

THE MENACE OF NOROVIRUS ON HUMAN HEALTH

By

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ABSTRACT

Norovirus formally known as Norwalk virus was named after Norwalk, Ohio, in the United States, where an outbreak of acute viral gastroenteritis occurred among children at Bronson Elementary School in November, 1968. Norovirus is the only species of the genus Norovirus, which belongs to the family calciviridae. Infection by Norovirus relies on the recognition of histo-blood group antigens (HBGAs) in the initial viral attachment and this key event most likely controls host susceptibility and resistance to Norovirus. The pathological effect due to infection with Norovirus begins with viral attachment to the cell receptor, the P2 subdomain of the VP1 binds to a sugar residue, mostly to the histo-blood group antigen (HBGA) carbohydrates in the case of human Norovirus but also to sialic acid or heparin sulfate. Diagnosis can be achieved through Electron Microscopy and Immune Electron Microscopy, Enzyme Linked Immunosorbent Assay and Nucleic Acid Hybridization Assay. Theoretically, any food item can potentially be infected with Norovirus through fecal contamination. Therefore, all food items should be handled in good hygienic manner

Keywords: Norovirus, Gastroenteritis, Norwalk virus, histo-blood group antigen, nucleic acid Hybridization

INTRODUCTION

Norovirus formally known as Norwalk virus was named after Norwalk, Ohio, in the United States, where an outbreak of acute viral gastroenteritis occurred among children at Bronson Elementary School in November 1968. Common names for the noroviruses are "winter vomiting disease," "cruise ship disease," and "stomach flu/virus." The "Norwalk agent" was the first virus to be conclusively associated with diarrhea in man. Subsequently, researchers began to recognize other viruses as causative agents of gastroenteritis (Roos-Weil *et al.*, 2011).

Despite the increasing attention given to this virus today and the significant morbidity and mortality associated with Norovirus gastroenteritis, no specific antiviral drugs or vaccines are yet available for treatment or prevention of illnesses associated with them. Only few years ago, a recombinant intranasal

vaccine entered a phase I clinical trial (El-Kamary *et al.*, 2010; Vinje, 2011).

The initial Norovirus challenge studies described a subset of volunteers who were resistant to the infection. Parrino *et al.*, (1977) reported that six of twelve individuals challenged with Norovirus developed clinical signs of infection, including vomiting and/or diarrhea, whereas the other six persons were asymptomatic. When the same twelve individuals were rechallenged with the same Norovirus inoculum 1–2 years later, the individuals who were ill previously suffered clinical disease again, whereas those who were asymptomatic after the initial challenge remained resistant to clinical disease. Thus, previous exposure to Norovirus that resulted in clinical illness did not confer protection against rechallenge in that study; yet, under the same conditions, resistance to clinical illness persisted in a subset of the volunteers (Hutson *et al.*, 2002). Subsequent Norovirus challenge

studies also found an absence of Norovirus infection in 12.5%–40% of volunteers (Herbst-Kralovetz *et al.*, 2013). A genetic control of susceptibility and/or resistance to Norovirus infection was proposed, but the basis for this control has remained elusive (Teunis *et al.*, 2008).

Progress in detecting and managing outbreaks of disease caused by these agents was hampered by the unavailability of sensitive and specific diagnostic tests that could be applied outside research settings (Vinjé *et al.*, 2011). Although outbreaks of nonbacterial gastroenteritis were

recognized as a public health concern, electron microscopy (EM) proved to be a tedious and insensitive method for routine examination for enteric viruses in stool specimens collected during outbreak investigations.

Norovirus S structure

The structure of Norovirus is round with rough surface and it is about 27-38 nm in size. The viral capsid encloses a single stranded RNA genome of about 7.3-7.7 kb. (Figure 1).

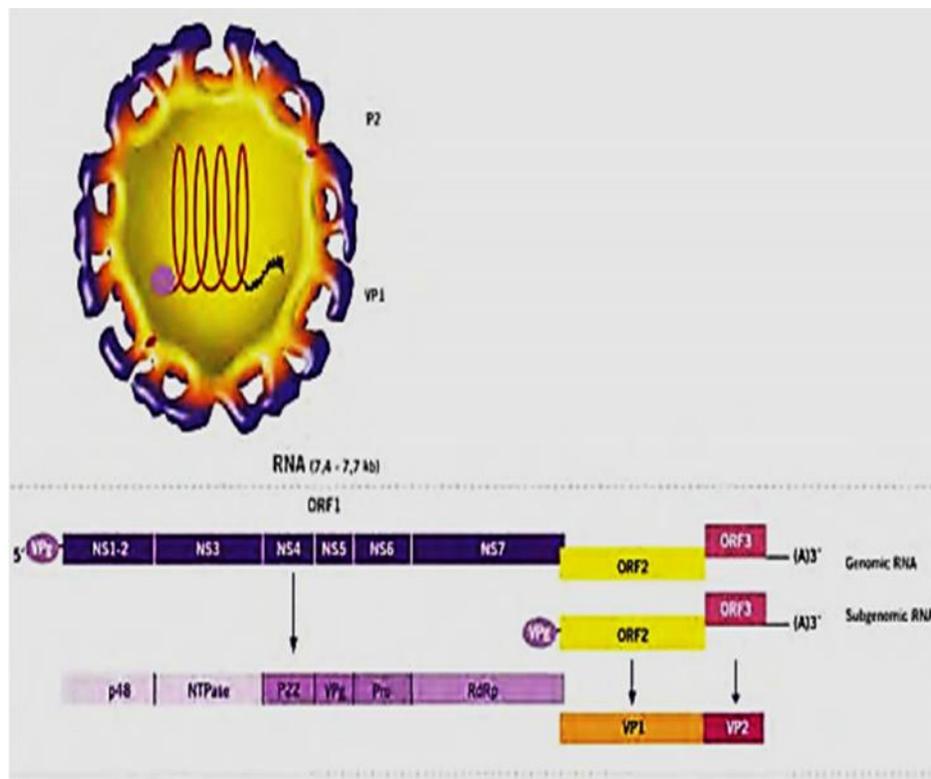


Figure 1: Schematic representation of Norovirus particle and genome organization of norovirus.

Source: Hadley (2013)

Noroviruses are today recognized as the leading cause of foodborne outbreaks and sporadic cases of gastroenteritis worldwide (Glass *et al.*, 2009; Patel *et al.*, 2009). Nowadays, they are even considered the second most important agent of severe childhood diarrhea after rotavirus (Patel *et al.*, 2008; Ramani & Kang, 2009) but the importance of

Norovirus in this age group is expected to increase in the upcoming years in most developing countries as a consequence of the non-availability and non-implementation of routine rotavirus vaccination (Koo *et al.*, 2010).

Taxonomy of Norovirus

The descendants of Norovirus are now called noroviruses because these viruses change shape slightly about every four years so there is no virus that is exactly the same as the one discovered in 1968, but still related (Hadley, 2013). Viruses are classified into family and genus Norovirus is the only species of the genus Norovirus, which belongs to the family Caliciviridae. Formerly, Norovirus was included in the genus "Norwalk-like virus" between 1999 and 2002, (Pringle, 1999; Mayo *et al.*, 2003) and the "Norwalk-like virus" was replaced with Norovirus on the 8th Report of the ICTV (Virus Taxonomy, 2005; Belgaumi, 2012).

Norovirus is the prototype strain of genetically and antigenically diverse single stranded RNA (Ribonucleic acid) viruses, previously called small round-structured viruses (SRSVs), that are classified in the genus Norwalk-like viruses in the family Caliciviridae (Green *et al.*, 2000; Kroneman *et al.*, 2013). Other genera in the Caliciviridae family include "Sapporo-like viruses," which also cause gastroenteritis among both children and adults, and Lagovirus and Vesivirus, neither of which are pathogenic for humans. NLVs can be divided into three distinct genogroups: GI, GII, and GIII (Chen, 2013). GI and GII NLVs infect humans and include 5 and 10 genetic clusters, respectively; GIII NLVs infect pigs and cows.

Pathogenesis of Norovirus

Susceptibility to Norovirus infection involves both acquired immunity and genetic resistance (Parrino *et al.*, 1977). Volunteer studies found that some individuals were repeatedly susceptible to Norovirus infection whereas others were repeatedly resistant (Parrino *et al.*, 1977). Although it was initially unclear why some subjects did not develop illness, current research suggests that host genotype is a prominent factor in the establishment of Norovirus infection since it depends on the presence of specific human histo-blood group antigen (HBGA) receptors in the gut of susceptible hosts. Infection by Norovirus

relies on the recognition of HBGAs in the initial viral attachment and this key event most likely controls host susceptibility and resistance to Norovirus infection (Hutson *et al.*, 2002; Marionneau *et al.*, 2002; Tan & Jiang, 2005).

HBGAs are complex carbohydrates linked to proteins or lipids on the surface of red blood cells and mucosal epithelia of the respiratory, genitourinary and digestive tracts, or present as free oligosaccharide in biological fluids such as milk and saliva. These antigens provide diversity within the human population and their biosynthesis is controlled by the enzyme products of alleles at the ABH, fucosyltransferase (FUT) 2, and FUT 3 loci (Hutson *et al.*, 2002).

A number of distinct binding patterns of Norovirus to HBGAs have been described according to the ABO, Lewis and secretor types of the human HBGAs. This explains the correlation between secretor status and susceptibility to Norovirus infection, where secretor individuals with a wild-type FUT2 gene (~80% of the population), who express HBGAs on gut epithelial cells and in body fluids, are susceptible to Norovirus infection, while nonsecretors, with a null FUT2 allele, are completely resistant (Hutson *et al.*, 2002).

The binding patterns of Norovirus to HBGAs are currently sorted into three major groups, the H, the A/B, and the Lewis binding groups (Tan & Jiang, 2011). While Norovirus strains display distinct HBGA binding properties, collectively they can infect nearly all individuals due to their high genetic variability. This highlights the highly adaptive nature of Norovirus and the likelihood of a long co-evolution of human Norovirus with their human host.

Replication of Norovirus

The replication of Norovirus has not been yet fully elucidated and most of the current knowledge is drawn by analogy with other positive-sense single stranded RNA [(+)ssRNA] viruses and studies with related animal caliciviruses. Norovirus is one of the positive-sense ssRNA viruses whose genome functions

directly as the mRNA, beginning the infectious cycle with the synthesis of a precursor that only gives rise to the nonstructural proteins, including the (RNA polymerase) RdRp enzyme that transcribes then one subgenomic mRNA encoding the structural proteins (VP1 and VP2) (Green, 2007). Like the other (+)ssRNA viruses, the replication of Norovirus occurs in the cytoplasm.

In the first step, the viral attachment of the virion to the cell receptor, the P2 subdomain of the VP1 binds to a sugar residue, mostly to the HBGA carbohydrates in the case of human Noroviruses but also to sialic acid or heparan sulfate (Tamura *et al.*, 2004; Stuart & Brown, 2007; Rydell *et al.*, 2009; Taube *et al.*, 2009). This interaction between VP1 and HBGA seems not to be enough for the entry of Norovirus in host cells and the involvement of a membrane protein as a receptor or co-receptor for subsequent penetration/entry is suspected (Tan & Jiang, 2011).

Norovirus enters the cell using a non-clathrin-, non-caveolin-mediated endocytic pathway but dependent on dynamin II and cholesterol (Perry & Wobus, 2010). Moreover, this entry step is pH-independent and no conformational changes in the capsid required for viral uncoating are observed with acidic intracellular pH (Perry *et al.*, 2009).

After the internalization in the cell and uncoating of viral genome, the translation of ORF1 of the viral genomic RNA produces a large protein, the so called nonstructural polyprotein.

The initiation of translation is dependent on the interaction of the VPg with the cellular translation initiation machinery (Daughenbaugh *et al.*, 2003; Daughenbaugh *et al.*, 2006). A co-translational processing releases the nonstructural proteins and their precursors (Sosnovtsev, 2010). The proteolytic processing is mediated by the viral protease (NS6) which is autocatalytically released from the polyprotein precursor (Putics *et al.*, 2010).

The replication of Norovirus is believed to occur in a replication complex (RC) formed by intracellular membranous structures which contain all

the viral nonstructural proteins along with host proteins that will help viral replication as well as the viral RNA intermediate, ssRNA and double stranded RNA (dsRNA) (Hyde & Mackenzie, 2010). The recruitment of host membranes (Endoplasmic reticulum, Golgi apparatus, endosomes) necessary for the formation of the RC, is induced by the viral non structural proteins p48 (NS1-2) and p22 (NS4), through a modulation of the host cell secretory pathway (Denison, 2008; Hyde & Mackenzie, 2010).

Once the RC is assembled, the RdRp (NS7) starts the synthesis of the antigenomic RNA (negative sense) from the genomic (positive sense) RNA template. The initiation of antigenomic RNA synthesis by the RdRp is dependent upon uridylylation of VPg that serves as a primer in the presence of the polyadenylated genomic RNA (Rohayem *et al.*, 2006a; Rohayem *et al.*, 2006b). This antigenomic RNA is then used as a template for synthesis of the new genomic RNA and of the subgenomic RNA.

The newly synthesized genomic RNA is either translated as a polyprotein precursor or used for packaging in the assembled viral protein core. The subgenomic RNA (positive sense) is translated as structural proteins, VP1 and VP2. Finally, the structural proteins are assembled and the genomic RNA packaged, followed by release of the mature virion from the cell. This late stages of replication are, however, poorly understood.

Efforts towards Development of Anti-Norovirus Drugs

The antiviral research of Norovirus is still in its infancy and there are only few reports of antivirals for Norovirus. Recently, a new chemical scaffold, the 2-styrylchromones, has shown anti-norovirus activity in the murine Norovirus (MNV) surrogate model, opening the door to the search of anti- Norovirus drugs among a wider range of novel compounds from different chemical families (Rocha-Pereira *et al.*, 2010). Additionally, nitoxanide, a prodrug used to treat protozoal gastroenteritis has been reported to reduce

the duration of Norovirus gastroenteritis in a clinical trial although the mechanism of action remains

unclear (Rossignol & El-Gohary, 2006).

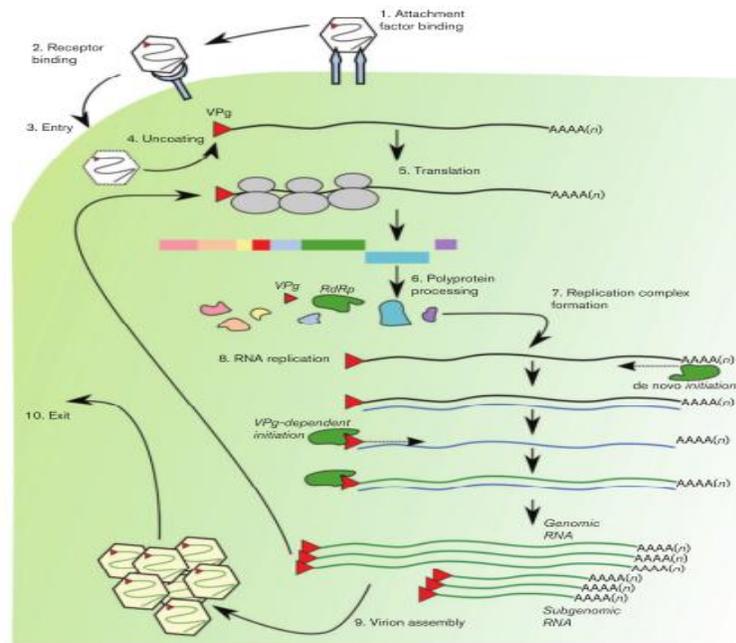


Figure. 3.2. Outline of the Norovirus life cycle.

Source: Thorne and Goodfellow, (2014)

Targeting cellular receptors of Norovirus

Human Noroviruses recognize HBGA carbohydrates present on cell surface, which are key players in the initial viral attachment, acting most likely as cellular receptors or co-receptors of norovirus (Marionneau *et al.*, 2002; Tan & Jiang, 2005, 2011). Norovirus present diverse binding patterns to HBGAs, being currently sorted into three major binding groups, the H, the A/B, and the Lewis binding group (Tan & Jiang, 2011). The interaction between Norovirus and HBGA is highly strain-specific rather than genogroup- or genotype-specific (Tan & Jiang, 2008). Hence, strains of the H, A/B and Lewis binding groups can be found in both two major genogroups of human Norovirus (GI and GII). The existence of only three HBGA-binding interfaces makes possible the design of antivirals against these targets in Noroviruses. Any compound that targets a given HBGA binding interface may be capable of blocking infection of all strains that bind same type of HBGA. Thus, only three different HBGA-binding interfaces would need to be targeted by

compounds to block nearly all Noroviruses in the GI and GII genogroups (Tan *et al.*, 2009).

The strategy of targeting this first step of virus-receptor interaction could be of great interest to use as prophylactic therapy, since it would be effective in preventing infections of individuals in high risk settings or that were in contact with an index case during an outbreak (Tan and Jiang, 2008). Furthermore, it would be expected that such compounds would be able to reduce the severity of symptoms in already infected individuals and likely reduce virus excretion, limiting its propagation to higher numbers of individuals (Tan and Jiang, 2008). Citrate, and other glycomimetics, showed to have the potential to block human Noroviruses from binding to HBGAs (Hansman *et al.*, 2011), providing a starting point for Norovirus inhibitors.

However, there are predictable difficulties to this strategy, one of which being the fact that the early steps of Norovirus life cycle are not yet fully disclosed. Recent findings of feline calicivirus (FCV)

and MNV binding to sialic acid (Rydell *et al.*, 2009; Stuart & Brown, 2007; Taube *et al.*, 2009) or heparan sulfate (Tamura *et al.*, 2004) broadens the spectrum of sugar residues that interact with these viruses. Moreover, the identification of a membrane protein, the junctional adhesion molecule A (JAM-A), as a receptor for FCV (Makino *et al.*, 2006) raises the hypothesis of this protein or other members of the Ig superfamily being also cellular receptors for caliciviruses, like they are for reoviruses and picornaviruses (Tan and Jiang, 2011). According to the proposed model for reoviruses, the virus interact firstly with a sugar residue like sialic acid, as a determinant of tropism that is responsible for initial virus attachment, and later use a membrane protein (JAM-1) to enter the host cell (Barton *et al.*, 2001). The role of these two molecules in viral attachment and entry is likely to occur also in FCV, and one could even speculate that it could be extended to Noroviruses since it is usual that viruses within the same family use similar cellular receptors (Tan and Jiang, 2011). Many remains to be unraveled in this field, therefore there are still doubts about the selection of the best target for chemical compounds to block or interfere with virus attachment.

Targeting entry and uncoating of Norovirus

In order to enter host cells, Noroviruses take advantage of cellular processes entering by an endocytic pathway, most commonly a clathrin-mediated endocytosis. Viral entry can also occur via caveolin mediated endocytosis, clathrin/caveolin-independent endocytosis, macropinocytosis, or phagocytosis (Marsh and Helenius, 2006). After entrance in the host cell, the uncoating of virus must occur in order to deliver the viral genome into the host cytoplasm. This event is often triggered by the acidic environment of endosomes but it can also occur after the binding of virus to cellular receptors (Tsai, 2007).

Studies with feline calicivirus (FCV) showed this virus enters cells by clathrin-mediated

endocytosis in a pH dependent manner (Stuart and Brown, 2007). However, it was demonstrated that the entry of MNV is clathrin/caveolin-independent but mediated by dynamin II and cholesterol (Perry and Wobus, 2010). In addition, studies with MNV show this virus is pH-independent and that a low intracellular pH does not trigger conformational changes in the capsid required for MNV uncoating (Perry *et al.*, 2009). This difference in sensitivity to low pH between FCV and MNV was suggested to be related with the different routes of infection of these viruses (Perry *et al.*, 2009). While MNV is an enteric virus that infects its host by the small intestine and retains infectivity for hours at a pH of 2 (similarly to the human Norovirus), FCV is a respiratory virus that significantly decreases infectivity at low pH (Cannon *et al.*, 2006). Further understanding of the cellular mechanisms of Norovirus entry and uncoating would bring out new antiviral targets.

Targeting structural proteins of Norovirus

The VP1 is the major structural protein of Norovirus and its P2 subdomain, which is located at the outmost surface of the viral capsid, comprises the binding surface for HBGA (Cao *et al.*, 2007; Bu *et al.*, 2008; Choi *et al.*, 2008). The function of VP2 is currently undefined and there is no sufficient information to address this minor protein as an antiviral target.

The search for compounds targeting VP1 of Norovirus was explored through a saliva-based enzyme immunoassay (EIA) that measures their capacity to block the binding of Norovirus VLPs to HBGAs present in such biological fluid (Feng & Jiang, 2007). Different chemical compounds were found to be strong inhibitors of this binding being potential candidates for further development as antivirals for Norovirus (Feng & Jiang, 2007), but one should be aware that a saliva-based EIA was used instead of a cellular system or animal model and this could be regarded as a handicap.

Antiviral drugs that target viral surface proteins of other RNA virus have been described, such as pleconaril, a picornavirus capsid-binding compound, but resulted in a not entirely successfully strategy (Field and Vere Hodge, 2008). The problem with this kind of drugs relies on their low genetic barrier since the viral surface proteins can undergo variations without compromising viral fitness, easily allowing resistant strains to emerge (Field and Vere Hodge, 2008). The same problem would most likely take place with Norovirus given its well-known antigenic variation. A strategy could be the use of combination therapy of this class of compounds together with drugs against well conserved nonstructural proteins with critical functions. This would avoid or at least delay the development of resistance.

Targeting nonstructural proteins

The six/seven nonstructural proteins of Norovirus are the p48/ N-terminal protein (NS1-2), the NTPase (NS3), the p22 (NS4), the VPg (NS5), the viral protease (Pro, NS6), and the viral RNA-dependent RNA polymerase (RdRp, NS7). Since Norovirus share some similarities with picornaviruses, the nomenclature and functions of these ORF1-encoded proteins of Norovirus was initially predicted through comparative sequence analysis of their picornavirus counterparts. Hence, the Norovirus RdRp was called 3D-like, the protease was called 3C-like, the p22 was called 3A-like, the NTPase was named 2C-like and the p48/Nterm was related to the 2B protein (Green, 2007). This resemblance could be important for the search of antivirals against Norovirus since the known strategies and targets for inhibiting the replication of picornavirus might be also effective for Norovirus.

Although a detailed knowledge of the role of nonstructural proteins is still not available, some important clues have raised from studies with MNV which showed that all these proteins play a role in Norovirus replication and are associated with the replication complex (RC) and the viral RNA intermediate dsRNA (Hyde *et al.*, 2009). There is also

evidence that the replication complex of MNV is associated with host membranes, namely of the endoplasmic reticulum (ER), the Golgi apparatus and endosomes which suffer virus induced rearrangements (Hyde *et al.*, 2009; Wobus *et al.*, 2004). These and other features of each nonstructural protein are described below, as well as how these could be used for the discovery of antiviral drugs against Norovirus.

Targeting the norovirus genome

The Norovirus RNA genome or viral transcripts also constitute an important target to inhibit the replication of Norovirus. Antisense oligonucleotides and siRNAs (small interfering RNA) can be designed to target conserved regions of the Norovirus genome with the aim of disrupting viral replication. For that, these oligonucleotides need to fulfill some requisites, namely the target sequence has to be involved in viral replication, accessible for oligonucleotide hybridization and conserved among different viral strains (Spurgers *et al.*, 2008).

The existence of conserved secondary structures among calicivirus genomes opens the possibility of designing an oligonucleotide that would present a broad spectrum activity among Noroviruses or throughout the family Caliciviridae. These conserved structures include 5' terminal stem-loops, 3' terminal hairpins, a stem-loop just upstream of the ORF1/2 junction in the antigenomic strand and a stem-loop at the 5' end of the polymerase coding region with a motif characteristic of picornavirus cis-acting replication elements (cre elements) that dictate VPg uridylation (Simmonds *et al.*, 2008). Some of these structures were found to be critical for the replication of MNV and for its infectivity (Simmonds *et al.*, 2008). By using MNV reverse genetics system it was demonstrated that the disruption of the 5'-stem loops or the 3'-hairpins strongly impaired MNV replication *in vitro* (Simmonds *et al.*, 2008). Moreover, a polypyrimidine tract located at the 3'-end of the genome has been incriminated in regulating viral

fitness and virulence of MNV in vivo (Bailey *et al.*, 2010). The important roles played by these conserved RNA structures in Norovirus replication and virulence makes them potentially good antiviral targets.

Epidemiology of Norovirus gastroenteritis

Norovirus gastroenteritis is generally acute and self-limiting, but in infants, elderly, and immune-compromised individuals it may be more severe and prolonged since they are more susceptible to complications due to dehydration (Green, 2007; Patel *et al.*, 2008). After an incubation period of 24–48 h, there is an acute onset of symptoms of nausea, vomiting, abdominal cramps, myalgias, and intense non-bloody diarrhea which usually resolves in 2–3 days (Green, 2007). The median duration of illness can be longer, lasting up to six weeks in infants and young children (Patel *et al.*, 2009).

Vomiting is relatively more prevalent among children, whereas a greater proportion of adults experience diarrhea. Patients can experience vomiting alone, a condition first identified as winter vomiting disease (Lopman *et al.*, 2014). Constitutional symptoms (e.g., headache, fever, chills, and myalgia) are frequently reported. Although rare, severe dehydration caused by Norovirus gastroenteritis can be fatal, with this outcome occurring among susceptible persons (e.g., older persons with debilitating health conditions). No long-term sequelae of Norovirus infection have been reported (Robilotti *et al.*, 2015).

Fecal-oral spread is the primary Norovirus transmission mode, although airborne and fomite transmission might facilitate spread during outbreaks (Caul, 1994; Otter *et al.*, 2014; Miyoshi *et al.*, 2015). Frequently during an outbreak, primary cases result from exposure to a fecally contaminated vehicle (e.g., food or water), whereas secondary and tertiary cases among contacts of primary cases result from person-to-person transmission (Becker *et al.*, 2000; Green, 2007; Lopman *et al.*, 2014). For 232 outbreaks of Norovirus gastroenteritis reported by CDC during July

1997–June 2000, foodborne sources was found to have the highest percentage and the waterborne source of the virus the least, as well as the setting of the outbreaks.

Previously, researchers believed that a person remained contagious 48–72 hours after recovery from Norovirus gastroenteritis (van Beek *et al.*, 2014). During a 1994 study of 50 volunteers exposed to Norovirus, 82% became infected; of these infections, 68% resulted in illness, whereas the remaining 32% were asymptomatic (Graham *et al.*, 1994; Simmons *et al.*, 2013). Viral shedding in stool began 15 hours after virus administration and peaked 25–72 hours after virus administration. Unexpectedly, viral antigen could be detected by ELISA in stool specimens collected 7 days after inoculation in both symptomatic and asymptomatic persons. In a later study of infected volunteers, viral antigen in stool was detected <2 weeks after administration of virus (Okhuysen *et al.*, 1995; Blatt, & Conner, 2013). Anecdotal evidence from outbreak investigations also demonstrates that viral shedding can occur for a prolonged period and in the absence of clinical illness (Daniels *et al.*, 2000; Lopman *et al.*, 2014). However, the epidemiologic significance of these findings is unclear. Additional research is required to determine whether the viral antigen that is detectable for prolonged periods after recovery from illness is evidence of infectious virus or just a soluble antigen and to assess the time of maximal viral shedding so that control measures can focus on the period during which the person is most likely to be contagious.

Characteristics of Norovirus facilitate their spread during epidemics. The low infectious dose of Norwalk like Virus (i.e., <100 viral particles) (Chiba *et al.*, 2012) readily allows spread by droplets, fomites, person-to-person transmission, and environmental contamination, as evidenced by the increased rate of secondary and tertiary spread among contacts and family members.

Until recently, humans were considered to be the only reservoir for Norovirus. However, the

characterisation of closely related viruses in pigs and cattle indicates interspecies transmission, and the potential for zoonotic transmission of Norovirus is under investigation. Concerns about a potential zoonotic transmission have been raised given the close genetic relatedness between Norovirus found in humans and animals and the presence of antibodies to animal strains in humans Nims & Plavsic, 2013).

Prolonged duration of viral shedding that can occur among asymptomatic persons increases the risk for secondary spread and is of concern in food handler-related transmission. The ability of the virus to survive relatively high levels of chlorine (Nims & Plavsic, 2013) and varying temperatures (i.e., from

freezing to 60°C) (Chiba *et al.*, 2012) facilitates spread through recreational and drinking water and food items, including steamed oysters (Bellou *et al.*, 2013). Because of the diversity of Norovirus strains, lack of complete cross-protection, and lack of long-term immunity, repeated infections can occur throughout life.

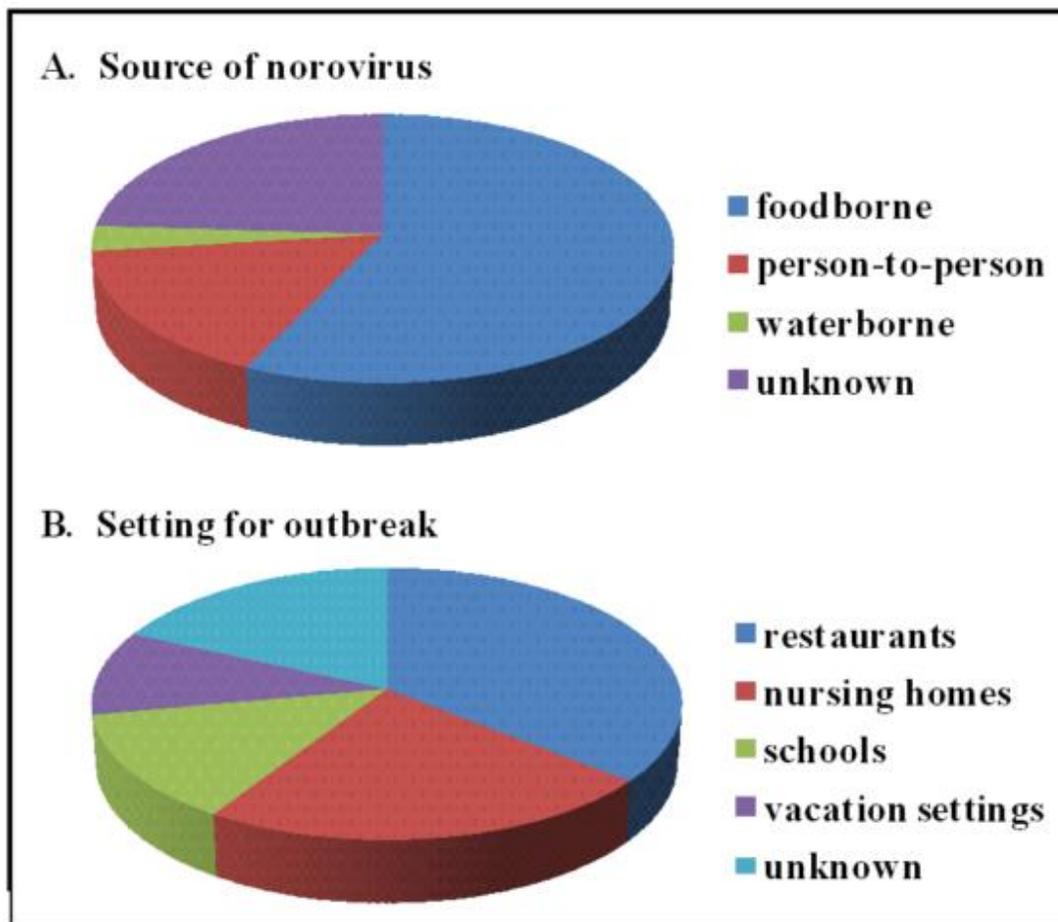


Figure 2.1 Distribution pattern of transmission

Source: Karst (2010)

Table 2: Characteristics of “Norwalk-like viruses” that facilitate their spread during epidemics

Characteristic	Observation	Consequences
Low infectious dose	<10 ² viral particles	Permits droplet or person-to-person spread, secondary spread, or spread by food handlers
Prolonged asymptomatic shedding	<2 weeks	Increased risk for secondary spread or problems with control regarding food handlers
Environmental stability	Survives <10 ppm chlorine freezing, and heating to 60 C	Difficult to eliminate from contaminated water; virus maintained in ice and steamed oysters
Substantial strain diversity	Multiple genetic antigenic types	Requires composite diagnostics; repeat infections by multiple antigenic types; easy to underestimate prevalence
Lack of lasting immunity	Disease can occur with reinfection	Childhood infection does not protect from disease in adulthood; difficult to develop vaccine with lifelong protection

Source: Karst (2010)

Although virtually any food may be implicated in Norovirus transmission, bivalve molluscs present a particularly high risk because of their ability to concentrate viruses from contaminated waters in their tissues. Due to the low infectious dose and the high concentration of viruses in stools, fresh products including frozen raw products and ready-to-eat foods (e.g., salads and deli sandwiches) also appear to present a particular risk following contamination by food-handlers infected with Norovirus. Another food group, which has emerged as a risk in regard to Norovirus infection, is fresh produce. Contamination of such products can occur at many stages including pre-, during and post-harvest.

Human health problems associated with bivalve shellfish are well-recognised internationally. The association of shellfish-transmitted infectious disease with sewage pollution became well-documented in the late 19th and early 20th century with numerous outbreaks of typhoid fever in several European countries, the US and elsewhere. In some years, the epidemiological evidence suggests that human enteric viruses, principally norovirus and hepatitis A virus, are now the most common aetiological agents transmitted by bivalve shellfish.

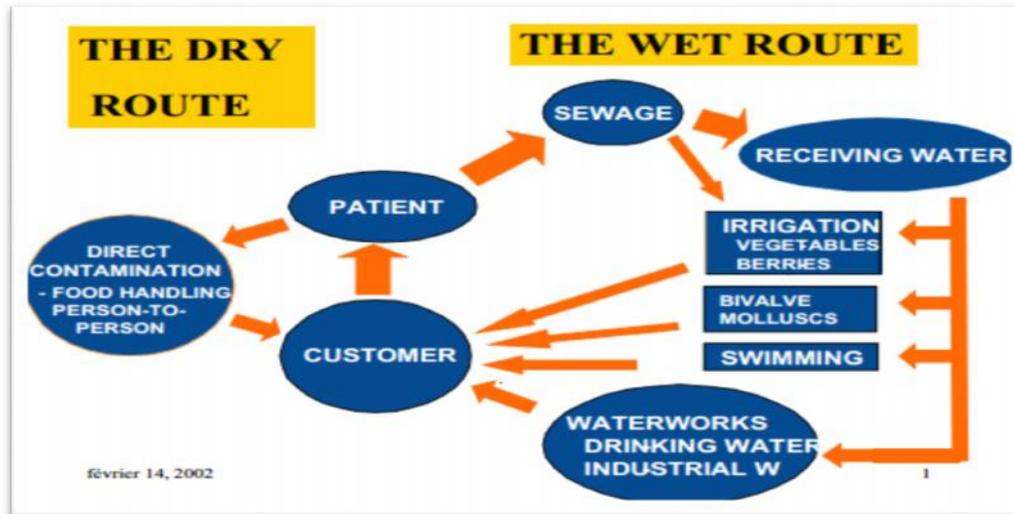


Figure 2: The epidemiology of Norovirus

Source: Fevrier (2002)

Diagnosis and Prevention of Norovirus infection

Detection rates improved with the development of immunologic assays (6–11), and 19%–42% of nonbacterial outbreaks were attributed to Norovirus in targeted studies conducted during the late 1970s and 1980s (Sukhrrie, 2014). However, because reagents for these assays came from human volunteers, the reagents were available only in limited quantities and only at certain facilities. Consequently, outbreaks were not fully investigated, and a substantial number were still labeled as being of unknown etiology.

Furthermore, because of the antigenic and genetic diversity of Norovirus and the inability to cultivate these viruses in cell lines, developing assays to detect the full spectrum of Norovirus associated with outbreaks of gastroenteritis was not possible. To circumvent these obstacles to laboratory diagnosis, clinical and epidemiologic criteria were developed that correlate with the presence of Norovirus in outbreaks of acute gastroenteritis (Sala *et al.*, 2014; Wikswa *et al.*, 2014). Advances in methods for detecting Noroviruses have changed our understanding of the epidemiology of these viruses. The following sections are the commonly available diagnostic methods (Atmar and Estes, 2001).

Electron Microscopy and Immune Electron Microscopy

Under the electron microscope, Noroviruses can be identified by their characteristic morphology (Knight *et al.*, 2013). Approximately 10^6 – 10^7 /ml of virus in stool is required for visualization by EM; therefore, this technique is useful only for specimens collected during the early stages of illness when substantial quantities of virus are shed. Even among experimentally infected volunteers, the virus can be found in only 10%–20% of fecal specimens collected on days 2 or 3 of illness. Immune electron microscopy (IEM) can improve the sensitivity of EM by 10- to 100-fold (Zhang *et al.*, 2013). In one type of IEM, convalescent-phase serum from patients is coated on the examination grid of the microscope before stool specimens are applied. The antibody on the grid traps homologous virus, thereby increasing diagnostic yield. However, IEM has certain disadvantages, the greatest of which is that success is highly dependant on the skill and expertise of the microscopist. Furthermore, the virus might be totally masked if a large excess of antibody is present, resulting in a false-negative test (Zhang *et al.*, 2013).

Enzyme Immunoassays

The expression in baculoviruses of the capsid proteins of Norovirus that self-assemble into stable virus-like particles has allowed the detection of these viruses by ELISAs. To develop assays to detect virus in fecal specimens, the expressed capsid antigens have been used to generate hyperimmune antibodies in laboratory animals. These assays have been reported to detect the presence of 10^4 – 10^6 viral particles/ml in clinical specimens. To date, these assays have been type-specific, but broadly reactive tests are under development (Richards *et al.*, 2003; Gray *et al.*, 2007).

The baculovirus-expressed viral antigen can be directly used for detection of antibodies to Norovirus in patient's sera by enzyme immunoassay. Because certain adults have preexisting immunoglobulin G (IgG) antibodies to Norovirus, a single serum specimen is insufficient to indicate recent infection. Sero-conversion, defined as a >4-fold rise in IgG antibody titer during acute- and convalescent-phase sera, is indicative of a recent infection. In outbreak settings, if at least half of affected persons seroconvert to a specific Norovirus, that viral strain can be designated as etiologic. Titers can begin to rise by the fifth day after onset of symptoms, peak at approximately the third week, and begin to fall by the sixth week. Hence, for IgG assays, the acute-phase serum should be drawn within the first 5 days and the convalescent-phase serum during the third to sixth weeks. In certain cases where diagnosis is critical (e.g., when a food handler is implicated as the source of an outbreak), single assays of serum immunoglobulin A (IgA) antibody can be successful if specimens are collected 7–14 days after exposure. In addition to potential difficulties in obtaining an adequate number of serum specimens during outbreaks, serologic assays are currently limited by the fact that the available array of expressed Norovirus antigens is insufficient to detect all antigenic types of Norwalk like viruses (Shigemoto *et al.*, 2014).

Nucleic Acid Hybridization Assays and RT-PCR

Nucleic acid hybridization assays and RT-PCR assays to detect Norovirus genome in clinical and environmental specimens have provided a sensitive and specific tool for Norovirus outbreak investigations. High sensitivity of these assays (i.e., ability to detect 10^2 – 10^4 viral particles/ml in stool) is both an asset and a liability because extreme care is required to avoid contamination in the laboratory. In addition, although the available primers for RT-PCR assays detect multiple strains of Norovirus, certain strains can escape detection (Ando *et al.*, 1995). Efforts are ongoing to develop universal or degenerate primers that would detect the majority of Norovirus strains that cause gastroenteritis outbreaks.

Prevention and control of Norovirus outbreaks

Although person-to-person spread might extend Norovirus gastroenteritis outbreaks, the initiating event is often the contamination of a common vehicle (e.g., food or water) (Shieh *et al.*, 2014). Consequently, efforts to prevent both the initial contamination of the implicated vehicle and subsequent person-to-person Norovirus transmission will prevent the occurrence and spread of Norovirus gastroenteritis outbreaks (Vinjé *et al.*, 2011).

Prevention of Foodborne Transmission

Theoretically, any food item can potentially be infected with Noroviruses through fecal contamination. However, certain foods are implicated more often than others in outbreaks of Norovirus gastroenteritis. Shellfish (e.g., oysters or clams) tend to concentrate in their tissues Norovirus that contaminate the waters from which they are harvested (Shieh *et al.*, 2000; Burkhardt and Calci, 2000), and even harvests meeting bacteriologic standards of hygiene can contain Norovirus. In addition, cooking (e.g., steaming) might not completely inactivate Norovirus (Bellou *et al.*, 2013). Until reliable indicators for routine monitoring of viral contamination of harvest waters and shellfish are

available, measures to prevent the contamination of harvest waters with human waste (e.g., surveillance of the shoreline for potential sources of fecal contamination and restricting boaters from dumping waste overboard) are probably a useful means of preventing shellfish-associated Norovirus gastroenteritis outbreaks.

Food contamination by infectious food handlers is another frequent cause of Norovirus gastroenteritis outbreaks. Because of the low infectious dose of Norovirus and the high concentration of virus in stool, even a limited contamination can result in substantial outbreaks. Ready-to-eat foods that require handling but no subsequent cooking (e.g., salads and deli sandwiches) pose greater risk. Previously, the exclusion of ill food handlers for 48–72 hours after resolution of illness was recommended to prevent outbreaks caused by food handlers (LeBaron *et al.*, 1990). Data from recent human volunteer and epidemiologic studies demonstrate that viral antigen can be shed for a longer duration after recovery from illness and in the absence of clinical disease. Although data are limited regarding whether this detectable viral antigen represents infectious virus, food handlers should be required to maintain strict personal hygiene at all times.

Prevention of Waterborne Transmission

Although waterborne outbreaks are far less common than foodborne outbreaks, Norovirus gastroenteritis outbreaks have been associated with sources of contaminated water, including municipal water, well water, stream water, commercial ice, lake water, and swimming pool water. Because current analytic methods do not permit direct monitoring of Norovirus in water, indicator organisms (e.g., coliform bacteria) have been used as proxy indicators of fecal contamination. However, because the size, physiology, and susceptibility to physical treatment and disinfection of bacterial indicators differ from those of Norovirus, inherent limitations of this approach exist.

Until reliable methods for assessing the occurrence and susceptibility to treatment of Norovirus are available, prevention methods should focus on reducing human waste contamination of water supplies. If drinking or recreational water is suspected as being an outbreak source, high-level chlorination (i.e., 10 ppm or 10 mg/L for >30 minutes) might be required for adequate disinfection; however, even this method might be insufficient in certain cases (Keswick *et al.*, 1985; Tian *et al.*, 2013).

Prevention of Person-to-Person Transmission

Person-to-person spread of Norovirus occurs by direct fecal-oral and airborne transmission. Such transmission plays a role in propagating Norovirus disease outbreaks, notably in institutional settings (e.g., nursing homes and day care centers) and on cruise ships. Although interruption of person-to-person transmission can be difficult, certain measures might help. Frequent hand washing with soap and water is an effective means of prevention (Biran *et al.*, 2012).

The recommended procedure is to rub all surfaces of lathered hands together vigorously for >10 seconds and then thoroughly rinse the hands under a stream of water. Because spattering or aerosols of infectious material might be involved in disease transmission, wearing masks should be considered for persons who clean areas substantially contaminated by feces or vomitus (e.g., hospital or nursing home personnel). Soiled linens and clothes should be handled as little as possible and with minimum agitation. They should be laundered with detergent at the maximum available cycle length and then machine dried. Because environmental surfaces have been implicated in the transmission of enteric viruses, surfaces that have been soiled should be cleaned with an appropriate germicidal product (e.g., 10% solution of household bleach) according to the manufacturer's instructions. In situations in which the epidemic is extended by periodic renewal of the susceptible population (e.g., camps and cruise ships), the facility

or institution might have to be closed until it can be cleaned appropriately.

CONCLUSION

Norovirus is a type of highly infectious, poorly immunogenic, stable virus that, although underreported, is one of the most commonly recognized causes of gastroenteritis in humans affecting people of all age groups. Progress in the characterization and control of NoV has been hampered by the inability to cultivate human NoVs in cell culture and the lack of a rapid as well as sensitive assay for use in clinical settings.

Norovirus infections occur both as sporadic cases and as outbreaks, including common-source international outbreaks. Norovirus typically spread by a faecal-oral route. While person-to-person transmission is the most common mode of spreading Norovirus infection, food- and waterborne outbreaks frequently occur. The potential for zoonotic transmission of Norovirus is currently under investigation. Progress has been achieved in regard to methodology for detecting Norovirus in bivalve molluscs, water and environmental samples, although improvement and standardization of methods are needed.

RECOMMENDATIONS

1. Norovirus should be routinely tested for in diagnostic laboratories.
2. The need for proper documentation in cases of infection of the virus or outbreak is necessary.
3. On the basis of lack of lasting immunity in individuals, efficacious norovirus vaccines should be developed.
4. Sensitization should be done to the general public, particularly food handlers in order to create awareness.

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