CHAPTER II

LITERATURE REVIEW

Acute gastroenteritis or acute diarrhea is an inflammation of the gastrointestinal tract involving both the stomach and small intestine. The spectrum of etiologic agents includes a variety of enteric bacteria, parasites, and viral pathogens (Tauxe et al., 2002). Most reports demonstrated that pathogenic enteric bacteria were associated with acute gastroenteritis, however with the recent improvement of molecular techniques, a large proportion of disease is related to the presence of gastroenteritis viruses (Fodha et al., 2006). Four major categories of viruses are now recognized as causing agents of acute gastroenteritis in infants and young children worldwide. These are rotaviruses, caliciviruses, astroviruses, and adenoviruses (Lyman et al., 2009).

A. Rotaviruses

1. Virion structure and genomes

Rotaviruses (RVs) are members of the *Rotavirus* genus of the *Reoviridae* family. The virus particle is an icosahedral, non-enveloped virion with the size of 70-75 nm in diameter that has a characteristic wheel-like appearance (Latin, rota means wheel) as observed under an electron microscope (Figure 1). The viral capsid consists of a double protein layer. The outer capsid is composed of two structural proteins VP7 and spike-like VP4, and the inner capsid is formed exclusively by the most abundant protein of the virus, VP6, which surrounds the core.

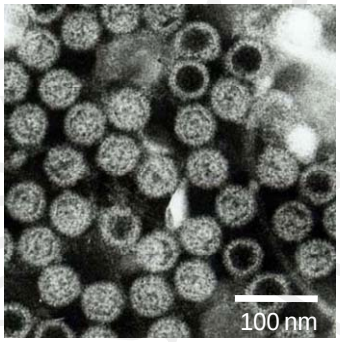

Figure 1 Negatively stained electron micrographs of rotavirus virions. (Adopted from http://web.centre.edu/bio/richey/ rotavirus.jpg)

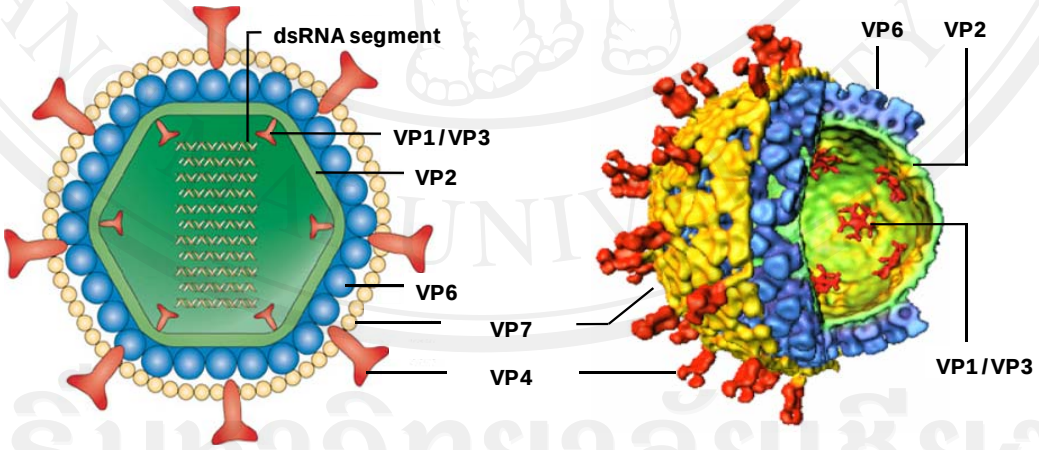

Figure 2 A schematic representation of a rotavirus virion (Left) and a cut-away of the viral structure (Right). (Adapted from Estes et al., 2001; Angel et al., 2007)

The core is comprised of VP2, which encloses two minor structural proteins (VP1 and VP3) and the viral genome of 11 segments double-stranded RNA (dsRNA), as shown in Figure 2. All of 11 segments of dsRNA genome have the molecular weight (MW) range from $2x10^5$ to $2.2x10^6$ daltons with the sizes of 0.6 to 3.3 kilobasepair (kb). A migration profile of these 11 segments in polyacrylamide gel electrophoresis, termed electropherotype, is widely used to characterize rotaviruses from stools and cell culture (Kapikian et al., 2001). Three markedly distinct profiles, long, short, and super-short RNA patterns are identified based on the relative migration rates of gene segments 10 and 11. In some human rotavirus strains, the RNA segment 11 migrates more slowly than usual and is thus located between segments 9 and 10, yielding a “short pattern”. Similarly in some rotavirus strains the segments 10 and 11 migrate faster, and therefore, yielding a “long pattern”. Moreover, several “super short” patterns of RNA migration, which the segment 11 migrates even more slowly than a short pattern, have been observed in the viruses recovered from humans, bovines, porcines, and lapines (Estes, 2001; Kapikian et al., 2001). The rotavirus genome segments encode 6 structural viral proteins (VPs); VP1, VP2, VP3, VP4 (VP5+VP8), VP6, and VP7, and 6 non-structural proteins (NSPs); NSP1, NSP2, NSP3, NSP4, NSP5, and NSP6. The VPs are found to make up virus particle whereas the NSPs are involved in some aspects of viral replication cycle or interact with host proteins to influence pathogenesis or immune response to infection. The functions of all proteins are summarized in Figure 3.

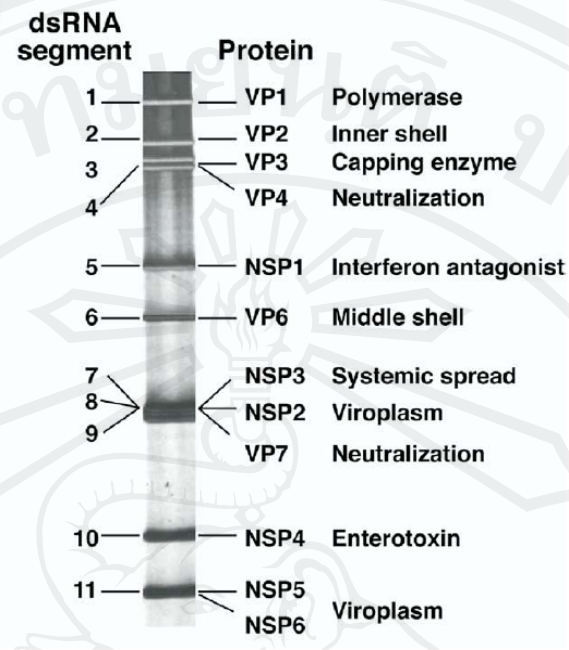

Figure 3 The viral genome of 11 segments of dsRNA is analyzed by polyacrylamide gel electrophoresis (PAGE). Each gene codes for at least one protein as shown with at least one major function of the protein indicated (Estes et al., 2001).

2. Classification

Rotaviruses are classified based on four important antigenic specificities: group, subgroup (VP6 genogroup), genotype, and NSP4 genetic group. The inner capsid protein VP6 allows rotavirus classification into seven distinct groups, A, B, C, D, E, F, and G. Among seven groups (A to G), group A rotavirus is recognized as the most common group that found in humans and in a wide range of mammalian and avian species with diarrhea (Estes et al., 2001). In addition, the VP6 protein also contains the subgroup specificities that allows the classification of group A rotaviruses into subgroups (SG), SGI, SGII, SG (I+II), and SG non-(I+II), as determined by reactivity with SG-specific monoclonal antibodies (MAbs) (Estes and

Cohen. 1989). More recently, molecular characterization based on VP6 nucleotide sequence analysis, only two VP6 genogroups are distinguished among group A rotaviruses, VP6 genogroup I (GI) and VP6 GII. All of the strains determined as SGI by ELISA were clustered together into GI cluster whereas the strains of SGII, SG (I+II), and SG non-(I+II) belonged to GII cluster. (Iturriza Gómara et al., 2002; Thongprachum et al., 2009).

The two outer capsid proteins, VP4 and VP7, are considered to be relevant to immune protection and vaccine development since both proteins elicit the production of neutralizing antibodies. Based on a dual classification system, rotaviruses are classified into G-serotypes (G stand for glycoprotein) and P-serotypes (P stand for protease-sensitive) as determined by specific monoclonal antibodies (mAbs) to VP7 and VP4 proteins, respectively. However, with this antigenic characterization (serotyping) is time-consuming and required virus collection and proper immunological reagents that are not available in all laboratories, (Estes et al., 2001) and therefore replaced by G- and P-genotypes which are classified according to the genetic diversity of VP7 and VP4 genes, respectively. More recently, a novel classification system based on the nucleotide sequences of all rotavirus gene segments has been proposed (Matthijnssens et al., 2008). Based on nucleotide identity cut-off percentages, the strains under investigation that shared above the cut-off value of that gene segment (Table 1) are considered to be the same genotype, whereas the strains that shared nucleotide sequence identity below the cut-off value are considered to be different genotype. Currently, 23G-genotypes and 31P-genotypes of rotaviruses have been identified from humans and various animal species (Schumann et al., 2009; Ursu et al., 2009).

Table 1 A summary of nucleotide percent identity cut-off values defining genotypes for 11 rotavirus gene segment (Matthijnssens et al., 2008)

Gene product	Percent identity Cut-off value (%)	Genotypes	Name of genotypes
VP7	80	19G	**G**lycosylated
VP4	80	27P	**P**rotease-sensitive
VP6	85	11I	**I**nner capsid
VP1	83	4R	**R**NA-dependent RNA
VP2	84	5C	**C**ore protein
VP3	81	6M	**M**ethyltransferase
NSP1	79	14A	Interferon **A**ntagonist
NSP2	85	5N	**N**TPase
NSP3	85	7T	**T**ranslation enhancer
NSP4	85	11E	**E**nterotoxin
NSP5	91	6H	p**H**osphoprotein

Group A rotavirus is generally described by dual classification system with G-type and P-type. However, the nonstructural glycoprotein NSP4 has also been studied extensively because of its multiple functions that involve in rotavirus morphogenesis, pathogenesis, and its enterotoxic activity (Estes et al., 2001). Analysis of NSP4 gene of human and animal rotavirus strains revealed the presence of six distinct NSP4 genetic groups (genogroups), KUN (A), Wa (B), RRV (C), Murine (D), Evian (E), and Porcine (F) (Cunliffe et al., 1997; Horie et al., 1997; Kirkwood et al., 1997; Ciarlet et al., 2000; Mori et al., 2002; Khamrin et al., 2008).

3. Global distribution of human rotaviruses

Based on several epidemiological studies, it is well-documented that all seven groups (A to G) of rotaviruses have been found to associate with infection in various animal species, but only group A, B, and C are associated with diarrhea in humans. Group A rotavirus is considered to be the main cause of viral gastroenteritis in infants and young children worldwide. In contrast, group B and C rotaviruses that confirmed as human pathogens have only rarely been identified in several areas of the world. Group B human rotavirus was first described in waterborne outbreaks in China, involving the cause of nationwide epidemics of diarrhea in adults (Adult diarrhea rotavirus: ADRV) as well as in children (Su et al., 1986). Group C rotavirus was first detected from piglet in 1980 (Saif et al., 1980) and has been responsible for some outbreaks occurred mainly in animals and affected humans occasionally.

On the basis of the dual classification (G- and P-types) of group A rotaviruses, numerous surveillances demonstrated that different G- and P-genotypes are predominant in particular area and the genotypes have changed overtime (Khamrin et al., 2006a; Rahman et al., 2007). The most frequent strain circulating worldwide is G1P[8], and the most common G-genotypes are G1, G2, G3, G4, and G9 in combination with P[4] and P[8] genotypes (Jin et al., 2008). Other genotypes which are relatively rare in human such as P[6] in combination with G1, G2, G3, and G4, P[9] with G1 or G3, P[4] with G1 or G4, P[8] with G9, and P[3] with G1 or G3 have also been reported (Santos et al., 1994; Ushijima et al., 1994; Silberstein et al., 1995; Steele et al., 1995). In recent years, epidemiological studies designed to monitor the appearance of novel or unusual strains of rotaviruses have been intensified throughout the world. Accordingly, the increasing data of atypical strains of rotaviruses isolated

from humans that share genetic and antigenic features of the viruses of different species have been reported. Those of rotavirus strains are likely represent the reassortants between human and animal rotavirus strains or even the viruses that directly transmitted from animal to human, including G3P[3], G3P[9], G3P[10], G3P[19], G5P[6], G6P[14], G10P[14], G11P[25], and G12P[8] (Maneekarn et al., 2006; Uchida et al., 2006; Khamrin et al., 2006b, 2007b, 2009a; Duan et al., 2007; Ghosh et al., 2007; Steyer et al., 2007).

In Thailand, the study of rotavirus infection was initially performed since 1979 (Maneekarn et al., 1980; Louisirirotchanakul et al., 1984). Later, rotavirus surveillance study has been reviewed by Maneekarn and Ushijima (2000). The overall picture of rotavirus infection in Thailand during the study period of 1977-1997 revealed that the peak seasonal distribution of rotavirus infection occurred in dry cool seasons, October to February. Rotaviruses were responsible for about 27% to 34% of diarrhea in hospitalized cases, of which G1 was the most predominant serotype in Thailand, followed by G2, G4, and G3, respectively. At least three G-serotypes, mostly G1, G2, and G4, were seen to coexist in Thailand each epidemic year, and in some studies all four G-serotypes were reported. Interestingly, although G1-G4 have been reported, G9 turned out to be the fifth most common in the later years. According to the study in 2002, Jirapongsa et al. (2005) had demonstrated that approximately half of rotavirus-positive samples were of genotype G9. Simultaneously, G9 has also been recognized as one of the most widespread emerging genotypes in several regions around the world, such as US, Australia, UK, Brazil, Africa, and Thailand (Griffin et al., 2000; Iturriza-Gomara et al., 2000; Zhou et al., 2001; Kirkwood et al., 2002; Santos et al., 2003; Steele et al., 2003). In Chiang Mai,

Thailand, the pattern of G9 distribution during 1989 to 2005 has been described by Khamrin et al. (2006a, 2009b). It was demonstrated that the G9 rotavirus in Thailand was first detected in year 1989 with a low prevalence rate, but in the year 2000 to 2002, it turned out to be the most predominant genotype which reached a peak at 100% of diarrhea cases in 2002. However, G9 rapidly decreased in the next three consecutive years and disappeared in 2006 (unpublished data). In addition, G2 reemerged in the epidemic season of 2003, whereas G1 became the most predominant circulating genotype in 2004 (Khamrin et al., 2007b). Overall, several G-genotypes of rotaviruses (G1-G4, G9) are circulating among pediatric patients in Chiang Mai each epidemic year for a long time and two P-genotypes, P[4] and P[8], were seen mostly to associate with those of G-genotypes with various combination patterns.

B. Caliciviruses

Human caliciviruses (HuCVs) are one of the leading etiologic agents of acute gastroenteritis in all age groups, and have been classified into two genera, noroviruses (NoVs) and sapoviruses (SaVs), based on the morphology and genome organization (Green et al., 1995).

1. Noroviruses

1.1 Virion structure and genome

Noroviruses (NoVs), formerly known as Norwalk-like virus (NLV), derived its name from an outbreak of ‘winter vomiting disease’ in 1968 at an elementary school in Norwalk, Ohio, US (Adler et a., 1969). The virus was originally discovered in fecal specimens obtained from infected volunteers by using immune electron microscopy (IEM) (Kapikian et al., 1972). The prototype strain Norwalk virus has its

unique appearance with a tiny size of 27 nm in diameter which also recognized as the small round structure virus (SRSV), as shown in Figure 4.

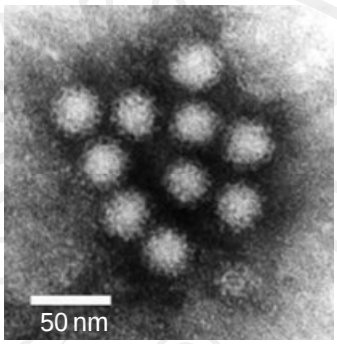

Figure 4 Negatively stained electron micrographs of norovirus virions. (Adopted from http://image3.examiner.com/image/blog/EXID31031/images/Norwalk_Norovirus.jpg)

NoVs are members of the genus *Norovirus*, family *Caliciviridae.* The viral particle is an icosahedral, nonenveloped virion with the size of 27-34 nm in diameter. A key characteristic of the calicivirus is the existence of 32 cup-shape depression on the surface of virus particle (Latin, calyx means cup or calici). The genome of NoV is a positive sense single-stranded RNA (ssRNA) of 7.6 kb, and is composed of three open reading frames (ORFs) includes ORF1, 2, and 3 which encode both the structural and non-structural proteins, as shown in Figure 5A.

ORF1 encodes a polyprotein, which is autoprocessed by a virally encoded protease to yield the non-structural viral replicase protein essential for replication. ORF2 encodes the major structural capsid VP1 protein, whereas ORF3 encodes for a minor basic structural VP2 protein that has been hypothesized to function the packaging the genome into virion (Glass et al., 2000). Further analysis of the VP1

major capsid protein has shown that it consists of two major domains, shell domain (S) and 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 NoV. The P domain which is further divided into subdomain, P1 and P2, forms the arch-like capsomer that protrude from the virion and amino acid of this domain contains the most variable region of the capsid protein, as shown in Figure 5B.

1.2 Classification

In the past, classification of NoV was based on cross-challenge studies in volunteers and cross-reactivity analysis by immune electron microscopy (Okada et al., 1990). Due to the fact that NoVs are genetically and antigenically diverse, these antigenic classification scheme had poor accuracy which were attributed to the cross-reactivity of antibodies. Consequently, reverse transcription-polymerase chain reaction (RT-PCR) and genomic sequencing have become the major means for characterizing the virus and to understand the relatedness of different strains (Ando et al., 2000). Based on DNA sequencing and phylogenetic analysis of the capsid region (ORF2), NoVs are classified into five distinct genogroups (GI, GII, GIII, GIV, and GV). Within each genogroups, NoV strains are subdivided further into several different genotypes, and at least 15 genotypes of GI, 18 genotypes of GII, 2 genotypes of GIII, and only one genotype of each GIV and GV have been identified (Shiota et al., 2007). Although NoV strains of three genogroups, GI, GII, and GIV, are found to infect humans, there is a porcine-specific virus within the GII genogroup (GII/11, GII/18, and GII/19), while GIII and GV are also found to infect cows and mice, respectively (Patel et al., 2009).

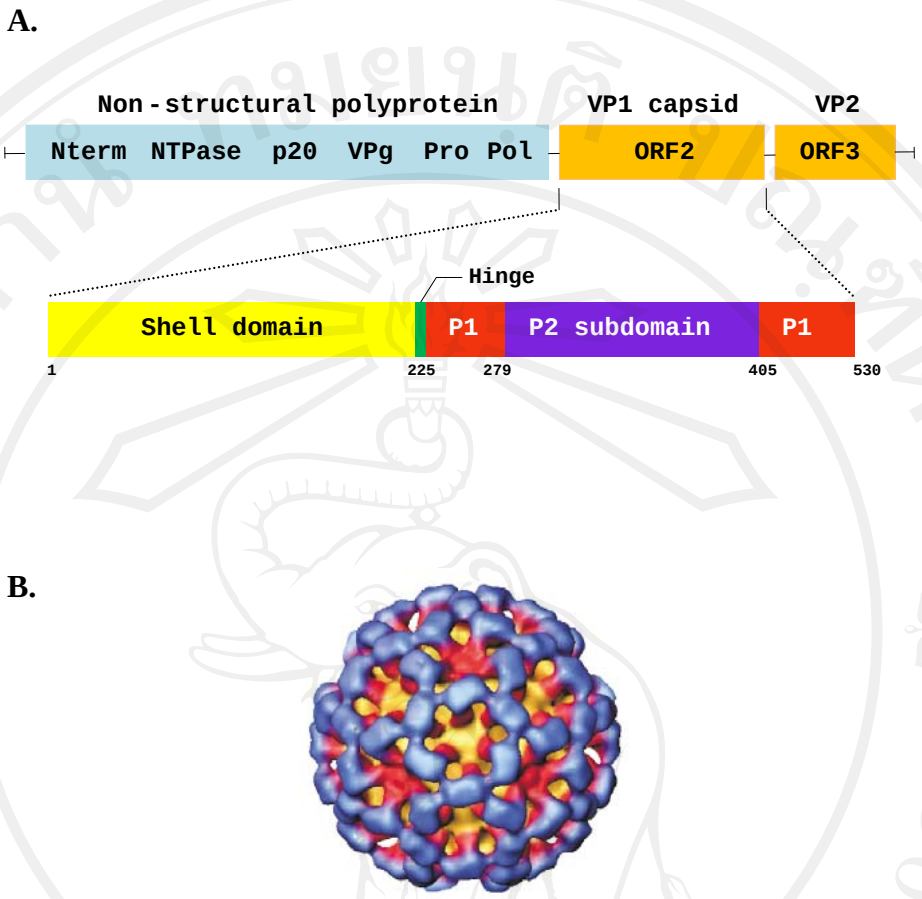

Figure 5 The norovirus genomic structure and capsid domain and the NoV-like particles structure observed by cryoelectron microscopy. (A) The norovirus genome is comprised of three open reading frames (ORFs), ORF1 encodes the non-structural protein (light blue), and ORF2 and ORF3 encode the structural proteins (yellow), including major VP1 and minor VP2 structural proteins. The non-structural polyprotein is processed into six mature proteins, includes N-terminal protein (N-term), nucleoside triphosphatase (NTPase), a protein of unknown function (p20), viral protein genome-linked (VPg), 3C-like proteinase (Pro), and viral RNA dependent RNA polymerase (Pol). The major capsid protein is further divided into the shell (s) and protruding (P) domains (P1 and P2). (B) Three-dimentional structure of NoV-like particle showed the S domain (yellow) forms the continuous surface, and the P domain; P1 and P2 (red and purple, respectively) that emanates from the S surface. (Adapted from Hutson et al., 2004; Donaldson et al., 2008)

1.3 Molecular epidemiology of noroviruses

Among different types of gastroenteritis viruses, NoV is also considered to be a significant global enteropathogen, being a major cause of acute gastroenteritis worldwide in all age groups (Phan et al., 2007c). Common-source of outbreaks frequently occur via ingestion of contaminated food, water, or by person-to-person transmission in semiclosed communities that posed an important public health problem (Kageyama et al., 2003). The CDC reported the sites of NoV outbreaks during January 1996-November 2000, as follows, 39% occurred in restaurants, 29% in nursing homes and hospitals, 12% in schools and daycare centers, 10% in vacation areas and cruise ship, and 9% in other settings (Parashar et al., 2001). During 1990-2005, data collected on foodborne outbreaks with a known food source indicated that several major outbreaks have been traced to consumption of uncooked and ready-to-eat foods, such as sandwiches, salads, fruits, vegetables, and shellfish (DeWaal et al., 2007). The epidemiological studies in various countries have shown that the detection rate of NoV infection appeared to vary among different geographical regions which was ranged from 3% to 60% or more, such as, 56% in Spain, 36% in UK, 43% in Hungary, 44.9% in Germany, and 4.5% in Bangladesh (Buesa et al., 2002; Lopman et al., 2003; Reuter et al., 2005; Ike et al., 2006; Dey et al., 2007). Those data revealed that NoV GI and GII together with more than 25 different genotypes that comprise these two genogroups account for the majority of human NoV cases in all parts of the world. Therefore, several genotypes have been shown to co-circulate, and GII/4 is the most prevail genotype while GI outbreaks have been occurred less frequently than GII (Ike et al., 2006). Regarding NoV GII/4 outbreaks, it is interesting to note that three epidemics have been recorded in 1995/6, 2002, and

2004 which were associated with the emergence of GII/4 viruses over a vast geographic range. NoV GII/4 outbreak was first recognized in the mid-1990s (Noel et al., 1999), and new variants of the GII/4 genotype have been detected in numerous populations. During1995-1996, strain US95/96 (subsequently classified as GII/4) was responsible for about 55% of the outbreaks in the US and 85% of the outbreaks in Netherlands (Vinje et a., 1997). Interestingly, during 2000-2004, the US95/96 strain was replaced rapidly by two new GII/4 variants. In the United States, Farmington Hills strain (subsequently classified as GII/4) was ultimately associated with about 64% of gastroenteritis occurred on cruise ship outbreaks and 45% in land-base outbreaks (Widdowson et al., 2004). Simultaneously, in Europe another GII/4 variant, GII/4b emerged and cause outbreaks during the winter, spring, and summer (Lopman et al., 2004). Especially in winter period of 2002-2003, the surveys conducted in the US and Europe reported that the NoV outbreak was increased and reached the highest on record (Ike et al., 2006; Norovirus-activity 2003; Vainio et al., 2006). Later in 2004, the Hunter GII/4 variant was first detected in Australia, and subsequently identified as the etiologic agent in large epidemic of gastroenteritis in various countries of Europe and Asia in 2004-2005 (Bull et al., 2006). However, this strain was replaced by three new co-circulating strains. One of these was Sakai GII/4 variant which was particularly associated with outbreaks in healthcare facilities in Southeast Asia (Okada et al., 2006), although the other two GII/4 strains Ehime and Chiba that cluster with Sakai have also been identified in the US and the Netherlands (Widdowson et al., 2004; Kroneman et al., 2004). The second and third strains of 2006 outbreak were Minerva and Laurens, which were as high as 76% (87 0f 114) of NoV detected from outbreaks in the US (Vinje et al., 2004).

In recent years, the changing pattern of genotypic distribution of NoV infection in children with acute gastroenteritis has been demonstrated in several countries, and some findings seemed to be more complicate than those of previous studies. For instance, in Japan, during 2005-2006 study period, Phan et al. (2007c) had reported that GII/3 was the most prevalent with a high frequency of 52.9% compared to the lower frequency GII/4 of 37.2% and GII/6 of 3.9%, which were the second and third prevailing genotypes, respectively. Additionally, there was a report described the multiple genotypes infection of NoV, all from shellfish-related specimens of the outbreak in 1999. Kageyama et al. (2004) had demonstrated that among four specimens obtained from four patients, one contained four genotypes including, GI/8, GII/4, GII/6, and GII/15. Another also contained four genotypes including, GI/2, GI/4, GI/5, and GII/15. The other two contained double genotype infection of GI/5 and GII/15, and GII/5 and GII/15, respectively.

In Thailand, even though few molecular epidemiological studies of caliciviruses have been conducted, various genotypes have been reported in different epidemiological settings. A survey in Chiang Mai, Thailand, from 2000 to 2002 demonstrated that NoVs co-circulated in this area in children admitted to hospital with diarrhea at 8.1% (Malasao et al., 2008). In that study, both NoV GI and GII have been detected with the prevalence rate of 29.2% and 70.8%, respectively. Among NoV GII strain, approximately half of them belonged to GII/4, followed by GII/3, GII/10, GII/1, GII/6, GII/8, and GII/15. During 2002-2004, the prevalence of NoV increase from 8.1% to 14.1%. Only NoV GII has been detected and GII/4 is the most predominant genotype, followed by GII/3, GII/1, GII/7, GII/2, and GII/16 (Khamrin et al., 2007c). One year later, the detection rate of NoV in Chiang Mai decreases from

14.1% to 6.8%. The GII/4 is still the most common genotype that had been found along with other genotypes, GII/15, GII/6, and GII/12 (Khamrin et al., 2010). Recently, survey conducted by Kittigul et al. (2010) has been demonstrated that NoV was detected with high frequency of 44.7% during 2006-2007 in Lopburi province and NoV GII/4 was the most predominant circulating genotype, followed by GII/3, GII/6, GII/17, and GII/2. As for GI strain, it is well-established that the NoV GI infections have been widely observed with sporadic cases of acute gastroenteritis and cause by miscellaneous genotypes which vary among different regions. In Thailand, the strains of NoV GI that have been published are comprised of GI/1, GI/2, GI/3, GI/4, GI/6, GI/7, and GI/13 (Malasao et al., 2008; Kittigul et al., 2010).

2. Sapoviruses

2.1 Virion structure and genome

As mentioned above, the caliciviruses derived from humans have been classified into two distinct types, the small round structured virus (SRSV) and the classical calicivirus, on the basis of their morphological features. The SRSV, currently known as norovirus, was found for the first time in the feces of a child with gastroenteritis in 1972 by electron microscopy (EM), as mentioned previously in B1. The classical calicivirus was originally discovered in an outbreak of gastroenteritis in an orphanage in Sapporo, Japan in 1977 (Chiba et al., 1979) that has a typical shape known as a “Star of David-like structure” (Figure 6). This virus is designated Sapporo virus (Hu/SV/Saporo virus/1977/JP) which currently known as the prototype strain of human sapoviruses. Sapovirus particle 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 is typically 41-48 nm in diameter with 32 cup-shape depressions and ten spikes on the outline which presented as a six-pointed star, as shown in Figure 7.

Sapoviruses (SaVs), formerly known as Sapporo-like viruses (SLVs), are the members in the genus *Sapovirus* within *Caliciviridae* family as NoV. SaV contains a positive sense single-stranded RNA (ssRNA) genome of approximately 7.5 kb surrounded by an icosahedral capsid. The genomic organization of SaV, which is distinct from that of NoV, contains either 2 or 3 main open reading frames (ORF1-ORF3). The SaV genogroup I (GI), GIV, and GV genomes, each is predicted to contain three main ORFs, whereas the SaV GII and GIII genome each has only two ORFs. ORF1 encodes a polyprotein that undergoes protease processing to produce several nonstructural proteins, including RNA-dependent RNA polymerase and a major capsid protein (VP1). ORF2 encodes a small basic protein, which is believed to be similar to VP2 of NoV (Hansman et al., 2007), and ORF3, which is an ORF overlapping with the 5'-end of the capsid gene (found only in the SaV GI, GIV, and GV), encodes proteins of yet unknown function (Schuffenecker et al., 2001), as shown in Figure 8.

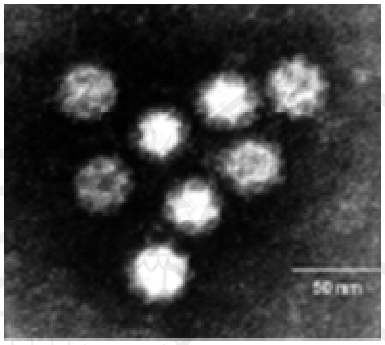

Figure 6 Negatively stained of electron micrographs of sapovirus virions. (Adopted from http://www.clinical-virology.org/gallery/images/em/sapovirus.gif)

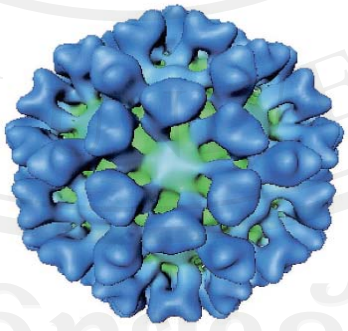

Figure 7 Three-dimensional structure of sapovirus particle (the Parkville virus rVLP, represented SaV GI) (Chen et al., 2004).

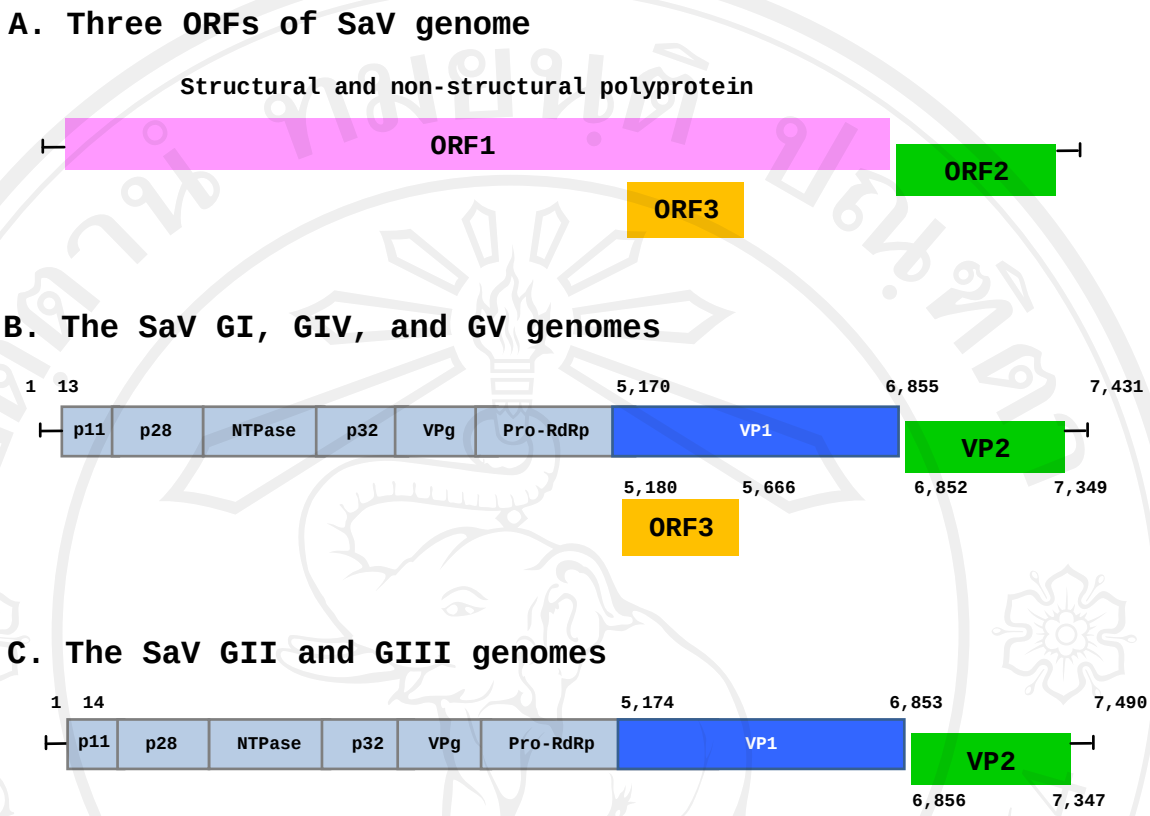

Figure 8 Genomic organization of sapoviruses. (A) The genomic organization of SaV are predicted to contain either two or three main ORFs, ORF1, OFR2, and/or ORF3. (B) Within the SaV genome, the main polyprotein genes, including 2C-like NTPase (NTPase), Vpg, 3C-like protease (Pro), and 3D-like RNA-dependent RNA polymerase, are fused with the major capsid gene (VP1) in a single ORF (ORF1). In addition, there are ORF overlapping capsid gene (capsid overlap), ORF3, contained only in the SaV GI, GIV, and GV genome. However, (C) the SaV GII and GIII genomes contain two ORFs include ORF1 and ORF2. (Adapted from Hansman et al. 2007)

2.2 Classification

According to the same classification system as that of NoV, SaV was initially classified based on the differences of the complete capsid amino acid sequences, into three major genogroups (G), GI (known as the Manchester virus), GII (known as the

London virus), and GIII (known as the porcine enteric calicivirus, PEC) (Schuffenecker et al., 2001). In 2004, two SaV strains, Hou7-118/90 and Argentina39, were identified and grouped into the new genogroups, GIV and GV, respectively. Moreover, another two SaV strains (Mex340/90 and Cruise ship/00) were also identified and grouped into the two new genetic cluster/genotypes within the London/92 genogroup (GII) (Farkas et al., 2004). On the basis of nucleotide sequence analysis of the capsid gene, SaV is divided into five genogroups, GI, GII, GIII, GIV, and GV. The SaV GI, GII, GIV, and GV strains infect humans, whereas GIII strains infect porcine species (Farkas et al., 2004; Wang et al., 2006; Hansman et al., 2007). Furthermore, those genogroups can be subdivided into genotypes and at least 8 in GI, 5 in GII, and 1 each in GIII, GIV, and GV have been identified (Akihara et al., 2005a).

2.3 Molecular epidemiology of sapoviruses

Epidemiological studies conducted in several countries, such as Australia, Canada, Finland, France, Japan, Mexico, Taiwan, Thailand, UK, US, and Vietnam revealed that SaV appears generally in sporadic cases in the community with no seasonality reported. The detection rate of SaV infections varies in each country and setting which are usually much less frequent than NoV (Hansman et al., 2007). In US, during 1997-1999, Zintz et al., (2005) had reported that SaV was detected at 1.4% in children hospitalized with acute gastroenteritis. Approximately half of the SaV sequences belonged to the London/92 virus (SaV GII), and the remaining belonged to the Manchester virus and Parkville virus (SaV GI). At the same time, the first report from Argentina, during 1997-1999, revealed that the SaV detected also belonged to the London/92 virus (SaV GII) and the Manchester virus (SaV GI)

(Bereciartu et al., 2002). Additionally, during 1988-1998, surveys of outbreaks and sporadic cases of SaV infection conducted in Europe (UK, Sweden, and Netherland) have been published. It was demonstrated that most of SaV strains belonged to Sapporo/82 virus (SaV GI/1) and the rests belonged to Parkville/94 virus (SaV GI/2), Stockholme/97 virus (SaV GI/3), and London/92 virus (GI/1) (Vinje et al., 2000). In Asia, surveys on diversity of SaV infection showed that SaV GI is detected more frequently than others and SaV GI/1 was the most common genotypes. According to the survey conducted in India, Rachakonda et al. (2008) had reported that all five Indian strains clustered into only one genotype, that was, GI/1. During 2004-2005, in Osaka, Japan, SaV was detected at 17.6% of infants and young children with acute gastroenteritis. All strains detected in that study belonged to GI. Nucleotide sequencing and phylogenetic analysis showed that all of those GI were classified into four distinct genotypes, GI/6, GI/8, GI/1, and GI/4 (Phan et al., 2007a). Even though many of earlier SaV outbreaks due to GI/1 infection, recent reports demonstrated that GI/1 was sometimes replaced by other genotypes. The first SaV outbreak of gastroenteritis in Taiwan reported that eight samples were positive for SaV by RT-PCR, and the phylogenetic analysis of capsid region revealed that all belonged to GI/2 cluster (Wu et al., 2008). In Japan during 2005-2007, the SaV detection rate was unexpectedly high at 19.2% and was found to be the secondary pathogen. Moreover, this surveillance notably demonstrated that the SaV strain belonged to GIV which was suddenly emerged in the year 2007 (Harada et al., 2009). Furthermore, evidence for mixed infections within the same individual or within the same outbreak has been reported. A study of SaV infection in Japan demonstrated that three isolates were positive for SaV, and one carried two strains of SaV GII/2 and GI/1. This was the

first report indicated multiple genogroups and genotypes infection in patients with oyster-associated gastroenteritis (Nakagawa-Okamoto et al., 2009).

In Thailand, the circulation of SaV is diverged genetically with the detection rate ranged from 0.8% to 15%. During 2000-2007, surveys conducted in several regions in Thailand demonstrated that SaV GI strains have been persisted consistently. Within SaV GI cluster, the GI/1 was the most prevalent genotype, while other strains including GI/2, GI/4, GI/5, GII/1, GII/2 GII/3, and GIV, circulating vary from time to time (Guntapong et al., 2004; Hansman et al., 2004; Khamrin et al., 2007c; Malasao et al., 2008; Kittigul et al., 2009; Khamrin et al., 2010).

C. Astroviruses

1. Virion structure and genome

Astroviruses (AstVs) were first described in 1975 during an outbreak of diarrhea and vomit among hospitalized children in Brighton, UK (Appleton and Higgins, 1975). The term ‘astrovirus’ was first used by Madeley and Cosgrove (1975) to describe particles with a star-shaped surface (Greek astron, meaning ‘star’). Negatively stained particles revealed a small, round, non-enveloped with characteristic of five- or six-pointed star-like surface structure, typically 28-30 nm in diameter, as shown in Figure 9.

Astroviruses (AstVs) are the members of the genus *Mamastroviruse* within the family *Astroviridae*. Its genome consists of a positive sense single-stranded RNA (ssRNA) of approximately 6.7 kb organized into three overlapping open reading frames (ORFs), ORF1a, ORF1b, and ORF2. The first two ORFs, ORF1a and ORF1b, which linked by a ribosomal frameshifting occurrence, encode a nonstructural protein,

serine 3C type viral protease (Pro) and viral RNA-dependent RNA polymerase (RdRp), whereas ORF2 encodes the precursor capsid protein of the virus, as shown in Figure 10 (Matsui et al., 2001).

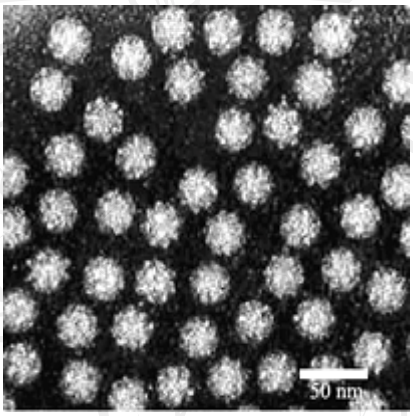

Figure 9 Negatively stained electron micrographs of astrovirus virions. (Adopted from http://www.oardc.ohio-state.edu/lsaiflab/Pictures/Astro%20virus%204x4.jpg)

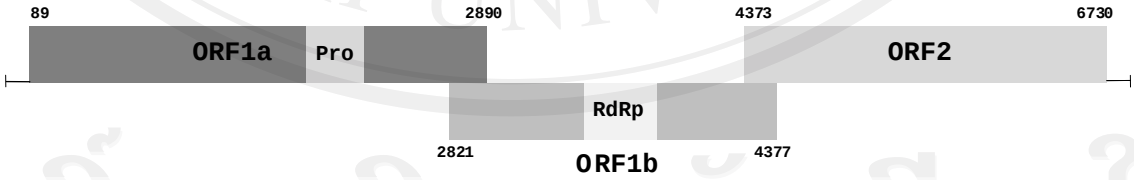

Figure 10 Genomic organization of human astroviruses. Open reading frame (ORF)1a includes serine protease (Pro) and ORF1b has a motif common to RNA-dependent RNA polymerase (RdRp). ORF2 encodes the capsid protein that assembles into the coat of the virus and cleaved to increase infectivity. (Adapted from Walter et al., 2003)

2. Classification

AstV is classified into eight serotypes according to the reactivity of the ORF2 capsid protein with type-specific mAbs. Later, an EIA using mAbs to the AstV antigen was developed for the detection of the virus in stools of patients with acute gastroenteritis (Herrmann et al., 1990). Additionally, AstV can also be classified into genotypes on the basis of partial nucleotide sequence of the capsid gene. Currently, there are eight published genotypes (AstV1-AstV8), which correlate perfectly with those of eight antigenic groups (serotypes) (Sakamoto et al., 2000). Newly, molecular analysis of partial nucleotide sequence at the 5'-end of the capsid gene revealed that AstV1 could be classified into four lineages, including 1a, 1b, 1c, and 1d (Guix et al., 2002; Gabbay et al., 2007).

3. Molecular epidemiology of astroviruses

Early, AstV appeared to be rare cause of acute gastroenteritis and the incident rate of AstV infection never exceed 4% among children suffering from diarrhea, as determined by EM (Kapikian, 1993). Currently, with molecular diagnostic method, AstV are recognized as one of the common cause of viral gastroenteritis in all age groups. However, children, the elderly and those that are immunocompromised are most affected. It is noteworthy that surveys had mainly concentrated on infections amongst children because they were thought to be at greater risk (Billiot et al., 1997). Estimates of the prevalence of AstV infection vary widely from 2% to 16% among hospitalized children with acute gastroenteritis (Oliver and Phillips, 1988), and the peak incidence of AstV infection is found in the winter in temperate climate and in rainy season in the tropical region (Matsui et al., 2001). Among eight genotypes

(AstV1-AstV8), numerous surveillances indicated that Astv1 is the most prevalent circulating strain in both developing and developed countries (Walter et al., 2003). AstV2 to AstV4 are common, and AstV5 to AstV7 are less common, whereas AstV8 is rare detected (Glass et al., 1996; Gaggero et al., 1998; Mitchell et al., 1999). In 2002, however, the surveys from several countries performed in Malawi, Egypt, France, Spain, and Australia clearly demonstrated that AstV8 appeared to be a frequent genotype. The explanations for these findings may be either due to the increased sensitivity of the assays utilized or AstV8 may be a newly emerging type. Additionally, the detection rate of AstV2, AstV3, AstV4, AstV5, AstV6, and AstV7 has also increased (Guix et al., 2002; Cunliffe et al., 2002; Chikhi-Brachef et al., 2002). Remarkably, Maldonado et al. (1998) has reported a high incidence of AstV infection among Mayan infants with the prevalent rate of 61% whereas rotavirus, which is recognized as the most important cause of acute gastroenteritis, was observed less frequently only at 4%. This finding seems to be in conflict with several studies that AstV is less frequently reported than those of RV and caliciviruses.

Based on a few number of reports published, it appears that AstV may be an uncommon cause of epidemic gastroenteritis in adult contacts. The best described outbreaks occurred in Japan. One large outbreak took place in 1991 among students and teachers at 14 primary and junior high schools in Osaka. Oishi et al. (1994) had demonstrated that 10 of 38 stool samples (26.3%) from the outbreak were positive for AstV when tested by a combination of assays, including electron microscopy, monoclonal antibody EIA, and a newly developed polymerase chain reaction test. That outbreak was believed to be caused by contaminated food prepared by a common supplier. However, other smaller disease outbreaks, the survey conducted

by Svenungsson et al. (2000) revealed that the detection rate of AstV was relatively low at 2% among Swedish adults with acute gastroenteritis. Later, Pager and Steele. (2002) examined fecal samples from adults and children with gastroenteritis in South Africa obtained in February 1998 and found that AstV infection in adults was detected at 3.1% which was similar to that reported in the prior group. AstV disease is generally milder than that caused by rotaviruses, however, there have been reported with incidence of severe diarrhea that frequent co-infection of AstVs with rotaviruses and caliciviruses in pediatric patients (Storr et al., 1986; Roman et al., 2003). In addition, it is interesting to note that some studies had been reported co-infection among AstV strains, which provided an opportunity for recombination to occur in a community. During 1989-1992, Walter et al. (2001) had demonstrated the first evidence that recombination occurs among human astroviruses (HAstVs). That was a novel recombinant strain which being closely related to HAstV3 in ORF1b, but closest to HAstV5 in ORF2.

In Thailand, the study of AstV was initially reported by Herrmann et al. in 1991. Serotyping by ELISA using mAbs specific for human astrovirus serotypes 1 to 5 revealed that AstV, in which co-circulated in children with and without gastroenteritis in Bangkok, was detected at 8.6% and 2.1%, respectively. Later, two surveillances performed in Ratchaburi province and Ramathibodi hospital in Bangkok reported that the detection rate of AstV had increased at 14% and 30%, respectively (Echeverria et al., 1994; Sirinavin et al., 2006). Recently, molecular epidemiological study was first described the distribution of AstV genotypes circulating in Chiang Mai, Thailand (Malosao et al., 2008). That study reported the prevalence of AstV at rather low rate of 2% and the strains detected belonged to AstV1, AstV2, AstV3, and

AstV5. However, surveillance study of viral agents associated with gastroenteritis in Lopburi province in 2006-2007, AstV was not detected in that study (Kittigul et al., 2009).

D. Adenoviruses

1. Virion structure and genomes

Adenoviruses (AdVs) were first isolated and characterized in 1953 during attempts to establish tissue culture lines from tonsils and adenoidal tissue surgically removed from children (Rowe et al., 1953), from which the name is derived. AdV represents the largest non-enveloped icosahedral particle approximately 80 nm in diameter (Figure 11). The outer capsid is composed of two main types of capsomer: 240 hexons make up the 20 triangular faces of the icosahedral, while 12 pentons form the 12 vertices. From each penton protrudes, and a fiber giving the virion the appearance of a “communications satellite” or “spike” that aid in attachment to the host cell receptor, as shown in Figure 12.

Human adenovirus (HAdV) belonges to the genus *Mastadenovirus* of the family *Adenoviridae*. Its genome, which is associated with an inner protein core, consists of a double-stranded linear DNA (dsDNA), of approximately 36-38 kb in size that allows the virus to theoretically carry 30 to 40 genes. By now, the complete sequences of at least eight HAdVs have been determined and revealed that the DNA sequences encoding most virion structural proteins, and non-structural proteins involved in viral replication and viral assembly. Moreover, an interesting feather of this viral genome is that it has a terminal 55 kilodalton (kDa) protein associated with

each 5'-terminus of the linear dsDNA, these are use as primers in viral replication and ensure that ends of the genome are adequately replicated.

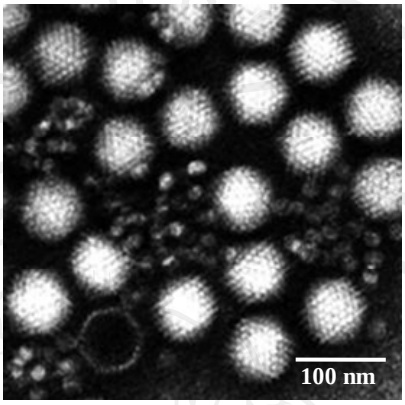

Figure 11 Negatively stained electron micrographs of adenovirus virions. (Adopted from http://www.cours.fse.ulaval.ca/ten20727/sitesdescours/0005ete2001/microbes56/virus.jpg)

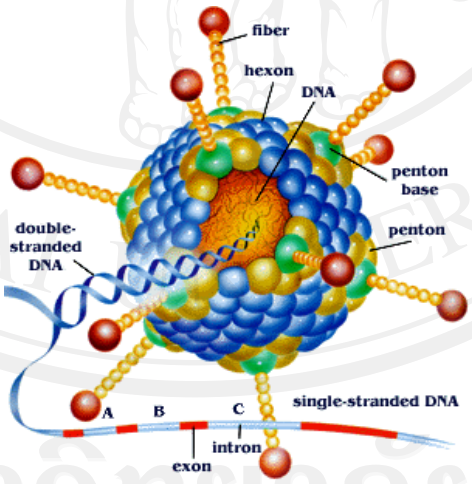

Figure 12 Diagrammatic representation of Adenovirus particle. Adenovirus is non-enveloped, double stranded DNA virus with icosahedral symmetry. The viral capsid is composed of hexon protein (shown in blue) and penton base proteins (shown in green). The fiber spike associated with each pentonbase allows the virus to attach to human cells during infection. (Adopted from http://www.medgadget.com/archive/img/bigadenovirus.gif)

2. Classification

Mammalian and avian adenoviruses comprise two distinct genera, designated *Mastadenovirus* and *Aviadenovirus*, respectively. In turn, the genus *Mastadenovirus* comprise numerous adenovirus serotypes specific for particular mammalian species, including all human adenoviruses. Currently, 51 human adenovirus serotypes have been distinguished on the basis of their resistance to neutralization by antisera to other known human adenoviruses. Various serotypes of human adenoviruses have been reported and are classified into six subgenera, A to F, as summarized in Table 2. This classification scheme is generally consistent with subgroupings of AdVs on the basis of their physicochemical, biological and genetic properties (De jong et al., 1993; Davison et al., 2003).

Table 2 Classification of human adenoviruses

Subgenera	Serotypes
A	12, 18, 31
B	3, 7, 11, 14, 16, 21, 34, 35, 50
C	1, 2, 5, 6
D	8, 9, 10, 13, 15, 17, 19, 20, 22-30, 32, 33, 36-39, 42-49, 51
E	4
F	40, 41

3. Molecular epidemiology of adenoviruses

Adenovirus is considered to be a significant enteropathogen in association with sporadic cases as well as outbreaks of acute gastroenteritis in kindergartens,

school, and hospitals (Chiba et al., 1983; Akihara et al., 2005b). In general, it is not as prevalent as rotavirus diarrhea, and the incidence of AdV infection differs considerably in different studies which accounts for 1-20% of cases of acute gastroenteritis and frequently affects infants and young children. Among six subgenera, subgenus F, which represented by two types, AdV type 40 (Ad40) and Ad41, is the most important in association with acute gastroenteritis worldwide (Akihara et al., 2005b; Shimizu et al., 2007). Surveys on the circulating AdV types have demonstrated a changing pattern of the dominant types. Two studies carried out during 1981 to 1989 in Netherland and 1987 and 1990 in Bangladesh, have shown a decrease in the prevalence rate of Ad40, whereas Ad41 becoming the predominant type (De Jong et al., 1993; Jarecki-Khan et al., 1993). During 1998-2001, epidemiological studies conducted in Japan, Korea, and Vietnam revealed that 4.4% were positive for AdV, of which more than half were Ad41, but Ad40, Ad2, Ad3, Ad8, and Ad31 were also detected (Li et al.,2005). Between 2002 and 2007, survey from the southern regions of Ireland revealed that all adenovirus isolates obtained from patients with acute gastroenteritis were Ad41 (Lennon et al., 2007). Another study of 337 children with acute diarrhea in Maizuru City, Japan conducted by Shimizu et al. (2007), reported that Ad41 was the most prevalent serotype, and other serotypes, including Ad1, Ad2, Ad3, and Ad5, were also detected while Ad40 was not detected in this study. In contrast, recent molecular epidemiology in Bangladesh conducted by Dey et al. (2009) had reported the disappearance of Ad41, while Ad40 predominated over other serotypes, Ad9 and Ad10. It is of note that those of data clearly indicated that “enteric adenoviruses”, Ad40 and Ad41, are the common cause of AdV infection in different part of the world. Most interesting, evidence of

interspecies transmission of AdV between human and animal has been documented. Phan et al. (2006) reported that one AdV strain detected in a fecal specimen collected from 1-year old female child with acute gastroenteritis in Japan carried a feline AdV gene. The data revealed that human AdV type 1 detected and feline AdV shared high identities of 100% and 97% at the amino acid levels of hexon and fiber genes, respectively. This finding emphasizes a possible zoonoses in human.

In Thailand, with few epidemiological data of AdV, it was found that the frequency of AdV detection rate ranged from 1.5% to 4.4% (Herrmann et al., 1988; Kittigul et al., 2009). Study of AdV infection was first reported in Bangkok, Thailand by Herrmann et al. (1988). The Ad40 and Ad41 were detected in children with and without gastroenteritis at 4.4% and 1.8%, respectively. Recently, molecular characterization of AdV had been studied in Lopburi province, Thailand, during January 2006 to February 2007 in patients hospitalized with acute gastroenteritis. Of these, of 262 fecal samples, 4 were positive for AdV. Two belonged to Ad41 while the other two belonged to Ad2 and Ad38 (Kittigul et al., 2009).