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Norovirus detection in environmental samples in norovirus outbreaks in closed and semi-closed settings

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SUMMARY

Background: Environmental surfaces are a potential vehicle for the transmission of norovirus outbreaks in closed and semi-closed settings. Testing of environmental samples may help control outbreaks.
Aim: To assess the level of environmental contamination by norovirus in acute gastroenteritis outbreaks in closed or semi-closed settings (nursing homes, schools, kindergartens, youth accommodations, hospitals and social health centres) in the Barcelona region between January 2017 and March 2019.
Methods: A prospective surveillance study was carried out. Environmental samples (529) were collected in 46 of the 50 outbreaks of acute norovirus gastroenteritis from environmental surfaces of common areas, bathrooms and kitchens in closed and semi-closed settings when the outbreak was notified and 10 days later. Instructions for taking environmental samples were distributed to public health inspectors. Norovirus was detected by reverse transcription polymerase chain reaction.
Findings: Environmental samples were positive for norovirus in 31 (67.4%) outbreaks. Norovirus was most frequently detected on elevator buttons (4/17, 24%), toilet handles (16/66, 24%) and handrail bars (7/34, 21%). Positive samples from the first sampling were mainly found in bathrooms and greater viral persistence in the second sampling was found on elevator buttons and TV remote controls. Nursing homes were the setting with the most

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types of environmental surfaces contaminated (82% in first samples and 55% in second samples).

Conclusion: The probability of virus detection is independent of the time between notification of the outbreak or symptom onset and sample collection. Our results suggest possible defects in cleaning protocols and disinfection in closed and semi-closed settings.

Introduction

Noroviruses are non-enveloped RNA viruses belonging to the calicivirus family with six genogroups (GI—GVI), although only genogroups I, II and IV are human pathogens [1,2]. Symptoms are generally characterized by nausea and vomiting, and the evolution is usually self-limiting, lasting about 48—72 h [3].

The viral capsid provides enhanced resistance to the external environment, high concentrations of chlorine [4], heat and cold [5], acidic pH and organic solvents [6,7], characteristics which mean norovirus has a high infective capacity and environmental persistence [8] and highly efficient transmission modes [9]. The low infective dose, with a mean of 18 viral particles [7], together with the lack of long-term immunity in infected people means norovirus very frequently causes outbreaks, which are particularly frequent in closed or semi-closed settings and may last for extended periods [10]. Infected individuals may excrete norovirus for up to 30—40 days and excretion may be especially prolonged in immunosuppressed people: up to 15 months in HIV-infected people and 898 days in transplant patients [11,12].

The direct fecal—oral route is the principal transmission mechanism of norovirus, although transmission by aerosols, generated by vomiting, also occurs. The principal indirect routes of transmission are food-borne and water-borne and contact with contaminated surfaces [3].

Contamination of objects in the environment by people excreting norovirus may facilitate transmission, and decontamination of surfaces with sodium hypochlorite at concentrations of 1000—2000 ppm (5000 ppm if not previously cleaned) is essential [13]. Recommendations for controlling outbreaks include the elimination of norovirus from environmental surfaces that may facilitate transmission [10].

Studies have suggested that environmental contamination facilitates the spread of outbreaks [14—16]. The objective of this study was to analyse the level of environmental contamination detected in acute gastroenteritis (AGE) outbreaks caused by genogroup I and II norovirus in closed or semi-closed settings (nursing homes, schools, kindergartens, youth accommodations, hospitals and social health centres) in the Barcelona region, distinguishing between outbreaks caused by direct person-to-person transmission and those caused by food-borne sources.

Methods

A prospective surveillance study was carried out. We investigated norovirus positivity in environmental samples from settings where there was an outbreak of AGE due to norovirus and viral persistence in samples after control actions had been carried out.

Environmental investigation

Outbreaks were reported by attending physicians to the epidemiological surveillance units of the Sub-Directorate General of Surveillance and Response to Public Health Emergencies (SgVRESP) of the Public Health Agency of Catalonia. When an outbreak of AGE compatible with norovirus was declared in a closed or semi-closed setting, an environmental sampling protocol was initiated to investigate the causal agent, consisting of taking up to eight samples from surfaces, with three obligatory samples from bathrooms, and the remaining five chosen according to inspectors' judgement from kitchens and common areas such as dining rooms, recreational spaces, living room televisions, reading rooms, meeting rooms and games rooms. Other points, such as diaper changing tables, bookcases, faucets and oven handles were also included when necessary. If any sample was positive for norovirus, a second sample from the same site was recommended to be taken after 10 days.

Outbreaks with person-to-person transmission were defined as those in which the temporal epidemic curve showed various incubation periods of norovirus infection between the first and last cases of the outbreak. Food-borne outbreaks were defined as those with a sudden onset of cases and in which most cases began symptoms between the minimum and maximum incubation period of norovirus infection and information on a possible common source of exposure epidemiologically linked to the outbreak was available.

The results of samples collected from kitchen surfaces were analysed separately according to the mechanism of transmission (person-to-person or food-borne).

Collection of environmental samples

Public health inspectors involved in the investigation of outbreaks were informed of the sample collection protocol in writing and by a video to facilitate harmonization. The protocol was based on the swab sampling method of Park *et al.* [17].

Environmental surfaces were sampled using swabs with flocked polyester tips moistened with Ringer ¼ solution. First, an area not exceeding 100 cm^2 was rubbed with a moistened swab and then the surface was rubbed again with a dry swab. Both swabs were placed in a 15-mL tube with 1.5 mL of Ringer ¼ solution for transport.

Extraction and purification of total nucleic acids

Before analysis, swabs were stored at 2—8°C for a maximum of 24 h or alternatively at -80°C for a maximum of 3 weeks.

The content of the swabs was resuspended in the volume of transport solution by homogenization for 1 min at 2000 rpm. Total nucleic acid extraction was made with the BioMérieux

Table I
Norovirus positivity in first and second environmental samples by areas and specific points

	Specific points	1st samples +/total 1st samples	2nd samples +/total 2nd samples	Total samples collected+/total samples	Reduction in norovirus positivity	P*
Common areas	Handrail bar	5/16 (31%)	2/18 (11%)	7/34 (21%)		0.21
	Plastic toy/table game	0/5 (0%)	0/1 (0%)	0/6 (0%)		1.0
	Television remote	1/11 (9%)	1/6 (17%)	2/17 (12%)		1.0
	Table	9/48 (19%)	0/16 (0%)	9/64 (14%)	48%	1.0
	Elevator button	2/9 (22%)	2/8 (25%)	4/17 (24%)		1.0
	Hoist	0/1 (0%)	0/1 (0%)	0/2 (0%)		1.0
	Others[a]	6/30 (20%)	1/14 (7%)	7/44 (16%)		0.40
Bathroom areas	Toilet handle	14/43 (33%)[b]	2/23 (9%)	16/66 (24%)[c]		0.06
	Door handle	2/27 (7%)	1/14 (7%)	3/41 (7%)	52%	1.0
	Toilet seat	18/93 (19%)	5/35 (14%)	23/128 (18%)		0.68
Kitchen areas	Fridge handle	5/35 (14%)	1/12 (8%)	6/47 (13%)	36%	1.0
	Worksurface	4/48 (8%)	1/15 (7%)	5/63 (8%)		1.0
Total		66/366 (18%)[Ref]	16/163 (10%)	82/529 (16%)[Ref]	45%	0.02

Ref, reference.
[b] P=0.015.
[c] P=0.055.
* P value between first and second samples in each specific point.
[a] Light switch, child's bed, diaper changer, wheelchair.

NucliSENS easyMag system using 1 mL of the suspension to which 10 μL of a mengovirus suspension (CEERAM Mengo Extraction Control, BioMérieux) was added to estimate the extraction efficiency (EE%). Nucleic acids were eluted in a volume of 100 μL and the eluates were stored at -80 °C until analysis for a maximum of 4 weeks.

Reverse transcription polymerase chain reaction for norovirus and mengovirus

Norovirus genogroups I and II were detected in a Roche LightCycler 480II using a one-step real-time reverse transcription polymerase chain reaction (RT-PCR) reaction. Five microlitres of the eluates were used in a total reaction volume of 25 μL. The primers, probes and the reaction mix, their concentrations and volumetry, the inhibition detection system that added external RNA controls in parallel reactions, and the thermocycling conditions, were those indicated in ISO 15216–2:2013 [18].

The estimation of the EE% of each sample was made in a Roche LightCycler 480II using a one-step real-time quantitative RT-PCR reaction. The reaction mix and the thermocycling conditions where those supplied and indicated by the product CEERAM Mengo Extraction Control (BioMérieux). The EE% of the samples was assessed to guarantee that the results were at least >1%.

Statistical analysis

The data were collected in an Access database for later debugging and analysis. To assess whether the differences between proportions were statistically significant, Pearson's Chi squared test, and Fisher's test, when indicated, were used, assuming an alpha error of 0.05.

Results

From January 2017 to the beginning of March 2019, 50 outbreaks of AGE caused by norovirus in closed and semi-closed settings were reported in the Barcelona region. Environmental samples were collected in 46 outbreaks (32 (68.5%) in nursing homes, seven [15.2%] in schools, three [6.5%] in children's and youth accommodations, three [6.5%] in kindergartens and one [2.17%] in a social health centre). In four outbreaks (two schools, one hotel and one hospital) no samples were taken for logistical reasons.

Environmental samples were positive for norovirus in 31 (67.4%) outbreaks. The most-frequently found genogroup was genogroup II (87%), followed by genogroup I (10%) and the combination of genogroups I and II (3%). Nursing homes were the setting with the most types of environmental surfaces contaminated both for the first (82%) and second samples (55%). Kindergartens were the setting with the second most types of environmental surfaces contaminated (18.2% in first samples and 27.3% in second samples) and youth accommodations and social health centres the third (9% in first samples and 0% in second samples in both). No surfaces positive for norovirus were found in schools (see Supplementary Tables S1 and S2).

A total of 529 environmental samples were collected (366 first samples and 163 second samples), distributed as follows: 235 (44%) on bathroom surfaces, 184 (35%) in common areas and 110 (21%) in kitchens. Forty-two (18%) samples in bathrooms, 29 (16%) in common areas and 11(10%) on kitchen surfaces were positive for norovirus.

Comparison of the 366 first environmental samples and the 163 second samples, showed a reduction of 48% in positive bathroom samples, 52% in common areas, and 36% in kitchens. The differences were not statistically significant (Table I).

Environmental surfaces

The specific points where the highest norovirus positivity was detected were elevator buttons (4/17, 24%), toilet handles (16/66, 24%) and handrail bars (7/34, 21%). The positivity for toilet handles was significantly higher compared with samples from other locations in the first sampling (33% vs 16%, P=0.01) but not in the second sampling (9% vs 10%; P=1) (Table I).

In the first sampling (366), the specific points where the virus was most frequently detected were toilet handles (14/43, 33%), handrail bars (5/16, 31%) and elevator buttons (2/9, 22%).

In the second sampling (163) the specific points where norovirus was most frequently detected were elevator buttons (2/8, 25%), TV remote controls (1/6, 17%) and toilet seats (5/35, 14%).

Overall norovirus positivity significantly decreased in the second sampling (18% vs 10%, P=0.02).

The greatest decreases in norovirus positivity were for table surfaces in common areas (9/48, 19% first samples; 0/16, 0% second samples, P=1) and toilet handles (14/43, 33% first samples; 2/23, 9% second samples, P=0.06). There was a non-significant increase in norovirus positivity in the second sampling in two specific points in common areas: elevator buttons (2/9, 22% in the first samples and 2/8, 25% in the second samples; P=1) and TV remote controls (1/11, 9% in the first samples, 1/6, 17% in the second samples; P=1).

In samples collected from kitchen surfaces in outbreaks with person-to-person transmission, 12% (11/95) of all samples were positive (9/74, 12% of first samples and 2/21, 10% of second samples). In contrast, in food-borne outbreaks, both the first and second environmental samplings were negative for norovirus (0/15, 0%) (Table II).

The surfaces with the lowest norovirus positivity were kitchen work surfaces (5/63, 8%) and bathroom doorknobs (3/41, 7%), with no decrease in positivity between the first and second samples (4/48, 8% vs 1/15, 7%; P=1; and 2/27, 7% vs 1/14, 7%; P=1) (Table I).

Influence of time after notification and from symptom onset.

To analyse the influence of the time from notification of the outbreak to the collection of the first environmental samples, 42 outbreaks in which the date of sample collection was known were studied. No significant differences in positivity were found between samples collected in the first 5 days after notification and those collected ≥6 days after notification (17/26, 65% vs 12/16, 75%, P=0.73) both for person-to-person outbreaks (14/22, 67% vs 11/14, 78%; P=0.46) and for food-borne outbreaks (3/4, 75% vs 1/2, 50%; P=1). Similar results were obtained in second samples collected in the first 10–20 days after notification and those collected ≥ 21days after notification (4/8, 50% vs 5/15, 33%; P=0.65) (Table III).

Analysis of the time from symptom onset of the first case to the date of the first sampling showed no significant differences in positivity between samples collected in the first five days and those collected after ≥6 days (73% and 67 %, respectively; P=1.0). Neither were significant differences found between second samples collected 10–20 days and ≥21 days after notification (50% vs 33%; P=0.65) (Table IV).

In food-borne outbreaks, 66% (4/6) first samples and 25% (1/4) second samples were positive (Table IV). Norovirus was not detected in environmental samples in food-borne outbreaks ≥21 days after notification or after symptom onset (Tables III and IV).

In outbreaks with person-person transmission, 69% (25/36) of the first environmental samples and 42% (8/19) of the second samples were positive for norovirus, with positivity being found up to 40 days after notification and up to 60 days after symptom onset of the first case (Tables III and IV).

Discussion

Our study shows that nursing homes are the type of institution most affected by AGE outbreaks caused by norovirus, which has become one of the leading causes of viral gastroenteritis worldwide, frequently affecting older residents of closed or semi-closed settings [19]. Costantini *et al.* found that, in the USA, long-term care facilities were the most common setting for norovirus outbreaks [20].

In the norovirus outbreaks investigated in this study, GII was the genogroup most frequently found in environmental samples. Stobnika *et al.* compared the prevalence of norovirus (GI and GII) with that of other viruses on different office surfaces and also found that GII was the most frequent genogroup (in 14.8% of samples collected) [21].

The results of the first and second environmental samplings collected on the surfaces of common areas, bathrooms and kitchens show that bathrooms and common areas had the most samples positive for norovirus and kitchen surfaces the least. This might be explained by the cleaning procedures of kitchen staff.

Jones *et al.* described consecutive AGE outbreaks in houseboats in Arizona in which samples were collected from

Table II
Norovirus positivity in first and second environmental samples according to specific sampling points in kitchens and transmission mechanism

Number of samples/Sampling point	Person-to-person transmission			Food-borne transmission			P
	1st sampling	2nd sampling	Total	1st sampling	2nd sampling	Total	
	Total samples+/total	Total samples +/total	Samples+/samples collected	Total samples+/total	Total samples+/total	Samples+/samples collected	
Fridge handle	5/31 (16%)	1/9 (11%)	6/40 (15%)	0/4	0/3	0/7	0.57
Worksurface	4/43 (9%)	1/12 (8%)	5/55 (9%)	0/5	0/3	0/8	1.0
Total	9/74 (12%)	2/21 (10%)	11/95 (12%)	0/9	0/6	0/15	0.35

*P-value comparing positivity in samples (first and second) in specific sampling points according to transmission mechanism.

Table III
Norovirus positivity in first and second environmental samples according to time after notification of the outbreak, globally, and by transmission mechanism

Days between notification and collection of first samples	Total outbreaks		Food-borne transmission		Person-to-person	
	No. outbreaks+/no. outbreaks	P*	No. outbreaks+/no. outbreaks	P*	No. outbreaks+/no. outbreaks	P*
0–5	17/26 (65%)	0.73	3/4 (75%)	1.0	14/22 (67%)	0.46
6–10	8/12 (67%)		1/2 (50%)		7/10 (70%)	
11–15	2/2 (100%)		0/0		2/2 (100%)	
16–21	2/2 (100%)		0/0		2/2 (100%)	
≥6	12/16 (75%)	Ref	1/2 (50%)	Ref	11/14 (78%)	Ref
Total	29/42 (69%)		4/6 (66%)		25/36 (69%)	
Days between notification and collection of second samples						
10–20	4/8 (50%)	0.65	1/2 (50%)	1.0	3/6 (50%)	1.0
21–30	3/9 (33%)		0/1 (0%)		3/8 (38%)	
31–40	2/4 (50%)		0/0		2/4 (50%)	
41–50	0/1 (0%)		0/1 (0%)		0/0	
51–60	0/1 (0%)		0/0		0/1 (0%)	
≥21	5/15 (33%)	Ref	0/2 (0%)	Ref	5/13 (38%)	Ref
Total	9/23 (39%)		1/4 (25%)		8/19 (42%)	

Ref, reference.
* *P* value comparing first samples collected between days 0–5 and ≥6 days and second samples collected between days 10–20 and ≥21 days.

bathrooms, kitchens, and doorknobs: 83% (5/6) of bathroom samples, 40% (2/5) of kitchen samples and 100% (3/3) of doorknob samples were positive for norovirus [14]. Gallimore *et al*. studied the presence of gastrointestinal viruses on surfaces and equipment of a unit for immunocompromised children by monitoring samples on the same surfaces for 6 months and found that norovirus was the most frequently detected virus on environmental surfaces, with bathroom faucets being the most contaminated sites [15]. Leone *et al*. analysed the presence of norovirus on bathroom surfaces in food establishments where AGE outbreaks had occurred and found 1.5% of samples collected were positive for norovirus, much lower than the 18% found in our study [22]. The specific surfaces that showed statistically significant differences in our study were toilet handles, both in all samples and the first sampling, underlining the need for bathroom cleaning and disinfection to prevent norovirus transmission in this type of setting.

Table IV
Norovirus positivity in first and second environmental samples according to time from symptom onset of first case, globally and according to transmission mechanism

Days between symptom onset and collection of first samples	Total outbreaks		Food-borne transmission		Person-to-person	
	No. outbreaks+/no. outbreaks	P*	No. outbreaks+/no. outbreaks	P*	No. outbreaks+/no. outbreaks	P*
0–5	8/11 (73%)	1.0	3/4 (75%)	1.0	5/7 (71%)	1.0
6–10	9/16 (56%)		1/2 (50%)		9/15 (60%)	
11–15	6/7 (86%)		0/0		6/7 (86%)	
16–21	4/6 (67%)		0/0		3/5 (60%)	
22–37	2/2 (100%)		0/0		2/2 (100%)	
≥6	21/31 (67%)	Ref	1/2 (50%)	Ref	20/29 (69%)	Ref
Total	29/42 (69%)		4/6 (66%)		25/36 (69%)	
Days between symptom onset and collection of second symptoms						
10–20	4/8 (50%)	0.65	1/3 (33%)	1.0	2/4 (50%)	1.0
21–30	1/6 (17%)		0/1 (0%)		2/6 (33%)	
31–40	3/6 (50%)		0/0		3/6 (50%)	
41–50	0/1 (0%)		0/0		0/1 (0%)	
51–60	1/2 (50%)		0/0		1/2 (50%)	
≥21	5/15 (33%)	Ref	0/1 (0%)	Ref	6/15(40%)	Ref
Total	9/23 (39%)		1/4 (25%)		8/19 (42%)	

Ref, reference.
* *P*-value comparing first samples collected between days 0–5 and ≥6 days and second samples collected between days 10–20 and ≥21 days.

The settings where outbreaks occurred received health hygiene recommendations including hand washing, exclusion and isolation of ill staff members until 48—72 h after symptom resolution and environmental disinfection with sodium hypochlorite [10,13] when the outbreak was notified and before samples were collected. Even so, environmental samples positive for norovirus were found in 67.4% of outbreaks, and the time between the onset of the first symptoms or the notification of the outbreak until the collection of the first samples did not influence the positivity of the samples collected. This suggests that cleaning routines were not effective in eliminating norovirus. Nenonen *et al*. found norovirus genogroup II in samples taken from dust particles for five months in rooms of patients affected and not affected by AGE. The high nucleotide similarity between the norovirus GII.4 strains of the patients and their surroundings in the hospital room provided evidence of the dispersion of GII.4 in the air and in dust, probably from aerosols that originated with patients' vomiting [16].

The second sampling showed that positivity significantly decreased compared with the first sampling, especially with respect to table surfaces in the common areas and toilet handles. These results are in line with those found by Brié *et al.*, who found that norovirus infectivity decreased with increasing temperature and disinfection with sodium hypochlorite [23]. We found that positive norovirus samples increased at two specific points (elevator buttons and TV remote controls). Again, this suggests cleaning routines might need improvement. Because flat areas (such as dining room tables, for example) are easier to disinfect than those with irregular surfaces or shapes (such as TV remote controls), cleaning routines should specifically include these types of surfaces.

The persistence of norovirus on dining room tables and elevator buttons was described in the study by Wu *et al*. on the importance of environmental contamination in the duration of norovirus outbreaks [8]. Likewise, a review by Cook *et al*. also described norovirus persistence on environmental surfaces such as ceramics, polyvinyl chloride, formica, and stainless steel [24].

Analysis of samples collected from kitchens according to whether they were food-borne or person-to-person outbreaks showed there were fewer positive samples in food-borne outbreaks. This suggests that cleaning and disinfection protocols in kitchens of this type of setting may be effective in food-borne outbreaks but not in those with person-to-person transmission, possibly due to a lack of staff training on personal hygiene and good practices [25].

Our study had some limitations. First, despite the standardization of the request for environmental samples and description of the target points, on some occasions, inspectors collected samples from surfaces other than the first sampling and therefore no comparison could be made, while in other cases no second sample was collected. Second, not all centres where outbreaks occurred could be inspected and environmental samples taken due to logistical problems. Third, the low viral load of the environmental samples was not enough to determine the RNA sequence by RT-PCR in most outbreaks; this was possible in only three outbreaks of which two showed that environmental and clinical samples clearly coincided (data not shown).

In conclusion, the results of this study underline the importance of contamination by norovirus of specific environmental surfaces in closed and semi-closed settings where outbreaks had occurred. Although norovirus positivity was significantly reduced in the second sampling after recommendations on cleaning and disinfection were given after the outbreak was notified, norovirus remained in some specific places. While evidence of the value of specific measures in controlling norovirus outbreaks in closed and semi-closed settings is scarce [26,27], our data suggests that cleaning protocols should incorporate more frequent cleaning and disinfection of specific points as elevator buttons, television remote controls and toilet handles.

Future research on the efficacy of biocides used in cleaning and disinfecting surfaces in these settings could provide information of great interest for the prevention and control of AGE due to norovirus.

Conflict of interest statement
None declared.

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Appendix A. Supplementary data

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