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Norovirus shedding among food and healthcare workers exposed to the virus in outbreak settings

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ABSTRACT

Background: Noroviruses (NoV) are highly contagious and the leading cause of nonbacterial outbreaks of gastroenteritis worldwide. Individuals who are infected asymptomatically may act as reservoirs and facilitate the transmission of NoV, but the likelihood of workers of becoming infected in outbreak settings has not been systematically studied.
Objectives: We evaluated the occurrence of norovirus infections among workers exposed to the virus in different outbreak settings.
Study design: We screened feces from food handlers and healthcare workers related with gastroenteritis outbreaks, and shedding concentrations over time were calculated from serial samples of infected individuals. Sequence analyses of the capsid P2 domain and region C were used to evaluate linkage between asymptomatic employees and outbreak cases.
Results: Of all employees, 59.1% were positive for NoV, and more than 70% of them were asymptomatic. Asymptomatic infections were significantly more frequent in foodborne compared to person-to-person transmitted outbreaks; and in restaurants and hotels, compared to nursing homes and healthcare institutions. Mean viral loads were similar between symptomatic and asymptomatic individuals, starting at 7.51 ± 1.80 and 6.49 ± 1.93 $\log_{10}$ genome copies/g, respectively, and decreasing to 5.28 ± 0.76 and 4.52 ± 1.45 $\log_{10}$ genome copies/g after 19 days.
Conclusions: The likelihood of becoming infected when a NoV outbreak occurs at the work place is high and similar between food handlers and healthcare workers, but asymptomatic infections are more frequently identified among food handlers. Since shed amounts of viruses in the absence of symptoms are also high, reinforcement of hygiene practices among workers is especially relevant to reduce the risk of virus secondary transmissions.

1. Background

Human norovirus (NoV) is a major cause of acute non-bacterial gastroenteritis worldwide [1]. Food handlers (FHs) and healthcare workers (HCWs) are considered important contributing factors in the spread of NoV both in foodborne outbreaks as well as in person-to-person transmitted outbreaks occurring in healthcare institutions [2]. Several case reports have confirmed the role of infected FHs in causing outbreaks [3–5], and the percentage of outbreaks in which FHs were involved has been reported to be 34% or 70% [6,7]. In healthcare settings, although NoV nosocomial transmission is mainly caused by symptomatic shedders [8], HCWs have also been shown to transmit several infectious diseases to patients and cause substantial morbidity, mortality and healthcare costs, even if they do not show symptoms [9]. NoV outbreaks in health-

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related institutions linked to infected staff have also been reported [10,11]. Sequence analysis of the most variable region of the capsid gene of viruses has helped in the identification of transmission routes [12–14].

Asymptomatic excretion of NoV among healthy individuals is common, with reported prevalences among healthy adults ranging between 12 and 16% [15,16]. Studies focused on FHs describe similar prevalence in Japan [17], but also lower (1–3.4%) in other countries such as South Korea [18,19]. Asymptomatic shedding is also possible for a short period of time before the onset of symptoms, and may continue between 13–60 additional days after symptoms disappearance [20–22]. Concentrations of shed NoVs are high both for symptomatic and asymptomatic infected individuals, and peak titers of shedding usually occur during the first 5 days after infection. Challenge studies with healthy volunteers have revealed that symptomatic individuals may shed significantly higher concentrations of viruses than asymptomatic individuals [21,22], but other studies involving subjects related to outbreak investigations have not confirmed this observation [20].

While infected FHs and HCWs may play a role in transmission, staff personnel may also be at a higher risk of acquiring NoV infections when an outbreak occurs at their workplace. Contact with a person with gastroenteritis is risk factor for viral acute diarrhea [23,24], but few reports have addressed whether working in the food sector may also pose a higher risk for NoV infections.

2. Objectives

The aim of this study was to analyze the occurrence of NoV infections among FHs and HCWs in outbreak settings. Percentages of subjects who were symptomatically or asymptomatically infected were determined for different types of outbreaks and concentrations of NoV shedding were analyzed in correlation with symptoms. Finally, sequence analysis was applied to confirm linkage between cases and asymptomatic individuals.

3. Study design

3.1. Definitions

Norovirus infection was defined as fecal virus excretion detected by RTqPCR [25]. Acute gastroenteritis was defined as diarrheal disease of rapid onset presenting together with nausea, vomiting, fever, or abdominal pain. Individuals fitting with this definition were considered symptomatic individuals. A confirmed case of acute gastroenteritis by NoV was defined as a patient with ≥2 loose stools and/or ≥2 episodes of vomiting within 24 h, with detection of norovirus in feces.

3.2. Sample collection

Fecal samples (n = 259) and epidemiological data (age, sex, and occurrence and type of symptoms) were collected from all food handlers (FHs) and healthcare workers (HCWs) related to 59 previously reported outbreaks caused by NoV [25]. This number represented all NoV outbreaks reported to the Public Health Agency of Catalonia during 2010–2012, for which samples from workers could be collected. Setting and type of transmission were known for 57 of them. Type of transmission was determined after analyzing the occurrence of an exposure source and the epidemic curve. In point-source outbreaks, cases which occurred after 50 h (maximum incubation period) of the common exposure event were considered secondary cases. Main features for these outbreaks are summarized in Table 1. FHs included cooks (24), kitchen assistants (159), waiters (3), and dining room instructors and assistants (30). HCWs included caregivers (57), entertainers (7), instructors (7), and other staff working at the institution (2). Thirty caregivers who assisted during lunch were simultaneously considered as FH and HCW. In addition, 49 samples were collected from FH and HCW related to 8 gastroenteritis outbreaks which tested negative for NoV.

3.3. NoV screening

All stool samples were screened for NoV by qRT-PCR assays as previously described [25]. In a subset of individuals who were willing to participate, serial fecal samples were collected in approximately 1 week intervals apart after providing informed consent, until NoV was no longer detected. Viral load was determined following the ISO/TS 15216-1:2013 method [25]. Standard curve was constructed using serial dilutions of a plasmid containing the qRT-PCR amplicon for NoV GII.4 [26].

3.4. Strain sequence analysis

Amplification and sequencing of region C and P2 domain were performed by nested PCR using previously described primers [12,25] and primers that were designed for this study (Table 2). Nested PCR conditions used have been previously described [25]. Detection of more than one peak at the same position in the sequence in high quality chromatograms were considered as heterogenic sites, which may indicate the existence of mixed sequences in the sample, and have already been observed by others [27,28]. Nucleotide sequence alignments were performed using Clustal Omega [29].

Strain characterization was performed based on the analysis of the P2 sequence, using the criteria suggested by Sukhrie and coworkers [12], which demonstrates that minor nucleotide

Table 1
Main epidemiological features of the 59 outbreaks used in the study.

Feature	Description[c]
Type of transmission[a]	Foodborne [35]; person-to-person [20]; waterborne [2]
Setting[a]	Restaurants/hotels [28]; nursing homes [13]; schools [11]; healthcare institutions [4]; private house [1]
NoV genotype[b]	GII.4 [29]; GII.1 [4]; GII.6 [3]; GII.12 [2]; GII.7 [1]; GII.10 [1]; GI.7 [1]; multiple genotypes [5]
Duration of outbreak in days: range (median)	1–71 (5)
Number of affected individuals: range (median)	3–166 (29)
Attack rate: average ± standard deviation	44.3 ± 28.4
Number of workers' samples collected per outbreak: range (median)	1–26 (3)
Days passed between the onset of the outbreak and date of sampling: range (median)	3–36 (10)

[a] Information was available for 57 outbreaks.
[b] Information was available for 46 outbreaks.
[c] Numbers in brackets indicate the number of outbreaks; numbers in parenthesis indicate median values.