CHAPTER 6

Discussion

Rotavirus is a leading cause of severe gastroenteritis in infants and young children under 5 years worldwide, and accounting for approximately 5% of child deaths annually (Agócs et al., 2014). Moreover, rotavirus also infects many animal species, especially pig (Saif et al., 1994). Close contact between humans and animals may facilitate interspecies infections and genetic reassortment during co-infection with rotavirus strains from different animal species and results in the generation of progeny viruses with novel or atypical genotypes (Palombo, 2002). Pigs are considered to be an important source of human rotavirus infections (Martella et al., 2008; Okada et al., 2000; Varghese et al., 2004). Therefore, this study has determined simultaneously the prevalence of human and porcine rotaviruses and to perform molecular characterization of rotavirus genotypes circulating in Chiang Mai and Lamphun provinces of Thailand.

Previous studies of epidemiological surveillance of group A rotavirus in Thailand indicated that the predominant of rotavirus genotypes are changing overtime. The present study demonstrates that the prevalence of group A rotavirus in pediatric patients with diarrhea in Chiang Mai province of Thailand during January 2013 through February 2014 is relatively high at 34.2% (137 of 401 samples) which is consistent with the previous studies reported in Thailand in Lop Buri during 2006-2007 (prevalence of 57.0%) (Kittigul et al., 2014b), in Chiang Mai during 2000-2001 (prevalence of 34.0%), 2002-2004 (prevalence of 37.3%), 2005 (prevalence of 29.3%), and 2007 (prevalence of 29.4%) (Khamrin et al., 2006a; 2007b; 2010; Chaimongkol et al., 2012b). Recently, group A rotavirus have not found in adult patients from the study by Saikruang et al. (2014) which screened for viruses causing diarrhea including group A and C rotaviruses, noroviruses GI and GII, sapovirus, Aichi virus, human parechovirus,

enterovirus, adenovirus and astrovirus. In addition, Kittigul et al. (2014b) have tested for group A rotavirus in children and compared with rotavirus infections in adults, the results indicated that rotavirus detected in children have a significantly higher prevalence (57.0%) than those in adults (27.0%). The data confirmed that rotavirus is the most important cause of severe diarrhea in infants while rotavirus in adults is uncommon (Kittigul et al., 2014b). In addition, the prevalence of porcine group A rotavirus detected in the present study in diarrheic piglets in Chiang Mail and Lamphun provinces of Northern Thailand during January 2011 to March 2014 is 23.0% which is consistent with the studies previously reported in the same area during 2000-2001 (Maneekarn et al., 2006), 2002-2003 (Chan-It et al., 2008), and 2009-2010 (Saikruang et al., 2013) with the prevalences of 22.3, 17.2, and 19.8%, respectively. Moreover, Saikruang et al. (2013) also investigated the prevalence of porcine rotavirus in non-diarrheic healthy piglets in Chiang Mail and Lamphun province, none of group A rotavirus was detected in healthy piglets. These results indicated that group A rotaviruses are the common causes of severe gastroenteritis in both humans and piglets in Chiang Mai and Lamphun provinces of Northern Thailand.

The seasonal pattern of human rotavirus infection observed in the present study is all-year-round with slightly higher in early winter to late summer which is different from the previous survey in developed counties which showed epidemic peak in cooler months of each year (Estes and Kapikian, 2007) and in other countries in Asia also reported the peak of rotavirus infection in the cooler months (Kawai et al., 2012). While the seasonal distribution of rotavirus gastroenteritis in children in Ivory Coast was highest in winter 2008 (Akoua-Koffi et al., 2014) as well as in Sydney, Australia during 2007-2010 were detected rotavirus infection peak in winter (Fletcher et al., 2013). The seasonal distribution of porcine rotavirus observed in the present study is also occurred throughout the year with the highest prevalence in the late rainy season to early winter which is in line with the previous report in Chiang Mai area in 2009-2010 (Saikruang et al., 2013). However, the seasonal pattern of porcine rotavirus in Iowa, USA was slightly higher in late summer and early autumn and highest in winter (Will et al., 1994). The seasonality of rotavirus infection varies from country to country around the world.

The diagnosis of rotavirus infection have been done by using several methods, initially by electron microscopy or ELISA (Brandt et al., 1981) and more recently by RT-PCR (Gouvea et al., 1990; Gentsch et al., 1992; Gómara et al., 2000; IturrizaGómara et al., 2004). ELISA is applied most frequently in the routine diagnostic laboratory due to the ease of use and less time consuming. Currently, RT-PCR has been used as the gold standard of diagnosis due to the highly sensitive, specific and also suitable for genotyping of rotaviruses by using specific primers (Richardson et al., 1998; Fischer and Gentsch, 2004; Iturriza-Gómara et al., 2004). Nevertheless, genetic variations by the accumulation of point mutations may affect the sensitivity and specificity of primers for genotyping and new primers need to be designed periodically (Iturriza-Gómara et al., 2000, 2004; Simmonds et al., 2008). Moreover, some genotypes without genotype-specific primers are available. Thus, in the present study a specific primer for P[13] genotype has been designed and used to identify rotavirus P[13] genotype in porcine stool samples. In addition, some rotavirus isolates of nontypeable strains could not be identified by RT-multiplex PCR by using specific primers and finally have to be identified by nucleotide sequence analysis. The results of nucleotide sequence analysis of some nontypeable strains revealed that the primer binding sites in VP7 and/or VP4 genes of those strains have been changed in some nucleotide positions. This results indicate that the failure to identify G and/or P genotypes of some rotavirus strains due to several reasons, such as no specific primer for genotyping, mutations at the genotype-specific primer binding sites, and insufficient of viral load in clinical specimens.

The previous review of WHO global rotavirus surveillance network reported that the most frequently observed rotavirus genotypes in children with diarrhea during 2009-2012 were G1P[8], G2P[4], G3P[8], G4P[8], and G9P[8], although regional differences were observed (Agócs et al., 2014). In Thailand, the review of the distribution of G genotypes during 15 years (1982-1997) indicated that G1 was the most predominant genotype at the prevalence rate of 36.8% followed by G2, G4, G3, and G9 at 21.0%, 7.5%, 2.5%, and 0.4%, respectively (Maneekarn and Ushijima, 2000). The follow-up studies in Thailand during 2000-2011, six different G genotypes, G1, G2, G3, G4, G9, and G12 were detected. In 2000-2004, G9 was found to be the most predominant genotype (40.8-91.6%). The G1 was reported as the most predominant genotype (49.4-

83.1%) during 2005-2009, while G3 was detected as the most predominant genotype in 2009-2011 at 60.4% (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). In the present study, five different G genotypes of human rotavirus circulated in pediatric patients with diarrhea in Chiang Mai were detected and G3 (50.3%) was the most predominant genotype, followed by G1 (21.9%), G2 (16.1%), G9 (2.9%), and G8 (2.2%). Interestingly, rotavirus G3 reemerges as the most predominant genotype which is different from several previous studies revealed that G1 was the most frequently observed rotavirus genotypes (Agócs et al., 2014; Chaimongkol et al., 2012b; Khamrin et al., 2010; Khananurak et al., 2010; Theamboonlers et al., 2008). However, G3 was the most prevalent genotype in Thailand during 2009-2011 (60.4%) (Maiklang et al., 2012) and in Japan in the same period (65.0%) (Thongprachum et al., 2013) as well as in the present study (50.3%; 2013-2014). For the distribution of P genotypes of human rotavirus, P[8] was detected as the most prevalent genotype (80.3%), follows by P[4] (18.3%), mix-infected of P[8] and P[4] (0.7%), and P[19] (0.7%). These findings are in consistent with several previous studies, P[8] is the only P genotype invariably existed as the most predominant genotype in Thailand during 2000-2011 with very high prevalence, ranging from 69.8-99.6% and follows by P[4] genotype (Chaimongkol et al., 2012b; Khamrin et al., 2006a; 2007b; 2010; Khananurak et al., 2010; Maiklang et al., 2012; Theamboonlers et al., 2008). The present study clearly demonstrates that the G-P combinations of human rotavirus circulating in Chiang Mai, Thailand consists of several combinations, G3P[8] is identified as the most predominant genotype (49.6%), follows by G1P[8] (23.4%), G2P[4] (13.9%), G1P[4] (4.4%), G8P[8] (2.9), G2P[8] (2.2%), G9P[8] (2.2%), and mix-infected of G3 in combination with P[8] and P[4], and G9P[19], each of 0.7%. The findings are similar to the previous studies in Japan (Thongprachum et al., 2013), China (Jin et al., 2008), Hong Kong (Mitui et al., 2011), Vietnam (Ngo et al., 2009), and Thailand (Maiklang et al., 2012). It should be noted that G9P[19] genotype detected in the present study is considered to be the unusual G-P genotype combination in human. The G9P[19] (Mc323 and Mc345) has been reported previously in Chiang Mai in children with diarrhea in 1989 (Urasawa et al., 1992). Later, the nucleotide sequence of VP7 and VP4 genes of these strains have been analysed and found to be more closely related to the porcine rotaviruses than to human

rotaviruses (Okada et al., 2000). The data from these studies indicates that interspecies transmission among human and porcine rotaviruses could be occurred in nature.

Porcine rotaviruses are important animal pathogens for pigs and being a large reservoir of genetic segments for human rotaviruses. Previous epidemiological studies of porcine rotavirus showed that the combination of G3, G4, and G5 genotypes with P[6] and P[7] were the most common genotypes in northern Thailand (Okitsu et al., 2011). In the present study, porcine group A rotaviruses of 5 different G genotypes, G4 (56.6%), G5 (20.3%), G3 (15.9%), G9 (5.4%), G11 (1.8%), and 5 different P genotypes, P[13] (40.6%), P[23] (34.4%), P[6] (15.1%), P[19] (4.5%), and P[7] (3.6%) have been detected in diarrheic piglets from several farms in Chiang Mai and Lamphun provinces of Northern Thailand during January 2011 to March 2014. However, there are two strains of P genotype remains unidentified. Of the G genotypes, G4 is the most predominant genotype which is consistent with the previous study in the same geological area with the prevalence of 58.5% and other G genotypes, G3, G9, and G5 are also detected (Saikruang et al., 2013). The majority of G4 genotypes are found in combination with P[13] and P[23] to form G4P[13] and G4P[23] with the prevalence of 29.2% and 14.1%, respectively. The other combinations, G5P[23], G4P[6], G3P[23], G5P[13], G3P[13], G3P[6], and G5P[6] are also detected with lower prevalence in the present study. In contrast, the previous study in Chiang Mai during January 2009 to August 2010 reported 11 G-P combinations, the G4P[6] was identified as the most prevalent genotype, followed by G4P[23], G3P[23], G4P[19], G3P[6], G3P[13], G3P[19], G9P[13], G9P[19], G5P[6], and G5P[6] (Saikruang et al., 2013). In the previous epidemiological studies of porcine rotaviruses in the same area from 2000 to 2003 revealed that the combinations of G3, G4, and G5 genotypes with P[6], P[7], P[13], and P[19] were the most common genotypes in diarrheic piglets in northern Thailand (Chan-It et al., 2008; Maneekarn et al., 2006). These findings imply multiple reassortment between G and P combination in nature and show the diversity of P[13] porcine rotavirus strains circulating in the piglets in Chiang Mai and Lamphun provinces of Northern Thailand. Moreover, our study clearly demonstrates that porcine rotaviruses are genetically diverse and various G-P combinations have been detected in the viruses circulating in pigs in northern Thailand. It should be noted that porcine

rotavirus G9P[19] genotype detected in the present study carry VP4 and VP7 nucleotide sequences closely related to those of human rotavirus G9P[19] detected in the same study as well.

In several previous studies in Chiang Mai, Thailand, unusual strains of G9P[19], Mc323 and Mc345, were initially detected in children hospitalized with diarrhea in Chiang Mai in 1989, and their VP7 and VP4 genes had been shown to be more closely related to those of porcine rotaviruses than to human rotaviruses (Okada et al., 2000; Urasawa et al., 1992). After that, both strains (Mc323 and Mc345) were successfully cultured in MA-104 cell and the whole genomes have been analyzed by nucleotide sequence analysis (Ghosh et al., 2012). Of these, the VP1-VP3, VP6, and NSP1-NSP5 genes of strains Mc323 and Mc345 were assigned to the R1, C1, M1, I5, A8, N1, T1, E1 and H1 genotypes, respectively. Moreover, all the 11 gene segments of strains Mc323 exhibited high nucleotide sequence identities to those of Mc345 and most of the genes of strains Mc323 (VP2-4, VP6-7, NSP1-4 genes) and Mc345 (VP2-4, VP6-7 and NSP1-5 genes) were found to be closely related to porcine rotavirus genes. Nevertheless, VP1 and NSP5 genes of Mc323 and VP1 gene of Mc345 could not be ascertained (Ghosh et al., 2012). The unusual rotavirus strains G9P[19] detected in human in the present study were characterized further of their full-length VP7, VP4, VP6, NSP4, and NSP5 genes and compared with those of G9P[19] detected in piglets. Comparing human and porcine rotaviruses G9P[19] genotype revealed that all of VP4, VP7, VP6, NSP4, and NSP5 gene segments of human rotavirus strain CMH-S070-13 show high level of nucleotide sequence identities to those of porcine rotavirus strain CMP-015-12. In addition, the phylogenetic analysis of human rotavirus strain G9P[19] indicates that VP4, VP7, VP6, NSP4, and NSP5 genes are found to be more closely related to those of porcine rotavirus reference strains than to human rotavirus reference strains. The genotype constellation of the human and porcine rotavirus G9P[19] strains carry at least 5 of 11 genes of the same genotype (G9-P[19]-I5-E1-H1). The finding demonstrates that human rotavirus strain CMH-S070-13 contains a porcine genetic backbone and is likely of porcine origin. It is interesting to note that human rotavirus G9P[19] (CMH-S070-13) isolated from a 3-year-old girl with diarrhea in 4 January 2013 lives in Lamphun province where porcine rotavirus G9P[19] (CMP-015-12) has been isolated from a

diarrheic piglet. The data support the hypothesis of interspecies transmission and reassortment events might have been occurred in nature between human and porcine rotaviruses circulating in northern Thailand.

In conclusion, the present study provides the information of wide variety of rotavirus genotypes circulating in human and piglets with diarrhea in northern Thailand. Moreover, this study also provides the indirect evidence to support the hypothesis of interspecies transmission and genetic reassortment between rotaviruses of porcine and human origins.