CHAPTER 2

Literature Review

Acute gastroenteritis is the common cause of morbidity and mortality of all age groups especially in children under 5 years of age (Glass et al., 2009). Acute gastroenteritis causes by wide variety of pathogens including bacteria, parasites, and viruses. Beside rotavirus, norovirus (NoV) and sapovirus (SaV) are identified as the main etiological agents of acute gastroenteritis in pediatric patients (Knipe et al., 2013; Patel et al., 2009).

2.1 Noroviruses

2.1.1 Norovirus biology

Human NoV was previously known as Norwalk virus which was first identified from stool samples collected from children in Norwalk city, Ohio, United States in 1968 and was described as the etiologic agent of "winter vomiting disease" (Adler et al., 1969). Later, in 1972, Norwalk virus was first visualized in stool materials obtained from the previous outbreak occurred in Norwalk city in 1968 by Kapikian et al. (1972) (Figure 2.1). Thus, Norwalk virus is considered to be the prototype strain of NoV (NoV/Hu/Norwalk/ 1968/US), and currently known as NoV.

NoV is classified into the family *Caliciviridae* which is comprised of five genera, including *Norovirus, Sapovirus, Lagovirus, Nebovirus*, and *Vesivirus.* The virus particle is a small (27-40 nm), non-enveloped, icosahedral capsid containing a linear, positive sense single-stranded RNA (ssRNA) genome of approximately 7.3-8.5 kb in length.

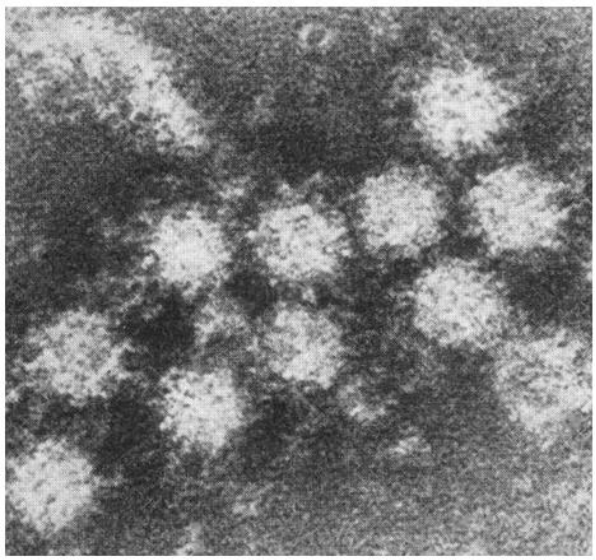

Figure 2.1 Norwalk virus particle identified by immunoelectron microscopy from Norwalk outbreak (Kapikian et al., 1972).

The human NoV genome consists of three open reading frames (ORFs), ORF1, ORF2, and ORF3 (Figure 2.2). At the 5' end, the genome is covalently linked to the viral protein (VPg) and 3' end is polyadenylated (Thorne et al., 2014). The ORF1 encodes for a polyprotein of six nonstructural proteins including p48, NTPase, p22, VPg, Pro (proteinase), and Pol (RNA dependent RNA polymerase; RdRp), which is processed into the mature nonstructural proteins (Jiang et al., 1993; Lambden et al., 1993; Liu et al., 1996). The ORF2 encodes for the major structural capsid protein (VP1) with molecular weights of 58-60 kDa (Hardy, 2005). The ORF2 overlaps the 3' end of ORF1 about 17-20 nucleotides (Bull et al., 2005). The ORF3 encodes for the minor structural protein (VP2) with calculated molecular weights of 22-29 kDa (Glass et al., 2000). From the studies of x-ray crystallographic structure by using Norwalk virus-like particles, it was demonstrated that VP1 contains 2 major subunit domains, a shell (S) and a protruding (P) domain (Prasad et al., 1999). The P domain is linked to the S domain which corresponds to the C-terminal part of the VP1. Moreover, the P domain is divided into P1 and P2

subdomains. By the electron microscope (EM) observation, the P domains interact with the dimeric contacts and increase the stability of the capsid (Tan et al., 2003). The P1 subdomain provides a platform to orient the P2 subdomain (Chen et al., 2004). The P2 subdomain is highly variable and consists of the putative neutralization sites and can interact with histo-blood group antigen (HBGAs) (Lindesmith et al., 2012). The VP2 locates inside the viral particle and involves in capsid assembly and genome encapsidation (Vinjé, 2015; Vongpunsawad et al., 2013).

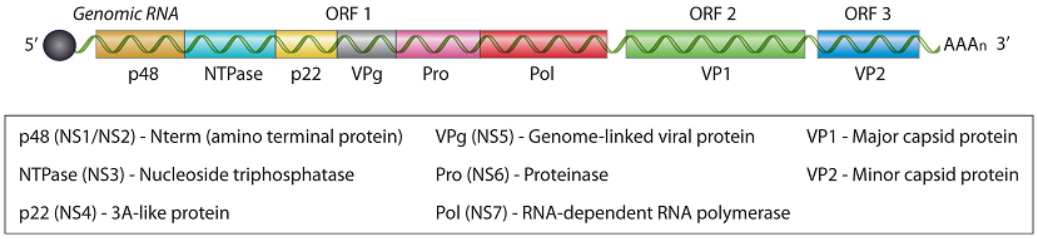

Figure 2.2 Human norovirus genome. The genome consists of three open reading frames (ORFs), designated ORF1, ORF2, and ORF3. ORF1 encodes seven nonstructural (NS) proteins, including important protein essential for replication such as protease and RNA-dependent RNA polymerase (RdRp). ORF2 encodes the major structural capsid protein or viral protein 1 (VP1). ORF3 encodes the minor structural protein or viral protein 2 (VP2) (Robilotti et al., 2015).

2.1.2 Norovirus classification

Because NoV cannot be cultured *in vitro*, except for murine NoV strains, so the classification into distinct serotypes is not possible. Currently, NoV classification is based on genome sequencing and can be classified into several genogroups and genotypes. Up to date, NoV is classified into 6 established genogroups (GI to GVI) (Knipe et al., 2013; Vinjé, 2015). Recently, Tse et al. (2012) discovered novel virus strain from fecal swab samples of dog in Hong Kong and proposed as a tentative GVII. Thus, NoV

is now classified into seven genogroups based on the diversity of amino acid sequence in the complete VP1 protein (Green, 2013) (Figure 2.3). The viruses that belong to GI, GII, and GIV infect human (Vennema et al., 2002). NoV GIII infects cows and sheep, whereas GV infects mice and rats (Karst et al., 2003). The GVI and GVII infect only canine species (Martella et al., 2009; Vinjé, 2015). The NoV GI and GII are responsible for the outbreaks of disease whereas GIV is rarely detected in human (Martella et al., 2009). Furthermore, each genogroup is divided into various genotypes by the difference of amino acid sequences of the major capsid protein (VP1). NoV GI is divided into at least 9 genotypes (GI.1 to GI.9) and GII into 22 genotypes (GII.1 to GII.22). The GII is the most prevalent genogroup and GII.4 is the most predominant genotype that has been reported to associate with diarrhea worldwide (Kroneman et al., 2008; Ramani et al., 2014; Siebenga et al., 2009; Thongprachum et al., 2014; Vinjé, 2015). Moreover, GII.3 is found as the second most common genotype, especially in children (Boon et al., 2011; de Graaf et al., 2016). The GIII is divided into three genotypes (GIII.1 to GIII.3). NoV GIV is divided into two genotypes (GIV.1 to GIV.2) of which GIV.1 have been detected in humans and GIV.2 in feline and canine species. Each of NoV GV and NoV GVI are also divided into two genotypes, GV.1, GV.2, and GVI.1, GVI.2, respectively. However, NoV GVII contains only one genotype (Table 2.1).

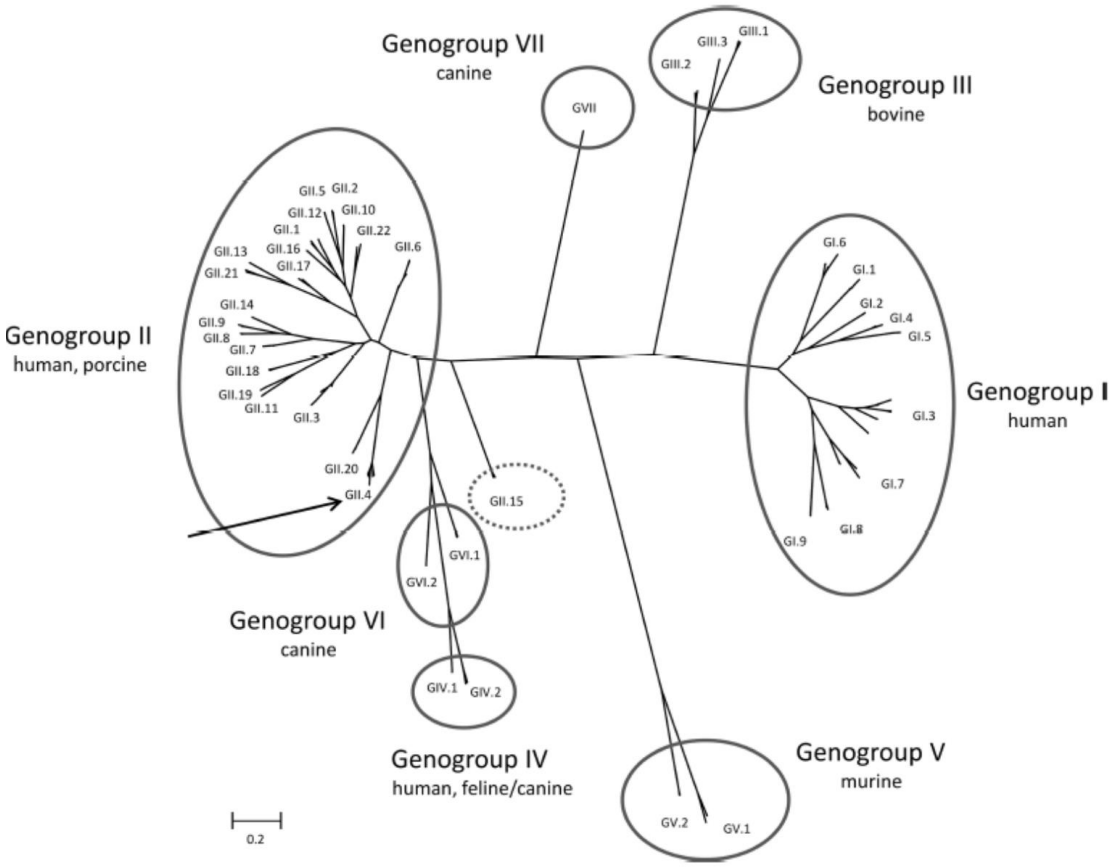

Figure 2.3 Phylogenetic tree of norovirus classification. NoV can be classified into 7 genogroups (GI-GVII) based on the difference of amino acid sequences in the VP1 (capsid) complete protein. NoV GII.4 (arrow) is the major strain of human infection worldwide. The GII.15 has been proposed as a tentative new genotype (dotted circle) (Vinjé, 2015).

NoV GII.4 is subdivided into several variants according to their nucleotide sequence variations. Novel pandemic of GII.4 variant has occurred every 2-3 years since the mid-1990s (Siebenga et al., 2009). The GII.4 variants including GII.4 US95/96 (occurred in 1995), Farmington Hills (in 2002), Hunter (in 2004), Den Haag (in 2006), New Orleans (in 2009), and Sydney (in 2012) have been detected thus far and associated with the large outbreaks globally (Fankhauser et al., 2002; Huhti et al., 2011; Kroneman et al., 2008, 2013; Leshem et al., 2013; Tuladhar et al., 2012; Verhoef et al., 2009) (Figure 2.4).

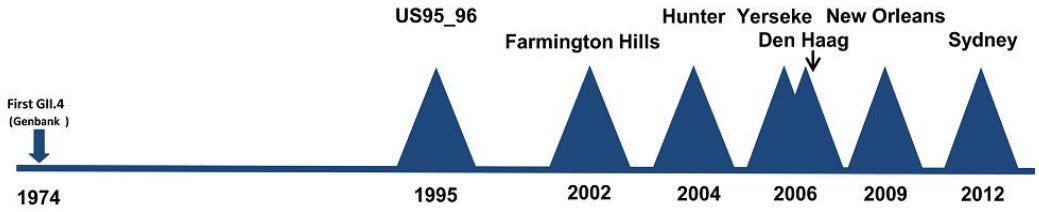

Figure 2.4 Norovirus GII.4 variants that caused a global outbreaks (Vinjé, 2015).

Another NoV genotyping system based on RNA-dependent RNA polymerase (RdRp) gene (ORF1) has been proposed (Bull et al., 2007; Katayama et al., 2002). The RdRp genotyping system of NoV is based on the genetic variations of RdRp gene. In order to differentiate RdRp genotype from VP1 capsid type, a capital P (for polymerase) is used before number of genotype such as GII.P4 genotype. The "GII.P4" is represented the genotype based on RdRp gene as GII.4 genotype. Until now, 14 RdRp genotypes of GI (GI.P1 to GI.P9 and GI.Pa to GI.Pf) and 32 RdRp genotypes of GII (GII.P1 to GII.P22 and GII.Pa to GII.Pn) have been described (Table 2.2) (Green, 2016).

Table 2.1 Norovirus genogroups and genotypes as determined by capsid (C) gene relatedness (Green, 2016).

Reference virus	Genogroup. C genotype	GenBank accession number
Hu/GI.1/Norwalk/1968/US	GI.1	M87661
Hu/GI.2/Southampton/1991/UK	GI.2	L07418
Hu/GI.3/DesertShield 395/1990/SA	GI.3	U04469
Hu/GI.4/Chiba 407/1987/JP	GI.4	AB042808
Hu/GI.5/Musgrove/1989/GB	GI.5	AJ277614
Hu/GI.6/BS5(Hesse3)/1997/DE	GI.6	AF093797
Hu/GI.7/Winchester/1994/GB	GI.7	AJ277609
Hu/GI.8/Boxer/2001/US	GI.8	AF538679
Hu/GI.9/Vancouver730/2004/CA	GI.9	HQ637267
Hu/GII.1/Hawaii/1971/US	GII.1	U07611
Hu/GII.2/Melksham/1994/GB	GII.2	X81879
Hu/GII.3/Toronto 24/1991/CA	GII.3	U02030
Hu/GII.4/Bristol/1993/GB	GII.4	X76716
Hu/GII.5/Hillingdon/1990/GB	GII.5	AJ277607
Hu/GII.6/Seacroft/1990/GB	GII.6	AJ277620
Hu/GII.7/Leeds/1990/GB	GII.7	AJ277608
Hu/GII.8/Amsterdam/1998/NL	GII.8	AF195848
Hu/GII.9/VA97207/1996/US	GII.9	AY038599
Hu/GII.10/Erfurt546/2000/DE	GII.10	AF427118
Po/GII.11/Sw918/1997/JP	GII.11	AB074893

Table 2.1 (Continued)

Reference virus	Genogroup. C genotype	GenBank accession number
Hu/GII.12/Wortley/1990/GB	GII.12	AJ277618
Hu/GII.13/Fayetteville/1998/US	GII.13	AY113106
Hu/GII.14/M7/1999/US	GII.14	AY130761
Hu/GII.15/J23/1999/US	GII.15	AY130762
Hu/GII.16/Tiffin/1999/US	GII.16	AY502010
Hu/GII.17/CS-E1/2002/US	GII.17	AY502009
Po/GII.18/OH-QW101/2003/US	GII.18	AY823304
Po/GII.19/OH-QW170/2003/US	GII.19	AY823306
Hu/GII.20/Luckenwalde591/2002/DE	GII.20	EU373815
Hu/GII.21/IF1998/2003/IR	GII.21	AY675554
Hu/GII.22/Yuri/2003/JP	GII.22	AB083780
Bo/GIII.1/Jena/1980/DE	GIII.1	AJ011099
Bo/GIII.2/Newbury-2/1976/GB	GIII.2	AF097917
Ov/GIII.3/Norsewood30/2007/NZ	GIII.3	EU193658
Hu/GIV.1/Alphatron 98-2/1998/NL	GIV.1	AF195847
Fe/GIV.2/Pistoia 387/2006/IT	GIV.2	EF450827
Mu/GV.1/MNV-1/2002/US	GV.1	AY228235
Rn/GV.2/HKU_CT2/2011/HK	GV.2	JX486101
Ca/GVI.1/Bari 91/2007/IT	GVI.1	FJ875027
Ca/GVI.2/Viseu/2007/PT	GVI.2	GQ443611
Ca/GVII/026F/2007/HK	GVII	FJ692500

Table 2.2 Norovirus genogroups and genotypes as determined by polymerase (P) gene relatedness (Green, 2016).

Reference virus	Genogroup. P genotype	GenBank accession number
Hu/GI.P1-GI.1/Norwalk/1968/US	GI.P1	M87661
Hu/GI.P2-GI.2/Southampton/1991/GB	GI.P2	L07418
Hu/GI.P3-GI.3/VA98115/1998/US	GI.P3	AY038598
Hu/GI.P4-GI.4/Chiba407/1987/JP	GI.P4	AB042808
Hu/GI.P5-unknown/07_1/2005/SE	GI.P5	EU007765
Hu/GI.P6-GI.6/BS5(Hesse)/1997/DE	GI.P6	AF093797
Hu/GI.P7-GI.7/Lilla Edet/2008/SE	GI.P7	JN603251
Hu/GI.P8-GI.8/890321/2008/US	GI.P8	GU299761
Hu/GI.P9-GI.9/Chatellerault709/2004/FR	GI.P9	EF529737
Hu/GI.Pa-GI.3/DesertShield/1990/SA	GI.Pa	U04469
Hu/GI.Pb-GI.6/WUG1/2002/JP	GI.Pb	AB081723
Hu/GI.Pc-GI.5/SzUG1/2000/JP	GI.Pc	AB039774
Hu/GI.Pd-GI.3/Vesoul576/2003/FR	GI.Pd	EF529738
Hu/GI.Pf-GI.3/Otofuke/1979/JP	GI.Pf	AB187514
Hu/GII.P1-GII.1/Hawaii/1971/US	GII.P1	U07611
Hu/GII.P2-GII.2/Melksham/1994/GB	GII.P2	X81879
Hu/GII.P3-GII.3/Toronto/1991/CA	GII.P3	U02030
Hu/GII.P4-GII.4/Bristol/1993/GB	GII.P4	X76716
Hu/GII.P5-GII.5/MOH/1999/HU	GII.P5	AF397156
Hu/GII.P6-GII.6/Saitama U16/2002/JP	GII.P6	AB039778

Table 2.2 (Continued)

Reference virus	Genogroup. P genotype	GenBank accession number
Hu/GII.P7-GII.6/Saitama U4/2002/JP	GII.P7	AB039777
Hu/GII.P8-GII.8/Saitama U25/2002/JP	GII.P8	AB039780
Po/GII.P11-GII.11/Sw918/1997/US	GII.P11	AB074893
Hu/GII.P12-GII.4/Sakai/04-179/2005/JP	GII.P12	AB220922
Hu/GII.P13-GII.17/Briancon870/2004/FR	GII.P13	EF529741
Hu/GII.P15-GII.15/Hiroshima66/2006/JP	GII.P15	AB360387
Hu/GII.P16-GII.16/ Neustrelitz260/2000/DE	GII.P16	AY772730
Po/GII.P18-GII.18/OHQW101/2003/US	GII.P18	AY823304
Hu/GII.P20-GII.20/Leverkusen267/2005/DE	GII.P20	EU424333
Hu/GII.P21-GII.2/PontdeRoide673/2004/FR	GII.P21	AY682549
Hu/GII.P22-GII.5/Hokkaido133/2003/JP	GII.P22	AB212306
Hu/GII.Pa-GII.3/SN2000JA/2004/JP	GII.Pa	AB190457
Hu/GII.Pc-GII.2/SnowMountain/1976/US	GII.Pc	AY134748
Hu/GII.Pe-GII.4/OC07138/2007/JP	GII.Pe	AB434770
Hu/GII.Pf-GII.5/S63/1999/FR	GII.Pf	AY682550
Hu/GII.PgGII.13/GoulburnValley/1983/AU	GII.Pg	DQ379714
Hu/GII.Ph-GII.2/OC97007/1997/JP	GII.Ph	AB089882
Hu/GII.Pj-GII.2/E3/1997/GR	GII.Pj	AY682552
Hu/GII.Pk-Unknown/OC96065/1996/JP	GII.Pk	AF315813
Hu/GII.Pm-GII.12/PunePC24/2006/IN	GII.Pm	EU921353
Hu/GII.Pn-GII.22 /Beijing53931/2007/CN	GII.Pn	GQ856469

2.1.3 Norovirus recombination

Viral genome recombination is one of the most important mechanisms that drives NoV evolution and creates major change in the viral genetic background (White, 2014). The NoV recombination could have significant implications for escape from human immune response. A simple mechanism for recombination in NoV was proposed by Bull et al., (2005). The recombination event is occurred during coinfection with two viruses in a single cell. The recombination is driven by RdRp during the transcription that the positive-sense RNA ((+)RNA) is transcribed into negative-sense intermediate ((-)RNA). This event provides templates for the transcription of genomic and subgenomic (+) RNA. The recombination occurs when the RdRp initiate the synthesis of (+) RNA at the 3' end of a (-) RNA but stalls at the subgenomic promoter and templates then switch to an available negative subgenomic RNA from a co-infection virus. Following the recombination event, a novel recombination virus strain has a mixed viral genome from both parental strains. The break-points of NoV recombination strains are mostly identified near to or within the junction point of ORF1 (polymerase) and ORF2 (capsid) (Bull et al., 2005; Vinjé, 2015) and these occur predominantly among virus strains of the same genogroup (Bull et al., 2005). The diagram of the mechanism of the NoV recombination is shown in Figure 2.5.

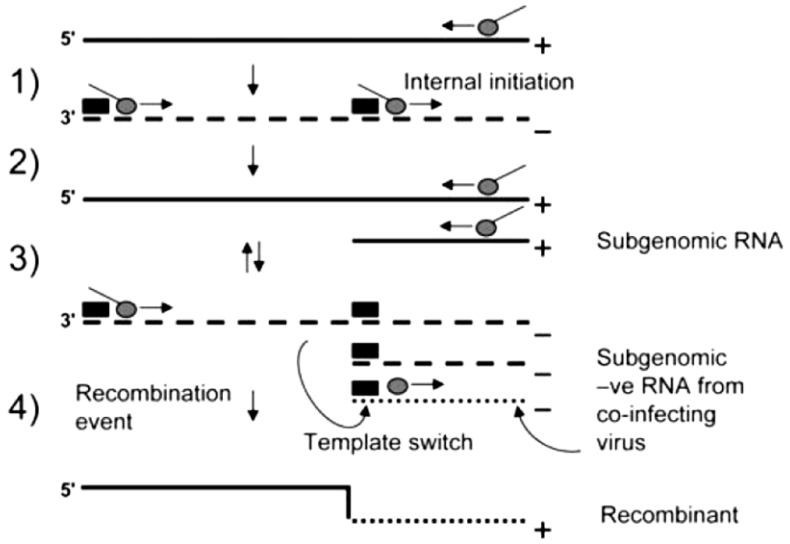

Figure 2.5 Diagram of the mechanism of norovirus recombination. 1) The RNA-dependent RNA polymerase (RdRp) (gray circle) generates negative-stranded intermediate (dashed line) from positive-stranded RNA by RNA transcription. 2) The RdRp binds to the RNA promoter sequences (filled boxes) and then generates positive-stranded (straight line) genomes, and subgenomic RNA. 3) A full-length negative genome and a negative subgenomic RNA are synthesized from 3' end of the templates (positive stranded RNA) 4) The RdRp enzyme initiates positive-strand synthesis at the 3´ end of the negative strand and stalls at the subgenomic promoter, then template switches to an available negative subgenomic RNA of a co-infecting virus. Thus, the recombination event occurs and recombinant virus is produced (Bull et al., 2005).

The first reported natural recombinant NoV strain was the prototype Snow Mountain that caused an outbreak of acute gastroenteritis in USA in 1976 (Morens et al., 1979). Viral genome characterization revealed that the Snow Mountain strain was a GII.Pc/GII.2 recombinant type (Lochridge et al., 2003). Thus far, there are three genogroups that have been reported as naturally occurring recombinant NoVs, including GI, GII, and GIII (Han et al., 2004; Hardy et al., 1997; Katayama et al., 2002). For example, a recombination within GI, GI.P4/GI.2, was detected in Japan in 2002 (Katayama et al., 2002). For the recombination of GII, GII.Pg/GII.12 and GII.P12/GII.3 recombinant strains were detected in China (Sang et al., 2014). For GIII, GIII.P1/GIII.2 recombinant type was detected from bovine species in USA (Han et al., 2004).

Up to date, the NoV recombinant strains have been largely reported and responsible for outbreaks and sporadic cases worldwide. For instance, a GII.Pb/GII.3 variant caused outbreaks of gastroenteritis across Europe between 1999-2002 (Ambert-Balay et al., 2005; Gallimore et al., 2005), Australia between 1997-2000 (White et al., 2002), and Japan during 2003-2004 (Phan et al., 2006a). In Belgium, multiple outbreaks of the GII.Pg /GII.1 strain were detected in 2010 (Mathijs et al., 2010). Furthermore, GII.Pg/GII.12 strain was found among sporadic cases in Italy during 2009-2010 (Giammanco et al., 2012). Very recently, GII.P16/GII.2 strain was observed as the predominant type in Germany during winter, 2016 (Niendorf et al., 2017). In mid-2016, Thongprachum et al. (2017) observed that GII.Pe/GII.2, GII.P16/GII.2, and GII.P17/GII.2 were involved as a major caused of gastroenteritis outbreak in Japan. Thus far, to the best of our knowledge, there is only one report of human NoV recombination in Thailand, of which three recombination patterns of GII.P21/GII.3, GII.P12/GII.3, and GII.P12/GII.1 were observed (Phumpholsup et al., 2015).

2.1.4 Molecular epidemiology of norovirus

NoV is genetically diverse and constantly changing overtime. Several studies demonstrated that the estimated prevalence of NoV in pediatric patients with diarrhea was the second most common virus after only the rotaviruses (Knipe et al., 2013; Mladenova, 2015; Nguyen et al., 2007; Patel et al., 2008; Tran et al., 2010). Globally, the NoV infection rate in acute gastroenteritis cases was variable, ranging from 10.7-64.9% (Chan-It et al., 2012; Chhabra et al., 2009; 2010; Lindell et al., 2005; Mladenova et al., 2015; Osborne et al., 2015; Page et al., 2017; Tan et al., 2015; Thongprachum et al., 2016; Timurkan et al., 2017; Zhang et al., 2016b; Zhirakovskaia et al., 2015). Moreover, the most common genogroup that causes the majority of outbreaks and sporadic cases worldwide is GII (Chhabra et al., 2009; Noel et al., 1999). The GII.4 and GII.3 were the most frequently detected genotypes with about 85% of the total NoV infections in children worldwide (Hoa Tran et al., 2013). In Asia, NoV is also a clinically important virus which causes acute gastroenteritis and GII.4 is the most predominant genotype. For instance, several studies throughout Japan revealed that NoV detection rates ranged from 11.0-64.9% of sporadic cases of acute gastroenteritis among pediatric patients between 1995 and 2013 (Thongprachum et al., 2016). Several studies of NoV in South Korea between 2001-2011 demonstrated that the prevalence of NoV infection in patients with acute gastroenteritis ranged from 9.7-15.0% (Lee et al., 2015).

In Thailand, most of NoV studies targeted on the hospitalized children (under 15 years old) with acute gastroenteritis and the prevalence ranged from 6.8-44.7% (Bodhidatta et al., 2015; Chaimongkol et al., 2012, 2014; Guntapong et al., 2004; Hansman et al., 2004; Khamrin et al., 2007, 2010, 2017; Kittigul et al., 2010; Malasao et al., 2008; Neesanant et al., 2013; Thongprachum et al., 2013). The NoV prevalence in acute gastroenteritis patients in Thailand is summarized in Table 2.3. The first epidemiological surveillance study of NoV infection in Thailand was reported by Hansman et al. (2004), the prevalence of NoV was detected at 8.6% during 2000-2001 in Chiang Mai. Later, the study of NoV infection had been conducted in pediatric patients with diarrhea between 2000-2002 in the same area and found that NoV was

detected at 8.1% and most predominant genotype was GII.4 followed by GII.3, GII.10, GII.1, GII.6, GII.8, and GII.15 (Malasao et al., 2008). In 2002-2003, a survey of NoV infection in five distinct geographical regions in Thailand (Tak, Sa Kaeo, Nong Khai, Chanthaburi, and Songkhla) had been conducted. The data revealed that the prevalence of NoV was 14%. Among these, 4% of NoV was mixed infection with SaV (Guntapong et al., 2004). The majority of detected strains belonged to GII.4, followed by GII.1, GII.3, GII.6, and one novel genotype (GII.new). Among the NoV positive specimens, NoV GI was also detected including GI.3, GI.4, GI.6, GI.7, and GI.13 genotypes. During 2002-2004, the prevalence of NoV in Chiang Mai was detected at 14.1% (Khamrin et al., 2007). Among the NoV positive cases in that study, majority of the strains belonged to GII.4 followed by GII.3, GII.1, GII.7, GII.2, and GII.16. In 2005, the prevalence of NoV was detected at 6.8% and majority of the strains belonged to GII.4, followed by GII.15, GII.6, and GII.12 (Khamrin et al., 2010). Later, in 2006, NoV was detected at 20.5% and the most predominant strain was GII.4 (Thongprachum et al., 2013). During 2005-2006, surveillance of NoV has been conducted in five distinct study locations in Thailand (Mae Hong Son, Ubon Ratchathani, Trang, Samut Songkhram, and Bangkok). It was found that NoV was detected at 23.8% among children with acute gastroenteritis in those areas (Neesanant et al., 2013). The most common strain found in that study was GII.4. In addition, a survey study conducted in Lopburi province among all age groups (ranged from < 1 to > 60 years old) with acute gastroenteritis during 2006-2007 demonstrated that the prevalence of NoV was 44.7% and most of the genotypes found in this study was also GII.4 followed by GII.6, GII.3, and GII.17. Moreover, two GI genotypes, GI.2 and GI.6, were found (Kittigul et al., 2010). In 2007, the prevalence of NoV infection in Chiang Mai area was found at 12.5% (Chaimongkol et al., 2012). In 2006 and 2008, Pongsuwanna et al. (2017) conducted the study of NoV infection in diarrheic children from four distinct provinces (Songkhla, Chanthaburi, Tak, and Nong Khai) and found that NoV was detected at 26.6% and GII was the most prevalence genogroup. Another report of NoV in a muti-site study including Bangkok,

Mae Hong Son, Ubon Ratchathani, Samut Songkhram, Ratchaburi, Nakhon Pathom, Suphan Buri, Phetchaburi, and Samut Sakhon, during 2004-2010 demonstrated that the prevalence of NoV infection was found at 38% (Bodhidatta et al., 2015). The GII.4 was also found to be the most predominant genotypes in this study. Then, the surveillance of NoV in Chiang Mai, during 2007 and 2010-2011, demonstrated that 15.9% of the specimens were positive for NoV. The GII.4 was the most predominant genotype, followed by GII.6, GII.16, GII.12, GII.3, GII.13, GII.7, GII.2, GII.1, and GI.14 (Chaimongkol et al., 2014). In 2015, Phumpholsup et al. reported the prevalence of NoV from Khon Kaen and Bangkok at 8.96% with the GII.4 genotype as the predominant genotype followed by GII.3, GII.1, GII.2, GII.5, GI I.6, GII.7, GII.12, GII.13, GII.14, GII.17, and GII.21 (Phumpholsup et al., 2015). Recently, a report of NoV surveillance in Chiang Mai during 2012-2014 demonstrated that NoV infection was at 17.3%. The most predominant genotype was GII.4 followed by GII.3, GII.13, GII.1, GII.6, GII.7, GII.17, GII.2, GII.15, and GII.21 (Khamrin et al., 2017).

Table 2.3 Summary of norovirus prevalence in acute gastroenteritis patients in Thailand

Locations	Years	Prevalence of NoV (%)	Reference
Chiang Mai	2000-2001	8.6%	Hansman et al., 2004
Chiang Mai	2000-2002	8.1%	Malasao et al., 2008
Tak, Nong Khai, Sa Kaeo, Chanthaburi, Songkhla	2002-2003	13.8%	Guntapong et al., 2004
Chiang Mai	2002-2004	14.1%	Khamrin et al., 2007
Chiang Mai	2005	6.8%	Khamrin et al., 2010
Chiang Mai	2006	20.5%	Thongpreachum et al., 2013
Mae Hong Son, Ubon Ratchathani, Trang, Samut Songkhram, Bangkok	2005-2006	23.8%	Neesanant et al., 2013
Lopburi	2006-2007	44.7%	Kittigul et al., 2010
Chiang Mai	2007	12.5%	Chaimongkol et al., 2012
Songkhla, Chanthaburi, Tak, Nong Khai	2006, 2008	26.6%	Pongsuwanna et al., 2017
Bangkok, Mae Hong Son, Ubon Ratchathani, Samut Songkhram, Ratchaburi, Nakhon Pathom, Suphan Buri, Phetchaburi, Samut Sakhon	2004-2010	9.4%	Bodhidatta et al., 2015
Chiang Mai	2007, 2010-2011	15.9%	Chaimongkol et al., 2014
Khon Kaen and Bangkok	2009-2014	8.9%	Phumpholsup et al., 2015
Chiang Mai	2012-2014	17.3%	Khamrin et al., 2017

2.2 Sapoviruses

2.2.1 Sapovirus biology

The prototype strain of sapovirus (SaV) was Hu/SaV/Sapporo/1982/JP. The virus was detected from a gastroenteritis outbreak in Sapporo, Japan, since October 1977 (Chiba et al., 1979). SaV was previously called “Typical human caliciviurses” or “Sapporo-like viruses”. SaV belongs to the family *Caliciviridae*, genus *Sapovirus*. The virus causes acute gastroenteritis in human of all ages, in both sporadic and outbreaks worldwide. Major clinical symptoms of SaV include diarrhea and vomiting with additional symptoms such as nausea, stomach/abdominal cramps, myalgia, and malaise. The clinical symptoms of SaV are indistinguishable from those by NoV (Oka et al., 2015).

The SaV particle is a small (30-38 nm in diameter), nonenveloped with icosahedral symmetry and has a cup-shaped depression, like the typical calicivirus morphology (Figure 2.6) (Madeley, 1979). Human SaV can be grown in cell culture such as green monkey kidney cells (Cubitt et al., 1979, 1984, 1981; Kjeldsberg, 1977; Spratt et al., 1978), however, no confirmed reproduction of these data is available (Oka et al., 2015). The infection trial of human SaV in mice was not succeed yet (Cubitt et al., 1979).

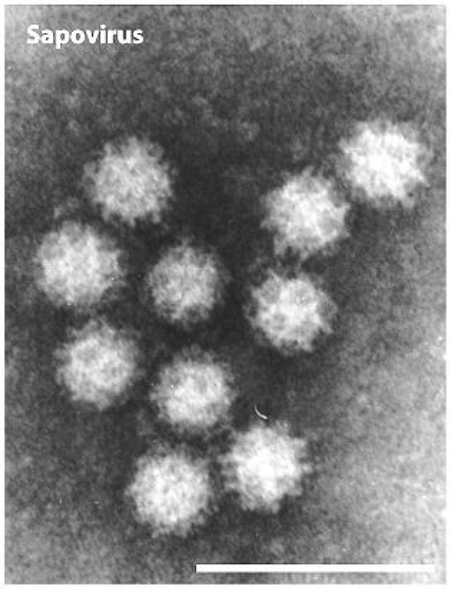

Figure 2.6 Electron micrographs of sapovirus particles (scale bar indicate 100 nm) (Oka et al., 2015).

The genome of SaV is a positive sense, single-stranded RNA with approximately 7.1-7.7 kb in length and has a 3'-end poly (A) tail. The SaV genome consists of two open reading frames (ORFs), ORF1 and ORF2, as shown in Figure 2.7. ORF1 encodes nonstructural polyprotein that consists of at least 6 nonstructural (NS) proteins (NS1, NS2, NS3, NS4, NS5, and NS6-NS7) and one structural capsid protein VP1 (Oka et al., 2005, 2011; Yokoyama et al., 2012). ORF2 encodes the minor structure protein VP2 (Chang et al., 2005). The VP1 protein is a major component of complete virion with molecular weight of approximately 60 kDa (Parwani et al., 1990; Terashima et al., 1983). The VP2 protein has not yet been identified for its function but believed to be similar to the VP2 of NoV (Atmar et al., 2001).

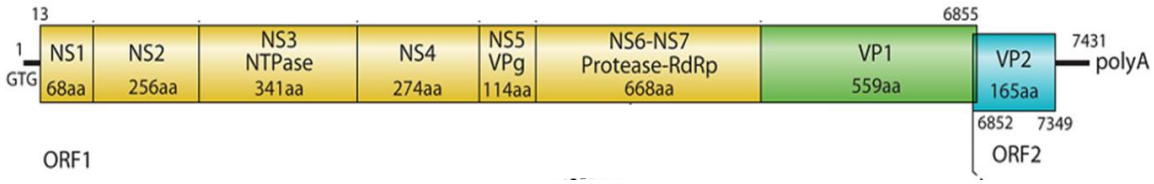

Figure 2.7 Sapovirus genome organization with two open reading frames (ORF1 and ORF2). The nonstructural proteins (NS1, NS2, NS3, NS4, NS5, and NS6-7 (protease-RNA dependent RNA polymerase)), and structural proteins VP1 and VP2 are indicated (Modified from Oka et al., 2015).

2.2.2 Sapovirus classification

Because the VP1 encoded region is the most diverse region, SaVs are divided into several genogroups and genotypes based on the complete VP1 nucleotide sequences. SaV is divided into five genogroups (GI to GV) (Farkas et al., 2004; Oka et al., 2012). Currently, four genogroups including GI, GII, GIV, and GV have been isolated from human (Oka et al., 2015), whereas GIII infects porcine species (Hansman et al., 2007a). The SaVs within GI, GII, and GV are also further classified into several genotypes (Hansman et al., 2007b; Kitamoto et al., 2012; Oka et al., 2009). Each of human SaV GI and GII is subdivided into seven genotypes (GI.1 to GI.7 and GII.1 to GII.7). The GIV comprises of a single genotype (GIV.1). Human SaV GV is subdivided into two genotypes (GV.1 and GV.2) (Oka et al., 2015).

2.2.3 Molecular epidemiology of sapovirus

SaV is an etiological agent of acute gastroenteritis. SaV infection is more frequently detected in young children than in adults. In addition, children at day-care centers and elementary schools are at greatest risk of SaV infection and transmission (Hansman et al., 2007a). Based on the epidemiological data from different detection methods such as electron microscopy, ELISA, and RT-PCR assays, the SaV detection rates ranged from 2.2-12.7% (Oka et al., 2015). In 2010, the largest foodborne outbreak of SaV has been recorded in Japan with 665 infected cases (Kobayashi et al., 2012). Moreover,

coinfections of SaV with multiple enteric viruses (such as NoV, rotavirus, astrovirus, enterovirus *etc*.) have been detected among the SaV outbreaks worldwide (Bon et al., 2005; Iizuka et al., 2010; Iritani et al., 2014; Nakata et al., 2000; Oka et al., 2015; Rasanen et al., 2010). Variety of SaV genotypes were identified in different geographical areas. For instance, SaV GI.1 was the predominant genotype of sporadic cases in Japan from 1999-2013 (Chan-it et al., 2010; Dey et al., 2010; Harada et al., 2009; Phan et al., 2005a, 2005b, 2006b; Shimizu et al., 2007; Thongprachum et al., 2015), and in the United Kingdom from 1989-2004 (Gallimore et al., 2006). The SaV GI.2 caused multiple outbreaks in the Netherlands between 2007-2009 (Svraka et al., 2010), in USA between 2002-2009 (Lee et al., 2012), and caused a large food borne outbreak in 2010 in Japan (Kobayashi et al., 2012). During 2011-2013, SaV GI.2 was reported as the predominant strains in sporadic cases in China (Zhang et al., 2016a). Recently, SaV GI.2 related to the gastroenteritis outbreaks during 2012-2013 in Japan (Iritani et al., 2016).

Based on the molecular epidemiological study of SaV in Thailand, the SaV detection rates ranged from 0.7-11% (Chaimongkol et al., 2012, 2014; Guntapong et al., 2004; Hansman et al., 2004; Khamrin et al., 2007, 2010, 2017; Pongsuwanna et al., 2017) (Table 2.4). The wide variety of SaV genotypes were found in Thailand, including SaV GI.1, GI.2, GI.4, GI.5, GII.1, GII.2, GII.3, and GIV. SaV was first reported in Thailand by Hansman et al. (2004) with the detection rate of about 4.8% in pediatric patients with diarrhea in Chiang Mai during 2000-2001. An epidemiology study from 2000-2002 demonstrated that SaV was detected at 3.4% in acute gastroenteritis pediatric patients in Chiang Mai and GI.1, GI.4, GI.5, GII.1, and GII.2 were detected (Malasao et al., 2008). During 2002-2003, a report from five distinct geographic regions in Thailand (Tak, Sa Kaeo, Nong Khai, Chanthaburi, and Songkhla) demonstrated the prevalence of SaV was about 11% in hospitalized children with acute gastroenteritis (Guntapong et al., 2004). A three years study in Chiang Mai during 2002-2004 revealed that SaV was detected at 1.2%, and GIV, GI.1, and GI.2 genotypes were detected (Khamrin et al., 2007). Then, in 2005, SaV was detected at 3.4% in pediatric

patients in Chiang Mai and the predominant genotypes were GI.1, GI.2, GI.5, and GII.3 (Khamrin et al., 2010). In 2006 and 2008, the surveillance of SaV was conducted in four distinct regions including Songkhla, Chanthaburi, Tak, and Nong Khai and it was found that SaV caused acute gastroenteritis in 1.1% of diarrheic children (Pongsuwanna et al., 2017). The epidemiological surveillance of SaV in acute gastroenteritis pediatric patients in Chiang Mai in 2007, 2010-2011 demonstrated that SaV was detected at 1.2% and only the GI.1 genotype was found in this study (Chaimongkol et al., 2012, 2014). Recently, during 2012-2014, the prevalence of SaV was reported at 0.7% in pediatric patients in Chiang Mai and 4 different genotypes including GI.1, GII.1, GII.4, and GIV.1 were detected (Khamrin et al., 2017).

Table 2.4 Summary of sapovirus prevalence in acute gastroenteritis patients in Thailand

Locations	Years	Prevalence of SaV (%)	Reference
Chiang Mai	2000-2001	4.8%	Hansman et al., 2004
Chiang Mai	2000-2002	3.4%	Malasao et al., 2008
Tak, Nong Khai, Sa Kaeo, Chanthaburi, Songkhla	2002-2003	11%	Guntapong et al., 2004
Chiang Mai	2002-2004	1.2%	Khamrin et al., 2007
Chiang Mai	2005	3.4%	Khamrin et al., 2010
Songkhla, Chanthaburi, Tak, Nong Khai	2006, 2008	1.1%	Pongsuwanna et al., 2017
Chiang Mai	2007, 2010-2011	1.2%	Chaimongkol et al., 2012, 2014
Chiang Mai	2012-2014	0.7%	Khamrin et al., 2017