.Y. 2014. Determination of the 50% human infectious dose for Norwalk virus. *J Infect Dis*, **209** (7), 1016-1022.

Bányai K., Estes M.K., Martella V. & Parashar U.D. 2018. Viral gastroenteritis. *Lancet*, **392** (10142), 175-186.

Barker J., Vipond I.B. & Bloomfield S.F. 2004. Effects of cleaning and disinfection in reducing the spread of Norovirus contamination via environmental surfaces. *J Hospital Infect*, **58**, 42-49.

Centers For Disease Control (CDC). 2003. Norovirus activity-United States, 2002. *MMWR*, **52** (3), 41-45.

Chong P.P. & Atmar R.L. 2019. Norovirus in health care and implications for the immunocompromised host. *Current Opinion Infect Dis*, **32** (4), 348-355.

Cromeans T., Park G.W., Costantini V., Lee D., Wang Q., Farkas T., Lee A. & Vinjé J. 2014. Comprehensive comparison of cultivable norovirus surrogates in response to different inactivation and disinfection treatments. *Appl Environ Microbiol*, **80** (18), 5743-5751.

Danzmann L., Gastmeier P., Schwab F. & Vonberg R.P. 2013. Health care workers causing large nosocomial outbreaks: a systematic review. *BMC Infect Dis*, **13**, 98.

de Graaf M., van Beek J., Vennema H., Podkolzin A.T., Hewitt J., Bucardo F., Templeton K., Mans J., Nordgren J., Reuter G., Lynch M., Rasmussen L.D., Iritani N., Chan M.C., Martella V., Ambert-Balay K., Vinjé J., White P.A. & Koopmans M.P. 2015. Emergence of a novel GII.17 norovirus – End of the GII.4 era? *Euro Surveill*, **20** (26), 21178.

Di Giannatale E., Alessiani A., Sauro F., Sbraccia F., Croce G., Nissim A., Carnevale A. & Migliorati G. 2013. Epidemiological study of an outbreak of Norovirus in a rest home in Italy. *Vet Ital*, **49** (2), 175-180.

Division of Viral Diseases, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention (CDC). 2011. Updated norovirus outbreak management and disease prevention guidelines. *MMWR Recomm Rep*, **60** (RR-3), 1-18.

Ho Z.J., Vithia G., Ng C.G., Maurer-Stroh S., Tan C.M., Loh J., Lin T.P. & Lee J.M. 2015. Emergence of norovirus GI.2 outbreaks in military camps in Singapore. *Int J Infect Dis*, **31**, 23-30.

Hofmann F.M., Olawumi E., Michaelis M., Stößel U. & Hofmann F. 2020. Significance of norovirus in occupational health: a review of published norovirus outbreaks in Central and Northern Europe. *Int Arch Occup Environ Health*, **93** (8), 911-923.

Keswick B.H., Satterwhite T.K., Johnson P.C., DuPont H.L., Secor S.L., Bitsura J.A., Gary G.W. & Hoff J.C. 1985. Inactivation of Norwalk virus in drinking water by chlorine. *Appl Environ Microbiol*, **50** (2), 261-264.

Kojima S., Kageyama T., Fukushi S., Hoshino F.B., Shinohara M., Uchida K., Natori K., Takeda N. & Katayama K. 2002. Genogroup-specific PCR primers for detection of Norwalk-like viruses. *J Virol Methods*, **100** (1-2), 107-114.

Lai C.C., Wang Y.H., Wu C.Y., Hung C.H., Jiang D.D. & Wu FT. 2013. A norovirus outbreak in a nursing home: norovirus shedding time associated with age. *J Clin Virol*, **56** (2), 96-101.

Lin H., Ng S., Chan S., Chan W.M., Lee K.C., Ho S.C. & Tian L. 2011. Institutional risk factors for norovirus outbreaks in Hong Kong elderly homes: a retrospective cohort study. *BMC Public Health*, **11**, 297.

Parrón I., Barrabeig I., Alseda M., Cornejo-Sánchez T., Guix S., Jané M., Izquierdo C., Rius C. & Domínguez À. 2020. The working group for the study of acute gastroenteritis outbreaks in Catalonia. Involvement of workers in closed and semiclosed institutions in outbreaks of acute gastroenteritis due to Norovirus. *Viruses*, **12** (12), 1392.

Rico E., Pérez C., Belver A., Sabaté S., Razquin E., de Benito

J., Coronas L., Domínguez A., Jané M. & Working Group for the Study of Outbreaks of Acute Gastroenteritis in Catalonia (PI16/02005). 2020. Norovirus detection in environmental samples in norovirus outbreaks in closed and semi-closed settings. *J Hosp Infect*, **105** (1), 3-9.

Rushton S.P., Sanderson R.A., Reid W.D.K., Shirley M.D.F., Harris J.P., Hunter P.R. & O'Brien S.J. 2019. Transmission routes of rare seasonal diseases: the case of norovirus infections. *Philos Trans R Soc Lond B Biol Sci*, **374** (1776), 20180267.

Wu H.M., Fornek M., Schwab K.J., Chapin A.R., Gibson K., Schwab E., Spencer C. & Henning K. 2005. A norovirus outbreak at a long-term-care facility: the role of environmental surface contamination. *Infect Control Hosp Epidemiol*, **26** (10), 802-810.

Yang L.C., Chiang P.C., Huang T.H., Chi S.F., Chiu Y.P., Lin C.S., Chou Y.F., Hsu S.C., Zhang X.S., Huang C.G., Kao C.Y. & Lin C.M. 2010. Residents had an increasing risk of norovirus gastroenteritis infection than health care workers during an outbreak in a nursing home. *J Am Med Dir Assoc*, **11** (8), 592-597.

Zarb P., Coignard B., Griskeviciene J., Muller A., Vankerckhoven V., Weist K., Goossens M., Vaerenberg S., Hopkins S., Catry B., Monnet D., Goossens H., Suetens C. & National Contact Points for the ECDC pilot point prevalence survey; Hospital Contact Points for the ECDC pilot point prevalence survey. 2012. The European Centre for Disease Prevention and Control (ECDC) pilot point prevalence survey of healthcare-associated infections and antimicrobial use. *Euro Surveill*, **17** (46), 20316.

Zheng Q.M., Zeng H.T., Dai C.W., Zhang S.X., Zhang Z., Mei S.J., He Y.Q. & Ma H.W. 2015. Epidemiological investigation of a norovirus GII.4 Sydney outbreak in a China elder care facility. *Jpn J Infect Dis*, **68** (1), 70-74.