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Noroviruses – State of the Art

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Abstract

Noroviruses are a common cause of both endemic and epidemic gastroenteritis. These highly infectious viruses usually cause self-limited disease, but chronic infections occur in highly immunocompromised patients and unusual manifestations are also being described in some populations. Histoblood-group antigen expression is now recognized as an important susceptibility factor for many norovirus strains, but a correlate of acquired immunity to infection or illness has not yet been identified. Currently, treatment and prevention strategies rely on non-specific measures. However, virus-like particles containing capsid antigens are undergoing evaluation as a vaccine candidate for illness prevention. This article reviews the biologic properties, epidemiology, clinical features, host susceptibility, diagnosis, and treatment and prevention of norovirus infection.

Keywords

norovirus; gastroenteritis; review; human; calicivirus

Introduction

Gastroenteritis is a major cause of morbidity and mortality worldwide. The World Health Organization (2008) estimates that there are as many as 4.6 billion episodes of diarrheal illness with approximately 2.2 million associated deaths worldwide each year. Noroviruses (NoVs) are now recognized as a major cause of both sporadic and epidemic gastroenteritis. A recent estimate is that NoVs may cause more than 1 million hospitalizations and up to 200,000 deaths in children less than 5 years of age on an annual basis (Patel 2008). This article reviews the biologic properties, epidemiology, clinical features, host susceptibility, diagnosis, and treatment and prevention of NoVs.

The Virus

Norovirus is a genus in the family *Caliciviridae*, a group of nonenveloped, icosahedral viruses that have a single-stranded, positive sense RNA genome. The viral genome is 7.5 to 7.7 kilobases in length and contains three open reading frames (ORFs) (Figure 1). ORF1 encodes a large polyprotein that is post-translationally cleaved into six nonstructural proteins that are involved in viral replication, including a N-terminal protein (designated p48 for Norwalk virus), a NTPase, a “3A-like protein” (designated p22 for Norwalk virus), a VPg, a viral protease, and a viral polymerase (Green 2007). ORF2 encodes the major structural protein (VP1) that forms the capsid, and ORF3 encodes a minor structural protein, VP2. The viral capsid contains 180 copies of VP1 protein and a few copies of VP2, and *in vitro* expression of the VP1 gene leads to the spontaneous formation of virus-like particles (VLPs). Virus replication takes place through a negative sense strand intermediate from which both positive sense genomic RNA and a positive strand subgenomic RNA containing ORFs 2 and 3 are produced (Figure 1). NoVs cannot be cultivated *in vitro*, with the exception of murine strains

(Duizer 2004; Wobus 2004). NoV VLPs that are expressed from ORFs 2 and 3 are antigenically and morphologically similar to intact NoV particles. Thus, the VLPs are often used as a surrogate in laboratory studies and VLPs are under evaluation as a vaccine candidate (see below).

NoVs are antigenically and genetically diverse. The lack of *in vitro* cultivation systems precludes classification of NoVs into distinct serotypes. Instead, NoVs are classified using genetic analysis. Currently there are five genogroups. Within a genogroup, NoVs are further divided into genotypes, or genetic clusters (Zheng 2006). Genogroup is designated by a capital "G" and a Roman numeral, and genotype is designated by an Arabic numeral. The prototype NoV strain, Norwalk virus, belongs to genogroup I and genotype 1, and is designated as GI.1. Different criteria for separating strains into genotypes have been proposed (Katayama 2002; Zheng 2006). At the Third International Calicivirus Conference held in Cancun, Mexico, in 2007, there was general agreement among attendees to accept the methods proposed by Zheng et al (2006) that require analysis of the entire VP1 capsid amino acid sequence for identification of a new genotype rather than analysis of shorter sequences, as originally proposed by Katayama et al. (2002). Using these criteria, there are at least 33 NoV genotypes, with 9, 19, 2, 2, and 1 genotypes belonging to genogroups I through V, respectively (Figure 2). Genogroup and genotype are often inferred by analysis of shorter capsid sequences, or by analysis of other portions of the genome (e.g., viral polymerase). The latter approach can lead to misclassification because of the propensity of NoVs to recombine, most commonly near the junction of the first and second ORFs (Bull 2007). Thus, a strain may have nonstructural genes that were originally associated with one genotype and a VP1 gene associated with a second genotype.

NoV strains that infect humans are found in genogroups I, II and IV. NoV genogroups III and V contain strains that infect cows and mice, respectively. Porcine strains are found in genogroup II (II.11, II.18, and II.19) and strains that infect feline and canine species are found in genogroup IV (IV.2). NoVs have tended to be species specific, although human NoV strains have been found in beef and porcine species by RT-PCR (Mattison 2007), and a human GII.4 strain is able to infect gnotobiotic pigs and calves (Cheetham 2006; Souza 2008). However, to date no infections with animal strains have been identified in people.

Epidemiology

The primary route of transmission of noroviruses is fecal-oral, although airborne transmission also occurs (Atmar & Estes 2006). Contaminations of food, water or fomites and direct person-to-person spread have all been implicated in outbreaks of NoV gastroenteritis. Certain genotypes are more likely to be associated with specific routes of transmission. For example, GII.4 strains are more commonly associated with person-to-person transmission, while GI strains are identified more frequently in shellfish-associated outbreaks (Siebenga 2007; Le Guyader 2006).

The incubation period ranges from 10 to 51 hours, and the infectious dose is low (Glass 2009). Teunis and colleagues (2008) estimated the infectious dose 50% for Norwalk virus to be between 18 and 1000 viral particles. Larger amounts of virus have routinely been found in shellfish implicated epidemiologically as causes of gastroenteritis (Le Guyader 2003, 2010). The low infectious dose also allows transmission of virus from persons prior to onset of symptoms or after recovery from illness (Patterson 1993; Lo 1994). As many as a third of persons shed virus prior to onset of illness, and peak fecal virus shedding may occur after gastroenteritis symptoms have resolved (Atmar 2008).

NoV-associated gastroenteritis occurs in a variety of settings. NoVs are most frequently recognized as causes of outbreaks of acute gastroenteritis, but sporadic illness also occurs

commonly. NoVs are responsible for 47–96% of outbreaks of acute gastroenteritis and for 5–36% of sporadic cases of acute gastroenteritis reported from countries around the world (Atmar & Estes 2006; Patel 2008). NoVs are the most common cause of acute gastroenteritis in the community (de Wit 2001). Among hospitalized patients with acute gastroenteritis, NoVs are second to rotaviruses among children <5 years of age, and they are second to *Campylobacter* spp. among adults as causes of hospitalization for acute gastroenteritis (Jansen 2008). A median of 12% of hospitalizations for acute gastroenteritis among children <5 years of age are attributable to NoVs (Patel 2008).

As noted earlier, food is a frequent vehicle for virus transmission. Contamination of the food with fecal material can occur at any step during its production. For example, contamination of shellfish usually occurs prior to harvesting. Contamination of berries (e.g., raspberries) may occur prior to harvesting (e.g., due to irrigation with fecally-contaminated water), during harvesting (contamination by infected field workers) or during processing prior to distribution (contamination in the factory by infected food handlers or by spraying with contaminated water) (Falkenhorst 2005). Other foods implicated in outbreaks, including salads, sandwiches, and deli meats, have been contaminated at the site of preparation by infected food handlers.

Secondary transmission of NoV infection is common (often >30%), allowing amplification of an outbreak, particularly in closed settings. Such outbreaks are commonly recognized in healthcare institutions (e.g., hospital or nursing home) and on cruise ships (Lopman 2004b; Verhoef 2008). Because NoVs are relatively resistant to inactivation by many common disinfectants, outbreaks in these settings may require closure of the unit or ship for more extensive cleaning and disinfection.

NoVs cause infection throughout the year, but in temperate climates there is a distinct winter seasonality (Lopman 2009). Genogroup II NoVs have been the most prevalent strains causing infection worldwide for at least the past 15 years. Among these, the GII.4 genotype is most prevalent in the majority of the years, and large increases in the number of outbreaks have coincided with the emergence of novel variants. The emergence of new variants is analogous to what is seen with influenza viruses, suggesting that population immunity may be driving the evolution of the GII.4 viruses (Siebenga 2009). A similar phenomenon of genetic drift has also been described for GII.2 strains, but only among strains that are not recombinants (Iritani 2008). It is not clear at this time whether antigenic drift driven by population immunity occurs among other NoV genotypes.

Clinical Features

Acute NoV-associated gastroenteritis is characterized by the sudden onset of vomiting, watery diarrhea or both symptoms. Additional constitutional symptoms that can be seen include nausea, abdominal cramping and pain, malaise, anorexia, fever, chills, headache and myalgias. Subclinical infection is common, being reported in up to one third of infected persons following experimental challenge (Graham 1994). Vomiting has been reported more frequently with GII.4 strains in nursing home outbreaks than with outbreaks caused by other strains (Friesema 2009). Illness typically is two to three days in duration, but symptoms commonly persist beyond four days in young children (under 12 years of age) and in the hospitalized persons over 80 years of age (Rockx 2002; Lopman 2004a).

NoV infection is usually a self-limited illness, and healthy persons typically recover without sequelae. Complications can occur and include volume depletion, electrolyte disturbances (e.g., hyponatremia), and renal insufficiency (Mattner 2006). Elderly and chronically ill persons are more likely to suffer these complications, and death has complicated norovirus outbreaks among elderly residents of nursing home facilities (CDC 2007).

Unusual presentations of NoV infection have been described in special populations. Disseminated intravascular coagulation complicated NoV-associated gastroenteritis in previously healthy soldiers exposed to severe environmental stresses (CDC 2002). A cluster of necrotizing enterocolitis was noted in a neonatal intensive care unit, and NoV infection was confirmed in half of the affected patients (Turcios-Ruiz 2008). NoV infection was associated with benign infantile seizures more commonly than was rotavirus infection among Chinese children hospitalized with acute gastroenteritis (Chen 2009), and infection has also been described in association with exacerbations of inflammatory bowel disease (Khan 2009). Although the association of NoV infection with these unusual presentations requires further confirmation, the impact of NoVs in immunocompromised patients is clear. Chronic diarrhea, with complications of volume depletion and malnutrition, can complicate infection in immunocompromised patients, particularly recipients of solid organ and stem cell transplants (Nilsson 2003; Roddie 2009; Westhoff 2009). Symptoms persist for years in some patients. NoV infection in small bowel transplant recipients can be difficult to distinguish from allograft rejection and should be considered in the differential diagnosis of diarrhea in this patient population (Kaufman 2005).

Host Susceptibility

There are two recognized mechanisms involved in resistance to NoV infection: genetic factors and acquired immunity. The potential role of genetic resistance was first recognized more than 30 years ago when experimental human infection studies with Norwalk virus showed that study participants were repeatedly susceptible or resistant to symptomatic infection following repeated virus challenge (Parrino 1977). Subsequently, VLPs from Norwalk virus were shown to differentially bind to histoblood group antigens (HBGAs), and the binding pattern correlates with susceptibility to infection and illness (Hutson 2002, 2005; Lindesmith 2003). For example, Norwalk virus VLPs do not bind well to the blood group B trisaccharide in vitro, and persons expressing a blood group B antigen are less likely to become ill following challenge with Norwalk virus (Hutson 2002, 2003). A number of enzymes are important in the synthesis of HBGAs, including fucosyl transferase-2 (FUT-2, secretor enzyme), FUT-3 (Lewis enzyme) and the A and B enzymes (Figure 3). Mutations in the genes encoding these enzymes can render them non-functional, altering expression of HBGAs at the mucosal surface.

The FUT-2 enzyme has a particularly important role in susceptibility to NoV infection. Persons who do not possess a functional FUT-2 enzyme (secretor-negative) are resistant to infection with GI.1, GII.3 and most GII.4 NoVs (Table 1). This observation has led to the proposal that the glycan produced by FUT-2, H type 1, serves as a viral receptor for Norwalk virus (Hutson 2004). Not all NoV strains are FUT-2 dependent. For example, secretor-negative individuals can be infected with Snow Mountain virus, a GII.2 strain (Lindesmith 2005), and with a GI.3 strain (Nordgren 2010). The binding pattern of NoVs to HBGAs varies between genotypes (Huang 2005; Shirato 2008), and even within a genotype (Lindesmith 2008). Thus, it is likely that every person is genetically susceptible to one or more NoV genotypes.

Acquired immunity is the other mechanism by which resistance to NoV infection occurs. Following symptomatic infection persons re-challenged with the same strain of NoV within 6–14 weeks do not develop illness (Parrino 1977). The immunity is not long lasting, in that symptomatic illness develops following re-challenge two to three years later. Immunity is also strain-specific; exposure to a serologically distinct strain leads to symptomatic infection (Wyatt 1974). The mediator of immunity has not yet been defined, although antibody that blocks VLP binding to HBGAs has been proposed as a potential candidate. On the other hand, antibody to the virus capsid measured by ELISA has not reliably predicted immunity to infection. In some studies high levels of antibody have been associated with a decreased likelihood of infection while in other studies the absence of antibody was associated with a lower frequency of

infection (Matsui & Greenberg 2001). This apparent paradox might be explained by lower levels of antibody in persons genetically resistant to infection with a specific strain and higher levels of antibody in persons recently infected with a related strain.

Diagnosis

The diagnosis of NoV infection has evolved over the past several decades. In the 1970s and 1980s, the primary means of diagnosis was by electron microscopy (Atmar & Estes 2001). This method is much less sensitive than the molecular assays currently in use today. RT-PCR assays first became available in the 1990s and are currently the most sensitive methods for detection of NoVs in clinical samples. These assays target conserved areas in the genome, including the polymerase gene (region A), the ORF1/ORF2 junction (region B), and areas in the VP1 gene (regions C and D). The specificity of the assays is confirmed by probe hybridization or sequencing of the amplicons. The sequence data using the latter approach can be used for genotyping and in molecular epidemiologic studies (Le Guyader 2008; Yee 2007). Real-time RT-PCR assays are also now available and allow more rapid evaluation of clinical samples. These assays identify and classify NoVs to the genogroup level (Kageyama 2004; Trujillo 2006).

Antigen detection assays are another approach to diagnosis of NoV infection. Kits using either an ELISA or immunochromatographic format are commercially available. The principal disadvantage of these kits has been their poor sensitivity, being as low as 32% in the identification of sporadic cases of NoV infection; sensitivity is also affected by NoV genotype, as some less common genotypes are not identified by these assays (Gray 2007). Application to multiple samples from an outbreak can improve the likelihood of obtaining a positive result (Duijzer 2007). Problems with specificity have also been reported in certain populations, further limiting the utility of these commercially-available kits (Wiechers 2008).

Serologic assays are not generally available for clinical use. However, infection can be diagnosed by identifying a four-fold or greater increase in antibody titer between acute and convalescent serum samples using NoV VLPs as antigen. ELISA-based assays are most commonly used, but HBGA-blocking assays also can identify infection. A serologic rise is more likely to be detected when VLPs closely related to the infecting strain are used as antigen. Heterologous antibody responses may also occur (e.g., antibody rise to a GII NoV VLP following a GI NoV infection), but are of a lower magnitude than that identified using a homologous antigen (Noel 1997; Treanor 1993). HBGA blocking antibody rises may be more specific than rises identified by ELISA to the capsid antigen. Increases in serum HBGA-blocking antibody levels appear to be restricted to capsid antigens within a genogroup, although heterotypic rises between genotypes within a genogroup still occur (Lindesmith 2010).

NoV infection can also be suspected as the cause of an outbreak of gastroenteritis based upon clinical and epidemiological criteria (Kaplan 1982). The criteria include the following: (1) vomiting in >50% of ill persons; (2) mean incubation period of 24–48 hours, (3) mean duration of illness of 12 to 60 hours, and (4) no bacterial pathogen identified. These criteria are highly specific (98.6%) and have a high positive predictive value (97.1%) (Turcios 2006). One of the specific tests described above is required to confirm the etiology of a NoV outbreak suspected on a clinical or epidemiological basis.

Treatment and Prevention

In most persons NoV illness is self-limited and mild, and either no treatment is needed or it is supportive (e.g., oral rehydration, antiemetics, analgesics, antimotility and antisecretory agents). For patients who become more severely volume depleted, intravenous fluid and electrolyte replacement may be required in the emergency center or in the hospital; usually no

treatment is required. No specific antiviral therapy is currently available. Ribavirin and interferons inhibit viral replication in a Norwalk viral replicon system, but no clinical data are available to evaluate their utility in active infection (Chang 2007). Immune globulin also has been proposed as a treatment (given either orally or intravenously), but only limited anecdotal information has suggested a potential beneficial effect of such therapy (Florescu 2008; Glass 2009).

Preventive methods are also currently non-specific in nature. These methods include appropriate hand hygiene, environmental decontamination, and furlough of symptomatic food handlers and healthcare workers during the period of illness and for up to 2–3 days after symptom resolution (Parashar 2001; Harris 2010). There are limited data that evaluate the effectiveness of each specific measure in controlling healthcare-associated outbreaks (Harris 2010). However, the use of alcohol-based hand sanitizers in an elementary school decreased absenteeism due to any gastrointestinal illness and was associated with a lower level of classroom contamination with NoVs (Sandora 2008). Similarly, another study showed that cruise ships with more thorough disinfection of public restrooms, as a measure of environmental hygiene, were less likely to have a subsequent NoV outbreak than ships with poorer scores (Carling 2010). These control measures may not be sufficient to halt an ongoing outbreak, and closure of a hospital unit, cruise ship, or other facility for more intensive environmental decontamination may be needed to stop replenishment of the pool of susceptible individuals.

Measures to identify contaminated foodstuffs are also used to prevent NoV transmission. For example, current regulations call for screening of shellfish-growing waters or shellfish for fecal coliform bacteria to identify exposure to sewage pollution (Le Guyader 2009). However, this approach has failed to identify viral contamination of shellfish associated with a number of NoV outbreaks. With the development of methods to directly detect viral contamination of foodstuffs implicated in foodborne NoV outbreaks, it should be possible to adapt these assays for screening high risk foods (Schwab 2000; Le Guyader 2003, 2004). Other approaches to inactivate NoVs contaminating food, including high-pressure processing and irradiation, are under evaluation (de Roda Husman 2004; Kingsley 2007).

NoV VLPs are being evaluated as a vaccine candidate. Preclinical studies have shown that the VLPs are immunogenic when administered orally, intranasally or parenterally (Estes 2001; LoBue 2006), and clinical studies have shown that the VLPs are safe and immunogenic when administered orally to people (Ball 1999; Tacket 2003). A number of barriers to the development of an effective vaccine remain, including the lack of an immune correlate of protection from illness, the short term duration of immunity following an episode of illness (less than 2–3 years), the genetic and antigenic diversity of NoVs, and the evolution of NoVs belonging a genotype that appears to be due to population-based immunity. On the other hand, studies of vaccine candidates and human challenge experiments may provide new information about correlates of immunity, including the importance of HBGA-blocking antibodies. The predominance of a few strains (e.g., GII.4 viruses) as the cause of the majority of outbreaks and the induction of cross-reactive blocking antibody following infection (or vaccination) may allow the development of a vaccine that will prevent illness caused by the most common strains.

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Figure 1.

Norovirus genomic organization and replication strategy. Norovirus is a positive strand RNA virus with three open reading frames (ORFs) encoding a large polyprotein from which non-structural proteins are produced by proteolytic cleavage and two structural proteins, VP1 and VP2. Replication takes place through a negative sense intermediate, from which the genomic RNA and a subgenomic RNA encoding the structural proteins are produced.

Figure 2.

Classification of noroviruses into genogroups and genotypes. The Norovirus genus is divided into 5 genogroups (GI-GV). Each genogroup has from 1 to 19 different genotypes. GI.9 is a newly designated genotype, and its prototype strain is NoV/IF2036/2003/Iraq (AY675555).

Figure 3.

Histoblood group synthetic pathway for blood group ABH and Lewis antigens. A type 1 precursor glycan is modified by fucosyltransferase-2 (FUT2) to produce the H type 1 glycan, which can be further modified by FUT3 (to produce Lewis-b [Le^b]) and by A and B enzymes (enz) to produce the A and B histoblood group antigens (HBGAs). If FUT2 is non-functional, a person is a non-secretor, although the H type 1 precursor can still be modified to produce Le^a in the presence of a functional FUT3. Persons with a functional FUT2 enzyme are called secretor positive because the ABH HBGAs are found in secretions and on mucosal surfaces.

Table 1

Susceptibility to norovirus infection by histoblood group antigen type

Genotype (strain)	Author (year)	Association with Protection from Illness or Infection		
		Secretor status (FUT2)	Lewis antigens (FUT3)	A or B antigens
GI.1 (Norwalk)	Hutson et al. (2002)	Not evaluated	Not evaluated	Risk of illness: OR=0, 95% CI 0–0.999, blood group B
GI.1 (Norwalk)	Lindesmith et al. (2003)	No infection in secretor negative persons. Risk of infection: OR= 0, 95% CI 0–0.11, secretor negative	Not evaluated	No
GI.1 (Norwalk)	Hutson et al. (2005)	No infection in secretor negative persons. Risk of infection: OR= 0, 95% CI 0–0.023, secretor negative	Not evaluated	Risk of illness: OR=0, 95% CI 0–0.37, blood group B (among secretor positive persons)
GI.3 (Kashiwa645- like)	Nordgren et al. (2010)	No	No	No
GII.2 (Snow Mountain)	Lindesmith et al. (2005)	No	No	No
GII.3 (not stated)	Tan et al. (2008)	No illness in secretor negative persons. Risk of illness: OR=0, 95% CI 0–1.4, secretor negative	Not evaluable (all persons Lewis positive)	No
GII.4 (not stated)	Thorven et al. (2005)	No illness in secretor negative persons. Risk of illness: OR=0, 95% CI 0–0.23, secretor negative	Not evaluated	Not evaluated
GII.4 (not stated)	Kindberg et al. (2007)	No illness in secretor negative persons. Risk of illness: OR=0, 95% CI 0–0.36, secretor negative	Not evaluated	Not evaluated
GII.4 (not stated)	Tan et al. (2008)	No illness in secretor negative persons. Risk of illness: OR=0, 95% CI 0–0.47, secretor negative	No	Risk of illness: OR = 2.7, 95% CI 1.2–5.9, blood group A (among secretor positive persons); OR= 0.34, 95% CI 0.14–0.85, blood group O (among secretor positive persons)
GII.4 (not stated)*	Klement & Halperin (2008)	Not evaluated	Not evaluated	Risk of illness: OR = 2.0, 95% CI 1.1–3.5, blood group O
GII.4 (Valencia/04)	Carlsson et al. (2009)	Risk of illness: OR=0.019, 95% CI 0.003–0.13, secretor negative	No	Not evaluated

* Based upon sequence data from polymerase gene