CHAPTER II

LITERATURE REVIEWS

A. Structures and molecular biology

Norovirus (NV, formerly known as Norwalk-like virus) is a member of the nonenveloped, single-stranded RNA viruses in the genus *Norovirus*, family *Caliciviridae* and causes acute gastroenteritis in humans worldwide. A key characteristic of the calicivirus is the existence of 32 cup-like depressions on the surface of virus particle (*calici* is derived from the Latin word *calyx*, or cup). Norwalk virus was originally described as 27 nm in its shortest diameter and 32 nm in its longest diameter based on an observation of particles in fecal specimens obtained from infected volunteers by immune electron microscopy (IEM) (Kapikian et al., 1972). The structure of Norwalk virus was initially identified from Norwalk virus recombinant virus-like particles (rVLPs) (Jiang et al., 1992) using electron microscopy technique. The electron cryomicroscopy and computer image processing of rVLPs demonstrates that the particles have a distinct architecture and exhibit T=3 icosahedral symmetry (Prasad et al., 1994). The viral capsid is composed predominantly of a single major capsid protein. The capsid contains 180 molecules of capsid protein which folds into 90 dimers that form a shell from which 90 archlike capsomers protrude at all the local and strict twofold axes (Prasad et al., 1999; 2000). These arches are arranged in such a way to form large hollows at the icosahedral five- and three-fold axes, and these hollows are seen as cuplike structure on the surface of the particle (Figure 1). Further analysis of capsid protein by x-ray crystallography has

shown that it consists of two major domains, shell domain (S) and a protruding domain (P). The S domain forms the inner part of the capsid that surrounds the genome and the amino acid sequence of this domain is relatively conserved among NV. The P domain forms the archlike capsomer that protrude from the virion and amino acid sequence of this domain contains the most variable region of the capsid protein (Figure 2).

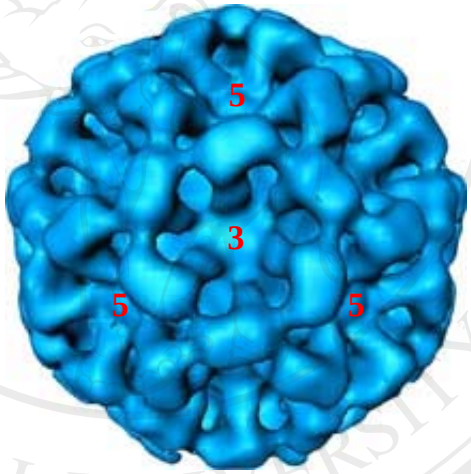

Figure 1. Three-dimensional structure of Norwalk virus rVLPs viewed along the icosahedral threefold axis at 22 Å resolution as determined by cryo-EM techniques. The Norwalk virus rVLPs have a distinct architecture, they exhibit T=3 icosahedral symmetry; the threefold and fivefold axes of symmetry are shown, and the cuplike depressions are evident at the threefold and fivefold axes (Prasad et al., 1994).

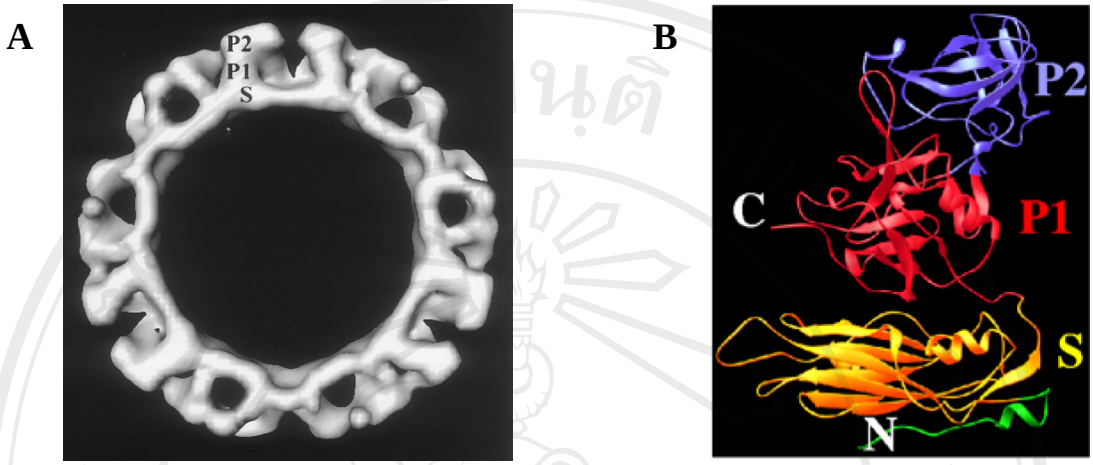

Figure 2. **A:** A central section of Norwalk virus rVLP capsid viewed along the icosahedral 3-fold axis. S domains associate to form contiguous shell of ~45 Å thickness from which arches containing subdomains P1 and P2 emanate. **B:** Ribbon representation of structure of Norwalk virus rVLP capsid protein. N-terminal arm, S domain, P1, and P2 subdomains are colored in green, yellow, red, and blue, respectively. N- and C-termini of capsid protein are indicated. C-terminus faces hollow, whereas N-terminal arm faces interior of capsid and P2 subdomain faces exterior of capsid (Prasad et al., 2000).

The genome of NV is a positive-sense, single-stranded RNA of approximately 7,300-8,300 nucleotides in length which has a genome-linked protein (VPg) at the 5′ terminus and a poly A tail at the 3′ terminus. The genome consists of three open reading frames (ORFs). A 5′-ORF (ORF1) encodes the non-structural polyproteins such as nucleoside triphosphatase (NTPase), genome-linked protein (VPg), proteinase and RNA-dependent RNA polymerase (RdRp). A second ORF (ORF2) encodes the

structural protein (major capsid protein, VP1) and a 3′-ORF (ORF3) encodes for a small capsid (VP2) protein (Bertolotti-Ciarlet et al., 2003).

Sapovirus (SV, formerly known as Sapporo-like virus) belongs to genus *Sapovirus*, family *Caliciviridae* and is a causative agent of acute gastroenteritis. The viral particle of SV has a typical calicivirus morphology with a “Star of David” appearance by EM. It has a feathery edge, a six-pointed star with a dark hollow in the center, and distinct surface hollows that appear round or oval. The virion has 41–48 nm in diameter with 32 cup-like depressions and ten spikes on the outline which presented as a six-pointed star. The genome of SV is a positive-sense, single-stranded RNA of approximately 7,300-8,300 nucleotides long and surrounded by an icosahedral capsid. The 5'-end of the genome has a genome-linked protein (VPg) and the 3'-end has a poly (A) tract. The genomic organization of SV is different from that of NV. The SV capsid gene is fused to the non-structural gene within a 5′-ORF (ORF1), which encodes the non-structural polyproteins and the structural protein (capsid protein) (Green et al., 2001). A 3′-ORF (ORF2) comparable to ORF3 of NV encodes a small protein of unknown function (Wilhelmi et al., 2003). In addition, there is an ORF overlapping with the 5′-end of the capsid gene (ORF overlapping capsid gene, only in the GI strains), which is not found in the NV genome. The presence of a conserved translation initiation motif (GCAAUGG) at the 5′end of this ORF suggests that a functional protein may be encoded in this ORF (Schuffenecker et al., 2001).

Human astrovirus (HAstV) is one of the members of the genus *Astrovirus*, family *Astroviridae*. In 1975, Madeley and Cosgrove had observed, by EM, small round viruses have a distinctive five- or six-pointed star-like appearance and

subsequently designated as astrovirus (Greek, astron means star). HAstV is a small, round, nonenveloped virus (28-30 nm in diameter). The genome is a positive-sense, single-stranded RNA of approximately 6,800 nucleotides in length excluding the 3′poly (A) tail. The genome contains three open reading frames, ORF1a and ORF1b encode for the non-structural proteins, including protease (Pro) and RNA-dependent RNA polymerase (RdRp), respectively, while ORF2 encodes for the capsid protein precursor (Schnagl et al., 2002).

B. Classification and nomenclatures

The Norwalk-like viruses (NLVs) are classified into the genus "*Norwalk-like viruses*" and recently renamed as the genus *Norovirus* within the family *Caliciviridae* (Green et al., 2000; Mayo, 2002). The current nomenclature system for identification of calicivirus strains is organized as follows: host species from which the virus is obtained/ genus abbreviation/ species abbreviation/ strain name/ year of occurrence/ country of origin; for example, Hu/NLV/I/Norwalk/1968/US (Green et al., 2001). NVs can be divided into three distinct genogroups, GI, GII, and GIII, each of which can be subdivided into several clusters/genotypes based on the sequence analysis. NVGI and NVGII infect humans, while GIII infects pigs and cow (Ando et al., 2000). NVs GI includes the Norwalk, Southampton, Cruise Ship, and Desert Shield virus clusters, GII includes the Bristol, Snow Mountain agent, Toronto, Hawaii, and Gwynedd virus clusters, while GIII includes Jena bovine enteric calicivirus (Ando et al., 2000). Kageyama et al. (2004) had classified NVGI and GII strains into 14 and 17 genotypes, respectively, based on the partial capsid sequence analysis. Later, Okada et al. (2005) had classified NVGI and GII strains into 15 and 18 genotypes,

respectively, based on the analysis of the partial 5′end sequences of ORF2. Recently, NV strains are classified into five genogroups based on molecular characterization of the complete capsid gene sequences (Zheng et al., 2006). NV strains of three genogroups, GI, GII, and GIV, are known to infect humans (except GII/11 strains infect pigs), while GIII and GV strains are found to infect cows and mice, respectively. To date, NV strains are classified into 29 genetic clusters/genotypes (8 in GI, 17 in GII, 2 in GIII, and 1 each in GIV and GV) based on the analysis of the complete capsid amino acid sequences (Zheng et al., 2006).

The Sapporo-like viruses (SLVs) are classified into the genus "*Sapporo-like viruse*" and recently renamed as the genus *Sapovirus* within the family *Caliciviridae* (Green et al., 2000; Mayo, 2002). According to the same nomenclature system as that of NV, a representative SV strain could be named as, Hu/SLV/Sapporo/1982/JP (Green et al., 2001). SV was initially classified, based on the differences of the complete capsid amino acid sequences, into 3 major genogroups, GI (known as the Manchester virus), GII (known as the London virus), and GIII (porcine enteric calicivirus, PEC). SV strains are classified further into 5 genetic clusters/genotypes (3 in GI, 1 each in GII and GIII) (Schuffenecker et al., 2001). In 2004, two SV strains (Hou7-118/90 and Argentina39) were identified and grouped into the new genogroups, GIV and GV, respectively. In addition, another two SV strains (Mex340/90 and Cruise ship/00) were identified and grouped into the two new genetic clusters/genotypes within the London/92 genogroup (GII) (Farkas et al., 2004). Recently, Akihara et al. (2005) reported that SV strains are classified into 5 genogroups and 16 genetic clusters/genotypes (8 in GI, 5 in GII, 1 each in GIII, GIV, and GV) based on the differences of the partial capsid amino acid sequences.

Human astroviruses (HAstVs) were initially isolated from natural infections in the United Kingdom (UK) in 1980s and classified into five serotypes by immunofluorescence, neutralization, and IEM using serotype-specific polyclonal antiserum (Lee et al; 1982; Kurtz et al., 1984; Hudson et al., 1989). During 1989 to 1992, HAstVs serotypes 6 and 7 were also identified in the UK (Cubitt et al., 1990; Lee and Kurtz., 1994). The eighth serotype of HAstV was subsequently detected in the UK, in 1999, based on nucleotide sequence analysis of a limited region of ORF2 (Noel et al., 1995; Belliot et al., 1997; 1999). Later, all eight serotypes of HAstVs were recognized by a single, group-specific monoclonal antibody (Herrmann et al., 1990; Lee et al., 1994). This monoclonal antibody is directed at a viral structural protein, most likely an epitope of the highly conserved N-terminal half of the ORF2 product (Jonassen et al., 1995; Belliot et al., 1999). An enzyme immunoassay (EIA) using monoclonal antibodies to the HAstV group antigen was developed for the detection of HAstVs in stools of patients with gastroenteritis (Herrmann et al., 1990). Following the partial and complete capsid gene, as well as entire genome of HAstV serotype 1, 2, 3, 4, and 5 had been sequenced in the early 1990s, the serotype-specific oligonucleotide primers were designed and used in reverse transcription-polymerase chain reaction (RT-PCR) for genotyping of HAstV isolates (Lee et al., 1994; Willcocks et al., 1994; Noel et al., 1995; Willcocks et al., 1995). A relative high concordance between results of genotyping and serotyping was observed (Matsui et al., 1998; Wang et al., 2001). To date, eight serotypes of HAstV have been reported, and HAstV-1 is detected most frequently worldwide, HAstV-2 to HAstV-4 are common, and HAstV-5 to HAstV-7 are less common, whereas HAstV-8 is rare serotype as compared to the others (Mitchell et al., 1999; Pang and Vesikari, 1999b).

C. Transmission, pathogenesis, and pathology

Transmission of human caliciviruses occurs through the ingestion of contaminated food (particularly oysters) and water, and person-to-person spread (CDC, 2001). Human caliciviruses remain uncultured *in vitro* and no animal models available other than experimental transmission to human volunteers. Virions are acid stable, consistent with an ability to survive during passage through the stomach. The site of primary replication for human caliciviruses is assumed to occur in the upper intestinal tract. Several studies carried out on volunteers, infection by Norwalk virus was observed to produce an expansion and shortening of the villi of the proximal small intestine, while the epithelial cells remain intact (Schreiber et al., 1974; Dolin et al., 1975). Infiltration of polymorphonuclear and mononuclear cells in the lamina propria, and cytoplasmic vacuolization are also observed. Norwalk virus can cause cytolytic infections in the villous enterocytes but not in the crypt enterocytes of the proximal small intestine, which leads to the destruction of the differentiated, absorptive villous enterocytes and resulting in villous atrophy and a malabsorptive diarrhea (Guo et al., 2001) However, the mechanism by which diarrhea is produced remains unclear, although it has been suggested that the delay in gastric emptying observed in Norwalk virus gastroenteritis may play a role (Meeroff et al., 1980)

HAstV is transmitted through the fecal-oral route and from person to person (Kurtz et al., 1979; Oshiro et al., 1981). In HAstV infection, very little is known about HAstV pathogenesis as well as host factors involved in viral clearance and disease resolution. The correlation of diarrhea in children with fecal shedding of HAstVs and the identification of viral particles in the intestinal epithelial cells

suggests that viral replication takes place in the intestinal tissue in humans (Phillips et al., 1982).

D. Clinical features, diagnosis, and treatment

NVs have been associated with infection and disease in all age groups, although the majority of gastroenteritis outbreaks occur in school-age children and adults. Recent studies demonstrate that NVs may also the causative agent of gastroenteritis among infants and young children (Pang et al., 1999a). The incubation period of NV-caused gastroenteritis is approximately 12-48 hours and the symptoms usually last 24 to 48 hours. The illness is characterized by acute onset of nausea, vomiting, abdominal cramps, and diarrhea. Vomiting is relatively more prevalent than diarrhea among children, whereas in adults diarrhea is more common. Constitutional symptoms, for example, headache, fever, chills, and myalgia are frequently reported (CDC, 2001). The illness is generally mild and self-limited, and usually no hospitalization or rehydration is required for adults. Although severe dehydration caused by NV gastroenteritis is relatively rare, it could be fatal among susceptible persons (e.g., older persons with debilitating health condition) (Green et al., 2001). The diarrheal stools are often liquid, but do not contain excess mucus, blood, or leukocytes (Dolin et al., 1976). In volunteer study, the virus shedding in stools was detected from 15 hours to 7 days post inoculation, with a peak between 25 and 72 hours, and it could persist up to 2 weeks (Okhuysen et al., 1995). Recently, virus shedding in stools was detected by RT-PCR and Southern hybridization for up to 28 days in patients naturally infected with NVGII (Hazelton et al., 2000). Diagnosis of NV infection has previously performed by detection of the virus in

stools of infected patients by electron microscopy (EM) or immune-electron microscopy (IEM), with some difficulty due to the small size of these viruses and the low numbers of virus shed in stool. Recently, RT-PCR as well as multiplex RT-PCR assay have been developed and improved simultaneous detection of NV and other enteric viruses (Ando et al., 1995; Rohayem et al., 2004). For large-scale epidemiological surveys, capture enzyme immunoassay (EIA) based on hyperimmune antiserum raised against recombinant capsid protein have been used. However, the samples that negative by EIA should be confimed by RT-PCR (Gallimore et al., 2004). The VLP-based antibody detection by EIA have been used in seroprevalence surveys and in longitudinal studies of antibody acquisition (Atmar and Estes, 2001).

Although SVs mainly infect infants and young children (Pang et al., 2000; Green and Kapikian, 2001), SV gastroenteritis outbreaks in adults have also been documented (Noel et al., 1997). In infected volunteers, the incubation period was 12-72 hours and illness lasted for 1-11 days. The duration of virus shedding paralleled the appearance of symptoms (Levy et al., 1976). The clinical symptoms for SV associated with acute gastroenteritis including vomiting, diarrhea, nausea, malaise, aching limbs, and headache (Cubid WD., 1994). The predominance of the symptoms varied between different outbreak, which may be related to the average infectious dose per patient and the age of the patients. SV-associated diarrhea is mild, but severe cases can occur (Sakai et al., 2001; Robinson et al., 2002). For detection of SV, purified native SV particles from stool specimens were used for producing antisera for immunoassays, including IEM and EIA (Sakuma et al., 1981; Nakata et al., 1985). However, the most widely used method for the detection of SV is RT-PCR, which has a high sensitivity (Okada et al., 2002).

HAstV diarrhea is principally seen in young children with the age of 6 months to 2 years, but HAstV infections in elderly, institutionalized patients and immunocompromised individuals have also been reported (Lewis et al., 1989; Grohmann et al., 1993; Matsui et al., 1994). Following a 1-4 days incubation period, the clinical symptoms present as anorexia, fever, vomiting, abdominal pain, and watery diarrhea that lasts for 2-3 days and resembles a mild rotavirus infection. Virus shedding is detectable for weeks despite resolution of symptoms. The disease is most often mild and does not result in severe dehydration (Greenberg et al., 1992). However, severe infections have been occurred in young adults infected with HAstV serotype 4 (Cubitt WD., 1990). HAstVs were previously detected in stool specimens by EM or IEM (Berthiaume et al., 1981; Kurtz and Lee, 1987). An EIA based on a group-reactive monoclonal antibody to capture viral antigen and polyclonal antiserum as a detecting antibody have been useful for rapid detection of HAstV antigen (Herrmann et al., 1988; 1990). Recently, RT-PCR have been used for detecting and typing of HAstVs in clinical samples and also used to confirm HAstV-positive samples detected by EIA (Noel et al., 1995; Mitchell et al., 1998).

Gastroenteritis caused by human Caliciviruses and HAstV is generally a mild, self-limiting illness and does not require specific therapy. In the young child or rare adult patient who becomes dehydrated, oral or intravenous fluid therapy may be necessary.

E. Molecular epidemiology

1. Molecular epidemiology of Norovirus (NV)

In 1968, an acute gastroenteritis outbreak among students and teachers of an elementary school in Norwalk was reported by CDC (Adler and Zickl, 1969). In 1972, Kapikian et al had demonstrated virus particles in stool samples collected during outbreak of gastroenteritis in 1968 in Norwalk, Ohio, by electron microscopy (EM), and subsequently named Norwalk virus (Kapikian et al., 1972). An outbreak and sporadic cases of NV-associated gastroenteritis occur in all age groups throughout the year, with a seasonal peak during the cooler months (Mount et al., 2000). NVs have a worldwide distribution since 1970s to 1980s (Greenberg et al., 1979; Sekine et al., 1989; Monroe et al., 1991), and the methods for detection of NVs in stool samples were limited to direct EM, IEM, Radioimmunoassay (RIA), and Enzyme-immunoassay (EIA). Recently, the molecular methods and DNA sequencing have been developed for the detection and characterization of NV strains (Green et al., 1994; Ando et al., 1995; Yan et al., 2003). Strains belonging to NVGI and GII genetic clusters/genotypes have been shown to cocirculate (Noel et al., 1997; 1999; Fankhauser et al., 1998). GII is currently globally predominant among NV strains (Jiang et al., 1995; Noel et al., 1999; Koopmans et al., 2000), while few GI strains are reported (Fankhauser et al., 1998; Maguire et al., 1999; Gonin et al., 2000).

In USA, more than 20 million cases of acute gastroenteritis have been reported, 220,000 were hospitalized and up to 40 deaths annually among children less than 5 years of age (Barnes et al., 1998). During 1993 to 1997, Noel et al. (1999) had identified the NV strain, 95/96-US (subsequently belonged to NVGII/4) as the predominant causative agent in 60 of 152 outbreaks of diarrhea occurred in multiple

settings (mostly in health-care settings) in the US. During 1996 to 2000, 348 outbreaks of NV gastroenteritis had occurred in multiple settings in the US with different frequencies, i.e. 39% in restaurants, 29% in nursing homes and hospitals, 12% in schools and day care centers, 10% in vacation settings, including cruise ships, and 9% in other settings were reported to Centers for Disease Control and Prevention (CDC, 2001). In November 1997 to December 1999, 1840 stool samples were collected over 2 years from symptomatic children up to 5 years of age at three pediatric hospitals in the US. Overall, 8.5% were calicivirus-positive, of which 7.1% were NV and 1.4% were SV as confirmed by sequence analysis. Phylogenetic analysis of 97 NV sequences showed that 7 and 86 strains belonged to GI and GII, respectively, whereas the other four strains were unclassified genogroup (Zintz et al., 2005). Between July 1997 and June 2000, stool samples from 284 outbreaks of nonbacterial gastroenteritis in multiple settings in the US were submitted to CDC for detecting of NVs. NVGII strains were the predominant causative agents (73%), while GI strains caused only 26% of all NV-positive outbreaks. NV strains of GII/4 were more commonly associated with outbreaks in nursing home than in other settings (Fankhauser et al., 2002). In 2002, Widdowson et al. (2004) had identified the NV strain, provisionally named “Farmington Hills strain” (belonged to NVGII/4) as the important cause of gastroenteritis occurred in 64% on cruise ship outbreaks and 45% in land-based outbreaks during the same period in the US. Recently, an outbreak of gastroenteritis occurring at a swimming pool in Vermont was reported and 50% of cases were attributed to NV (CDC, 2004).

NVs of GII are also the most common strains in association with gastroenteritis outbreaks in Europe (Fankhauser et al., 1998; Smit et al., 1999).

During 1991-1999, the seroprevalence of Norwalk virus antibody was detected among all age groups (less than 6 months to more than 90 years) in England. Overall, the seroprevalence of Norwalk virus antibody was 73.3% among all age groups. It was observed that the prevalent rate was highest (75.2%) in infants with the age of less than 6 months and lowest (24.6%) in infants with the age of 6 to 11 months and increased to 50.5% in children with 1-4 years of age. The prevalence continued to increase to 81.4% in adults and 89.7% in persons over 60 years old (Gray et al., 1993). The distribution of various genotypes varies year by year, from town to town and from one country to another. Six-year period (1992 to 1998) of NV surveillance in West and North Yorkshire and Humberside, UK, had demonstrated an annual variation in the distribution of genotypes (Lopman et al., 2002). Overall Grimsby-like virus (GII/4) was the most prevalent genotype in each year, except in 1993 Mexico-like virus (GII/3) emerged and became the most common genotype (Lopman et al., 2002). In the Netherlands, during 1991 to 1999, 82% outbreaks of gastroenteritis were associated with NV, of which NVGII strains had been detected more frequently than GI strains. In 1994, NVGII/3 (Mexico-like virus) was the most common strain, however, from 1995-1996 NVGII/4 (Lordsdale-like virus) became the most common strain. In the following years, the Mexico-like virus had been detected infrequently, whereas the Lordsdale-like virus was still commonly found along with other genotypes (Koopmans et al., 2000; Koopmans M, 2001). Moreover, NVGII/4 (Grimsby-like virus) was also reported as the predominant strain associated with gastroenteritis outbreaks in Germany during 2000-2002 (Oh et al., 2003). Between 1995 and 2002, Lopman et al (2004) had collected the data of gastroenteritis outbreaks from nine European regions (Denmark, England and Wales, Finland,

France, Germany, Hungary, the Netherlands, Slovenia, and Spain). The data revealed an impressive increase of NV outbreaks in 2002 compared with those in the previous years in 3 regions (England and Wales, Germany, and the Netherlands). The findings coincided with the emergence of a new NVGII/4 variant, which had a consistent mutation in the polymerase gene from AACTTG to AATCTG. In addition, eight of nine regions had an annual peak in 2002, except for Spain, however, the new NVGII/4 variant was detected in all regions. In 2000, Mounts and colleagues (2000) had investigated the seasonal pattern of NV-associated gastroenteritis in 8 countries (Australia, Canada, Denmark, England, Japan, The Netherlands, United States, and Wales) by analysis of 12 surveys conducted during a 21-period (1978 through 1998). A remarkable finding in all studies and in all countries was that NVs were reported throughout the year, except in one study from Australia, where no NVs were detected in the summer months. The reports of NV-associated disease, both sporadic cases and outbreaks, were low in the warm months of summer but peaked in the winter in 10 of 12 surveys. In one survey from Yorkshire, England, and in one from southeastern Australia, NVs were reported predominantly in cold weather months but peaked in spring or early summer (Mount et al., 2000).

NVGII/4 strain (represented by Camberwell virus, Australian strain, belongs to the same cluster as Lordsdale virus) was the most common genetic cluster/genotype associated with sporadic cases and outbreaks of acute gastroenteritis in Australia and New Zealand (Greening et al., 2001; Marshall et al., 2003; Kirkwood et al., 2005). In addition, NVGII/3 (represented by Auckland virus, closely resembling Mexico virus) was first identified in 1995 (Regli et al., 1995) and GII/7 (represented by Gwynedd/Napier virus) was subsequently identified in the following year from

outbreaks associated with consumption of local oysters (Greening et al., 2001). Between 1995 and 1999, seven NV strains were responsible for the majority of outbreaks occurred in New Zealand. In that period, NVGII (GII/4, GII/3, GII/7, and GII/2) were the most common strains detected, except in 1998, NVGI (GI/2 and GI/3) strains had predominated (Greening et al., 2001). In Melbourne, Australia, Kirkwood et al. (2005) had detected calicivirus infection in 9.2% of hospitalized children under 5 years of age, in which 95% of cases were attributed to NV and the remaining to SV. NVGII/4 strain was the most common genotype detected during 1998-1999 and 2001-2002, while NVGII/5 was identified as the predominant genotype only in 2000. The studies on NV periodicity in southeastern Australia (Wright et al., 1998; Marshall et al., 1999) and New Zealand (Greening et al., 1999; 2001) showed that NV incidence tended to occur more commonly in spring/summer. However, there was no distinct seasonality of the sporadic cases of acute gastroenteritis observed in Kirkwood's study (Kirkwood et al., 2005).

In Africa, the studies of viral gastroenteritis are limited. In Kenya (1991 to 1994), NV infection was investigated in children younger than 6 years old with acute gastroenteritis. The prevalence of Norwalk virus (NVGI) and Mexico virus (NVGII) antigen was 0.1% and 0%, respectively. However, the prevalence of Norwalk virus and Mexico virus antibody was 58% in infants with 12 to 23 months of age and increased to 70% in preschool-age children (Nakata et al., 1998). Another report from South Africa, NV was demonstrated in only 3.3%, predominantly from children less than 4 years of age (Wolfaardt et al., 1997).

There are several epidemiological studies of NV in Asian countries, particularly in Japan. In Japan, during 1987-1989, Numata et al., 1994 and Honma et

al., 1998 had investigated rNV (recombinant NV) antigen and rMXV (recombinant Mexico virus, GII) antigen among children under 10 years of age with sporadic acute gastroenteritis in Sapporo and Ehime prefecture and they found that only 0.6% was positive for rNV antigen, while 1.3% was positive for rMXV antigen. NV antigens were also investigated among 245 patients involved in 42 outbreaks of acute gastroenteritis, of which 7.1% was positive for rNV antigen and 4.8% was positive for rMXV antigen. In the same study, 534 serum samples collected from cord blood, healthy children and adults in Hokkaido were tested for antibody to rNV. The prevalence of antibody in cord blood was rather high (71%) and decreased to 6-11% in 4-month to 6-year age group, then increased to 35% in school to early adult age group. The positive rate still increased to 70-76% in adulthood and to 98% in the over 50-year-old age group. However, the prevalence of antibody to rMXV was different from that of rNV, in which 53.3% was found in cord blood, and decreased to 45%, 6.7% in 0-3 month and 1-2 year age group, respectively. The prevalence of antibody remained low for the first 3 years of age, showed a steep rise during nursery school age, reaching 50%, and another steep rise during adolescent, reaching 80% and steadily increased to almost 100% by the age of 60. During 1976-1995, 6 of 36 outbreaks of acute gastroenteritis occurred in an infant home in Sapporo, Japan, were attributed to NVGII which was closely related to Lordsdale virus (NVGII/4), Hawaii virus (NVGII/1), and Mexico virus (NVGII/3) and 1 of those was attributable to both NVGII and SVGI (Nakata et al., 2000). Between 1988-1993, the various genotypes of NVs were found in Kyushu, Japan, however, the Mexico virus (NVGII/3) was dominant in 1989 (Otsu et al., 2003). From 1996-2000, a study of sporadic NV infection among diarrheal children had conducted in Osaka city, Japan, and revealed

that NV strains of OC96-00 cluster (similar to the Lordsdale virus (NVGII/4), with 96.3 to 100% amino acid sequence identity) were detected as the most common strain during 4-year study period, however, Arg320-like strains (NVGII/3) which appeared to be recombinants (as the capsid region was more similar to that of Mexico virus cluster (NVGII/3) while the RdRp region was similar to that of Lordsdale virus cluster (NVGII/4)) suddenly appeared and spread during 1999 to 2000 (Iritani et al., 2003). Between 1997 and 2002, sixty-six outbreaks occurred in Saitama prefecture, Japan, and 61.5% were attributed to NV. In outbreaks, multiple genotypes of GII and GI accounted for 55% and 14% of infections, respectively (Kageyama et al., 2004). Between 1999 and 2004, NV was detected in 23.5% of sporadic cases of children associated with acute diarrhea in Chiba prefecture, Japan. The total positive samples for NVGII, NVGI, and both genogroups were 80%, 13%, and 7%, respectively. The dominant genotype was NVGII/4 throughout the 5-year study period, except in 2001 and 2002, NVGII/5 (represented by Hillingdon virus) became the dominant genotype instead of GII/4 (Okada et al., 2005). Interestingly, NVGII which was closely related to the Mexico virus (NVGII/3) (with 94.9% nucleotide sequence identity) was detected among 70.2% of cases in the recent outbreaks (2003) of gastroenteritis in Nagasaki city, Japan (Hirakata et al., 2005). From 1990 to 1994, Phan et al. (2004) had identified NV in 9.9% of Pakistani children with acute diarrhea, in which NVGII was the causative agent in 76.5% and NVGI in 23.5% of cases. In China, between 1994-1996, Qiao et al. (1999) had identified calicivirus in 7.6% of children under 5 years of age hospitalized with acute diarrhea. In Indonesia, during 1997 to 1999, the prevalence of NV infection was 20.6% among all age groups (mostly less than 13 years) of patients with acute gastroenteritis, in which Taunton virus (serotype 4) and

Hawaii/Snow Mountain virus (NVGII/1 and NVGII/2) were detected as 42% and 58%, respectively (Subekti et al., 2002a). Later, the similar study was performed among infants and children (aged 0-12 years) with diarrhea, NV was demonstrated in 30% and the common serotypes/genotypes were the Taunton virus and the Hawaii/Snow Mountain virus similar to those observed in the previous study (Subekti et al., 2002b). In Ho Chi Minh City, Vietnam, during 1999 to 2000, the overall annual detection rate of NV was 5.4% among children hospitalized with acute sporadic gastroenteritis. Phylogenetic analysis revealed that the most common genogroup, was GII 94.4% and the remaining was GI 5.6%. The majority of GII strains (78%) belonged to GII/4 genotype (Lordsdale cluster). Other NVs such as GI/4, GI/8, GI/11, GII/1, GII/3 (Saitama U201 cluster, a recombinant NV), GII/7, GII/10, and GII/14 were also cocirculating, but these were infrequent (Hansman et al., 2004a). In Hong Kong, NV infection in sporadic cases was examined in one year period from July 2001 to June 2002. Lau et al. (2004) reported that NV infection had occurred among 19.5% of children less than 5 years of age. The predominant strains belonged to the Bristol virus (NVGII/4) which was closely related to the 95/96-US subset pandemic strain reported in USA (Noel et al., 1999) were detected during the first 6 months. In the latter 6 months of the study, strains belonged to other genotypes of NVGII were seen more commonly (Lau et al., 2004). NV infection often occurs in the winter months (Qiao et al., 1999; Phan et al., 2004), although many studies showed no seasonal prevalence (Nakata et al., 1998; Subekti et al., 2002a).

2. Molecular epidemiology of Sapovirus (SV)

In 1977, SV was originally isolated from an infant in an outbreak of gastroenteritis in Sapporo, Japan (Chiba et al., 1980). Several studies have noted that SV detection rates were less frequent than those of NV (Pang et al., 2000; Kirkwood et al., 2001; Buesa et al., 2002). Recently, the molecular methods and nucleotide sequencing were developed for the detection and strain characterization (Yan et al., 2003; Farkas et al., 2004; Akihara et al., 2005). Most of SV infections in human are caused by SVGI among outbreaks and sporadic cases (Schuffenecker et al., 2001; Okada et al., 2002; Phan et al., 2006).

In USA, during 1997 to 1999, SV was detected in 1.4% of hospitalized children up to 5 years of age, in which about half of the SV sequences identified belonged to the London/92 virus (recently belonged to SVGII) and the remaining belonged to the Manchester virus and the Parkville virus (recently belonged to SVGI) (Zintz et al., 2005). In 2002, twelve outbreaks of acute diarrhea on cruise ships in the US were reported and only one outbreak was attributed to SVGI and GII (Widdowson et al., 2004). In South America such as Argentina (1997-1998), the first report of the calicivirus infection showed that 24.2% had occurred in children under 3 years of age. Sequence analysis of 4 calicivirus positive samples found that 3 of 4 strains were SVGII/1 (the London/92 virus) and SVGI/1 (the Manchester virus) and only one strain was NV (Bereciartu et al., 2002).

In Europe, the rate of SV detection was 2.2 cases per 1000 person/year (0.01% of all cases) (Wheeler et al., 1999). Fecal specimens collected during a 10-year period (1988 to 1998) from outbreaks and sporadic cases in Europe (United Kingdom, Sweden, and The Netherlands), were analyzed for SV infection. Most of SV strains

identified were classified into Sapporo/82 virus (SVGI/1) and the remaining to Parkville/94 virus (SVGI/2), Stockholm/97 virus (SVGI/3), and London/92 virus (SVGII/1) (Vinje et al., 2000). From 1998 to 2004, Bon et al. (2005) had identified 7% for SV associated in sporadic cases of gastroenteritis in France among the 2- to 5-year age group. Several studies reported that SV was accounted for the cause of gastroenteritis in 9.0%, 5.0%, and 1.3% among infants and young children in Finland, the Netherlands, and Spain, respectively (Pang et al., 2000; de Wit et al., 2001; Buesu et al., 2002).

In Africa, detection of SV infection conducted in Kenya during 1991 to 1994 revealed that SV antigen was detected in 2.2% of children younger than 6 years old with acute gastroenteritis and constantly detected every year without seasonality. In addition, about 50% of individuals with 1-2 years of age had acquired antibody to SV and the rate of positivity was maintained at 70% and 90% from young children to adults. (Nakata et al., 1998).

In southeastern Australia, during 1986-1996, SVs were detected in 5.2% among patients in all age groups with gastroenteritis, however, mostly (62.5%) were detected in infants and children (Wright et al., 1998). Later, during 1998-2002, Kirkwood et al. (2005) had detected and identified 5 strains of SV (0.4%) as the cause of sporadic acute gastroenteritis among young children in Melbourne. Sequence analysis of these strains, revealed that 3 strains were classified into GI (2 strains were Sapporo-like virus and 1 strain was Manchester-like virus), and one each of GII and GV, respectively.

In Asia, SV is also frequently detected in feces of infants hospitalized with diarrhea, however, it is less common than NV. SVGI (represented by Sapporo virus)

is more frequently detected than SVGII (Nakata et al., 2000; Okada et al., 2002; Akihara et al., 2005). In Pakistan, Phan et al. (2004) had identified SV in 3.2% of infants and young children with acute diarrhea from 1990 to 1994. The highest incidence of SV infection fell into 1991 and the lowest in 1993. Analysis of these sequences revealed that 70.6% of SV strains detected in that study belonged to SVGI (represented by Manchester virus), which were classified further into GI/1 (33.3%), while other uncommon genotypes, i.e., GI/4, GI/5, GI/6, and GI/7 were also identified. The SVGII (represented by London virus) was detected in 23.5% of which two were GII/1 and another two were GII/4. In addition, one belonged to GIV (represented by Hou7-1181 virus) (Phan et al., 2004; 2005). In 1999 to 2000, another study in Vietnam, reported that SV was detected at 0.2% from infants and children with acute gastroenteritis. SV strain detected was further classified into GI (Manchester virus) (Hansman et al., 2004a). Between 1990 and 2000, in Tokyo, Japan, SV was detected in 2.3% among infants with acute gastroenteritis in day care center, both isolates were GIV genogroup (Akihara et al., 2005). During 2004-2005, in Osaka, Japan, SV was detected at 17.6% of infants and children with acute gastroenteritis. All SV strains detected in the study belonged to GI and were classified further into 4 genotypes, including GI/6, GI/8, GI/1, and GI/4. The SVGI/6 was the most predominant genotype and the epidemic confined within a period of 5 months. Moreover, two strains of SV were the recombinant viruses of GI/8 capsid and GI/1 polymerase genes (Phan et al., 2006).

3. Molecular epidemiology of Human Astrovirus (HAstV)

HAstVs were discovered in fecal specimens from children with diarrhea by EM in 1975 (Appleton and Higgins, 1975; Madeley and Cosgrove, 1975). HAstV infections have been found worldwide, primarily in young children with diarrhea (Ashley et al., 1978; Matsui et al., 2001; Walter and Mitchell et al., 2003). However, sporadic outbreaks of gastroenteritis caused by HAstV have also been reported among elderly patients (Oshiro et al., 1981; Lewis et al., 1989; Matsui et al., 1994). Recently, the molecular methods and nucleotide sequencing have been developed for the detection and characterization of HAstV (Sakamoto et al., 2000; Lukashov and Goudsmit, 2002). Human Astrovirus serotype 1 (HAstV-1) is the strain detected most frequently worldwide, HAstV-2 to HAstV-4 are common, and HAstV-5 to HAstV-7 are less common, whereas HAstV-8 is rare as compared to the other serotypes (Glass et al., 1996; Gaggero et al., 1998; Mitchell et al., 1999; Pang and Vesikari, 1999b; Guix et al., 2002). According to several reports, HAstV infections in different geographical areas are detected in different seasons, e.g. in winter in Australia (Palombo and Bishop, 1996), spring in Japan, which peaks in March and April (Utagawa et al., 1994), and rainy season in Guatemala (Cruz et al., 1992), etc.

In Arizona, USA, between 1981-1983, Lew et al. (1991) had identified HAstV at 4% among children with diarrhea in day care center, in which HAstV detection rate in children under 18 months of age was significantly greater than in older children. In Middle America, the study conducted in a rural community in Guatemala among children <1-3 years old, 7.3% of diarrheal episodes were associated with HAstV (Cruz et al., 1992). During 1994 to 1995, a study in Mexico revealed that the prevalence of HAstV infection in children under 5 years of age with diarrhea was

4.6%; most predominant genotype was AstV-1 (29%), followed by S3, S8, S2, and S4 (Méndes-Toss et al., 2004). The other study in Mexico, Maldonado et al. (1998) had reported the detection of HAstV 61% among infants enrolled in an oral polio vaccine immunogenicity study.

In Europe, the incidence of HAstV infection was 7.3% among children less than 5 years of age with diarrhea in Ireland (Foley et al., 2000). In the Netherlands, seroprevalence of HAstV was investigated among people of all age groups, revealed that overall percentage of persons with neutralizing antibodies to HAstV was highest for HAstV-1 (91%), followed by S3 (69%), S4 (56%), S5 (36%), S2 (31%), S6 (16%), and S7 (10%), respectively (Koopmans et al., 1998). In Hungary, Jakab et al. (2003) reported that 5 genotypes/serotypes of HAstVs were circulating among hospitalized children with gastroenteritis during a 4-year period of 1995-1999. HAstV-1 was the most predominant serotype detected (76%) throughout a 4-year period, but other genotypes were also detected, including S5 (8%), S8 (8%), S3 (4%), and S4 (4%). Later, the following-up study in Hungary in 2002 by the same investigators (Jakab et al., 2004) demonstrated that HAstV-1 remained the most predominant genotype (50%), followed by S4, S3, and S8, but S5 was not detected.

In South Africa, twenty two HAstV-positive samples collected between 1996-2000 were classified and the distribution of serotypes/genotypes of these HAstV strains were as following: S1 (63%), S3 (13%), S5 (8%), S6 (8%), and S8 (8%), except S2, S4, and S7 were not found in the study (Nadan et al., 2003).

In Melbourne, Australia, thirteen HAstV-positive samples from infants hospitalized with diarrhea between 1980-1985 were retrospectively identified and found that S1 strains were the most common serotype. In 1995, HAstV was detected

in 4.2% in children under 5 years of age hospitalized with diarrhea. S1 strains predominated again, and S4 strains were also detected (Palombo and Bishop, 1996). In Sydney, the total of 464 specimens from children aged less than six years presenting with gastroenteritis were investigated for HAstV infection during 1997-1998. HAstV-1 (57.9%) strains predominated ,particularly in winter and spring of 1997, followed by S4 strains (36.8%) were mostly detected between January to June 1998, and a single S3 strain (5.3%) was found in winter 1998 (McIver et al., 2000).

In Japan, Konno et al. (1982) first described an outbreak of HAstV infection among children and teachers of a kindergarten. In 1991, one remarkable large outbreak had occurred in Osaka prefecture, in which more than 4000 school-children, teachers, and cooks were affected. In that outbreak, the causative agent was HAstV as determined by EM, PCR, and EIA (Oishi et al., in press). From 1982 to 1991, a total of 1720 specimens were collected and 10 specimens from each year were randomly selected for detecting of HAstV. Of the 100 specimens from patients with sporadic cases of diarrhea tested by EIA, 10% were positive for HAstV. HAstV was most frequently detected in infants less than 1 years of age (Utagawa et al., 1994). Sakamoto et al. (2000) had identified HAstV in 5.9% of children with diarrhea in Japan between 1995-1998 which could be classified into S1 (80.5%), S3 (17.1%), and S4 (2.4%). During 1990-1994, the study in Pakistan showed that HAstV infection was 11.2% among children with acute gastroenteritis. HAstV-1 was the most frequenctly detected and occurred during September and October 1990 (Phan et al., 2004). In 2000, the first report of HAstV in Korea revealed that HAstV infection had occurred 1.5% among children and adults with gastroenteritis. The predominant

genotype/serotype was HAstV-1 (60%) with intragenotypic variation of 0.7% (Kang et al., 2002).

4. Molecular epidemiology of NV, SV, and HAstV in Thailand

Epidemiological studies of NV, SV, and HAstV are less frequently reported than those of RV. In 1980, Echeverria and colleages (1983) had conducted a serological study in Thailand and revealed that 70% of children resided in the northeastern area had already acquired antibody to Norwalk virus during the first 4 to 5 years of age. During January, 1982 to May, 1983, the small round virus-like particles (or NV) were first detected by EM in stools of diarrheal children aged 1 month to 5 years who admitted to two hospitals in Bangkok (Wasi et al., 1984). In 1990, Herrmann et al. (1991) used an indirect double-antibody ELISA with monoclonal antibody to detect HAstV in stool specimens from diarrheic infants and children under 5 years of age who attended to the clinics in Bangkok and HAstVs were detected at 8.6%. Later, in 1991, Echeverria et al. (1994) reported the detection of HAstV in stool specimens of children under 4 years of age who admitted to hospital and clinics with acute diarrhea in the western part of Thailand; HAstV was identified in 14% of diarrheic children. Gungtapong et al. (2004) had detected NV (14%), SV (11%), and NV/SV (4%) mixed infections in children hospitalized with acute gastroenteritis collected from 4 regions of Thailand. Then, they analyzed the partial capsid sequences of NV or SV and revealed that the detected NV strains were classified into GII, in which the majority of them belonged to NVGII/4 (64%), while GII/1, GII/3, GII/6, and GII/New (recently classified into GII/16 by Okada et al., 2005) were also detected with low frequency. Most of SV strains (83%) belonged to

SVGI. Moreover, they also found one strain belonged to SVGII new cluster and another one SV strain that represented a novel genogroup (GV) within the SV genus. In 2004, a report from Bangkok demonstrated the relevance of HAstV as a cause of neonatal gastroenteritis outbreak occurred in nursery of a maternity ward of Ramathibodi Hospital. HAstV was detected, by ELISA, in 4 of 13 (30.7%) of diarrheic neonates and in 1 member of nursery staff who had diarrhea (Sirinavin et al., 2006). The preliminary study of NV and SV infections among infants and children under 5 years of age had performed in Chiang Mai in 2002 (Hansman et al., 2004b). NV and SV were found at 7.6% and 3.8%, respectively, and 0.95% was NV/SV mixed infection. Most of NV belonged to NVGII, including GII/1 (3), GII/3 (1), GII/10 (1), and GII/new (1) whereas the remaining 3 strains belonged to NVGI including GI/3, GI/8, and GI/9. In addition, SV strains detected in this study were SVGI and SVGII.