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Norovirus genotypes causing gastroenteritis outbreaks in Finland 1998–2002

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Abstract

Background: Outbreak investigation methods for enteric viruses were improved in 1990s when gene amplification techniques were established in viral laboratories.
Objectives: The objective of the study was to determine the causative agents for Finnish viral gastroenteritis outbreaks. Our aim was also to further characterise the norovirus strains, reveal the temporal occurrence of norovirus (NV) genotypes and to study some epidemiological aspects concerning the outbreaks.
Study design: A total of 416 Finnish viral gastroenteritis outbreaks that occurred during 5 years (1998–2002), excluding those among hospitalised children, were investigated for enteric viruses. Stool samples were screened by electron microscopy as well as analyzed by specific noro- and astrovirus RT-PCR tests. Amplicon sequence analysis was used to find out norovirus genotypes.
Results: Noroviruses caused 252 (60.6%) of the outbreaks; other viruses, astro- or rotavirus, caused four epidemics. Norovirus epidemics occurred in all kinds of settings, most often in hospitals (30.6%) and in restaurants and canteens (14.3%). Both NV genogroups were found every year, but NV GGII outbreaks always outnumbered those of GGI. All but one outbreak at hospitals and nursing homes were of genotype GII. Polymerase sequence analysis revealed a variety of NV genotypes; six GI and at least eight GII genotypes. The GI.3 Birmingham-like and GII.4 Bristol-like genotype appeared every year, whereas the other types were circulating for shorter periods or sporadically. During the study period the genotypes GII.4 (Bristol), GII.1 (Hawaii), an emerging genotype GIIb, and a new variant of GII.4 predominated in that order. Indication for rapid genetic changes in the genotype GII.4 was also noticed.
Conclusions: Noroviruses were the most prevalent causative agents in the outbreaks. Many NV genotypes were circulating, and a shift in the predominant genotypes was evident between epidemic seasons.

Keywords: Norovirus; Genotype; Gastroenteritis; Molecular epidemiology; Outbreak

1. Introduction

The enteric viruses commonly causing human gastroenteritis are adeno-, astro-, noro- (NV, previously Norwalk-like viruses), parecho-, rota- (groups A and C) and sapoviruses (SV, previously Sapporo-like viruses; Knipe, 2001). The majority of population-based diarrheal outbreaks are caused by NVs in all age groups (Koopmans et al., 2000), while group A rotavirus is the most frequently found, in physician based studies, to be the causative agent in children. NV-caused diarrheal outbreaks are very common in the community, especially in institutions like nursing homes, daycare centers, schools and hospitals. Noroviruses are easily spread among individuals, but they can also be transmitted by food- and waterborne routes, since they survive well in the environment (Green et al., 2001). The entity "winter vomiting disease", well known to physicians for decades, appears to be caused by NVs.

Nonenveloped small-round-structured viruses (SRSVs) 27–40 nm in diameter were often visible by electron microscopy in stool specimens from diarrheal patients (Green

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et al., 2001). Since attempts to grow NVs in cell culture have thus far been unsuccessful, molecular and genetic techniques have been necessary. It soon became evident that the Norwalk virus was only one member of a genetically heterogeneous group of noroviruses that cause outbreaks with similar symptoms. Noroviruses belong to the Caliciviridae family as do the sapoviruses, which attack mostly children.

The genome of NV is a positive-sense, single-stranded, polyadenylated, approximately 7.6 kb RNA. Three ORFs occur: ORF1 encodes a large polyprotein containing several nonstructural proteins including the polymerase, ORF2 encodes the structural capsid protein (approximately 57 kDa) and the basic protein ORF3 is a minor structural protein in the virion (Glass et al., 2000). Human NVs can be divided into two main genogroups: GGI and GGII, and these can be further divided into at least seven (GGI.1–GGI.7) and eight genotypes (GGII.1–GGII.8; Green et al., 2001). Establishment of new genotypes will be necessary in the near future, since the number of diverging sequences is increasing.

Detection of NVs by diagnostic methods has been demanding because of their heterogeneity and a lack of knowledge of all existing NV strains. Although in recent years antigen-detection assays have been developed, the technique most widely used involves part of the NV polymerase gene containing genetically conserved stretches being amplified by polymerase chain reaction (PCR; Atmar and Estes, 2001). During recent years, much sequence data from the NV polymerase region have enabled the development of broadly reacting primers and probes for PCR and hybridization (Vennema et al., 2002; Vinje et al., 2003). In epidemiological studies, detailed genotype or sequence data are necessary in the search for links between causative agents from patients and the environment. Hybridization and an amplicon sequence determination provide a rapid way to discover preliminary information about the genotype. Strictly, NV genotypes can be reliably determined only based on the entire capsid gene sequence. In 2002, Katayama et al. (2002) reported that part of the capsid gene, the N terminal and the S region known to form the "shell" of the virus particle, would be the most suitable region for genotype determination. Rarely, typing based on polymerase or capsid sequences may produce with different genotypes for the same strain, since gene recombination, possibly between the polymerase and capsid genes encoded in different frames, has been reported for NV (Jiang et al., 1999; Vinje et al., 2000). In our hands, amplification of the polymerase region has been the most convenient method to provide information about NV outbreaks rapidly and easily, so long as these limitations are kept in mind.

Thorough, prolonged surveillance of the epidemics and NV strains in various countries have been possible only from the mid-1990s. The EU project for Food-borne viruses in Europe (contract QLK1-1999-00594) started in 1999 has enabled collection of NV sequence data at the European level (Koopmans et al., 2003). A year earlier, a new outbreak surveillance system began in Finland. Municipal authorities were required to rapidly report suspected food-borne outbreaks to the National Public Health Institute and encouraged to gather stool samples also for viral investigation, including RT-PCR analysis for NV and astroviruses in addition to the bacterial analyses. Here we report the results from viral investigations of stool samples obtained from Finnish diarrheal epidemics between 1998 and 2002.

2. Materials and methods

2.1. Clinical specimens

Stool samples from 2614 patients in 416 gastroenteritis epidemics were analyzed in the Department of Virology of the Helsinki University Central Hospital. Samples from gastroenteritis outbreaks were received during the time period 1998–2002 from various parts of Finland. The recommendation was to submit 3–10 samples from each epidemic.

2.2. Criteria for an outbreak caused by one particular virus

The criteria for a viral outbreak was that at least 4 of 10 samples or, when fewer samples were obtained, half or more of the samples in one outbreak were positive for one particular virus. If only two samples were available, both had to be positive to fulfil the criteria. Epidemics with only one stool sample were excluded. Epidemiological data (site of the epidemic) regarding the outbreaks was mainly based on the information in the report obtained together with the patient samples. In 354 of the 416 outbreaks (85.1%), the site was mentioned.

2.3. Electron microscopy

The stool sample was suspended in PBS or in 0.05 M Tris–0.1 M NaCl–1 mM $CaCl_2$ pH 7.4 (10%, w/v). A grid was prepared for negative staining (2% potassium phosphotungstate, pH 5.5–6.0), and the presence of virus particles (small round viruses, rota- or adenoviruses) was determined by electron microscopy (EM).

2.4. RNA extraction and RT-PCR assay

RNA was extracted from a 100 μl volume of stool suspension (5–10% (w/v) in 0.05 M Tris–0.1 M NaCl–1 mM $CaCl_2$, pH 7.4) with a phenol and guanidium thiocyanate-containing Tripure reagent (Roche, Mannheim, Germany), and the volume was reduced by ethanol precipitation (detailed description in Maunula et al., 1999). RNA was suspended in 20 μl of sterilized distilled water.

RNA (6 μl) was transcribed into cDNA in a separate 20 μl reaction in the presence of the RT expand enzyme (Roche) and the primer Nvp110 (5′-ac(a/t/g)at(c/t)tcatcatcaccata-3′: 4865–4884, antisense; Le Guyader et al., 1996). The entire RT-reaction volume was used for PCR amplification