CHAPTER II

LITERATURE REVIEW

Gastroenteritis or diarrhea is a major cause of children morbidity and mortality worldwide, especially in developing countries. Although pathogenic enteric bacteria are important etiologic agents, other agents such as viruses have also been recognized as an important cause of acute gastroenteritis in infants and young children.

Viral gastroenteritis is one of the most common illnesses in humans of all age especially in infants and the elderly. There are a number of viruses from different family cause diarrhea in humans and other animal species. Rotavirus (family *Reoviridae*) has been recognized as the main causative agents for this disease, followed by norovirus and sapovirus, (family *Caliciviridae*), adenovirus (family *Adenoviridae*), and astrovirus (family *Astroviridae*), respectively. Other viruses in the family *Picornaviridae* (enterovirus, human parechovirus, and Aichi virus) have also been recognized as the causative agents of diarrhea.

A. Gastroenteritis viruses in *Reoviridae* family

Rotavirus

Rotavirus (RV) is the most common cause of severe diarrhea among infants and young children under 5 years old worldwide. Global surveillance performed in 2004 indicated that rotavirus caused diarrhea in 29% of hospitalized children and 527,000 (range 475,000–580,000) deaths (Parashar et al., 2009). RV is a member of

the *Rotavirus* genus of the *Reoviridae* family. The virus particle is an icosahedral, non-enveloped virus with the size of 70 - 75 nm in diameter. The particle has a characteristic of wheel-like appearance (Latin, rota means wheel). The viral genome consists of 11 segments of double-stranded RNA (dsRNA), which are packaged within a triple-layer capsid, encoding six structural viral proteins (VPs), (VP1, VP2, VP3, VP4 (VP5+VP8), VP6, and VP7) and six non-structural proteins (NSPs), (NSP1, NSP2, NSP3, NSP4, NSP5, and NSP6). The outer capsid is composed of two structural proteins, VP7 and VP4 (spike protein), which have been discriminated genetically as G genotype (G-type) and P genotype (P-type) of group A rotavirus (RVA), respectively. The middle capsid consists of a single protein VP6, which used to classify RV into group and subgroup, and surrounds the core particle. The inner capsid protein or core protein consists VP2 encloses two structural proteins (VP1 and VP3), which contains the viral genomes.

Generally, classification of RV is based on four antigenic specificities, which are carried by the two outer capsid proteins (VP7 and VP4), the middle capsid protein (VP6) and non structural protein 4 (NSP4) (Estes and Kapikian, 2007). The two outer capsid proteins, VP7 and VP4, form the basis of the current dual classification system of RVA into G (glycoprotein) and P (protease-sensitive protein) genotypes, respectively. The middle capsid protein VP6 allows rotavirus classification into seven distinct groups, group A to G. Moreover, the VP6 protein is responsible for subgroup (SG) specificities, allowing classification of RVA into subgroups (SG), SG I, SG II, SG (I+II), and SG non (I+II) (Estes and Kapikian, 2007). In addition, the non-structural protein 4 (NSP4) encoded by gene segment 10 has been reported to be associated with viral morphogenesis, pathogenesis, and its enterotoxin-like activity.

Analysis of NSP4 gene of RVA strains can be classified into six genetic groups (genogroups), including KUN (A), Wa (B), RRV (C), Murine (D), Avian (E), and Porcine (F) genetic groups (Cunliffe et al., 1997; Horie et al., 1997; Kirkwood et al., 1997; Ciarlet et al., 2000; Mori et al., 2002; Khamrin et al., 2008). Recently, a novel classification system based on the nucleotide sequences of all RV gene segments has been proposed by the Rotavirus Classification Working Group (RCWG) (Metthijnssens et al., 2008). Based on nucleotide sequence identity cut-off percentages, the strains under investigation that shared above the cut-off value of that gene segments 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 (Table 1). Currently, at present 27 G genotypes and 35 P genotypes of RVA have been reported (Matthijnsses et al., 2011).

Table 1 A summary of nucleotide percentage identity cut-off value defining genotype for 11 rotavirus gene segments (Matthijnsses et al., 2008)

Gene product	Percent identity Cut-off value (%)	Genotypes	Name of genotypes
VP1	83	9R	**R**NA-dependent RNA polymerase
VP2	84	9C	**C**ore protein
VP3	81	8M	**M**ethyltransferase
VP4	80	35P	**P**rotease-sensitive
VP6	80	16I	**I**nner-capsid
VP7	80	27G	**G**lycosylated
NSP1	79	16A	Interferon **A**ntagonist
NSP2	85	9N	**N**TPase
NSP3	85	12T	**T**ranslation enhancer
NSP4	85	14E	**E**nterotoxin
NSP5	91	11H	p**H**osphoprotein

Based on several epidemiological studies, it is well-documented that all seven groups (A to G) of rotaviruses have been found associated with infection in various animal species. Among them, only group A, B, and C rotaviruses are associated with diarrhea in humans. RVA is the major etiological cause of acute viral gastroenteritis in infants and young children worldwide, while group B and C rotaviruses are minor pathogens causing diarrhea in children and adults. Group B rotavirus was first described in waterborne outbreaks in China, involving the cases of nationwide epidemics of diarrhea in adults 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. Moreover, RVA is also known to be the cause of acute gastroenteritis in adults, which has been described as epidemic outbreaks, travel-related gastroenteritis, infections transmitted from children to adults, and endemic cases (Hrdy, 1987; Anderson and Weber, 2004). There are many reports of epidemic outbreaks among RVA in adults in closed communities, such as long-term health-care facilities and homes for the elderly, although their frequencies are relatively lower than outbreaks that caused by norovirus and other viral agents (Halvorsrud and Orstavik, 1980; Feeney et al., 2006; Iijima et al., 2006). In North America, the prevalence and characteristics of RV has been investigated in adults and the prevalence ranged from 20% to 63.6% (Pietruchinski et al., 2006; Carraro et al., 2008). In Europe, RVA was detected at the prevalence of 3.2% in adults with diarrhea in Sweden (Rubilar et al., 2005). In Asia, RVA is also frequently detected in adults with diarrhea at 5% to 10.1% (Sanekata et al., 2003; Uchida et al., 2006; Wang et al., 2007; Paul et al., 2008; Aung et al., 2009; Wang et al., 2009; Tatte et al., 2010).

In Thailand, most of the studies of RV infection were done mainly in children with diarrhea but not in adults. Several studies carried out in children with diarrhea and revealed that RVA was responsible for about 27% to 34% of diarrhea in hospital cases (during 1977-1997) (Maneekarn and Ushijima, 2000; Jiraphongsa et al., 2005). For the epidemiological studies of RV in Chiang Mai province, Thailand during 2000-2007, the data demonstrated that the prevalence of RVA infection were 34%, 37.3%, 29.3%, 25.6%, 27.5% in 2000-2001, 2002-2004, 2005, 2006, and 2007, respectively (Khamrin et al., 2006, 2007b, 2010; Chaimongkol et al., 2012). In addition, in 2007-2009, Khananurak et al. (2010) reported the distribution of RVA circulating in infants and young children with diarrhea admitted to four hospitals in Bangkok, Khon Kaen, Nakhon Ratchasima and Tak provinces. RVA was detected at 28.4%. From the literature reviews above, the surveillances of RVA has been conducted extensively in children, while the epidemiology of RV in adults in Chiang Mai has not been conducted.

B. Gastroenteritis viruses in *Caliciviridae* family

The virus in the family *Caliciviridae* is a small, icosahedral, non-enveloped virus with the size of about 27 - 40 nm in diameter. Genome of the virus is a positive-sense, single-stranded RNA (ssRNA). Four genera comprise in this family are *Norovirus*, *Sapovirus*, *Vesivirus*, and *Lagovirus*. The major viral pathogens cause acute gastroenteritis in this family are *Norovirus* and *Sapovirus* which are responsible for outbreaks in various epidemiological settings, including restaurants, schools, day-care centers, hospitals, and nursing homes (McIntyre et al., 2002; Akihara et al., 2005).

Norovirus

Norovirus (NoV) formerly called ‘‘Norwalk-like virus” is an important cause of acute gastroenteritis in infants, young children, and adults in developed and developing countries. In 1929, Zahorsky et al. (1929) first described “winter vomiting disease”, an illness characterized by the sudden onset of self-limited vomiting and diarrhea that typically peaked during the colder months. Later, Kapikian and others discovered the etiology of this syndrome by immune electron microscopic (IEM) examing of stools collected from elementary school students affected by an outbreak of gastroenteritis in 1968 (Green, 2007). A key characteristic of the calicivirus is the existence of 32 cup-like depression on the surface of virus particle (calici is derived from the Latin word calyx, or cup). The viral particle is an icosahedral, non-enveloped virion with the size of 27-34 nm in diameter. The NoV genome is approximately 7.4 - 7.7 kb in length that contains three major open reading frames (ORFs). ORF1 encodes the nonstructural polyprotein that is cleaved by viral 3C-like protease into probably 6 proteins, such as nucleotide triphosphatase (NTPase), genome-linked protein (VPg), proteinase, and RNA-dependent RNA polymerase (RdRp) which are viral replicase proteins essential for replication (Green, 2007). ORF2 and ORF3 encode the major (VP1) and minor (VP2) capsid proteins, respectively (Jiang et al., 1990, 1992; Green et al., 2000).

NoV is classified into the genus *Norovirus* within the family *Caliciviridae* (Green, 2007). Currently, the nomenclature system for identification of calicivirus strain 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/ GII-4/ Hokkaido5/ 2007/ JPN. NoVs can be divided into five

distinct genogroups (G), GI, GII, GIII, GIV, and GV based on the diversity of the capsid sequence, and only GI, GII, and GIV have been found in humans (Kageyama et al., 2004; Zheng et al., 2006), while GIII and GV have been found in bovine and murine, respectively (Oliver et al., 2003; Wobus et al., 2004). In addition, NoV genogroups can be subdivided into several genotypes. NoV GI and GII are divided into 15 and 19 genotypes, while each of GIII and GIV comprised of 2 genotypes, whereas only 1 genotype in GV has been reported (Glass et al., 2009).

NoV has been reported as the causative agent associated with acute gastroenteritis in infants, children, and adults worldwide (Fankhausers et al., 2002). During 1996-2000, the NoV outbreaks were occurred in multiple settings in the USA, such as 39% in restaurants, 29% in nursing homes and hospitals, 12% in schools and day-care centers, 10% in vacation areas and cruise ship, and 9% in other settings (Parashar et al., 2001). Moreover, an outbreak of acute gastroenteritis from a swimming pool in Vermont was reported and about 50% of cases were attributed to NoV (CDC, 2004). The epidemiological studies in various countries have shown that the detection rate of NoV infection in children with diarrhea appeared to be varied from different geographical regions, such as 56% in Spain, 36% in UK, 43% in Hungary, 44.9% in Germany, 35% in Brazil, 4.5% in Bangladesh, and 14.5% in Poland (Buesa et al., 2002; Lopman et al., 2003; Reuter et al., 2005; Ike et al., 2006; Dey et al., 2007; Ferreira et al., 2010; Oldak et al., 2011). The predominant strains of NoV circulating worldwide belong to GII, and GII/4 has been reported as the most prevalent genotype (Fankhauser et al., 2002; Blanton et al., 2006).

In Thailand, the epidemiological studies of NoVs are less frequently reported than that of RV. Previous study of NoV infection in Chiang Mai revealed that NoV

was detected at 8.1% from children admitted to hospital with diarrhea during 2000-2002 (Malasao et al., 2008). Among these NoV strains, both NoV GI and GII were found at 29.2% and 70.8%, respectively, and several NoV genotypes were identified, including GII/4, GII/3, GII/10, GII/1, GII/6, GII/8, GII/15, GI/3, GI/4, GI/6, GI/7, and GI/13. Later, in 2002-2004, the detection rate increased from 8.1% to 14.1%. NoV GII/4 was the most predominant genotype, followed by GII/3, GII/1, GII/7, GII/2, and GII/16 (Khamrin et al., 2007a). In the following year of 2005, the detection rate of NoV in Chiang Mai province decreased from 14.1% to 6.8%. The GII/4 was still detected as the most common genotype along with other genotypes, GII/5, GII/6, and GII/12 (Khamrin et al., 2010). Most recently, the epidemiological study of NoV in Chiang Mai revealed that the prevalent rate of NoV infection in pediatric patients was 13.8%. Nucleotide sequence and phylogenetic analyses showed that eight distinct genotypes of GII namely GII/2, GII/3, GII/4, GII/6, GII/7, GII/13, GII/16, GII/new, and one isolate of GI/14 were detected (Chaimongkol et al., 2012).

Sapovirus

Sapovirus (SaV), previously known as "Sapporo-like virus" (SLV), is also the member in the family *Caliciviridae* as NoV. The particle of SaV has a typical "Star of David" appearance by electron microscope. Sapovirus is a non-enveloped RNA virus with a single stranded positive sense genome of approximately 7.4 kb. The viral genome consists of either two or three open reading frames (ORF1-ORF3) depends on the genogroup of the virus. The genome of SaV GI and GV contain all three ORFs (ORF1, ORF2, and ORF3) while SaV GII and SaV GIII contain only two ORFs (ORF1 and ORF2). The ORF1 encodes a polyprotein which undergoes protease

processing to produce several nonstructural proteins and major capsid protein (VP1). The ORF2, which is located at the 3' end, encodes a putative VP2 minor structural protein (Wilhelmi et al., 2003), and ORF3, which is an ORF that overlaps with the 5' end of VP1 gene, encodes protein of unknown function (Schuffenecker et al., 2001).

SaV belongs to genus *sapovirus* within the family *Caliciviridae* (Green, 2007). SaV is classified, based on the differences of the complete capsid amino acid sequence, into 3 major genogroups, GI (known as the Manchester virus), GII (known as the London virus), and GIII (porcine enteric calicivirus, PEC). In 2004, two SaV strains were identified and grouped into the new genogroups GIV (Hou7-118/90 strain) and GV (Argentina39 strain). Currently, those genogroups can be subdivided into genotypes and at least 8 genotypes in GI, 5 in GII, and 1 each in GIII, GIV, and GV have been identified (Akihara et al., 2005).

SaV was originally isolated from an infant in an outbreak of gastroenteritis in Sapporo, Japan in 1997 (Chiba et al., 1980). Several studies have noted that SaV detection rate was less frequent than those of NoV (Hansman et al., 2007). Most of SaV infections in human are caused by SaV GI among outbreaks and sporadic cases (Phan et al., 2006). In Europe, the detection rates of SaVs have been reported in various countries, such as 1.3% in Finland, and 7% in France (Buesu et al., 2002; Bon et al., 2005). In Asia, SaV is also frequently detected in feces of infants hospitalized with diarrhea. In Japan, during 2004-2005, SaV was detected at 17.6% of infants and young children with acute gastroenteritis, all strains detected in that study belonged to GI (Phan et al., 2007). Afterward, in 2005-2007, SaV detection rate in Japan was reported at 19.2% (Harada et al., 2009). In Taiwan, a first SaV outbreak of gastroenteritis caused by GI/2 was reported in the year 2007 (Wu et al., 2008).

In Thailand, even though few epidemiological studies of SaV have been conducted, the data demonstrated that SaV GI has been persisted consistenly and SaV GI/1 was the most prevalent genotype. Moreover, other strains including GI/2, GI/4, GI/5, GI/6, GII/1, GII/2, GII/3, and GIV were also reported to circulating from time to time (Guntapong et al., 2004; Hansman et al., 2004; Malasao et al., 2008; Kittigul et al., 2009; Khamrin et al., 2010). Recently, a survey conducted in Chiang Mai province revealed that SaV GI/1 was detected with the prevalent rate of 3.1% in children with gastroenteritis in 2007 (Chaimongkol et al., 2012).

C. Gastroenteritis viruses in *Adenoviridae* family

The virus in the family *Adenoviridae* is an icosahedral, non-enveloped virus with the size of about 90 - 100 nm in diameter. Genome of the virus is a double-stranded DNA (dsDNA) of approximately 26 – 45 kb in length (Davison et al., 2003). This family consists of four genera *Mastadenovirus*, *Aviadenovirus, Atadenovirus,* and *Siadenovirus* (Wold and Horwutz, 2007).

Adenovirus

Adenovirus (AdV) was first isolated and characterized in 1953 during attempted to establish tissue culture from tonsils and adenoidal tissue surgically removed from children (Rowe et al., 1953), from which the name were derived. AdV causes various diseases such as conjunctivitis, respiratory infectious disease, diarrhea in infants and young children (Wadell, 1984; Moura et al., 2007; Aoki et al., 2008).

AdV is a member of the genus *Mastadenovirus* of the family *Adenoviridae.* The virus particle is approximately of 80 nm in diameter. The AdV genome is a double stranded DNA of approximately 36-38 kb in length and associated with an

inner protein core. By now, the complete sequences of at least seven AdV genotypes have been determined and revealed that the DNA sequences encoded most virion structural proteins, and non structural proteins involved in viral replication and viral assembly (Wold and Horwitz, 2007).

AdV is classified into seven subgroups, A to G (Jones et al., 2007) based on the serology. To date, at least 51 different adenovirus serotypes (and 5 proposed types, AdV52 to AdV56) have been distinguished on the basis of their resistance to neutralization by antisera (Robinson et al., 2011), as summarized in Table 2**.**

Table 2 Classification of adenoviruses

Subgroup	Serotypes
A	12, 18, 31
B	3, 7, 11, 14, 16, 21, 34, 35, 50, 55
C	1, 2, 5, 6
D	8, 9, 10, 13, 15, 17, 19, 20, 22, 23, 24, 25, 26, 27, 28, 29, 30, 32, 33, 36, 37, 38, 39, 42, 43, 44, 45, 46, 47, 48, 49, 51, 53, 54, 56
E	4
F	40, 41
G	52

The AdV subgroup F (AdV40 and AdV41) has been recognized as an important etiologic agent of gastroenteritis in children worldwide (Cruz et al., 1990; Shimizu et al., 2007). The data clearly indicated that subgroup F is “enteric adenoviruses”, in addition to subgroups A and C which have also been implicated in the etiology of diarrhea (Brown et al., 1996), while other subgroups have been reported to be frequently associated with respiratory illnesses and epidemic conjunctivitis (Gordon et al., 1996; McNeill et al., 1999). During 1998-2001, epidemiological studies conducted in Japan, Korea, and Vietnam in children with diarrhea revealed the prevalent rate of 4.4%, of which more than half were AdV41, while AdV40, AdV2, AdV3, AdV8, and AdV31 were also detected (Li et al., 2005). In Japan, the detection of AdV in children with acute diarrhea revealed that AdV41 was the most prevalent serotype, and other serotypes including AdV1, AdV2, AdV3, and AdV5 were also detected while AdV40 was not detected in this study (Shimizu et al., 2007). In contrast, the epidemiological surveillance in Bangladesh reported the disappearance of AdV41, while AdV40 predominanted over other serotypes (Dey et al., 2009). Moreover, the epidemiological studies of AdV infection in children with gastroenteritis during 2003-2006 in South-Western Hungary found that the detection rate was 8.1% during four years study, with a gradual decrease from 11.7% in 2003 to 5.7% in 2006. Molecular characterization of AdV-positive samples found that AdV40 strains were identified only in 2003 and 2004, while AdV41 strains were identified throughout the study period (Banyai et al., 2009).

In Thailand, the frequency of AdV detection rate in children with diarrhea ranged from 1.8% to 4.4% (Herrmann et al., 1988; Kittigul et al., 2009; Chaimongkol et al., 2012). The AdV40 and AdV41 were detected in Bangkok in children with and

without gastroenteritis at 4.4% and 1.8%, respectively (Herrmann et al., 1988). During 2006-2007, molecular characterization of AdV had been performed in children hospitalized with acute gastroenteritis in Lopburi province. It was found that 4 out of 262 fecal samples were positive for AdV, of which two strains belonged to AdV 41 while the other two strains belonged to AdV2 and AdV38 (Kittigul et al., 2009). Recently, a survey in Chiang Mai province in 2007, AdV was detected with the prevalent rate of 3.1% in children with gastroenteritis and the genotypes detected were AdV1, AdV3, AdV41 (Chaimongkol et al., 2012).

D. Gastroenteritis viruses in *Astroviridae* family

The virus in the family *Astroviridae* is a small round, icosahedral, non-envelope virus with the size of 28-32 nm in diameter. The viral genome is composed of a positive-sense, single-stranded RNA (ssRNA) of approximate 6.7-7.9 kb. This family includes human and animal astroviruses, and the viral particle appears as a characteristic star-like surface structure when observed by electron microscope (EM). Astrovirus was first described as a human pathogen in 1975 during an outbreak of acute gastroenteritis among infants in the UK (Madeley and Cosgrove 1975). Astroviruses are recognized as one of the most common cause of viral gastroenteritis in infants and young children worldwide (Glass et al., 1996; Giordano et al., 2001).

Astrovirus

Astrovirus (AstV) is one of the members of genus *Astrovirus* within the family *Astroviridae*. AstV is a distinctive five or six pointed appearance designated as astrovirus (Greek, astron meaning ‘star’). The AstV genome contains three open reading frames (ORFs), ORF1a and ORF1b encode for the non-structural protein,

including protease (Pro) and RNA-dependent RNA polymerase (RdRp), while ORF2 encodes for the capsid protein.

AstV can be classified into serotypes according to the reaction of the capsid protein with type-specific MAbs which have been developed for the detection of the virus in stools of patients with acute gastroenteritis (Hermann et al., 1991). Moreover, AstV can also be classified into genotype on the basis of partial nucleotide sequence of the capsid gene (ORF2) using reverse transcription-polymerase chain reaction (RT-PCR) (Noel et al., 1995). There is a good correlation between results of genotype and serotype (Sakamoto et al., 2000). Currently, AstV can be grouped into eight serotypes (AstV1 to AstV8) based on the reactivities of the capsid proteins with polyclonal and monoclonal antibodies (Mendez-Toss et al., 2000).

Currently, AstV appeared to be rare cause of acute gastroenteris in human. Epidemiological studies around the world have demonstrated that serotype 1 is the predominant type, followed by serotypes 2, 3, 4, and 5, which are less common, while serotypes 7 and 8 are relatively rare serotypes compared to other serotypes (Mustafa et al., 2000; Jeong et al., 2010). Disease cause by AstV is generally milder than that cause by RV and NoV. However, there have been reported with incidence of severe diarrhea that frequent co-infection of AstV with RAV and calicivirus (norovirus and sapovirus). The prevalence of AstV infection vary widely from 2% to 16% among hospitalized children with acute gastroenteritis (Oliver and Philips, 1988), and the peak incidence of AstV infection found in winter in temperate climate and in rainy season in the tropical regions (Matsui et al., 2001). In Japan, Oishi et al. (1994) had demonstrated that 10 of 38 stool samples (26.3%) from the outbreak were positive for AstV when tested by ELISA and polymerase chain reaction tests. Later, in 1998,

Steele et al. (1998) examined fecal samples from adults and children with gastroenteritis in South Africa, and found that AstV infection in adults was detected at the rate of 3.1%.

In Thailand, the epidemiological study of AstV infection was initially reported in 1991 by Hermann et al. AstV was detected at 8.6% and 2.1% in children with and without gastroenteritis in Bangkok, respectively. Later, in 2004, a report from Ratchaburi province and Ramathibodi hospital demonstrated the relevance of AstV as a cause of gastroenteritis and found that AstV was detected at the rate of 30.7% (Sirinavin et al., 2006). In 2000-2002, molecular epidemiological study described the prevalence of AstV circulating in Chiang Mai, Thailand at the rate of 2% (Malasao et al., 2008). The strains detected belonged to AstV1, AstV2, AstV3, and AstV5. Most recently, Chaimongkol et al. (2012) reported the detection of AstV circulated in children with gastroenteritis in Chiang Mai in 2007 at a low rate of 0.6% and the genotypes detected belonged to AstV1 and AstV2.

E. Gastroenteritis viruses in *Picormaviridae* family

The virus in the family *Picornaviridae* is non-enveloped virus with a positive sense, single-stranded RNA (ssRNA) genome. Special characteristics of viral genome in this family are a VPg, 5'UTR, leader protein, structural protein, non structural protein, and a 3'UTR. The 5'UTR is important in translation and the 3'UTR involves in negative strand synthesis. The family *Picornaviridae* contains several important human and animal pathogens which are grouped into twelve genera, *Aphthoviruses, Avihepatoviruses, Cardioviruses, Enteroviruses, Erboviruses, Hepatoviruses,*

Kobuviruses, Parechoviruses, Sapeloviruses, Senecaviruses, Teschoviruses, and Tremoviruses (Racaniello, 2007).

Kobuvirus was classified as a new genus, *Kobuvirus*, in 1999. The name 'kobu' was derived from the Japanese word for bump or knob, which is the characteristic morphology of the virus particle observed by EM. The genus kobuvirus consisted of 2 species, Aichi virus (AiV) and bovine kobuvirus, and one candidate kobuvirus species is a porcine kobuvirus (Reuter et al., 2008, 2009a). The major viral pathogen for human in this genus is the AiV. The AiV was first isolated from a person with acute gastroenteritis in 1989 in Japan (Yamashita et al., 1991). Later, AiV associated with acute gastroenteritis in human has been described in Asia and Europe (Yamashita et al., 2000; Sdiri-Loulizi et al., 2008; Pham et al., 2007).

Aichi virus

AiV is a small, non-enveloped virus with the size of 30 nm in diameter (Yamashita et al., 1991). The AiV genome is approximately 8.2 kb in length. The genome encodes a single polyprotein (2432 amino acid), which undergoes a cleavage cascade performed by virus encoded enzymes to give the final virus proteins. Predicted cleavage sites are similar to those in other picornaviruses, all being cleavaged between a glutamine (Q) and another amino acid, frequently glycine (G), alanine (A) or serine (S) and less frequently histidine (H), cysteine (C) or threonine (T). All kobuviruses share essentially the same genome organization. Nonstructural protein L (Leader), is encoded at the N-terminus of the polyprotein followed by three structural viral proteins (VPs), VP0, VP3, VP1 and seven nonstructural viral proteins (NSPs), NSP2A, NSP2B, NSP2C, NSP3A, NSP3B, NSP3C, and NSP3D (Yamashita et al., 2000).

AiV is classified into the genus *Kobuvirus* in the family *Picornaviridae*. To date, AiV can be divided into three genetically distinct genotypes including genotypes A, B, and C, based on the 519 base RNA sequences at the C terminus of 3C and N terminus of 3D (3CD region) (Yamashita et al., 2000; Ambert-Balay et al., 2008).

Initially, Yamashita et al. (1993) had reported the AiV infection in patients with gastroenteritis in Japan, at the rate of 18.8%. In addition, in 2000, Yamashita demonstrated the prevalence of AiV infection in patients with gastroenteritis by reverse-transcription PCR (RT-PCR) at the rate of 20.5%. Later, in 2003-2005, the epidemiological studies of AiV performed in patients with diarrhea reported the prevalent rate of 6.5% in Japan, 2.5% in Bangladesh, 1.6% in Vietnam, and 1.5% in Hungary (Pham et al., 2007; Reuter et al., 2009b). Moreover, AiV was also isolated from sporadic cases of gastroenteritis in children and adults, such as in Pakistan, France, which was detected at the rate of 2.2% and 0.9%, respectively, from children with gastroenteritis (Yamashita et al., 1995; Sdiri-Loulizi et al., 2008).

In Thailand, a total of 107 fecal specimens collected from patients with acute gastroenteritis were tested for AiV and only one fecal specimen was positive for AiV. This was the first report of AiV detection in a child with acute gastroenteritis in Thailand (Pham et al. 2007).

Enterovirus

Enterovirus (EV) is a small, non-envelope virus with the size of about 30 nm in diameter. The EV genome is approximately 7.0-8.5 kb in length which consists of the 5' and 3' untranslated regions (UTRs), single open reading frame (ORF), and the 3' poly (A) tail (Steil and Barton, 2009). A single open reading frame encodes a long viral protein which undergoes post-translational cleavage into the mature viral

proteins. These viral proteins include the four viral structural proteins (VPs), VP1, VP2, VP3, VP4, and seven nonstructural proteins (NSPs), NSP2A, NSP2B, NSP2C, NSP3A, NSP3B, NSP3C and NSP3D, of which the NSP3D is RNA dependent RNA polymerase (Kitamura et al., 1981). EV has been shown to associate with wide variety of clinical features, including common cold, hand-foot-mouth disease, acute hemorrhagic conjuncitivitis, myocarditis, aseptic meningitis, and acute gastroenteritis (Pallansch and Roos, 2007).

EV is a member of the genus *enterovirus* of the family *Picornaviridae.* The genus *enterovirus* was originally classified into four speices, A to D, which included four groups of viruses, *Polioviruses*, *Coxsackieviruses* A, C*oxsackieviruses* B, and *Echoviruses* (Pallansch and Roos, 2007), as summarized in Table 3. The reverse transcription-polymerase chain reaction (RT-PCR) using primers designed to target the 5' untranslated region (5'UTR) or VP1 region of the EV genome has been used for the detection of EV (Muir et al., 1998; Oberste et al., 2003). Recently, genotyping of EV is based on analyses of the sequence encoding the VP1 major capsid protein. The EV strains that have 75% of nucleotide or 85% amino acid sequence of complete or partial VP1 sequence are considered to represent the same genotype (Oberste et al., 2005).

Table 3 Classification of enteroviruses

Species	Serotypes
A	EV71, EV76, EV89, EV90, EV91,EV92 CV-A2, CV-A3, CV-A4, CV-A5, CV-A6, CV-A7, CV-A8, CV-A10, CV-A12, CV-A14, CV-A16
B	CV-B1,CB-2, CV-B3,CV-B4, CV-B5, CV-B6, CV-A9, CV-A23 EV69, EV73, EV74, EV75, EV77, EV79, EV80, EV81, EV82, EV83, EV84, EV85, EV86, EV87, EV88, EV93, EV97, EV98, EV100, EV101, EV106, EV107 E1, E2, E3, E4, E5, E6, E7, E9, E11, E12, E13, E14, E15, E16, E17, E18, E19, E20, E21, E24, E25, E23, E26, E27, E29, E30, E31, E32, E33
C	CV-A1,CV-A11, CV-A13, CV-A17, CV-A19, CV-A20, CV-A21, CV-A22, CV-A24 EV95, EV96, EV99, EV102, EV104, EV105, EV109 PV1, PV2, PV3
D	EV68, EV70, EV94

CV-A= coxsackievirus A, CV-B= coxsackievirus B, EV= enterovirus, E= echovirus, PV= poliovirus.

A surveillance for EV in Japan in infants and children with acute gastroenteritis during 2007-2008 revealed that the EV was detected at the rate of 16.6% (Pham et al., 2010). In contrast, the detection rate of EV in children with acute gastroenteritis in Chiang Mai, Thailand in 2007 was much lower at 3.8% (Chaimongkol et al., 2012).

Human Parechovirus

Human parechovirus (HPeV) is a small, non-enveloped virus with the size of about 24-30 nm in diameter. The HPeV genome is approximately 7.3 kb in length, and contains a large open reading frame encoding for a single polyprotein flanked by 5' and 3' untranslated regions (UTRs). The polyprotein is post-translationally cleaved into three structural viral proteins (VPs), VP1, VP2, VP3 and seven nonstructural proteins (NSPs), 2A to 2C and 3A to 3D. The genus *Parechovirus* consists of two species, *Human parechovirus* and *Ljungan virus*. HPeVs were first isolated in 1956 in the USA from stool specimens from children with diarrhea, previously known as echovirus 22 (HPeV1) and echovirus 23 (formerly HPeV2), respectively (Hyypia et al., 1992). Later, they were renamed and reclassified into their own genus in 1999 based on differences in genome organization and structure, as well as divergence of encoded proteins and biological properties (Hyypia et al., 1992; Stanway and Hyypia, 1999). HPeV is associated with mild gastrointestinal tract infection in children (Baumgarte et al., 2008; Chen et al., 2009; Pham et al., 2011a, 2011b) and animals (Shan et al., 2010). More severe consequences also have been ascribed to human parechovirus infections, including acute flaccid paralysis (AFP) (Ito et al., 2004), encephalitis (Legay et al., 2002), aseptic meningitis (Stanway et al., 2000),

myocarditis (Russell and Bell, 1970), neonatal sepsis (Boivin et al., 2005), and Reye syndrome (Watanabe et al., 2007).

HPeV is a member in the genus *Parechovirus* of the family *Picornaviridae*. At present, HPeVs can be classified into 14 genotypes based on VP1 sequence. HPeV1 and HPeV2 infections are most commonly associated with mild respiratory or gastrointestinal infections. Epidemiogical studies of HPeV infections revealed the prevalences at 16% from Brazilian children (Drexler et al., 2009) and 11.6% from patients in Germany (Baumgarte et al., 2008). Moreover, Benschop et al. (2008) had reported the prevalence of HPeV infection rate at 17.5%, 13.2%, and 18.2% of children with gastroenteritis in Netherland in 2004, 2005, and 2006, respectively. Recently, Pham et al. (2010) reported HPeV infection in infants and children hospitalized with acute gastroenteritis in Sri Lanka during 2005-2006 at the rate of 8.3% and several HPeV genotype were identified, including HPeV1, 3, 4, 5, 10, and 11. Additionally, Pham et al. (2011a) demonstrated that HPeV infection in children with acute gastroenteritis in Japan during 2007-2008 was detected at 8.1%.

In Thailand, Pham et al. (2009) have screened for HPeV by RT-PCR in 82 fecal specimens from children with acute gastroenteritis which were known to be negative for RV, AdV, NoV, SaV, and AstV. HPeV was detected in 12 of 82 specimens tested (14.6%). The HPeV strains detected in that study clustered into four different genotypes, HPeV1 to HPeV4. This was the first study of HPeV in children with acute gastroenteritis in Thailand (Pham et al., 2010b).