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Environmental testing for norovirus in various institutional settings using catering companies as sentinels for norovirus prevalence among the general population

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ABSTRACT

Noroviruses (NoV) are among the most common causes of viral gastroenteritis (GE) worldwide and can be transmitted from person-to-person, via food or contaminated surfaces. The present study aimed to examine the prevalence of NoV RNA on surfaces in food preparation and sanitary areas in different health care settings and to compare the outcomes with the prevalence in nearby located catering companies, mainly restaurants, for general public, as sentinels.

For this purpose, 1087 environmental swabs were taken for NoV analyses from surfaces in 241 institutional departments and 123 catering companies in The Netherlands without a recently reported outbreak of gastro-enteritis in high NoV season only. NoV RNA was detected in 15.1% of the 73 non-hospital health care institutions, 11.1% of the 54 hospital central kitchen departments, 14.9% of the 114 decentralized hospital kitchens (in-patient units) and 4.1% of the 123 nearby located catering companies. Twenty-five of the 49 positive environmental samples were genotyped by sequence analyses. In 7% of the investigated hospitals (4/58), NoV was detected in two or more departments. NoV prevalence was significantly lower in food preparation areas than in sanitary facilities ($p < 0.05$), but only in hospital central kitchen departments and non-hospital health care settings, and not in de-centralized hospital kitchens in in-patient units or in catering companies for the general public. This data suggests that there is a need for education on risks of NoV transmission by food handling of healthcare workers using in ward kitchen facilities.

1. Introduction

Noroviruses (NoV) are among the most common causes of viral gastroenteritis (GE) worldwide. In the Netherlands, the incidence of NoV illness in 2009 was estimated to be 3800 cases per 100.000 inhabitants, with an estimated burden over 1600 disability-adjusted life years (Verhoef, Koopmans, et al., 2013). The virus is highly infectious (Teunis et al., 2008) and often seen in outbreaks with high attack rates in health care settings, cruise ships, catered events, (EFSA, 2011; FAO/WHO, 2008; Meakins, Adak, Lopman, & O'Brien, 2003; Widdowson et al., 2004). NoV outbreaks are often prolonged, lasting longest in hospitals (19 days, range 6–92 days) and in nursing homes (16 days, range 3–44 days) in comparison to non-health care settings (7 days, range 1–26 days) (Harris, Lopman, & O'Brien, 2010). Outbreaks are recurring due to high levels of shedding in stool or vomitus and sometimes chronic shedding, especially in hospitals where people are vulnerable and there is a constant turnover of new susceptible people during outbreaks. Closure of wards is one of the control measures to control NoV spread in hospitals (Greig & Lee, 2012). Spreading is thought to be through person-to-person transmission, although environmental

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contamination has been implicated in this transmission (Lopman et al. 2012).

NoV have been demonstrated on different types of surfaces (floors, tables, doorknobs, handles, medical equipment, bed rails, carpets and curtains) in health care facilities, schools and food production facilities (Boone & Gerba, 2007; Gallimore et al., 2006; Morter et al., 2011; Wu et al., 2005). Recently, results of environmental sampling in catering companies suggested that health care institutions were more likely to be NoV contaminated than catering companies for general public (Boxman et al., 2011). This initiated further studies to examine the prevalence of NoV in different food preparation areas in various health care settings as part of assessment of risk of food contamination, and including an inventory on food handler's knowledge and working practices (Verhoef, Jaramillo Gutierrez, Koopmans, Boxman, 2013). The aim of the present study was to determine the NoV environmental presence during the winter season in healthcare settings. For the purpose of direct comparison, sampling in catering companies, mainly restaurants, nearby the institutions was performed. This was also done to reflect the background epidemiology of NoV in the general population, because of yearly differences in NoV activity (Lopman et al., 2004; Verhoef et al., 2008). In contrast to food handlers in institutions who work in a semi-closed settings prone to NoV outbreaks, food handlers in catering companies are part of the general public. Catering companies were therefore considered as sentinels for the NoV activity in the general population in this paper. In order to gain a deeper understanding of the potential sources of food-borne NoV outbreaks, outcomes were compared between food preparation areas and sanitary facilities within central and decentralized kitchens in health care institutions, and in comparison with outcomes of sampling in nearby public catering companies.

2. Materials and methods

2.1. Study rationale

In the periods January 2010–April 2010 and February 2011–March 2011, inspections were performed by officers of the Netherlands Food and Product Safety Authority (NVWA) in health care institutions, as well as in catering companies in the vicinity of these institutions, during the winter seasonal peak of viral gastrointestinal disease. The rationale for this was to capture environmental presence of NoV during the period of the year with most illness, in environments that may be relevant for the epidemiology of these viruses in health care settings. The healthcare settings included 58 hospitals (44.3%), 54 homes for the elderly (41.2%) as well as 11 nursing homes (8.4%). In hospitals, samples were collected in 54 central kitchen departments (32.1%) and 114 decentralized kitchens (67.9%). This was done to be able to discriminate food-handler introductions at these sites, that likely differ in the level of knowledge and stringency of food handling practices: staff in central kitchen department prepares food which is subsequently distributed to kitchens in-patient units (de-centralized kitchens), where other nursing staff reheat food to adequate temperatures, and serve meals and drinks. Sampling in nearby catering companies, mainly restaurants, was done to reflect the background epidemiology of NoV.

2.2. Sampling protocol

At each setting, two mixed surface swab samples were taken from surfaces in food preparation areas and one mixed surface swab sample was taken from the bathroom for NoV analyses, as described previously (Boxman et al., 2009; 2011). In brief, the first swab was used to collect a surface sample from a food preparation equipment, such as the grip of a refrigerator, knife or serving spoon, and the handle of a cutting or mixing machine in central kitchens, and surfaces from the microwave or the cutlery drawer in de-centralized kitchens. The second swab was used to collect a surface sample from the soap dispenser, from the pepper-and-salt set or from a food storage box in the refrigerator. In hospital in-patient units the handle of the dish washer was given as an alternative surface. The third swab was used to collect a surface sample from the flushing chain or knob and the toilet seat (both upper and undersurfaces) in the men's (employees') bathroom only, which was chosen for reasons of systematic sampling.

Sampling was done by inspectors who had no knowledge about possible outbreaks related to the sites that they visited to avoid oversampling of known contamination events. Environmental swab samples were either kept at 4 °C or kept frozen at −20 °C during transport and stored frozen at −20 °C until they were processed at the laboratory of the NVWA. Analyses were performed on coded samples.

2.3. Extraction and analyses of swab samples

Environmental swabs were extracted and analysed for the presence of NoV RNA as described previously (Boxman et al., 2009; 2011) with some modifications. In brief, RNA extractions were performed using 30 μl glassmilk (MP Biomedical) per swab extraction with no difference in detection (data not shown), as normal non-magnetic silica (BioMérieux) was no longer available. RNA samples were analyzed using two-step reverse transcription (RT) real time PCR. The N2pol assay targeting the polymerase region was used for the detection of NoV GII types as described (Boxman et al., 2011). For the detection of NoV GI types, the N1cap assay targeting the ORF1/ORF2 region was used in a two-step RT real time PCR format instead of the N1cap nested real-time RT-PCR assay (Boxman et al., 2009).

2.4. Sequencing and phylogenetic analyses

NoV presumptive positive samples, as judged by the presence of a typical S -curve in the amplification plot, were re-amplified and sequenced as described previously (Boxman et al., 2011). NoV sequences detected in the present study were genotyped using the NoV typing tool (Kroneman et al., 2011) (http://www.rivm.nl/en/Topics/N/NoroNet/Databases/) and compared to Dutch NoV outbreak strains detected in humans involved in outbreaks and for which clinical samples had been sent in for diagnosis at the Institute for Public Health and the Environment in the Netherlands. Sequence comparisons were done if an overlapping region of at least 100 nucleotides (nt) in region 5373–5577 for ORF2 of GI types and in region 4299–4494 for ORF1 of GII types was available (Kroneman et al., 2011). A multiple alignment of selected sequences was made for each genotype or, if genotype II.4 was involved, each variant. The pairwise distance was computed using a 2-parameter Kimura nucleotide substitution model.

2.5. Statistical analyses

Statistical analyses were performed using Fisher-P one tailed analyses to identify differences between settings and swabbing locations. If cell counts in a cross table did not include any observations, calculations were performed by artificially setting the number of the concerning cell to one instead of zero.