Norovirus: an overview

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Summary

Although noroviruses (NoVs) were the first viral agents linked to gastrointestinal disease, for a long time they have been considered secondary cause of gastroenteritis, second to rotaviruses as etiologic agents. The development of molecular techniques in diagnosing NoV provided a clearer insight into the epidemiological impact of these viruses, which are currently recognized not only as the leading cause of non-bacterial gastroenteritis outbreaks, but also as a major cause of sporadic gastroenteritis in both children and adults. This review focuses on the required knowledge to understand their morphology, genetics, transmission, pathogenesis, and control. Since no vaccine is available, prevention of NoV infection relies mainly on strict community and personal hygiene measures.

Keywords: Norovirus; gastroenteritis; diarrhea.

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Introduction

Acute gastroenteritis is one of the most common diseases in humans; in the United States, it is the second leading cause for reporting, followed by respiratory infections[1]. A billion cases of acute diarrhea are estimated to occur yearly in children and adults worldwide[2]. Gastroenteritis is usually expressed as a mild diarrhea, but it can be seen as a severe form with enhanced symptoms (nausea and vomiting), possibly leading to dehydration and death. The annual mortality associated with gastroenteritis has been estimated as four to six million people[2].

The etiology of diarrheas can involve several agents, such as viruses, bacteria, and parasites. Bacterial agents are relatively more important in developing countries, whereas viral agents are more relevant in industrialized countries. The importance of these agents is related to hygiene and sanitation conditions for the population[1]. In 1972, a 27-nm viral particle was discovered in an infectious filtrate of human fecal samples over a gastroenteritis outbreak in Norwalk, Ohio[3]. Since then, the number of viral agents associated with gastroenteritis has progressively increased, with rotaviruses[4], astroviruses[5], and Norwalk-like viruses[6] being identified.

Currently, most gastroenteritis in children are considered to be caused by viruses included in four different families: *Reoviridae* (rotavirus), *Caliciviridae* (norovirus and sapovirus), *Astroviridae* (astrovirus), and *Adenoviridae* (adenovirus)[7].

Viral particle structure of noroviruses

Virions consist of a capsid and a nucleic acid measuring about 27 to 30 nm in diameter. They have no envelope. The nucleocapsid is rounded and exhibits an icosahedral symmetry. The surface structure reveals a regular model with distinct features[8]. The capsomere arrangement is clearly visible (Figure 1).

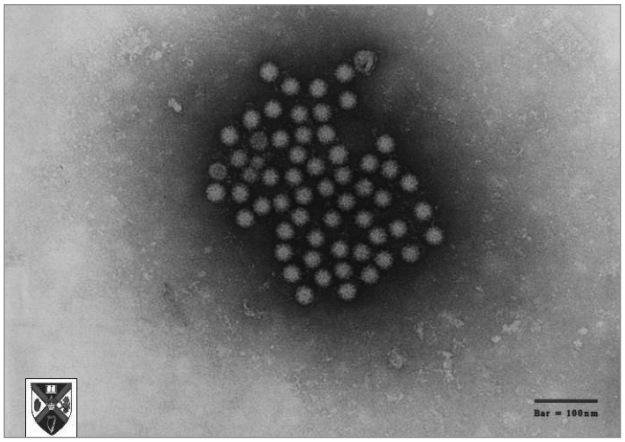

Figure 1 – Bovine calicivirus, viral particles extracted from stool sample (Stewart McNulty, Veterinary Sciences, Queen's University, Belfast). Negative dye technique by direct electron microscopy. Magnification 33,000 X. Available at: http: //www.qub.ac.uk

The virus genome consists of a linear molecule of single-strand RNA with a positive polarity. The genomes with these features serve as mRNA. As soon as they enter the target-cell, they are bound to cell ribosomes and protein translation occurs. The genome RNA serves as a template for a complementary negative strand being transcribed into genome RNA through the viral polymerase. The complete genome contains approximately 7.5 kb and consists of 45%-56% of cytosine + guanine (C + G). The genome 5' end presents the VPg protein, having an essential role in virus infectivity and initial translation; in the 3' end, the poli A tail addition occurs after the gene synthesis and its function is giving stability to the molecule and helping translation[8].

The three open read frames (ORF) of the virus genome can be observed in Figure 2: the first ORF encodes a 194-kDa polyprotein which is cleaved by the virus protease 3C into six likely proteins, including RNA-dependent RNA polymerase. Thus, the 5'-end in the genome encodes a precursor of non-structural proteins involved in the virus transcription and replication[9]. The second ORF encodes a 60-kDa capsid protein (VP1), a structural protein with a major role in virus replication[10]. The third ORF is considered the most variable region in the genome and encodes the 23-kDa basic protein (VP2) interacting with the genome RNA when the virion formation occurs[11].

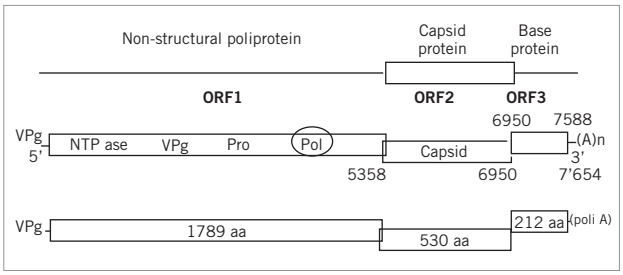

Figure 2 – Norovirus genomic organization. Localization of the three ORFs and the Pol region used for the design of the primer pool used in the RT-PCR for the identification of genogroups and genotypes. From Atmar & Estes, 2001.[29]

Nomenclature and classification

Because of low viral (NoV) load in feces and difficult spread in both cell culture and laboratory animals, the virus classification was defined only from 1990. Since then, some calicivirus genomes have been sequenced, allowing framing most of these viruses into the *Caliciviridae* family[12].

In 2005, a new classification system was established and based on ORF2 phylogenetic analyses of 164 NoV sequences. NoVs can be subdivided into five genogroups (GI, GII, GIII, GIV, GV), consisting of at least 31 genetic clusters or genotypes: 8 genotypes in GI genogroup, 17 in GII, 2 in GIII, 1 in GIV, and 1 in GV[13]. Across all of them,

GI and GII are the genogroups presenting the largest genetic diversity; six new genotypes were new genotypes defined and described: GI/8 in serogroup GI and GII/13-17 in genogroup GII[14]. NoVs with genogroups GI, GII, and GIV are found in humans, except for the sample of NV S11/GII found in swine; genogroups GIII and GV are found in cattle and mice, respectively[15]. Recently, molecular epidemiology studies have demonstrated 70% of NoV outbreaks are caused by the variant genotype GII.4[16,17].

Pathogenesis and replication

Human caliciviruses cause infection predominantly by the oral route. Virions are stable in acid and they can survive after passing through the stomach. NoVs are highly infectious due to the combination of low infecting dose (DI 50 < 20 virus particles), high virus excretion level (10^8 to 10^{10} copies of RNA per gram of feces) and extended excretion after clinical recovery[18,19]. The virus is replicated in the enterocyte cytoplasm, where the positive polarity RNA acts as mRNA. Studies showed the virus elimination can continue for over 2 weeks after the symptomatic phase of the disease, as well as cases of asymptomatic infection [20] with an implication on outbreaks caused by food transmission diseases[21]. There is little evidence that NoVs can cause a chronic infection in a normal host; however, a study performed in immunocompromised children and adolescents by Levett et al.[22] reported NoV GII shedding over at least 8 months.

Transmission

NoVs are the main cause of acute nonbacterial human gastroenteritis, being transmitted from food or from person to person via a fecal-oral route, affecting adults and children all over the world[23]. Indirect evidence in epidemiological studies suggests the virus transmission can be airborne, such as in explosive vomiting occurred during the disease[24]. Transmission can also occur via water reservoir when groundwater is contaminated[25]. They are highly contagious, possibly occurring in either sporadic cases or in great acute diarrhea outbreaks in wards, hospitals, schools, universities, camping places, cruises, hotels, and restaurants[26]. Food quality control is often based on bacterial contamination; thus, virus contamination may not be reported often[27]. Virus transmission in hospital wards is difficult to identify[26]. Filtering animals living in contaminated waters and which are eaten raw, such as oysters, are major transmission routes[28].

Clinical features are characterized by nausea, abdominal pain, vomiting, mild, self-limited, and non-bloody diarrhea. However, some patients can have severe forms, with symptoms linked to nausea and vomiting, followed by copious diarrhea, which can result in dehydration and occasionally death. Incubation period is 24 to 48 hours, with the symptoms lasting 12 to 60 hours[29]. Low fever and abdominal pain can also be associated with a virus infection, with the term "stomach flu" being used to describe the disease, although no biological association with influenza virus can be found. Approximately 10% of people with NoV require a medical visit, including hospitalization and dehydration treatment. Deaths caused by NoV are more frequently reported in elderly people. Around 30% of infections from NoV are asymptomatic; however, these individuals can transmit the virus, although in lower levels than symptomatic individuals[30].

Immunity

Because of a lack of animal model and resources to grow this virus in cell cultures, *in vitro* neutralization tests are not feasible, and data of immunity development after NoV infection is obtained from human studies with volunteers[31]. Studies indicate approximately 50% of people exposed to the virus acquired short-term homologous immunity, which is correlated with the serum antibody level[32]. However, people with preexisting high antibody levels to NoV may become ill if exposed to the virus[26,32]. A candidate vaccine has been developed, although it is not known whether the vaccine induces homotypic or heterotypic immune protection[31,33]. As no suitable prevention and control method is available, the development of a vaccine to NoV could be the best solution for this infectious disease[34]. Studies suggest there is a short-term immunity after infection, and some individuals are susceptible to symptomatic infection, whereas others never develop symptoms, even after a direct contact[32].

Prevention and treatment

Stopping transmission is the first strategy for prevention, especially in hospitals and day-care centers. A number of precautions, such as hand washing with water and soap before and after contacting the patient or objects used by him/her, must be taken when caring for a patient diagnosed with an acute gastroenteritis. It is also required to clean all surfaces with 2% hypochlorite[35], as NoV persist in dry inanimate surfaces over eight hours to seven days[36]. To avoid secondary transmissions, prevention of food contaminations during the preparation by a continuous hand washing is required. Those who handle food must wear plastic gloves when preparing raw food[37]. Affected workers must not prepare food for a minimum period of three days after the disease to avoid gastroenteritis outbreaks[38].

As there is no strengthened antivirus agent to treat norovirus diseases, the focus consists of prevention and treatment of the secondary dehydration. Fluid therapy is usually maintained orally with isotonic fluids. Hospitalization in cases of a severe dehydration may be required, although this is rare. Symptoms such as headache, myalgia, and nausea can be treated with analgesic and antipyretic drugs[39].

In 2006, Rossignol[40] analyzed a new drug, the nitazoxanide, indicated to treat diarrhea caused by virus gastroenteritis. In this study, the drug efficacy in several patients with symptoms and positive diagnosis for rotavirus, enteric adenovirus, norovirus, and astrovirus was observed. However, higher drug effectiveness was found against rotavirus, compared with other viral pathogens.

Laboratory diagnosis

The classic diagnosis method is electron microscopy (EM), detecting virus particles with 27 to 30 nm in diameter, the so-called SRSV. This method is used in public health laboratories in many countries; however, it requires a highly qualified microscopist and very expensive equipment, making epidemiological or clinical studies impracticable[41].

The immunoenzymatic method (ELISA) to detect the virus antigen uses norovirus capsid proteins expressed on baculovirus as a reactant in immunoenzymatic tests[29]. This method has been recently made commercially available to diagnose NoV directly from feces (Dako Cytomation, Ely, UK 2001; Denka Seiken, Tokyo, Japan, 2002; R-Biopharm AG, Germany 2004). These kits have low diagnostic sensitivity, as reported by Bull et al.[42]. However, the development of new kit generations, such as RIDASCREEN 3rd Generation kit (R-Biopharm AG, Darmstadt, Germany), which is more sensitive and specific, entailed benefits for NoV quick diagnosis, mainly targeting outbreaks[43].

The RT-PCR molecular technique, developed to identify NoV, is sensitive and specific, enabling epidemiological studies to identify gastroenteritis outbreaks[44]. International collaborative studies[45] demonstrated that, among several primer pools developed for regions ORFs 1, 2, and 3, those showing the best results were primers in POL region of ORF 1 (preserved region). Phylogenetic analysis of 145 nucleotides in the POL gene region was used as a pattern to identify genotypes. NoV sequencing has assisted in epidemiological investigations relating clinical cases to determine a common source and to differentiate outbreaks that could be wrongly related[45].

REAL-TIME TaqMan[46,47] RT-PCR and SYBR Green[48] techniques quantify specific DNA or RNA sequences in clinical samples and the gene expression from emitted fluorescence detection since the first amplification cycle. These methods have advantages over regular PCR, such as higher specificity, sensitivity, and reproducibility, in addition to allowing real-time monitoring; quicker cycling; lower RNA amount in RT-PCR reactions; and elimination of post-PCR product handling, thus reducing contamination[45].

Epidemiology

NoVs are considered the most common viral etiologic agents in outbreaks of virus gastroenteritis transmitted by water and food[49]. The epidemiology of diarrheal diseases transmitted by water and food is quickly changed from the human behavior changes concerning global economy, the industry, and microbiological adaptations. The highest disease incidence is among children under five years of age[50]. However, the highest economic impact is among elderly residing in nursing homes[51]. Although diarrhea outbreaks can occur all over the year, some seasonality patterns have been observed. These patterns are different in Northern and Southern hemispheres. In Northern hemisphere, gastroenteritis caused by NoV is more commonly seen in winter and early spring[22]. In the Southern hemisphere, outbreaks are more frequent over the spring and summer[52].

From 1999 to 2002, 170 NoV outbreaks occurred in Spain. By employing EM, RT-PCR and sequencing techniques, the genogroup GII was observed as predominating[53]. In England, Lopman et al.[51], by analyzing samples from outbreaks occurred between 1995 and 2002, observed NoV had a peak infection over the summer, in contrast with the reports from that time, describing higher occurrence in the winter.

Chapin et al.[54] found NoV transmission from food contamination crosses borders; the virus from the United States reached Guatemala and Mexico, and the samples were analyzed by RT-PCR and sequencing, with NoV genogroup GI being identified in 65% of positive cases.

In the state of São Paulo, during the summer of 1995, a gastroenteritis outbreak occurred, affecting around 3,500 people (CVE data). EM analysis of fecal samples from this outbreak detected virus particles with SRSV morphology identified as Norwalk-like by immunoelectron microscopy (IEM)[55]. These samples were further analyzed by RT-PCR and sequencing and characterized as calicivirus SMA (Snow Mountain Agent) type, currently termed GII genogroup NoV[56].

From 2005 to 2008, a surveillance NoV study was conducted in the state of Rio de Janeiro. A total 1,087 fecal samples were analyzed and about 35% were positive for NoV, with a 96% prevalence of GII genogroup and 80% GII.4; the requirement of implanting NoV diagnosis in the surveillance laboratories was described[57].

In the state of São Paulo, also in 2005, several gastroenteritis outbreaks occurred in children and adolescents, and the sample analysis demonstrated the circulation of different viruses around the same region. NoVs were detected in 21.4% of samples, followed by rotavirus, with 14.5%, astrovirus with 13.2%, and adenovirus with 2.1%[58].

Conclusion

The Nucleus of Enteric Diseases of Instituto Adolfo Lutz (IAL) is a macroregional reference for rotavirus and norovirus vigilance in the Acute Diarrheal Disease Monitoring Program (PMDDA), aiming at the early detection of diarrhea outbreaks all over the country. In 2008, systematic

implementation for NoV detection was initiated to detect NoV in IAL[59]. In 2009, with the enhancement of conducting, 15 identified NoV outbreaks were detected in Catanduva, Mongaguá, Piracicaba, Pitangueiras, Praia Grande, Ribeirão Preto, Sabino, São Paulo, Taubaté, Votuporanga[60]. In January 2010, Guarujá, in Baixada Santista, faced a severe diarrhea outbreak with NoV (28%) and rotavirus (20%) (data from IAL). The easiness with which noroviruses are transmitted and the low infecting dose required to set an infection results in extensive outbreaks. The accurate diagnosis of virus gastroenteritis is essential to reduce the impact of the disease on the society.

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