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Additional value of typing Noroviruses in gastroenteritis outbreaks in Amsterdam, The Netherlands

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Abstract

Background: In Amsterdam, 17 of the 55 gastroenteritis (GI) outbreaks reported from January 2002 to May 2003 were confirmed to be caused by noroviruses (NV).
Objective: In this study, we describe the molecular epidemiology of a group of nine outbreaks associated with a catering firm and two outbreaks, 5 months apart, in an Amsterdam hospital. All outbreaks were typed to confirm their linkage, and the hospital-related cases were studied to see if the two outbreaks were caused by one persisting NV strain or by a reintroduction after 5 months.
Results and conclusions: For the outbreaks associated with the catering firm one NV genogroup I strain was found which was identical in sequence among customers and employees of the caterer. This was not the strain that predominantly circulated in 2002/2003 in and around Amsterdam, which was the NV genogroup II4 "new variant" (GgII4nv) strain. In the Amsterdam hospital, the two outbreaks were caused by this predominant GgII4nv type, and we argue that NV was most likely reintroduced in the second outbreak from the Amsterdam community.

Keywords: Norovirus; Outbreak; Genotyping; Molecular epidemiology

1. Introduction

In The Netherlands, 4 million people (283/1000) suffer from gastroenteritis (GI) each year, and around 500,000 (25%) of these cases are caused by noroviruses (NV) (Koopmans, 2002). Since 1999, all major outbreaks of infectious diseases in institutions must be reported by law to the Municipal Health Service (MHS). In Amsterdam, available samples from GI outbreaks suspected to be caused by NV are send to the Amsterdam Municipal Health Laboratory (AMHL) and tested by PCR as NV cannot be cultured.

Noroviruses are ubiquitous human pathogens that cause epidemics of acute viral GI in people of all ages. The symptoms are in general mild and the disease is self-limiting. Vomiting and diarrhoea are predominant symptoms, and dehydration is the most common complication. NV are highly infectious, notably via the faecal-oral route or by aerosols generated by vomiting. They are commonly associated with food- and waterborne outbreaks (Koopmans et al., 2000; Lopman et al., 2002). Noroviruses can phylogenetically be divided into five genogroups of which groups I, II and IV contain human strains.

In this paper we describe a group of outbreaks associated with a catering firm in April 2003 and we tried to confirm the epidemiological link between these outbreaks by genotyping analysis of those that were PCR positive. We also studied two outbreaks in a general hospital in Amsterdam that occurred 5 months apart, first on one ward in 2002 and later on in 2003 on several wards in different buildings of the hospital. Using genotyping methods, we tried to determine whether both outbreaks were caused by the same persisting NV strain or whether the second outbreak was caused by reintroduction of NV from the community. All contemporal NV-positive

Abbreviations: NV, norovirus; GgII4nv, genogroup II4 "new variant"; MHS, Municipal Health Service; AMHL, Amsterdam Municipal Health laboratory; RT-PCr, reverse transcriptase polymerase chain reaction; nt, nucleotides; pt, patient; emp, employee; cust, customer

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outbreaks diagnosed in our laboratory were used as reference cases.

2. Participants and methods

2.1. Participants

From January 2002 to May 2003, 55 institutions reported GI outbreaks involving vomiting and diarrhoea to the MHS department of Infectious Diseases. Among these were 26 day-care centres, 11 nursing homes, 6 hospitals (including hospital A twice), 2 river-cruising ships, 2 companies, 1 catering firm and after investigation a group of 7 more outbreaks that were found associated with the catering firm. Criteria for institutional reporting of GI outbreaks include vomiting and diarrhoea in 1of five persons of the ward or group (in day-care centres, one of three persons). Response to outbreaks typically includes active surveillance, hygienic advice, and a request for at least five stool samples. We used the "Kaplan criteria" (Kaplan et al., 1982) to define noroviral outbreaks: a mean incubation period of 24–48 h, acute symptoms (including vomiting and/or diarrhoea) in >50% of cases, with an average duration of 12–60 h. Additional information on high attack rate, and stool samples that test negative for bacterial pathogens was also taken into consideration. This study concerns the NV PCR-positive outbreaks, each named after the institution followed by a letter (e.g. day-care centre A; day-care centre B; hospital A; nursing-home A).

2.2. Specimens and RNA isolation

From 29 of 55 GI outbreaks (53%), stool samples were sent to the AMHL. Stool samples were aliquoted as a 10–20% suspension in phosphate buffered saline. One milliliter of suspension was centrifuged for 3 min at 2.000 g and was stored at −80 °C until isolation. Of the faeces suspension, 100 μl was used for RNA isolation using TriPure Isolation Reagent (Roche Diagnostics, Almere, The Netherlands), according to the manufacturer's protocol. Isolated RNA was resuspended in 100 μl Tris–HCl (10 mM, pH 8.0) with RNasin (0.2 μg/μl, Roche) and stored at −80 °C until use in reverse transcriptase (RT)-PCR. The molecular detection of NV in stool samples has become a standard diagnostic procedure in the AMHL since 2001.

2.3. Nested multiplex RT-PCR

A nested PCR was performed and primers were located in the RNA polymerase gene (ORF1) spanning nucleotide positions 4490–5127 in reference sequence M87661 (GenBank, www.ncbi.nlm.nih.gov). Access RT-PCR (Promega, The Netherlands) was used for reverse transcription and for the first-round PCR. The primers used for the outer PCR were CAL-32 (5′-ATGAATATGAATGAGGATGG-3′) (Schreier et al., 2000) and MO3-N (5′-TCAGATGGGTCTTCATGATTGG-3′) (this study), which amplify a 629-bp outer product. The inner PCR primer sequences were JV-12 (5′-ATACCACTATGATGCAGATTA-3′) (Vinjé and Koopmans, 1996) and ACAL-36 (5′-GACAAAACAGAAGGACCAAT-3′) (Schreier et al., 2000), which amplify a 428-bp inner product. All primers were synthesized by Invitrogen, Life Technologies (Maarssen, The Netherlands).

Amplification was performed in a PTC-200 DNA Engine Thermal cycler (MJ Research, BioZym, The Netherlands). The first-round RT-PCR cycling conditions were as follows: 48 °C/45 min (RT-step), 94 °C/2 min, followed by 35 cycles of 94 °C/30 s, 37 °C/30 s and 68 °C/50 s, followed by one extension step of 68 °C/7 min. For the second-round PCR cycling conditions were: 94 °C/3 min followed by 30 cycles of 93 °C/30 s, 37 °C/15 s and 72 °C/30 s plus one extension step of 72 °C/5 min. The inner PCR products were analyzed by electrophoresis on a 10% acryl amide gel stained with ethidium bromide and UV visualized.

2.4. Sequencing and phylogenetic analysis

Sequencing was performed directly on the second round PCR products using the inner primers and Big Dye Terminator chemistry v3.0 (PE Biosystems, Nieuwerkerk a/d IJssel, The Netherlands). Sequencing products were analyzed on an ABI 310 automated sequencer (PE Biosystems, Nieuwerkerk a/d IJssel, The Netherlands) and the sequences were aligned with the BioEdit Sequence Alignment Editor computer program (Hall, 1999) using 387 nt of the inner PCR product sequence of the RNA polymerase gene (ORF1) covering positions 4573–4960 in the reference strain M87661. Molecular Evolutionary Genetics Analysis version 2.1 computer software (Kumar et al., 2001) was used to generate the phylogenetic tree, which was constructed based on the Kimura-2 parameter substitution model. Reproducibility of the tree was tested with 1000 bootstraps.

2.5. Nucleotide sequence accession numbers

The reference types used were obtained from GenBank (www.ncbi.nlm.nih.gov).

The new sequences accession numbers from this study are: AY766260–AY766300.

3. Results

3.1. Norovirus outbreaks in Amsterdam 2002/2003

In the present study, we present a picture of the molecular epidemiology of NV in Amsterdam, particularly of a caterer-associated group of outbreaks and two outbreaks in hospital A.

From January 2002 to May 2003, 55 GI outbreaks were reported to the MHS Department of Infectious Diseases in Amsterdam. They occurred 26 times in day-care centres, 11