Chapter 2

Review of literature
2.1. Discovery

In 1929, Zahorsky first described "winter vomiting disease," an illness characterized by the sudden onset of self-limited vomiting and diarrhea that typically peaked during the colder months [Zahorsky, 1929:391-395]. In October 1968, Center for Disease Control (CDC) investigated specimens collected from an outbreak of gastroenteritis, which occurred in an elementary school in Norwalk, Ohio [Adler and Zickl, 1969:668-673]. Of 232 students and teachers in the school, 50% were affected and 32% of family contacts of primary cases also developed illness. The predominant symptoms were vomiting and nausea although diarrheal episodes also occurred. Laboratory investigations failed to reveal any etiological agent [Adler and Zickl, 1969:668-673]. The supernatant of a rectal swab suspension recovered from an ill individual was administered to adult volunteers as a bacteria-free filtrate and serially passaged to other volunteers [Dolin et al., 1971:307-3012; Dolin et al., 1972:578-583]. In 1972, Kapikian et al. discovered spherical virus like particles in fecal specimens of these volunteers by Immune Electron Microscopy (IEM) [Kapikian et al., 1972:1075-1081]. These particles measured 27 nm in their shortest and 32 nm in their longest diameter. The virus was named Norwalk Virus (NV) after the location of outbreak. The virus was the first enteric virus to be visualized and identified under electron microscope (Fig. 2.1).

Following the discovery of NV, many related viruses like Hawaii Virus, Toronto Virus, Snow Mountain virus and Montgomery County virus were also reported from different geographic locations and named accordingly [Caul 1988:139-163]. In 1982, Caul and Appleton classified these viruses as 'small round structured viruses'. (SRSV) [Caul and Appleton, 1982:257-265]. These were also known as Norwalk-like viruses. NV became the prototype strain of the group. International Committee of Taxonomy of Viruses in 2002 approved the genus name Norovirus (NoV) under the family Caliciviridae, which now possesses four genera NoV, Sapovirus, Lagovirus, and Vesivirus that represent four distinct genetic clades in the family [Green et al., 2000:S322-330]. Members of this family possess a nonenveloped icosahedral capsid of 27 to 40 nm in diameter surrounding positive-sense polyadenylated RNA genome [Green et al., 2000:S322-330].
2.2. Physicochemical Properties

The physicochemical properties of NV are well studied. NV has a buoyant density of 1.33 to 1.41 g/cm$^3$ in cesium chloride (CsCl) [Kapikian et al., 1973:874-877]. It retains infectivity in pH 2.7 for 3 hrs at room temperature and after treatment with 20% ether at 4°C for 18 hrs and incubation at 60°C for 30 min [Dolin et al., 1972:578-583]. It is resistant to inactivation following treatment with 3.75 to 6.25 mg/L of chlorine (free residual chlorine of 0.5 to 1.0 mg/L) [Keswick et al., 1985:261-264]. However, is inactivated following treatment with 10 mg/L of chlorine (used for decontamination of water supply systems).

2.3. Genome organization

The molecular characterization of NoVs has been greatly hindered due to the failure to grow the virus in cell culture, the lack of an animal model and the small amounts of virus shed in feces [Green et al., 1994:1883-1888]. In 1990, Jiang et al. used NV purified from stool specimens of volunteers to generate a library of cDNA clones of the viral genome [Jiang et al. 1990:1580-1583]. Subsequently, the genomes of NV and related viruses were successfully cloned using primers designed from the original NV sequence [Green et al., 1994:1883-1888; Lew et al., 1994a:319-325; Lew et al., 1994b:535-542]. Comparison of these sequences showed that NoVs could be divided into 2 discrete genogroups [Green et al.,
NV and Southampton virus (SV) with genome size 7,645 and 7,708 nt (excluding polyA tail), respectively were considered as genogroup (G) I viruses [Jiang et al., 1993:51-61; Lambden et al., 1993:516-519]. On the other hand, Bristol, Lordsdale and Camberwell viruses with genome size 7,598, 7,555 and 7,555 respectively i.e. ~200bp smaller than Gl viruses were placed in GII [Dingle et al., 1995:2349-2355; Clarke and Lambden, 2000:S309-316].

The genome of the NoVs contains three overlapping open reading frames (ORFs). The nonstructural proteins are encoded by the genome region near to the 5' end in ORF1 and the structural proteins -VP1 and VP2 are encoded by the genome region at the 3' end in ORFs 2 and 3, respectively. The ORF-1 and ORF-2 sequences overlap by 17 and 20 nucleotide base pairs in genogroup I and II respectively whereas the stop codon of ORF-2 overlaps with the start codon of ORF-3 by 1 nucleotide in both the genogroups [Altmar and Estes, 1996:1041-1059].

1. ORF-1 is the longest ORF with 1738 amino acids. The first initiation codon (AUG) is found at nucleotide position 5 and this is followed by two additional in-frame initiation codons. The ORF encodes for a large polyprotein precursor of approximately 2.0x10^5 Da in size, which undergoes proteolytic cleavage by the virus-encoded proteinase (as observed in Southampton Virus) releasing intermediate precursors and final nonstructural protein products NS1-2°^{N-term}, NS3^{NTPase}, NS4°^{p20}, NS5^{VPg}, NS6°^{Pro} and NS7°^{Pol} [Liu et al., 1996:2605-2610; Liu et al., 1999:291-296; Bok et al., 2009:11890-11901] (Fig 2.2).

![Figure 2.2: Genome organization of Norwalk Virus](image-url)
2. **ORF-2** encodes a structural protein VP1 of 530 amino acids that forms a viral capsid protein of approximately 60 kDa in size. The VP1 protein forms two domains: P (further divided in protruding P1 and P2) and S (shell). Most of the cellular interactions and immune reactions are thought to be located in the P2 sub-domain, which carries highest sequence divergence in the genome (Fig 2.2).

3. **ORF-3** of NoV encodes a minor structural protein VP2. This protein is highly basic in nature, constitutes 212 amino acids and has an apparent molecular weight of about 35 kDa [Wirblich et al., 1996:7974-7983; Glass et al., 2000:6581-6591; Sosnovtsev and Green, 2000;193-203; Glass et al., 2003;3569-3577] (Fig 2.2). The protein contains determinants that regulate the expression and stability of the VP1 protein [Bertolotti-Ciarlet et al., 2003:11603-11615]. It also plays a role in encapsidation of viral RNA and nucleic acid binding [Glass et al., 2000:6581-6591].

### 2.4. **Virion Structure**

X-ray crystallography studies determined two major domains in the VP1 gene: the Shell (S) and the protruding arm (P) (Fig. 2.3).

1. **The S domain:** The S domain is responsible for maintaining the icosahedral contacts of the T=3 structure and surrounds the RNA genome [Prasad et al., 1999:287-290] (Fig 2.3). The entire S domain (1-225 aa) corresponds to the relatively conserved N-terminal region of the capsid protein [Prasad et al., 1999:287-290]. A classic eight-stranded antiparallel beta-sandwich fold is formed by amino acids at positions 50-225 (Fig. 2.3). The amino acid residues at positions 10-49 form the NH₂-terminal (N) arm that faces the interior of the capsid [Prasad et al., 1999:287-290]. The highly conserved S domain may function as an icosahedral scaffold with the N-terminal arm providing a switch to facilitate the appropriate curvature [Prasad et al., 1999:287-290; Chen et al., 2004:6469-6479; Chen et al., 2006:8048-8053].
Figure 2.3: A: A central section of the NV rVLP perpendicular to the icosahedral threefold axis. The arch is composed of the protruding (P) domain (divided into subdomains P1 and P2) and the shell (S) domain. B: Three-dimensional ribbon representation of an individual subunit from the rNV capsomere derived from x-ray crystallography studies of Norwalk rVLP at 3.4 Å. The S domain (amino acids 1–225) and the P1 (amino acids 225–278 and 406–519) and P2 (amino acids 279–405) subdomains are shown in blue, red, and yellow, respectively [Prasad et al., 1999:287-290]

2. The P domain: The P domain forms the arch-like protrusions that emanate from the shell and contains the dimeric contacts [Prasad et al., 1999:287-290]. The P domain is divided into the P1 subdomain (226 to 278 and 406 to 520 aa) and the P2 subdomain (279 to 405 aa) (Fig. 2.3). P1 forms the sides of the arch of the capsomeres while P2 is positioned at the top of the arch (Fig. 2.3). Thus, P2 subdomain of the VP1 gene possesses highest sequence variability and is considered as an important site in conferring antigenic and receptor binding specificity to the NoVs [Tan et al., 2003:12562-12571; Nilsson et al., 2003:13117-13124; Lochridge et al., 2005:2799-2806] (Fig. 2.3). It has been proposed that the P domain may be a replaceable module for conferring strain differences and antigenic specificity [Prasad et al., 1999:287-290; Chen et al., 2004:6469-6479; Chen et al., 2006:8048-8053].

Electron cryomicroscopy and computer image processing studies showed differences in the capsid structures of GI and GII NoVs [Prasad et al., 1999:287-290; Chen et al., 2004:6469-6479]. At a resolution of 22Å viewed along the icosahedral threefold axis, NV (genogroup (G) I) presented smoother edges and broader arches.
as compared with the Grimsby virus (GrV) (GI) although both represented a similar architecture (Fig 2.4).

![Figure 2.4: Surface representations of the structures of A) rNV and B) rGrV at a resolution of 22Å viewed along the icosahedral threefold axis [Chen et al., 2004:6469-6479].](image)

## 2.5. Viral replication

The replication strategy of NoVs shares many features with those of other positive stranded RNA viruses. The entry of the virus into the cell usually occurs when the virion interacts with the host cell via virus-specific receptors. Receptor (mostly carbohydrates) recognition is essential during early interactions [Kreutz et al., 1994:19-34; Maeda et al., 2002:291-300; Marionneau et al., 2002:1967-1977; Tamura et al., 2004:3817-3826]. A low pH also facilitates viral entry into cell [Kreutz and Seal, 1995:63-70]. The genome-linked VPg protein of the incoming positive strand interacts with cellular translation machinery (translation initiation factors eIF3 and eIF4E) to initiate the translation process [Gutierrez-Escolano et al., 2000:8558-8562; Daughenbaugh et al., 2003:2852-2859; Goodfellow et al., 2005:968-972] (Fig 2.5). The ORF1 is translated to produce the nonstructural polyprotein. The viral proteinase (NS6PRO) at several essential cleavage sites processes the polyprotein into precursors and products [Sosnovtsev et al., 2002:7060-7072].

The synthesis of negative strand RNA from the genomic RNA template begins at the 3' end of the genomic positive strand RNA [Gutierrez-Escolano et al., 2003:759-766]. The process probably involves interactions with cellular proteins
This negative strand RNA serves as a template for a full-length genomic and a subgenomic RNA [Neill and Mengeling, 1998:59-72]. The subgenomic RNA acts as a template for the synthesis of the structural proteins, VP1 and VP2 [Herbert et al., 1996:123-127; Neill and Mengeling, 1998:59-72]. The sequences near the end of the VP1 coding region contain a signal for translation of VP2 (termination or reinitiation mechanism) from the subgenomic RNA [Meyers, 2003: 34051-34060]. The VP2 is required for the production of infectious particles [Sosnovtsev et al., 2005:4012-4024]. The presence of VPg-linked genomic and subgenomic RNA in particles of distinct densities suggests that they are not packaged together in the same virion [Meyers et al.,1991:677-686; Neill, 2002:89-93] (Fig 2.5). The release of viral particles is facilitated by the apoptosis-associated change in cellular membranes [Sosnovtsev et al., 2003:1-10] (Fig 2.5).

![Figure 2.5: The replication cycle of NoVs involves the following stages: (1) Entry, (2) Uncoating, (3) Translation, (4) RNA replication, (5) Packing and maturation and (6) Release](image-url)
2.6. NoV Classification

Earlier, classification of NoVs was based on cross-challenge studies in volunteers and cross reactivity analysis by immune electron microscopy (IEM) [Wyatt et al., 1974:709-714; Okada et al., 1990:1244-1248; Green et al., 1995b:271-283; Lewis et al., 1985:501-504]. In one such classification scheme proposed by Okada et al. in 1990, NoVs were tentatively classified into nine antigenically distinct groups, SRSV-1 (S-1) to SRSV-9 (S-9) by cross-immune electron microscopy (IEM) [Okada et al., 1990:1244-1248]. Serotyping based on the neutralization assays was not possible due to non-availability of in vitro (cell culture) system and in vivo (animal) models. The sensitive molecular techniques such as reverse transcription polymerase chain reaction (RT-PCR) and genomic sequencing became the major tools for characterization of NoVs [Jiang et al., 1990:1580-1583; Jiang et al. 1993:51-61; Ando et al. 2000:S336-348; Katayama et al., 2002:225-239]. Classification schemes based on partial RdRp and capsid sequences were proposed that failed to classify some strains into their proper clusters [Fankhauser et al., 2002:1-7; Kageyama et al., 2004:2988-2995].

![NoV classification diagram](image)

Figure 2.6: NoV classification and proposed strain nomenclature as described by Zheng et al. in 2006.
To avoid the confusion, Zheng et al in 2006 proposed a classification scheme based on deduced amino acid sequences of complete major capsid protein (VP1) of 164 strains available in the GenBank at the time of study for standardization of NoV nomenclature [Zheng et al., 2006:312-323]. Accordingly, the genus NoV is divided into 5 major genogroups – GI–GV (Fig. 2.6). The strains of GI, GII and GIV infect primarily humans (except for GII, genotype I1 that infects porcine species) while GIII and GV strains infect bovine and murine species, respectively. GI, GII and GIII have been subdivided in 8, 17 and 2 genetic clusters (C)/genotypes, respectively whereas GIV and GV carry one cluster each (Fig. 2.6). The genotypes or Cs include closely related strains (S). The uncorrected pairwise amino acid distance ranges for S, C and G are 0-14%, 14.3-43.8% and 44.9-61.4%, respectively [Zheng et al., 2006:312-323].

In the recent years, recombinant NoV strains occurring naturally have been reported from around the world with unique polymerase and capsid combinations and breakpoint at the ORF1/2 overlaps [Bull et al., 2005:1079-1085]. Thus, genotyping based solely on capsid sequences is not sufficient to characterize the recombinant NoVs with different capsid and polymerase types. Also, some new or unclassified polymerases with >15% nucleotide differences from their parent types are identified in the recent years, which are usually found in combination with known capsids [Bull et al., 2007:3347-3359]. These new polymerases are classified as GII.a, GII.b, GII.c and GII.d types [Gallimore et al., 2004:2271-2274; Bull et al., 2007:3347-3359].

Recently, a new classification scheme has been proposed [Phan et al. 2007:1388-1400] to accommodate newly identified strains that do not cluster in the known genetic clusters. However, the classification scheme proposed by Zheng et al in 2006 is accepted worldwide for the characterization of NoV strains.

2.7. Modes of transmission

NoVs are highly contagious and even a very low dose (<10 viral particles) can cause serious outbreaks of AGE [Teunis et al., 2008:1468-1476]. The virus spreads rapidly through several modes of transmission (Fig. 2.7). The predominant mode is food- or water-borne transmission [Fankhauser et al., 2002:1-7]. Food borne
outbreaks were associated with the ingestion of Oysters or salad and contaminated cake frosting [Kuritsky et al., 1984:519-521] (Fig. 2.7). Consumption of contaminated sandwiches has been documented to cause an outbreak in India [Girish et al., 2002:603-607].

Figure 2.7: Various routes of NoV transmission (pathport.vbi.vt.edu/.../pathogens/Sapovirus.html)

In water borne outbreaks, contaminated municipal water supply, semi-public water supply, stored water on cruise ship, ice-containing beverages and recreational swimming are found to be the major causes [Morens et al., 1979:964-966; Dolin et al., 1982:184-189; Kapikian et al., 1996:783-870]. Another route of transmission can be person-to-person spread via aerosolized vomit following projectile vomiting [Chadwick and McCann, 1994:251-259]. Primary and secondary person-to-person transmissions are also observed in some outbreaks [Kaplan et al., 1982:756-761]. There are evidences that asymptomatic food handlers cause infection [Reid et al., 1988:321-323]. Direct transmission via contaminated surfaces, especially carpets and toilet seats, is also now considered a significant route [ESR data sheet, New Zealand].
2.8. Pathogenesis and Immunity

2.8.1. Incubation period

Virions are acid stable and able to survive passage through the stomach. Studies conducted in volunteers suggest that incubation period for NV ranged from 10 to 51 hrs while that of Snow Mountain virus (SMV) ranged from 19 to 41 hrs [Dolin et al., 1971:307-3012; Blacklow et al., 1972:993-1008; Dolin et al., 1972:578-583; Steinhoff et al., 1980:1495-1499; Kaplan et al., 1982:756-761]. Acute illness usually lasts about 24 to 48 hrs.

2.8.2. Site of replication in the host

Unavailability of a suitable animal model for human NoVs has hampered pathogenetic studies. Human volunteer studies provided limited information on pathogenesis of NoVs [Schreiber et al., 1974:705-708]. However, gnotobiotic (Gn) pigs have been used in recent studies to understand pathogenesis [Cheetham et al., 2006:10372-10381] (Fig. 2.8).

Figure 2.8: Apoptotic cells in small intestinal tissues of human NoV-inoculated Gn pig and mock-inoculated Gn pig. (A) More cells with dark-staining apoptotic nuclei (arrows) were observed in human NoV -inoculated duodenum. (B) Fewer apoptotic cells and more goblet cells (not evident in the infected pig) in a mock-inoculated control Gn pig. Bars, 50 μm. [Cheetham et al., 2006:10372-10381]

Acute infection with NoVs results in reversible histopathological lesions in the jejunum but not from the stomach or rectum [Agus et al., 1973:18-25; Schreiber et al., 1973:1318-1323; Schreiber et al., 1974:705-708; Dolin et al., 1975:761-768; Meeroff et al., 1980:370-373]. Pathological changes appear within 24 hours of viral
challenge, remain through the peak period of the illness, and persist for a variable time after illness. Intestinal villi appear blunt, but the mucosa remains intact [Meeroff et al., 1980:370-373] (Fig. 2.8).

Diarrhea is associated with transient malabsorption of D-xylose and fat, and brush bordered enzymes (e.g., alkaline phosphatase, trehalase) show reduced activity and levels return to normal within 2 weeks [Blacklow et al., 1972:993-1008; Schreiber et al., 1973:1318-1323; Agus et al., 1973:18-25; Levy et al., 1976:321-325]. A marked delay in gastric emptying was observed [Meeroff et al., 1980:370-373]. This abnormal gastric motor function is supposed to be responsible for the nausea and vomiting associated with NoV infections [Meeroff et al., 1980:370-373].

2.8.3. Immunity

In the absence of in vitro system for NoVs, most of the information about immunity is based on volunteer-studies. Short term and long term immunity to NV have been established [Dolin et al., 1972:578-583; Wyatt et al., 1974:709-714; Parrino et al., 1977:86-89]. Short-term immunity followed serotype specific pattern. Volunteers challenged with NV showed resistance to rechallenge with the same virus for about 6 to 14 weeks. However, they became ill when challenged with the strain (Hawaii Virus) other than NV. A similar pattern was observed when the volunteers challenged with Hawaii virus were exposed to NV [Wyatt et al., 1974:709-714]. However, long-term immunity studies showed that those volunteers, who developed illness in the initial exposure to NV, developed it again when re-exposed to the virus after 27 to 42 months later [Parrino et al., 1977:86-89]. Those who were asymptomatic in the initial exposure showed resistance to re-exposure also [Parrino et al., 1977:86-89]. Further, studies on oral immunization with NV-like particles (VLPs) and infection with SMV in volunteers depicted an increase in T-interferon (IFN) and IL-2, suggesting a dominant Th-1 pattern of cytokine production [Tacket et al., 2003:241-247; Lindesth Smith et al., 2005:2900-2909]. Acquired immunity plays a role in protection at mucosal level. The primary exposure to NV was observed to develop rapid mucosal IgA response and resistance to infection subsequent to the challenge with the same or related viruses. [Lindesmith et al., 2003:548-553].
2.8.4. Host resistance to NoV infections

The evidence for the mechanism involving ABH and Lewis carbohydrate antigens present on gut epithelial cells for long-term resistance to NV has been provided in some recent studies [Hutson et al., 2004:279-287; Tan et al., 2005: 285-293]. In 2002, Marionneau et al. discovered that Norwalk VLPs can bind to histo-blood group antigens expressed on human intestinal epithelial cells and suggested the role of histo-blood group antigens in mediating susceptibility to infection [Marionneau et al., 2002:1967-1977]. This finding was supported by other studies that showed the relationship between a person's ABO histo-blood group type and the risk of NV infection and symptomatic disease. It was observed that individuals with an O phenotype were more likely to be infected with NV, whereas people with blood group B antigen were at decreased risk of infection and symptomatic disease [Hutson et al., 2002:1335-1337; Lindesmith et al., 2003:548-553]. However, a study on SMV showed no correlation between ABH secretor status and susceptibility [Lindesmith et al., 2005:2900-2909].

2.9. Cell culture and animal model for human NoVs

2.9.1. Cell culture

Attempts have been made to grow NoV in vitro in various cell lines (A549, AGS, Caco-2, CCD-18, CRFK, CR-PSEC, Detroit 551, Detroit 562, FRhK-4, HCT-8, HeLa, HEC, Hep-2, Ht-29, HuTu-80, I-407, IEC-6, IEC-18, Kato-3, L20B, MA104, MDBK, MDCK, RD, TMK, Vero and 293) on solid or permeable surfaces. Despite numerous efforts, routine cell cultures have failed to yield replicating NoV [Duizer et al, 2004:79-87]. Asanaka et al. in 2005 reported production of NV particles after transfection of cultured kidney cells. However, in 2007 Straub et al. proposed 3-dimensional (3-D) organoid model of human small intestinal epithelium for growth of NoVs [Straub et al. 2007:396-403]. Thus, it is hypothesized that by mimicking the exact stages of cell differentiation of the intestinal epithelial cells along with the matching of the microenvironment in a cell culture system the growth of NoVs can be achieved in vitro [Duizer et al 2004:79-87].
2.9.2. Animal Model

NV failed to induce illness in mice, guinea pigs, rabbits, kittens, calves, baboons, rhesus monkeys, marmosets, owl monkeys, patas monkeys, and cebus monkeys [Dolin et al., 1971:307-3012; Blacklow et al., 1972:993-1008; Dolin et al., 1972:578-583; Wyatt et al., 1977:1857-1870; Wyatt et al., 1978:89-96]. The chimpanzees developed a serologic response after inoculation with NV however, without any symptoms of the illness [Wyatt et al., 1978:89-96; Hardy et al.1996:252-261]. Recent studies on newborn pigtail macaques and gnotobiotic piglets have reported the diarrheal illness caused by human NoVs [Subekti et al., 2002:400-406]. Also, there was evidence for replication of the virus in intestinal enterocytes of gnotobiotic piglets [Cheetham et al., 2006:10372-10381].

2.10. Clinical manifestations

Diarrhea (passage of ≥ 3 loose or watery stools a day) is usually the most common clinical manifestation associated with NoV gastroenteritis. Vomiting occurs more frequently than diarrhea in children, whereas in adults the reverse was observed [Kaplan et al., 1982:756-761]. However, nausea, abdominal cramps, headache, fever (above 99.4°F), chills, myalgias, sore throat, anorexia and abdominal discomfort were also observed in many outbreaks and volunteer studies [Wyatt et al., 1974:709-714; Kaplan et al., 1982:756-761].

2.11. Laboratory diagnosis

2.11.1. Electron Microscopy

Electron microscopy played an important role in discovery of NoVs. However, it is of limited value for direct examination of unconcentrated stool material for the routine detection of NoVs due to their low concentration and difficulties in distinguishing them from other small round objects present in stool [Green, 2007:950-979]. Nevertheless, IEM or solid-phase immune electron microscopy (SPIEM) is used for the detection of NoVs present in stools [Kapikian et al. 1980:70-83].
2.11.2. Immunoassays

Immunoassays for the detection of NoVs in clinical specimens use hyperimmune antisera prepared against VLPs. In past few decades, efforts have been made for the development of NoV-specific and cross-reactive monoclonal antibodies for the detection of NoV antigen in clinical specimens by ELISAs [Herrmann et al., 1995:2511-2513; Hardy et al., 1996:252-261; Hale et al., 2000:1656-1660; Kitamoto et al., 2002:2459-2465; Yoda et al., 2003:2367-2371; Parker et al., 2005:7402-7409]. Few commercial diagnostic ELISA kits are also available but have many limitations [Burton-MacLeod et al., 2004:2587-2595]. Sero-epidemiologic studies based on ELISAs using rVLPs to detect antibodies to Gl and GII viruses have demonstrated that the prevalence of antibody to the GII viruses (Mexico, Hawaii or Lordsdale) was greater than that of the Gl viruses [Parker et al., 1995:194-200; Dimitrov et al., 1997:115-118; Cubitt et al., 1998:135-139; Smit et al., 1999:227-231; Jiang et al., 2000:3349-359]. However, it is impossible to identify the antigenic type of an infecting NoV strain by serologic analysis because of the cross-reactive antibodies detected by ELISA [Noel et al., 1997:372-383; Belliot et al., 2001:4288-4295].

2.11.3. Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

RT-PCR is the most successful and widely used technique for detection of NoVs. Using sequence information of NoV strains available online many researchers have designed primers targeting four important regions of NoV genomes (A, B, C and D) for detection and genotyping of NoV strains [Ando et al., 1995:64-71; Green et al., 1995:197-202; Le Guyader et al., 1996:2225-2235; Noel et al., 1997:372-383; Jiang et al., 1999:145-154; Vinje et al., 2000:223-241; Fankhauser et al., 2002:1-7; Kojima et al., 2002:107-114; Vinje et al., 2004:235-241; Fankhauser et al., 2002:1-7; Kojima et al., 2002:107-114; Vinje et al., 2004:109-117] (Fig. 2.9). Of these region A and region B primers target RNA polymerase gene and are used worldwide for the detection of NoVs from clinical specimens [Vinje et al., 2004:109-117]. However, region C and region D primers target 5' and 3' ends of VP1 gene respectively and are used for genotyping of NoV [Vinje et al., 2004:109-117] (Fig. 2.9). The technique coupled with sequence analysis of the amplicons, has extensively been used to detect and characterize NoVs worldwide and has given new directions to NoV research. With this method, NoVs can be detected in clinical
specimens (feces or vomitus) and contaminated food, water, or fomites [Atmar and Estes, 2001:15-37]. In recent years, the real-time quantitative (q) RT-PCR is widely used as it allows rapid detection as well as comparison of viral RNA levels [Kageyama et al, 2003:1548-1557].

Figure 2.9: Schematic presentation of NoV genomic regions- A to D that are commonly used in RT-PCR for NoV detection and genotyping [Vinje et al., 2004:109-117]

2.12. Epidemiology

2.12.1. Seroprevalence

The development of immunoassays using rVLPs to detect antibodies to GI and GII NoVs demonstrate that the prevalence of antibodies is high among children <5 years of age, and thus, indicates exposure to the virus early in life [Nakata et al., 1988:2001-2005; Cubitt et al., 1998:135-139; Nakata et al., 1998:3160-3163; Pelosi et al., 1999:93-99; Talal et al., 2000:117-124]. It has been estimated that antibody to NoVs is lowest during the first years of life and that it rises after 2 years of age [Dai et al., 2004:4615-4619; Pease et al., 2004:2027-2036]. However, antibody prevalence to either genogroup has been noted to increase more gradually with age in developed than in developing countries [Jiang et al., 2000:S349-359]. The prevalence of antibody to the GII viruses was found greater than that of the GI viruses in most of the studies reflecting the predominance of circulating GII strains [Parker et al., 1995:194-200; Dimitrov et al., 1997:115-118; Cubitt et al., 1998:135-139; Smit et al., 1999:227-231; Jiang et al., 2000:S349-359].
2.12.2. Epidemic infections

NoVs are the single most common cause of nonbacterial gastroenteritis outbreaks worldwide [Greenberg et al., 1979:564-568; Vinje and Koopmans, 1996:610-615; Dedman et al., 1998:139-149; Inouye et al., 2000:270-274]. The Centers for Disease Control and Prevention (CDC) reported that 93% (217/233) of the total nonbacterial gastroenteritis outbreaks investigated between July 1997 and June 2000 were associated with NoVs [Fankhauser et al., 2002:1-7]. However, during 2006-07, there was a significant increase in gastroenteritis outbreaks (106 in 2006 as compared to 23 in 2005 in Wisconsin; 333 in 2006 as compared to 76 in 2005 in New York) reported by many state health departments in United States [Jenkins et al. 2007:842-846]. Ninety percent of these outbreaks were found associated with NoVs. A large proportion of these outbreaks occurred among residents of long-term-care facilities [Jenkins et al. 2007:842-846].

Of 3,714 nonbacterial gastroenteritis outbreaks investigated during 1995-2000 in Europe, 85% were associated with the NoVs [Lopman et al., 2003:90-96]. Again, there was an increase in NoV associated outbreaks in the year 2002 (155 in 2002 as compared to 68 in 1996 (128% increase) in Netherlands; 161 in 2002 as compared to 83 in 1999 (94% increase) in Germany; 614 in 2002 as compared to 347 in 1995 (77% increase) in England and Wales] [Lopman et al., 2004:682-688]. Common settings for NoV outbreaks were hospitals, camps, recreational areas, elementary schools, daycare centers, cruise ships, retirement centers, nursing homes, colleges, restaurants; social events with catered meals, families and the military camps [Fankhauser et al., 2002:1-7].

NoVs were identified in 30 of 59 (50.8%) gastroenteritis outbreaks in Victoria, Australia in 2001. Twenty seven percent of these outbreaks occurred in hostels/nursing homes, 13% in hospitals, 3% with youth refuges, 27% in social gathering, 13% in school outings/camps and 17% in pre-school/child minding centers [Marshall et al., 2001:321-331].

Outbreak investigation study conducted on 416 fecal specimens from 66 outbreaks that occurred in Saitama Prefecture, Japan, between January 1997 and May 2002 suggested 61.5% (256/416) NoV positivity by real time PCR [Kageyama, 2004:2988-2995]. The outbreaks occurred in a variety of the settings- restaurants
(49%), semiclosed communities [schools, nursing homes and dormitories] (21%),
catered lunches (12%), hotels (9%) and private homes (9%).
There is only one report of a food borne outbreak caused due to NoVs from India.
This outbreak occurred in a nurses' hostel of a civil hospital in Delhi after a farewell
party. One hundred and thirty nurses and some housekeeping staff were affected.
Six of the 6 stool specimens tested were positive to GII NoVs. All the affected
persons had consumed salad sandwiches that were supposed to be contaminated
with NoVs [Girish et al. 2002:603-607].

2.12.3. **Endemic/Sporadic infections**

In many countries NoVs are recognized as the second most common cause
of AGE after rotavirus in pediatric populations [Green, 2007:950-979]. NoV infections
are reported in all age groups [Rockx et al., 2002:246-253]. The variable rates (5.5-
44%) of NoV infections have been reported in AGE cases from different parts of the
world [O’Ryan et al., 2000:1519-1522; Parashar et al., 2004:1088-1092; Kirkwood
et al., 2005:96-101; Lindell et al., 2005:1086-1092; Nguyen et al., 2007:582-590].
The studies from Asian countries report 6-25% NoV positivity in sporadic cases of
AGE [Hansman et al., 2004:1673-1688; Dove et al., 2005:522-527; Liu et al.,
2006:69-72]. NoVs have been reported to account for 12% of severe gastroenteritis
among children <5 years of age worldwide [Patel et al. 2009:1-8]. The studies
carried out in adults demonstrate the NoV prevalence rate of 1.8%-17.6% [O’Neill et
al., 2002:335-343; Sagalova et al., 2006: 17-23; Chen et al., 2008:265-267]. NoVs
have also been documented as important agents of gastroenteritis in military
populations in several different areas of the world [McCarthy et al., 2000:5387-391].
Among U.S. military personnel deployed to South America or West Africa, NV
infection was detected in 10% of ill personnel, second to enterotoxigenic Escherichia
coli [Bourgeois et al., 1993:243-248].

A study from Vellore, South India conducted in children admitted to the
hospital and children in the community has reported 15.1% and 6.6% NoV
prevalence, respectively [Monica et al., 2007:544-551]. Other epidemiologic studies
conducted in New Delhi, North India and Kolkata, East India reported 15.9% and
18.9% norovirus prevalence, respectively [Rachakonda et al., 2008:42-48; Nayak et
al., 2009:223-229].
2.13. Seasonality

Seasonal variation in norovirus infection is poorly understood phenomenon. NoV infections are usually associated with winter months seasonality [Xi et al., 1990:1580-1583; Mounts et al., 2000:S284-287; Inouye et al., 2000:S270-274; Liu et al., 2006:69-72]. However, many recent reports associated them with spring or summer months [Boga et al., 2004:2668-2674; Marshall et al., 2005:321-331; Eric et al., 2007:2205-2211]. Although, higher infections of NoVs have been noted in relatively low humidity or dry weather conditions, there are reports on increase in NoV activity in rainy season also [Lopman et al., 2009:e6671; Dey et al., 2007:218-223; Nguyen et al., 2008:102-113].

2.14. Molecular epidemiology

Molecular epidemiology of NoVs suggests that GII is the most common genogroup circulating with variants of genotype 4 causing the majority of outbreaks and sporadic infections worldwide [Noel et al., 1999:1334-1378; Castilho et al., 2006:3947-3953]. The evolutionary mechanisms governing the persistence and emergence of new GII.4 NoV strains have been studied recently by Lindesmith et al 2008. Six sub-clusters within GII.4 cluster on the basis of their capsid diversities have been described as per their predominance period [Lindesmith et al., 2008:e31]. These include- Camberwell cluster (1987-95), Grimsby cluster (1995-2002), Farmington Hills cluster (2002-04), Hunter cluster (2002-04), Sakai cluster (2004-06) and DenHaag cluster (2006b variants) [Lindesmith et al., 2008:e31].

Apart from GII.4 strains, recombinant NoV strains also play a major role in causing outbreaks and sporadic infections worldwide [Ambert-Balay et al., 2005:5179-5186; Bull et al., 2005:1079-1085; Bull et al., 2007:3347-3359]. Among NoVs, three types of recombination viz. intergenogroup, intergenotype and intragenotype have been reported of which Intergenotype is commonly detected worldwide [Phan et al., 2007:1388-1400]. To date, recombinant NoVs are described to occur naturally within GI, GII and GIII [Katayama et al., 2002:225-239; Han et al., 2004:5214-5224; Phan et al., 2007:1388-1400]. The study of recombination events in NoVs has become more complicated with the addition of new recombinants with known capsid types and unclassified polymerases (GII.a, GII.b, GII.c and GII.d)
The polymerase type GII.b is most prevalent and usually found in combination with GII.3 [Gallimore et al., 2004:2271-2274, Ambert-Balay et al., 2005:5179-5186]. Recombinant NoV GII.b/GII.3 has emerged recently as the main causative agent for many outbreaks across Europe, Australia and Asia [Ambert-Balay et al., 2005:5179-5186; Bull et al., 2005:1079-1085; Phan et al., 2006:971-978; Phan et al., 2007:1388-1400].

Knowledge on the molecular epidemiology of NoV infections in India is limited to few cities from different regions of India [Kang et al., 2000:1-3; Girish et al., 2002:603-607; Monica et al., 2007:544-551; Nayak et al., 2008:117-123; Nayak et al., 2009:223-229]. A study from Vellore, South India conducted in children admitted to the hospital and children in the community reported GII.3 and GII.4 as predominant genotypes, respectively [Monica et al., 2007:544-551]. While GII.4 was identified as the predominant genotype circulating in Kolkata, eastern India, an intergenogroup (GI.3/GI.4) along with 3 novel intergenotype (GII.b/GII.7, GII.4/GII.8 and GII.5/GII.12) recombinants were also identified [Nayak et al., 2008: 117-123; Nayak et al., 2009:223-229]. Involvement of genotype GII.3 was identified in a food-borne outbreak occurred in nurses' hostel, New Delhi in 2000 [Girish et al. 2002:603-607].

2.15. Prevention and Control

2.15.1. Measures to control Infections

Specific methods for the prevention of norovirus infection focus on the prevention of spread of containment to other areas by ill or exposed individuals and effective environmental decontamination [Chadwick et al., 2000:1-10; Kuusi et al., 2002:133-138; Khanna et al., 2003:131-136; Albers, 2004:21-26]. Measures like surveillance of shorelines to identify possible sites of contamination of water, prohibiting overboard dumping of fecal wastes from boats to avoid contamination of waters in oyster-harvesting areas are taken for preventing oyster-associated NoV outbreaks [Parashar et al., 2001:1-17].

In view of the frequent occurrence of food-borne outbreaks, special care needs to be taken during the processing of food [Koopmans and Duizer, 2004:23-41]. Personal hygiene and the proper disinfection of environmental surfaces are critical.
for prevention of food-handier associated transmission. It is recommended that food-
handlers be excluded from work during and for 48–72h after recovery from NoV
gastroenteritis to prevent transmission of virus [Parashar et al., 2001:1-17].

To prevent person-to-person transmission of NoVs, strict personal hygiene is
recommended to sick individuals. The contact with ill person should be avoided and
appropriate disinfection of environmental surfaces contaminated with vomitus and
feces should be done [Parashar et al., 2001:1-17]. Hypochlorite at 5,000 ppm,
hydrogen peroxide-based cleaners, and phenolic-based cleaners are effective
disinfectants [Doultree et al., 1999:51-57; Barker et al., 2004:42-49].

2.15.2. Treatment (Clinical management)

As first-line therapy for diarrheal disease oral rehydration solutions are
administered to replace fluid loss [Green, 2007:950-979; Patel et al. 2009:1-8]. This
provides essential electrolyte replacement plus sugar (glucose or sucrose). Parenteral
administration of fluids is administered if severe vomiting or diarrhea
occurs with symptoms of significant dehydration [Green, 2007:950-979].

Patients with cardiovascular disease, those receiving immunosuppressive
therapy, the elderly, and organ transplant recipients are at higher risk of a severe
clinical outcome from norovirus infection in a hospital setting [Mattner et al., 2006:69-
74]. Deaths are reported in elderly patients and in immunocompromised children
with norovirus gastroenteritis [Kaplan et al., 1982:756-761]. However, oral
administration of bismuth subsalicylate after onset of symptoms significantly reduces
the severity and duration of abdominal cramps in adults [Steinhoff et al., 1980:1495-
1499].

Recent advances in understanding the genomic structure, individual viral
proteins, RNA replication strategy and virus-host interaction of the NoVs have
provided new strategies in the development of antinoroviral agents that could inhibit
viral attachment to host cells through carbohydrate receptors, viral protease and
polymerase functions and viral replication [Tan and Jiang, 2008:146-151].

2.15.3. Attempts for vaccine development

A safe and effective vaccine could reduce the incidence of epidemic viral
gastroenteritis and number of episodes of gastroenteritis in infants and children
[Green, 2007:950-979]. However, many challenges to developing NoV vaccines exist, including an incomplete understanding of the immune correlates of protection, lack of persistent long-term and cross-protective immunity, and the existence of multiple genetic and antigenic types of virus [Tacket et al., 2003:241–247]. The inability to cultivate NoVs in cell culture has prevented an analysis of the role of neutralizing antibodies. Recombinant VLPs expressed in baculovirus or in transgenic plants was safe and immunogenic when administered orally to volunteers and thus can be a good candidate for subunit vaccine development [Ball et al., 1999:40-48; Tacket et al., 2003:241-247; Tacket, 2005:1866-1869]. Another approach for norovirus vaccine development under consideration is based on alphavirus vectors, such as Venezuelan equine encephalitis (VEE) virus [Baric et al., 2002:3023-3030].