CHAPTER 2

Literature review

Rotaviruses (RVs) are the major cause of severe diarrhea in infants, young children, and in young animals of many species worldwide (Kapikian et al., 2001). Rotavirus was first recognized as a causative agent of diarrhea in human in 1973 (Bishop et al., 1973). Before that RVs had been discovered in diarrheic mice (Adams and Kraft, 1963), monkeys (Malherbe and Harwin, 1963) and cattle (Mebus et al., 1969). Later, the virus has been detected in wide variety of mammalian species including pig (Khamrin et al., 2007a; Maneekarn et al., 2006; Saikruang et al., 2013), cat (Martella et al., 2000; Taniguchi et al., 1994), dog (Ciarlet et al., 2000), sheep (Chen et al., 2009), horse (Matthijnssens et al., 2012a), bats (Estes and Greenberg, 2013; He et al., 2013) and also in birds (Kindler et al., 2013; Otto et al., 2012; Trojnar et al., 2009). RVs are the major cause of deaths in children less than 5 years of age, particularly, in developing countries in Asia and Africa. (Parashar et al., 2006; Parashar et al., 2009; Tate et al., 2012). Worldwide, it is estimated that RVs cause the deaths of 527,000 children (range 475,000-580,000) in 2004 (Parashar et al., 2009) and 453,000 children (range 420,000-494,000) in 2008 (Tate et al., 2012). Apart from high mortality, RVs infection annually results in an estimated of 457,000-884,000 hospitalizations and 2 million outpatient visits in children younger than 5 years of age (Tate et al., 2009). In addition to its impact on human, RVs also infect many animal species, including pig. Group A rotavirus (RVA) is one of the most frequently detected enteric pathogens associated with diarrhea in piglets between 1 and 8 weeks of age (Saif et al., 1994).

2.1 General characteristics and genome structure of rotaviruses

A mature rotavirus particle is a non-enveloped triple-layered icosahedral capsid of about 70-75 nm in diameter. The rotavirus particle shows a wheel-like appearance when viewed under the electron microscope, hence, the wheel in Latin means "rota" (Flewett et al., 1974), as demonstrated in Figure 2.1. The viral genome consists of 11 segments of double-stranded RNA (dsRNA) of 667-3,302 bp in length (Estes and Cohen, 1989; Ramig, 1997). The rotavirus genome segments encode 6 structural proteins (VP1-VP4, VP6, and VP7) and 6 nonstructural proteins (NSP1-NSP6). The genes are monocistronic, except for segment 11, which encodes for two nonstructural proteins, NSP5 and NSP6 (Estes and Kapikian, 2007). The NSPs are synthesized in infected cell and function in some aspects of the viral replication cycle or interact with host proteins in the process of pathogenesis or immune response to infection. The rotavirus protein VP4 and VP7 are the outer capsid protein which induce neutralizing antibodies and used to classify rotaviruses into P (protease sensitive protein) (VP4) and G (glycoprotein) (VP7) genotypes. The middle capsid is formed exclusively by the most abundant protein of VP6, which surrounds the inner core. The inner layer comprises of VP2 (inner shell), VP1 (RNA-dependent RNA polymerase), and VP3 (methyltransferase) proteins, as shown in Figure 2.2. The individual protein encoded by each genome segment and its function are listed in Table 2.1 (Matthijnssens et al., 2008; Mlera, 2012). All of 11 segments of dsRNA genome have the molecular weight (MW) range from 2.0×10^5 to 2.2×10^6 daltons with the sizes of 0.6 to 3.3 kilobasepair (kbp) (Estes and Kapikian, 2007). The clinical manifestations of rotavirus range from mild to severe gastroenteritis with diarrhea, vomiting, and lethal dehydration. The body temperature is often ranging from moderate to high. Vomiting precedes diarrhea and lasts for a short period (3-5 days). Death from RV disease is mainly due to severe dehydration and cardiovascular failure. Rotavirus is transmitted by fecal-oral route and possibly by contaminated surfaces and hands of caregivers (Mlera, 2012; Vesikari et al., 2006).

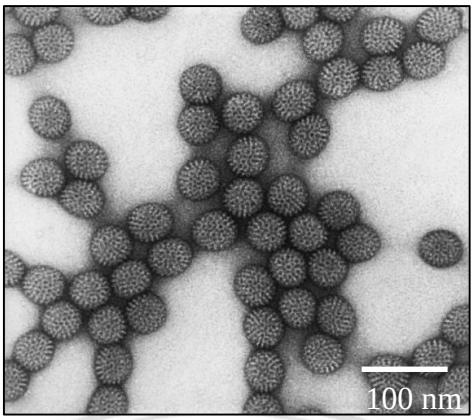

Figure 2.1 Electron micrograph of intact rotavirus particles.
(Adapted from http://pathmicro.med.sc.edu/virol/rota-cdc2.jpg)

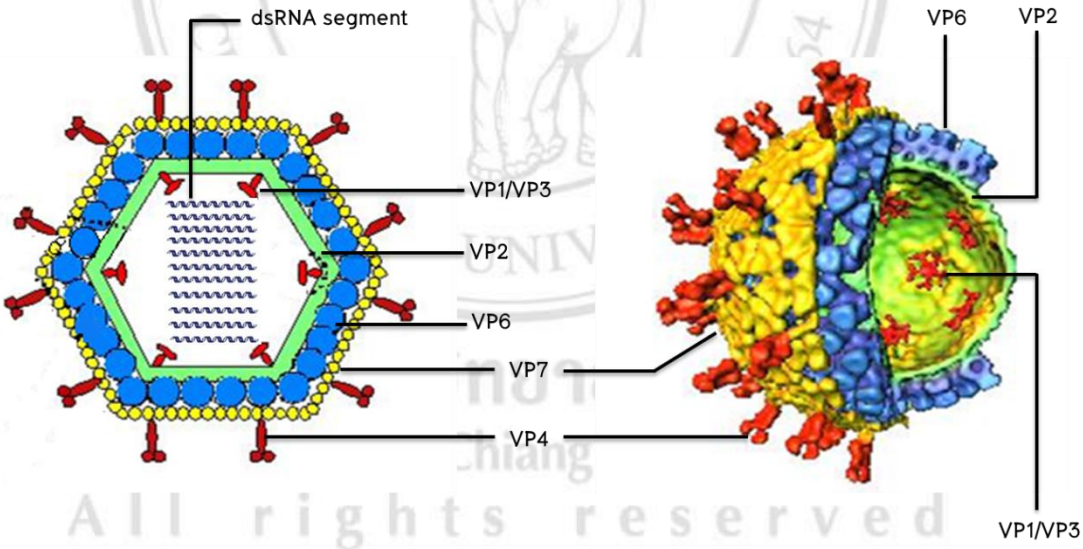

Figure 2.2 A schematic representation of the rotavirus triple-layered particle (Left) and cut-away view of the rotavirus structure (Right).
(http://www.reoviridae.org/dsRNA_virus_proteins/rotavirus%20figure.htm)

Table 2.1 The rotavirus genome segments, encoded proteins, and functions

GS	Size (bp)	Protein	Size (kDa)	Location	Genotype	Protein function
1	3302	VP1	125	Core	R	RNA-dependent RNA polymerase; 3‘-mRNA binding; forms transcription complex with VP3
2	2690	VP2	102	Inner capsid	C	Inner capsid structural protein; non-specific ssRNA and dsRNA binding; myristoylated; required for replicase activity of VP1
3	2591	VP3	98	Core	M	Guanylyltransferase and methyltransferase; non-specific ssRNA binding; part of transcription complex with VP1
4	2362	VP4	VP4: 88 VP5*: 60 VP8*: 28	Outer capsid	P	Outer capsid spike protein; P-type neutralization antigen; virulence determinant; cell attachment protein; trypsin cleavage of VP4 into VP5* and VP8* enhances infectivity; VP5* permeabilises membranes; VP8* contains the heamagglutinin domain (in some strains)
5	1611	NSP1	59	Cytoplasm	A	Associates with cytoskeleton; high degree of sequence variation; role in suppression of host interferon response
6	1356	VP6	48	Middle capsid	I	Major virion protein; middle capsid structural protein; homotrimeric structure; group- and subgroup-specific antigens
7	1105	NSP3	35	Cytoplasm	T	Homodimer; virus-specific 3'-mRNA binding; binds elongation factor eIF4G1 and circularises mRNA on translation initiation complex; involved in translational regulation and host shut-off

Table 2.1 (continued)

GS	Size (bp)	Protein	Size (kDa)	Location	Genotype	Protein function
8	1059	NSP2	37	Cytoplasm	N	NTPase and helicase activity; non-specific ssRNA binding; major component of the viroplasm; binds NSP5 and VP1; essential for dsRNA synthesis and formation of infectious viral progeny
9	1062	VP7	37	Outer capsid	G	Outer capsid structural glycoprotein; G-type neutralization antigen; N-linked high mannose glycosylation and trimming; RER transmembrane calcium-binding
10	751	NSP4	20	Cytoplasm	E	Viral enterotoxin; receptor for double-layer particle budding through ER membrane; Nlinked high mannose glycosylation; modulates intracellular calcium levels essential for viral RNA replication and formation of infectious viral progeny
11	667	NSP5/6	NSP5: 22 NSP6: 11	Cytoplasm	H	NSP5: viroplasm formation; multimerizes; O-linked glycosylation; hyper-phosphorylated; autokinase activity; enhanced by NSP2 interaction; binds ssRNA; component of viroplasm NSP6: interacts with NSP5 and localises to viroplasms

*GS, genome segment; ds, double-stranded; ss, single-stranded; ER, endoplasmic reticulum; RER, rough ER

(Matthijnssens et al., 2008; Mlera, 2012).

2.2 Classification of rotaviruses

Rotaviruses (RVs) belong to the genus *Rotavirus*, one of 15 genera of *Reoviridae* family, which is subdivided into the sub-families of the Sedoreovirinae (genera Ardoreovirus, Mimoreovirus, Orbivirus, Phytoreovirus, Rotavirus, Seadornavirus) (Mertens et al., 2004). RVs have been classified based on the serological reactivity and genetic variability of VP6 capsid protein, at least eight different groups, A-H, have been identified. Based on genetic variability of VP6 amino acid sequence, cut-off at 53% are proposed as a baseline value for defining rotavirus groups (Matthijnssens et al., 2012b). Group A rotaviruses are the most important enteric viruses in humans and in various animal species (Estes and Kapikian, 2007).

The rotaviruses contain two outer capsid proteins, VP4 and VP7 and both capsid proteins are involved in virus penetration into cells. A dual system of classification of rotaviruses is based on the genetic variability of VP4 and VP7 genes, which are used to classify rotavirus into VP4 or P (P for protease-sensitive) genotypes and VP7 or G (G for glycoprotein) genotypes (Estes and Kapikian, 2007). The nucleotide cut-off percentages of rotavirus strains sharing >80% for VP4 and VP7 amino acid sequence identities belong to the same P genotype and G genotype, respectively, while those of rotavirus sharing with <80% VP4 and VP7 amino acid sequence identities belong to different P genotype and G genotype, respectively (Khamrin et al., 2007a; Matthijnssens et al., 2008). To date, at least 37 P-genotypes (P[1]-P[37]) and 27 G-genotypes (G1-G27) of group A rotaviruses have been reported in humans and animals (Matthijnssens et al., 2011; Trojnar et al., 2013). Among these, at least 12 G-genotypes and 15 P-genotypes have been identified in humans and more than 120 different G/P combinations have been detected worldwide (Matthijnssens et al., 2009).

Based on the classification of rotavirus nonstructural glycoprotein NSP4, which encoded by gene segment 10, the NSP4 gene sequence analyses showed that there are six distinct NSP4 genotypes: A (KUN), B (Wa), C (AU-1), D (EW), E (avian-

like), and F (porcine NSP4). Genotypes A, B, C, D, and F have been detected from mammal, while genotype E has been reported from avian. Only three genotypes (A-C) have been identified in human (Ciarlet et al., 2000; Estes and Kapikian, 2006; Lin and Tian, 2003; Mori et al., 2002). Rotavirus strains isolated from cows, horses, rabbits, and pigs generally cluster according to rotavirus host species and all of porcine rotavirus strains identified belong to NSP4 genotype B and F (Ciarlet et al. 2000; Martella et al., 2006a). For the function of NSP4, the protein is an intracellular receptor that mediates the acquisition of a transient membrane envelope as subviral particles bud into the endoplasmic reticulum for maturation. In this process, NSP4 serves as an intercellular receptor by interacting with VP6. Moreover, the NSP4 plays an important role in rotavirus morphogenesis, pathogenesis, and enterotoxic activity (Ball et al., 1996; Taylor et al., 1996).

The classification of NSP5 has been proposed as genotype H. Up to now, 11 genotype H (H1-H11) have been identified. In humans, genotypes H1, H2, and H3 have been described, and H1 has also been detected in pig (Martella et al., 2010; Matthijnssens et al., 2011). NSP5 encoded by segment 11 (667 bp) is hyperphosphorylated and O-glycosylated, and it consists of 198 amino acids, with abundance of serine and threonine (Afrikanova et al., 1998; Berois et al., 2003). NSP5 is essential for establishment of viroplasms in the virus replication cycle. NSP5 interacts with NSP2 to form viroplasms, inside of which RNA replication and morphogenesis of new viral particles occur (Carreño-Torres et al., 2010; Eichwald et al., 2004; Estes and Kapikian, 2007;). The segment 11 also contains the coding sequence of NSP6 in a second ORF, whose function is still unknown.

Most recently, a more complete classification and nomenclature system has been proposed by the Rotavirus Classification Working Group (RCWG) based on the assignment of genotypes to all 11 RVA genome segments. The notation of Gx-P[x]-Ix-Rx-Cx-Mx-Ax-Nx-Tx-Ex-Hx, representing genotypes of VP7-VP4-VP6-VP1-VP2-VP3-NSP1-NSP2-NSP3-NSP4 and NSP5, respectively, which x indicating the numbers of the corresponding genotypes (Arabic numbers starting

from 1) (Matthijnssens et al., 2011). Based on the classification system, at least 27 G (VP7) genotypes (G1-G27), 37 P (VP4) genotypes (P[1]-P[37]), 18 I (VP6) genotypes (I1-I18), 15 E (NSP4) genotypes (E1-E15), and 11 H (NSP5) genotypes (H1-H11), have been defined (Matthijnssens et al., 2008; Desselberger, 2014). A summary of the novel classification system for all 11 segments is shown in Table 2.2.

Table 2.2 Percentage of nucleotide identity cut-off values for defining the genotypes of 11 rotavirus gene segments (Matthijnssens et al., 2008; Desselberger, 2014)

RV protein	Identity cut-off values (%)	Number of genotypes	Name of genotype
VP7	80	27G	**G**lycosylated
VP4	80	37P	**P**rotease-sensitive
VP6	85	18I	**I**nner capsid
VP1	83	9R	**R**NA-dependent RNA polymerase
VP2	84	9C	**C**ore protein
VP3	81	8M	**M**ethyltransferase
NSP1	79	19A	Interferon **A**ntagonist
NSP2	85	10N	**N**TPase
NSP3	85	12T	**T**ranslation enhancer
NSP4	85	15E	**E**nterotoxin
NSP5	91	11H	**P**hosphoprotein

2.3 Molecular epidemiology of rotaviruses

Rotaviruses are classified into eight groups (A to H) based on the antigenicity of VP6. Amino acid sequence identity of 53% of VP6 protein is proposed as a cut-off value for defining rotavirus groups (Matthijnssens et al., 2012b). Groups A, B, and C are associated with infection in human and various animal species, especially Group A rotaviruses representing the major cause of acute gastroenteritis in infants and young children worldwide. Groups B and C are also important in human and many animal species, including lambs, pigs, cattle, goats

and rats (Chasey and Banks, 1984; Eiden et al., 1986; Marthaler et al., 2012; Munoz et al., 1995; Parwani et al., 1996). Groups D, F, and G are associated with infections in chickens and birds, while group E rotavirus was identified in pigs (Bányai et al., 2009; Devitt and Reynolds, 1993; Pedley et al., 1986; Trojnar et al., 2010). Group H rotavirus was detected recently in humans and pigs (Alam et al., 2007; Marthaler et al., 2014; Matthijnssens et al., 2012b).

2.3.1 Molecular epidemiology of human rotaviruses

Rotaviruses are the leading cause of severe gastroenteritis in infants and young children worldwide, particularly, group A rotavirus is the major cause of deaths in children younger than 5 years of age. In 1985, a literature review estimated that rotavirus accounted for 25% of severe cases of diarrhea among children with the age under 5 years in developing countries (Zoysa and Feachem, 1985). Subsequencently, the studies published during 1986-1999 indicated that rotavirus accounted for 22% of severe diarrhea cases among young children and caused 440,000 of annual deaths in children younger than 5 years of age worldwide (Parashar et al., 2003). During 2000-2004, rotavirus detection rate among children with severe diarrhea was 39% (Parashar et al., 2006), and World Health Organization (WHO) estimate of that diarrhea-related childhood deaths yielded a substantially of 611,000 global deaths from rotavirus disease. Later, the estimate of rotavirus gastroenteritis related to deaths were 527,000 (range 475,000-580,000) in 2004 (Parashar et al., 2009), and 453,000 (range 420,000-494,000) in 2008 (Tate et al., 2012). Among these, the infection causes approximately 527,000 deaths per year worldwide and more than 85% of those deaths occurring in developing countries in Africa and Asia (CDC, 2008; 2011). An updated review of the WHO has coordinated the Global Rotavirus Surveillance Network during 2011-2012, the mean percentage of rotavirus detection among 75,353 tested children was 36%, with the largest percentage of positivity (42%) in infants aged 6 to 11 months (Agócs et al., 2014). In Asia, the estimated total number of deaths

located in India (80,981 cases), followed by Pakistan (23,227 cases), Indonesia (14,340 cases), Myanmar (8,681 cases), Bangladesh (5,724 cases) and China (4,716 cases) (Kawai et al., 2012).

The two outer capsid proteins (VP7 and VP4) are the basis of the dual classification which define G genotype and P genotype, respectively. The numerous surveillances showed that different G and P genotypes are predominant in particular area and the genotypes have changed overtime (Khamrin et al., 2007b; Rahman et al., 2007). Currently, at least 27 G genotypes and 37 P genotypes have been identified (Desselberger, 2014; Matthijnssens et al., 2011; Trojnar et al., 2013). The most frequently observed rotavirus genotypes during 2009-2012 were five globally prevalent genotypes such as G1P[8], G2P[4], G3P[8], G4P[8], and G9P[8] (Agócs et al., 2014). Other G genotypes which are relatively rare in human such as G5, G6, G8, G10, G11, G12, and G20 are found in various combinations with P[1], P[3], P[6], P[9], P[10], P[11], P[13], P[14], P[19], P[25], and P[28] types and the viruses have only been detected in some regions of the world (Matthijnssens et al., 2011). In the recent years, more and more unusual rotavirus strains with uncommon genotype combinations have been reported and epidemiological studies designed for monitoring the appearance of novel or unusual strains of rotavirus have been intensified throughout the world. Furthermore, the increasing data of rotavirus unusual strains isolated from human that share genetic and antigenic features of rotavirus from different species have been reported. A high diversity of circulating rotavirus strains within the largest population due to the segmented genome structure facilitating reassortment in cells co-infected with different rotavirus strains of the same host species (Ghosh and Kobayashi, 2011). For example, G3P[3], G3P[9], G3P[10], G3P[19], G5P[6], G6P[14], G8P[8], G10P[14], G11P[25], and G12P[8] have been sporadically reported as reassortants between human and animal rotaviruses (Duan et al., 2007; Ghosh et al., 2007b; Khamrin et al., 2006b; 2007c; 2009b; Maneekarn et al., 2006; Steyer et al., 2007; Uchida et al., 2006).

In Thailand, the epidemiology of rotavirus was initially performed since 1977 (Jayavasu et al., 1982). Afterwards, a review of rotavirus studies in Thailand reported the prevalence of rotavirus infection rates during 1977-1996 was 27-34% and the peak seasonal distribution of rotavirus infection among children hospitalized with diarrhea occurred in the dry cool season (October to February) (Maneekarn and Ushijima, 2000). The surveillance study in 2001-2002 at 6 general hospitals located in different geographical regions of Thailand (North, Northeast, East, Central, and South regions) demonstrated that 43.0% (range 40.0-50.0%) of children admitted to the hospitals with acute gastroenteritis caused by rotavirus (Jiraphongsa et al., 2005). Several rotavirus surveillances performed in Chiang Mai, Thailand during 2000-2001, 2002-2004, 2005, and 2007 indicated that the prevalence of rotavirus in pediatric patients with acute gastroenteritis were 34.0, 37.3, 29.3, and 29.4%, respectively (Khamrin et al., 2006a; 2007b; 2010; Chaimongkol et al., 2012b). Later, in 2007-2009, the prevalence of rotaviruses circulating in infants and young children with diarrhea admitted in four hospitals in four provinces of Thailand (Bangkok, Khon Kaen, Nakhon Ratchasima, and Tak) was reported at 28.4% (Khananurak et al., 2010). Recently, the prevalence of rotavirus infection in infants and children with acute gastroenteritis admitted to two hospitals from Chum Phae Hospital in Khon Kaen and Chulalongkorn Hospital in Bangkok during 2009-2011 was reported at 44.5% (Maiklang et al., 2012).

The overall distribution of G genotype of rotavirus strains circulated in Thailand during 1982-1997 revealed that G1 was the most predominant genotype at 36.8% followed by G2, G4, G3, and G9 at 21.0, 7.5, 2.5, and 0.4%, respectively (Maneekarn and Ushijima, 2000). Recently, the distributions of G and P genotypes in pediatric patients hospitalized with acute gastroenteritis in Thailand during 2000-2011 have been summarized and reviewed by Maneekarn and Khamrin (2014). During the 12 years period of the surveillances in Thailand, 6 different G genotypes, G1, G2, G3, G4, G9, and G12 were detected. The G9 was found to be the most

predominant genotype in 2000-2004 with the prevalent rate ranging from 40.8-91.6 %. In addition, G9 was co-predominated with G1 in 2002-2004 with the prevalence of 40.8% and 30.6%, respectively. In 2005-2009, the G1 was reported as the most predominant genotype at the prevalent rate ranging from 49.4-83.1%, while G3 was detected as the most predominant genotype only in 2009-2011 at 60.4%. The G4 was detected only in 2001-2003 in one study only but not in others as well as G12 was detected only in 2007-2009 study (Chaimongkol et al., 2012b; Jirapongsa et al., 2005; Khamrin et al., 2006a; 2007b; 2010; Khananurak et al., 2010; Maiklang et al., 2012; Theamboonlers et al., 2008). For the P genotype, various P genotypes were detected in Thailand in children admitted to hospitals with diarrhea. The data are summarized and reviewed by Maneekarn and Khamrin (2014). Since 2000-2011, the P[8] was the only P genotype that invariably existed as the most predominant P-genotype with very high prevalence, ranging from 69.8-99.6%, while the P[4] was the second runner following P[8] genotype. Moreover, variety of other P genotypes were also detected, including P[3], P[4], P[6], P[8], P[9], P[10], and P[19]. A number of G-P combinations of RVA were found in Thailand during 2000-2011, the G1P[8] was the most common rotavirus strain, followed by G9P[8], G2P[4], and G3P[8], respectively. Additionally, the uncommon strains, G2P[8], G3P[3], G3P[9], G3P[10], G3P[19], G12P[6], and G12P[8] were also detected in this study period (Chaimongkol et al., 2012b; Khamrin et al., 2006a; 2007b; 2010; Khananurak et al., 2010; Maiklang et al., 2012; Theamboonlers et al., 2008).

In Chiang Mai, Thailand, the initial study performed in 1987-1988 found that G1 was co-predominated with G2, after that in 1988-1989 G1 became the most predominant genotype followed by G2 and G4. In addition, the emergence of G9 was first reported in Chiang Mai, Thailand with low prevalence of 1.98% (Urasawa et al., 1992). Then, prevalence of G9 increased to 16.2% in 1996-2000 (Zhou et al., 2001). In 2000-2001, G9P[8] was reported as the most predominant genotype with high frequency at

91.6% (Khamrin et al., 2006a). In 2002, G9P[8] turned out to be the predominant genotype which reached a peak of 100%. Then, the prevalence rate of G9 rapidly decreased during the years of 2003-2005 to 16.7, 32.1, and 4.7%, respectively (Khamrin et al., 2007b; 2009a). In addition, G2P[4] reemerged in the epidemic season of 2003, whereas G1P[8] became the most predominant strain in the years of 2004 and 2005 (Khamrin et al., 2007b; Khamrin et al., 2010). A recent study in 2007 found that G1P[8] was the most predominant genotype with the prevalence of 72.3% followed by G2P[4] at 19.2%, and G3P[8] at 8.5% (Chaimongkol et al., 2012b). Overall, several G genotypes including G1, G2, G3, G4, and G9 and two P genotypes, P[8] and P[4] are circulating in pediatric patients with acute gastroenteritis in Chiang Mai, Thailand and the predominant genotypes have changed in each epidemic year. Moreover, those of the rotavirus strains carrying uncommon genotype combinations are likely represent interspecies transmission between human and animal rotavirus strains or even a direct transmission from animal to human as in the case of human G9P[19], G3P[3], and porcine G3P[19] rotavirus strains previously detected in Chiang Mai area (Okada et al., 2000; Maneekarn et al., 2006; Khamrin et al., 2006b).

2.3.2 Molecular epidemiology of porcine rotaviruses

Porcine rotaviruses are important animal pathogens for pigs as it is a significant economic impact through loss in production due to death of pigs, poor growth performance, and costs of diagnostic testing and treatment. Among these, porcine rotaviruses are the most prevalent pathogen in neonatal pigs (<7 days) and piglets at the time of weaning (21–28 days) (Katsuda et al., 2006; Svensmark et al., 1989). Recently, five of the eight rotavirus groups, RVA, RVB, RVC, RVE, and RVH, have been detected in pigs (Estes and Greenberg, 2013; Molinari, 2014; Saif et al., 1980; Wakuda et al., 2011). Group A rotavirus (RVA) was the most common and pathogenic pathogen in swine. It was first identified in 1975 and had been

documented as an important cause of diarrhea in pigs (Amimo et al., 2013; Ciarlet et al., 2002; Miyazaki et al., 2011; 2013). Group B rotavirus (RVB) was detected 5 years after identification porcine group A rotavirus while the reports of RVB have been sporadically published, until recently, group B rotavirus has been considered not an important cause of diarrhea in pigs (Bridger and Brown, 1985; Kuga et al., 2009; Marthaler et al., 2012; Theil et al., 1985). In 1979, Group C rotavirus (RVC) was first discovered in piglet with diarrhea. This group can cause subclinical to severe gastrointestinal infections and can cause either sporadic cases or large outbreaks (Saif et al., 1980; Bohl et al., 1982; Collins et al., 2008). Group E rotavirus (RVE) has only been identified from a single porcine sample, however, the prevalence or importance of RVE in piglet is still unclear (Chasey et al., 1986). In 1999, group H rotavirus (RVH) was first detected in piglet with diarrhea in Japan (Wakuda et al., 2011). Later, 3 additional group H rotavirus strains were isolated in 2012 from Brazil (Molinari et al., 2014). Recently, the widespread of group H swine rotavirus was also reported in the US (Marthaler et al., 2014), nevertheless, its role in pathogenesis is still unknown. The most common genotypes of group A rotavirus found in pigs are G3, G4, G5, G9, and G11 in association with P[6], P[7], P[13], P[19], and P[23]. In addition, several G-types, such as G1, G2, G6, G8, G10, G12, and G26 and P-types, such as P[5], P[8], P[11], P[14], P[26], P[27], P[32], and P[34] have also been detected sporadically in pigs (Matthijnssens et al., 2011).

Porcine rotavirus G4 and G5 (Gottfried) were first detected in 1975 (Hoshino et al., 1984). Later, in 1983, outbreak of group A rotavirus in piglets was occurred in Italy, 23 rotavirus strains identified were analyzed and all of these isolates exhibited the G5P[6] genotype, which displayed the typical characteristics of rotaviruses of bovine origin (Martella et al., 2001). During 1991 to 1992, one isolate of porcine rotavirus carried the mixture of genotypes G1 and G5 specificity in combination with human P[8] strain was detected in Brazil (Santos et al., 1999). In 1999, porcine rotaviruses

genotype G4, G6, and G8 were found in combination with P[1] and P[6] had been reported in Argentina (Parra et al., 2008). In addition, porcine rotavirus G9 strain had been initially reported in 1988 in the United States (Zaberezhny et al., 1994). Later, G9 was reported as the most predominant genotypes among porcine associated with outbreak of diarrhea in young pigs in Japan between 2000-2002 and nucleotide sequence analysis of G9 VP7 gene showed that the porcine G9 strains were more closely related to human G9 strains reemerging globally since the mid-1990s (Teodoroff et al., 2005). The porcine G9 strains were in combination with unusual porcine P genotypes, P[13] and P[23], and two strains with P[6], of which the nucleotide sequence were closely related with human AU19 strain than those of porcine Gottfried strain, indicated that G9 was widely spread both in porcine and human populations (Teodoroff et al., 2005). A report of porcine group A rotavirus in South Korea demonstrated that the most predominant combination of G and P genotypes was G5P[7], followed by G8P[7], G9P[7], G9P[23], and G8P[1] (Kim et al., 2010). Recently, a wide range of G genotypes of G2, G3, G4, G5, G9, and G11 and P genotypes of P[6], P[7], P[8], P[13], P[23], and P[32] were reported in UK pigs during 2010-2012 (Chandler-Bostock et al., 2014).

For the epidemiology and distribution of porcine rotavirus strains in Thailand, serotype G3 was initially identified in 1988 from pigs with diarrhea in Rachaburi province, the Central region of Thailand (Pongsuwanne et al., 1989). In 1995, the surveillance of group A rotavirus in Nakorn Pathom, Rachaburi, and Chonburi provinces demonstrated that 4.1% were positive for group A rotavirus in diarrheic piglets. In addition, serotyping by ELISA showed that 3 and 14 isolates were serotype G3 and G10, respectively (Pongsuwanna et al., 1996). Several studies of epidemiology and molecular characterization of porcine rotavirus have been performed in Chiang Mai, Thailand since the year 2000 and the reports come out continually. During 2000-2001, the surveillance of group A porcine rotavirus in diarrheic piglets was detected at 22.3%. Of these,

G4P[6] was the most prevalent genotype, followed by G3P[19], G3P[6], G5P[13], G4P[13], and G9P[7], respectively (Maneekarn et al., 2006). Later, the surveillance of group A rotavirus in diarrheic piglets carried out in 2002-2003 revealed that the prevalence was decreased to 17.2% and G3P[13], G4P[13], and G5P[13] strains were detected in this study (Chan-It et al., 2008). In 2006-2008, the prevalence of porcine rotavirus was 10.7% and G3P[13], G3P[23], G9P[23] were detected (Okitsu et al., 2011). Most recently, the surveillance of porcine rotaviruses in diarrheic piglets in Chiang Mai was performed and reported the prevalence at 19.8%. Of these, a wide range of porcine rotavirus strains were detected in this study, including G3P[6], G3P[13], G3P[19], G3P[23], G4P[6], G4P[19], G4P[23], G5P[6], G5P[13], G9P[13], and G9P[19] genotypes. It is interesting to note that G4P[19] and G9P[19] were reported for the first time as the novel G-P combinations (Saikruang et al., 2013). Genetic characterization of human rotaviruses of some genes that closely related with the genes of porcine rotavirus suggesting the possibility of interspecies transmission of porcine rotaviruses that cross the species barrier to infect human or vice versa (Maneekarn et al., 2014; Theamboonlers et al., 2014).

2.4 Evidences for interspecies transmission of rotaviruses

Rotavirus is a common pathogen of acute gastroenteritis in infants and young children and in young animals of a large variety of species (Kapikian et al., 2001). In many cases, genetic analysis of rotavirus has clearly demonstrated the genetic relatedness of gene segments of rotavirus strains isolated from different species. It is possible that interspecies transmission may occur frequently in nature (Fujiwara and Nakagomi, 1997; Iizuka et al., 1994; Nakagomi et al., 1990). Close contact between humans and animals may facilitate interspecies infections and genetic reassortment during co-infection with rotavirus strains from different animal species and result in the generation of progeny viruses with novel or atypical genotypes (Palombo, 2002). The increased detections of rotaviruses strains bearing unusual combinations of human and animal rotavirus genotypes have

been well documented (Khamrin et al., 2006b; 2009b; Maneekarn et al., 2006). Genetic reassortment between group A rotaviruses of human and animal origins has been reported, including human-porcine (Maneekarn et al., 2006), human-simian (Khamrin et al., 2006b), human-bovine (Cooney et al., 2001), human-feline/canine (Isegawa et al., 1939), human-caprine (Khamrin et al., 2006b), human-lapine (Matthijnssens et al., 2006a), human-ovine (Bányai et al., 2010), and human-equine rotaviruses (Malasao, et al., 2014).

Animal-like group A rotaviruses, either as sporadic cases or as large epidemics have been detected in several epidemiological studies. There is evidence that group A rotaviruses of porcine origin or natural porcine-human group A rotavirus reassortments may have occurred and spread successfully thoughout human populations in many occasions (Martella et al., 2010). In Europe, analyses of P[6] of the Spanish (during 2001-2003) and Italian (during 2003-2004) porcine rotavirus strains revealed that all the P[6] strains were more homologous to human P[6] strains than to the prototype porcine P[6] Gottfried strain (Martella et al., 2006b). During 2004-2005, an unusual G3P[6] rotavirus strain SI-MB6 has been detected in children with diarrhea in Slovenia. Sequencing of four genes (VP7, VP4, VP6, and NSP4) segments showed that these genes were more closely related to porcine than to human rotavirus strains, suggesting that this might be the result of zoonotic transmission of rotavirus from pig to human (Steyer et al., 2008). In addition, G3P[6] rotavirus 128/07-34 strain which was isolated from a child hospitalized with acute gastroenteritis in Italy in 2006, showed the VP7 and VP4 genes closely related to rotavirus of porcine origin (Martella et al., 2008).

In Asia, G9P[6] rotavirus VN904/2003 strain isolated in Vietnam has been shown as a human-animal reassortant virus. The VP4 and VP7 genes of this strain show great identity with porcine G4P[6] rotavirus strains, suggesting interspecies transmission of rotavirus between porcine and humans (Nguyen et al., 2007). In addition, G11P[25] rotavirus CAU12-2 strain, was detected in a 16-year-old female with fever and diarrhea during the 2012 in South Korea. Phylogenetic and sequence analysis of the VP1 gene of this strain showed that it was most closely

related to the VP1 gene of porcine and human-porcine reassortant G11 rotaviruses. The VP6 gene was found to belong to the new genotype I12. This study indicates that the G11-P[25]-I12 genotype was introduced into the South Korean population by interspecies transmissions of human and animal rotaviruses, followed by multiple reassortment events (Than et al., 2013). In Ho Chi Minh City, Vietnam, a novel combination of rotavirus strain G26P[19] has been detected in hospitalized pediatric patients during 2009-2010. Sequencing of all 11 gene segments showed that 4 out of 11 (VP7, VP4, VP6, and NSP1) gene segments were most closely related to these of porcine rotavirus. This study indicated that this G26P[19] strain is the product of zoonotic transmission coupled with one or more reassortment events occurring in human and/or animal reservoirs (My et al., 2014). Moreover, P[19] is also infrequently reported, and has been detected in pigs and a limited number of symptomatic humans in Asia. Porcine rotavirus P[19] strain had been initially identified in China (Burke et al., 1994), and later P[19] was identified in rotavirus strains isolated from human infections in Thailand (G9P[19]) (Okada et al., 2000). Cumulative evidences from genetic investigations and the sustained detection of P[19] in humans and pigs support the theory that P[19] may have originated in pigs and was introduced into human rotavirus strains via human-porcine RV reassortment (Burke et al., 1994; Ghosh et al., 2012; Maneekarn et al., 2006; Mukherjee et al., 2011; Okada et al., 2000; Theamboonlers et al., 2008; Urasawa et al., 1992; Varghese et al., 2004; Wu et al., 2011; Zade et al., 2009).

In Thailand, an unusual strains G9P[19], Mc323 and Mc345, were initially isolated from children hospitalized with diarrhea in Chiang Mai in 1989, and had been shown by RNA-RNA hybridization, nucleotide and amino acid sequence analyses of VP7 and VP4 genes to be more closely related to the porcine rotavirus than to human rotaviruses (Okada et al., 2000; Urasawa et al., 1992). Additionally, the surveillance of porcine rotaviruses in piglets with diarrhea in Chiang Mai demonstrated that G3P[19] strains were detected in the same geographical area with those strains. By nucleotide sequence analysis, the VP4 gene of these porcine rotavirus strains were most closely related to the human

P[19] Mc323 and Mc345 strains isolated in 1989 from the same geographical area (Maneekarn et al., 2006). In addition, an unusual strain of human rotavirus G3P[3] (CMH222) was isolated from a 2-year-old patient during the epidemiological survey of rotavirus in Chiang Mai in 2000-2001. The molecular characterization of VP4, VP6, VP7, and NSP4 gene segments of CMH222 showed that the VP4 gene sequence was most closely related to P[3] rotavirus of caprine origin, while VP7 sequence showed highest identity with those of simian G3 rotavirus. In addition, the VP6 and NSP4 sequences were most closely related to those of simian and caprine rotaviruses, respectively (Khamrin et al., 2006b). Moreover, another two unusual and uncommon human rotavirus strains G3P[9] (CMH120/04 and CMH134/04) were detected in children hospitalized with acute gastroenteritis in Chiang Mai in 2004 (Khamrin et al., 2007c). Characterization and analysis of VP4, and VP7 genes of these two strains exhibited nucleotide sequences more closely related to P[9] of feline rotaviruses as well as feline-like human rotavirus AU-1 strain, which was reported as a reassortant strain resulting from reassortment between the viruses of cat and human origins (Nakagomi et al., 1987; 1989). Altogether, the findings of human rotaviruses with genetic characteristics of some genes that closely related with the genes of animal rotaviruses imply that interspecies transmission among human and animal rotaviruses may occur in nature (Maneekarn and Khamrin, 2014).

2.5 Mechanisms of genetic variation of rotaviruses

The diversity of rotavirus is generated by several mechanisms, including genetic reassortment, antigenic shift, point mutations, antigenic drift, genetic rearrangement, and intragenic recombination (Ramig, 1997; Palombo et al., 2002).

2.5.1 Genetic reassortment or antigenic shift

The prominent feature of rotavirus is a double-stranded RNA genome of 11 segments. The segmented nature of rotavirus genome allows reassortment

(exchange of genome segments between strains) of genome segments during mixed infections with other strains in the same cell. In addition, genetic reassortment can occur between strains of unrelated genogroups during co-infection, which is the major distinguishing feature of rotavirus genetics (Ramig, 1997; Ward et al., 1990). The isolation of unusual strains possessing the gene segments of human and/or of heterologous animal rotaviruses suggesting interspecies transmission and reassortment between the viruses of humans and animals, as well as between those of different animal species in nature (Cook et al., 2004; Iizuka et al., 1994; Isegawa et al., 1992; Khamrin et al., 2006b; Li et al., 1993; Nagagomi et al., 1993; Palombo et al., 2002, Tsugawa and Hoshino, 2008).

2.5.2 Point mutations or antigenic drift

In general, the genome of RNA viruses is unstable due to high mutation rates of approximately 10^{-3}–10^{-5} per nucleotide per replication cycle (Elena et al., 2006). Evolution of rotavirus is partly driven by high mutations rates due to the error-prone of RNA dependent RNA polymerase (Chen et al., 1994; Elena et al., 2006; Flores et al., 1988; Sanjuán et al., 2010). Some of the mutations are conserved and passed onto the progeny viruses in which these point mutations are accumulated. Such mutations can be used to identify the genetic lineages and sublineages, which have epidemiological meaning and are useful for classification of rotaviruses (Iturriza-Gómara et al., 2000). In addition, some of the mutations may be lethal or creates minor population variants that might affect infectivity of rotaviruses (González-López et al., 2004).

2.5.3 Genetic rearrangement or intragenic recombination

The mechanism of genome segment rearrangement is thought to involve in re-entry of the 3'-end of the negative strand into the catalytic core (forming a loop) and the RNA dependent RNA polymerase making a mistake by

switching template (Matthijnssens et al., 2006b). Genome segment recombination, a region from a genome segment of one specific gene can combine with another region of a different gene to form a single full-length genome segment, has also been reported. The phenomenon of genetic rearrangements has been shown to occur between two strains of the same rotavirus genotype and also between strains of a different rotavirus genotype (Jere et al., 2011; Parra et al., 2004). Although different genetic rearrangements have been documented in human and animal viruses, this mechanism probably plays only a minor role, if any, in generating significant variation in wild-type rotaviruses (Palombo, 2002; Ramig, 1997). The reports about the detections of genome segment recombinations that generate rotavirus diversity is rare so far, probably due to limited detection methods.

2.6 Rotavirus vaccines

Acute gastroenteritis is a significant cause of morbidity and mortality of infants in developing countries and morbidity of the same age group in developed countries. High rotavirus incidence, economic burden and loss of human life emphasize the need for a safe and effective rotavirus vaccine, particularly in developing countries. In 1998, the first live oral rotavirus vaccine being licensed was RotaShield® (Hochwald and Kivela, 1999). Whilst after administration of approximately 1.5 million doses, RotaShield® was withdrawn from the market because of an unacceptable incidence of 1:10,000 associations with intussusception, a form of bowel obstruction (Abramson et al., 1999; CDC 1999a; 1999b; Chang et al., 2001; Kramarz et al., 2001; Murphy et al., 2001; Simonsen et al, 2001). Currently, two safe and effective RV vaccines, RotaTeq® (Merck Inc., USA) and Rotarix™ (GlaxoSmithKline Biologicals, Belgium) have been licensed and used worldwide since 2006 (Angel et al., 2007; Dennehy, 2008). Rotarix™ is a monovalent vaccine containing a human G1P[8] vaccine strain that was attenuated for infants by multiple blind passaging and tested for safety and efficacy in more than 60,000 infants both in Europe and Latin America. This

vaccine, the virus replicates in the intestine and is shed by many infants following the first dose. However, on the second dose, if the infant has been effectively immunized, very little shedding can be detected (Linhares et al., 2008; Ruiz-Palacios et al., 2006). Another vaccine, RotaTeq® is a pentavalent vaccine which composed of VP4 or VP7 from G1-G4 and P[8] of human strains, while the genetic backbone of other genes derived from bovine rotavirus WC3 strain. This vaccine was also tested in >60,000 infants in the United States and Europe. Since the bovine parent strain does not replicate well in humans, the titer of virus in the vaccine is much higher but is hardly shed after immunization (Vesikari et al., 2006). Both vaccines are effective against the development of severe acute gastroenteritis disease. The clinical trials of both commercially available vaccines indicated that efficacies of 85-98% in the prevention of severe gastroenteritis are observed in the European and North American countries (Ruiz-Palacios et al., 2006; Vesikari et al., 2006). However, in African countries the efficacy is substantially lower when compared to developing countries in Latin America (Ruiz-Palacios et al., 2007). The efficacy differences between the developed and developing countries may be due to differences in gastrointestinal microbial composition, malnutrition in poor countries, the maturity of the immune system and possibly immunosuppression due to infectious pathogens (Gladstone et al., 2011).