CHAPTER 6

Discussion

NoV and SaV are recognized as the etiological agents that cause acute gastroenteritis. The viruses belong to the *Caliciviridae* family (Green, 2013; Oka et al., 2015; Patel et al., 2008). In Thailand, most of the epidemiology data of both NoV and SaV infections had been conducted in children hospitalized with acute gastroenteritis since 2000 (Bodhidatta et al., 2015; Chaimongkol et al., 2012, 2014; Guntapong et al., 2004; Hansman et al., 2004; Khamrin et al., 2007, 2010, 2017; Malasao et al., 2008; Neesanant et al., 2013; Phumpholsup et al., 2015; Pongsuwanna et al., 2017; Thongprachum et al., 2013). The findings from those studies revealed that the prevalence of NoV and SaV infections ranged from 6.8-17.3% and 0.7-4.8%, respectively. However, more detail analysis of genetic recombination of these viruses in Thailand has been conducted recently in 2015 and 2017 (Khamrin et al., 2017; Phumpholsup et al., 2015). Thus, the aim of this study was to determine the prevalence of NoV and SaV infections in children hospitalized with diarrhea in 2015-2016 and identified the NoV recombinant strains circulating in Chiang Mai during 2005-2015.

The present study revealed that the prevalence of NoV was 20.2%, which is relatively higher than the previous studies in the same geographical area (6.8-17.3%). The epidemiological surveillance of NoV in Chiang Mai was firstly reported in 2000-2001 by Hansman et al. (2004) with the prevalence of 8.6%. The other studies were then conducted continuously in 2000-2002 by Malasao et al. (2008), 2002-2004 by Khamrin et al. (2007), 2005 by Khamrin et al. (2010), 2007 by Chaimongkol et al. (2012), 2010-2011 by Chaimongkol et al. (2014), and 2012-2014 by Khamrin et al. (2017) showed the prevalence of NoV infections at 8.1, 14.1, 6.8, 12.5, 15.9, and 17.3%, respectively. Noteworthy, the prevalence of NoV infection in pediatric patients is gradually increased from 15.9% in 2010-2011 to 17.3% in 2012-2014 and 20.2% in the present study.

However, a study on NoV infection in diarrheic children in four other different regions of Thailand (Songkhla, Chanthaburi, Tak, and Nong Khai) during 2006 and 2008 reported the prevalence of NoV infection at 26.6% (Pongsuwanna et al., 2017). Furthermore, Kittigul et al. (2010) reported the prevalence of NoV infection in patients of all age groups with acute diarrhea in Lopburi province of Thailand at 44.7% during 2006-2007. The discrepancy of NoV prevalence of these studies could be explained by the differences of the year, target group of the study, and method of detection. Nevertheless, in the present study when the prevalence of NoV infection was analysed in a yearly basis, the prevalence in 2015 was at 13.4% which is in the range of those reported previously and in 2016 the prevalence of NoV infection is abruptly increased to 24.9% which may be due to the emergence of NoV GII.2 (10.4%), GII.6 (2.5%), and GII.13 (4.3%), where these genotypes are not detected in 2015.

The prevalence of SaV infection in this study is 1.8% which is relatively low compare to those of NoV, however, it is in good agreement with those reported previously in the same geographical area during 2000-2001 by Hansman et al. (2004), 2000-2002 by Malasao et al. (2008), 2002-2004 by Khamrin et al. (2007), 2005 by Khamrin et al. (2010), 2007 by Chaimongkol et al. (2012), and 2010-2011 by Chaimongkol et al. (2014), with the prevalence ranging from 1.2-4.8%. However, most recent surveillance of SaV infection during 2012-2014 in Chiang Mai reported the prevalence of SaV at 0.7% (Khamrin et al., 2017) which is relatively low when compare with the present study. In contrast, a relative high detection rate of SaV at 11% was reported in a study conducted in Tak, Nong Khai, Sa Kaeo, Chanthaburi, and Songkhla in 2002-2003 (Guntapong et al., 2004). Again, the different in detection rates may relate to the differences of target group, geographical areas and period of the study.

When look at the monthly distribution of NoV and SaV infections, the seasonal pattern NoV and SaV infections are observed all year-round with a high peak in dry and cold seasons (December 2015 to March 2016) which is similar to the distribution pattern of NoV in Europe such as France (Tran et al., 2010), Belgium (Mathijs et al., 2011), and Russia (Zhirakovskaia et al., 2015) and of SaV in Netherland (Svraka et al., 2010) and Canada (Pang et al., 2009). In addition, the other counties in Asia such as Japan (Dey et al., 2012; Thongprachum et al., 2015) and South Korea (Lee et al., 2015) reported a high

number of NoV and SaV detections in winter especially in November to January. Our data are also in good agreement with those reported previously in Chiang Mai (Chaimongkol et al., 2014; Khamrin et al., 2017; Malasao et al., 2008). Chaimongkol et al. (2014) demonstrated that the distribution of NoV was found in all year-round during 2007 and 2010-2011 and the detection increased in dry season (May to June and November to December). Malasao et al. (2008) also reported the detection of NoV and SaV mostly in dry season (January to May) during the year 2000-2002. Later, Khamrin et al. (2017) demonstrated the peak distribution of NoV in November to February during 2012-2014. Likewise, several studies in different area of Thailand also reported the detection of NoV and SaV in dry season (Bodhidatta et al., 2015; Pongsuwanna et al., 2017). Bodhidatta et al. (2015) reported that NoV GII infection was higher between October 2004 and February 2006. Pongsuwanna et al. (2017) demonstrated that NoV GII infection was found in all year-round, mainly in September and February between 2006 and 2008.

For NoV genotype distribution, it is interesting to observe that in the present study NoV GII.2 is emerged as the second most common genotype. Although, the NoV GII.2 was not detected in 2015 but it was detected with high prevalence (14.3%, 17 out of 119) and become the second most predominant genotype in 2016 after only NoV GII.4 genotype. From literature search, NoV GII.2 was initially detected in Chiang Mai since 2007 and only one strain (CMH-095-07) was detected at that time (Chaimongkol et al., 2014). Since then, NoV GII.2 disappeared for about 5 years, even though the NoV surveillance has been done annually, until recently one more strain of NoV GII.2 was detected again in Chiang Mai in 2013 (CMH-S099-13) (Khamrin et al., 2017). Thus, the NoV GII.2 strains detected in the present study have been characterized and compared with the NoV GII.2 detected in Chiang Mai in 2007 and 2013. A total of 16 out of 18 GII.2 strains detected in 2016 and those of 2007 and 2013 could be amplified by RT-PCR and analysed for their RdRp/capsid genes. Of 16 GII.2 strains detected in 2016, 8 were GII.P16/GII.2 recombinant strains while the other 8 strains were GII.P2/GII.2 genotype. Moreover, the GII.2 strains detected in 2007 and 2013 were also the GII.P16/GII.2 recombinant strains. By phylogenetic analysis based on RdRp region, the data revealed that GII.P16 strains detected in 2007 and 2013 are cluster together separated from the clusters of GII.P16

strains recently detected in 2016 suggesting that GII.P16 detected in 2016 may not originate from the GII.P16 genotype of 2007 and 2013.

The emergence of GII.P16/GII.2 recombinant strains have been reported during the winter of 2016-2017 in sporadic and outbreak cases around the world, including Japan (Thongprachum et al., 2017), China (Ao et al., 2017; Lu et al., 2017), France (Bidalot et al., 2017), and Germany (Niendorf et al., 2017). In Thailand, the emergence of GII.P16/GII.2 recombinant strains with high prevalence has never been reported previously and this is the first report of NoV GII.P16/GII.2 recombinants in Thailand. The emerging of GII.P16/GII.2 in several settings worldwide suggests that small numbers of amino acid substitutions in the polymerase may have driven the re-emergence of GII.2 during 2016-2017 (Tohma et al., 2017). So far, it is unclear whether the new recombinant GII.P16/GII.2 is associated with more severe symptoms and can replace the GII.Pe/GII.4 genotype of 2012. Continuous surveillance is needed to explore the epidemiological and clinical features of this GII.P16/GII.2 strain.

Recombination is one of the mechanisms for the evolution of many single-stranded RNA viruses, including NoV (Bull et al., 2005). A number of studies have reported the recombination sites occurred within ORF1 (Waters et al., 2007), ORF2 (Eden et al., 2013; Lindesmith et al., 2008; Rohayem et al., 2005), ORF 1/2, and ORF 2/3 overlaps (Eden et al., 2013). However, the most common recombination breakpoint has been identified at the positions close to or within the ORF1/2 junction (Bull et al., 2005, 2007). In the present study, wide variety of intergenotype NoV recombinant strains were identified and the most common recombination breakpoints located within ORF1 (15 strains), ORF2 (2 strains), and within the ORF1/2 overlapping region (4 strain). In Thailand, the recombination patterns of NoV GII strains reported previously from distinct areas (Bangkok and Khon Kaen) were GII.P21/GII.3, GII.P12/GII.3, and GII.P12/GII.1 during 2009-2014 and the most common recombination breakpoint was found within the C-terminus of ORF1 (Phumpholsup et al., 2015).

Comparison of the recombination patterns found in this study and the previous study, the recombination patterns reported by both studies were totally different apart from the detection of GII.P21/GII.3 strain in both studies. The discrepancy of the results could be explained by the difference in the study periods, target groups, and study locations

included in the two studies. The study period of this study ranged from 2005-2015, while the other study was 2009-2014 (Phumpholsup et al., 2015). The age of patients included in this study ranged from 2 months to 15 years old children, while the other study (Phumpholsup et al., 2015) was infants and children less than 5 years of age. Our study was performed in the northern part of Thailand (Chiang Mai province) while the other study (Phumpholsup et al., 2015) was performed in the central (Bangkok) and northeastern (Khon Kaen province) parts of Thailand. Although both studies targeted on the same genome region (ORF1/2), different sets of primers were used for recombinant analysis. Primers used in this study were targeted on the nt positions 4,295 to 5,389 whilst the primers used in the previous study targeted on the nt positions 5,050 to 5,389, corresponding to nt position of Lordsdale strain.

For the detection of GII.P21/GII.3 recombinants by both studies in (2005, 2009, and 2014), it is suggested that the GII.P21/GII.3 has been circulating in pediatric patients hospitalized with acute gastroenteritis in Thailand for a decade, at least from 2005-2014. The GII.P21/GII.3 variant (previously known as GII.Pb/GII.3) was first detected in 2002 from the outbreaks and sporadic cases of acute gastroenteritis in Spain (Buesa et al., 2002). Since then, it has been reported from several countries around the world, including Brazil (Fumian et al., 2016); Russia (Zhirakovskaia et al., 2015); South Africa (Mans et al., 2016); China (Jia et al., 2014); South Korea (Kim et al., 2015) and Thailand (Phumpholsup et al., 2015). The analysis of the RdRp sequences of 5 strains of GII.P21/GII.3 recombinants (CMH-145-05, CMH-N112-14, CMH-S026-14, CMH-S028-14, and CMH-S127-14) detected in this study revealed that the CMH-145-05 detected in 2005 was closely related to the GII.P21 variant found in France (AY682549_Hu/FR/2004/GII.P21/Pont de Roide) with RdRp nt sequence identity of 95.8% whereas the other 4 strains (CMH-N112-14, CMH-S026-14, CMH-S028-14, and CMH-S127-14) detected in 2014 were closely related to GII.P21 reported from Vietnam (KM198511_Hu/GII.P21/C2H-24/2011/VN) with RdRp nt sequence identity ranging from 98.6-99.0%. Additionally, these strains were also clustered together with other Thai recombinant strains detected in 2014 (KR007954_Hu/GII.P21/B387/2014/TH and KR007956_Hu/GII.P21/B433/2014/TH). These findings suggest that the GII.P21 circulating in Thailand may be originated from two different ancestors.

The NoV recombinants detected as the second most common recombination pattern were the GII.P16/GII.13 strains (CMH-S063-12, CMH-S003-13, CMH-N180-12, and CMH-N070-13). This recombinant has been reported previously from Spain in 2010 (Arana et al., 2014); Germany in 2012 (Mäde et al., 2013); Italy in 2010 and 2012 (Medici et al., 2014); China in 2013-2014 (Wu et al., 2015); Australia and New Zealand in 2013-2014 (Lim et al., 2016). The data imply that GII.P16/GII.13 probably emerged in 2010 and has been circulating in humans worldwide since 2010 up to at least 2014 in several countries in Europe, Australia, and Asia. The GII.P16/GII.13 strains detected in the present study showed great similarities to each other with regards to the RdRp sequences and were also similar to the strain from Taiwan (KM036380_Hu/GII.P16/13-BA-1/2013/TW). Analysis of the VP1 sequences of these strains revealed that they all were identical to each other with the nt sequence identity of 100% and were also closely related to the GII.13 strain reported from USA (AY113106_Hu/GII.13/Fayetteville/1998/US).

For the GII.Pe/GII.4 recombinants, the viruses were identified from the samples collected in 2014 and 2015 as the second most common recombinant strains (CMH-N052-14, CMH-ST023-15, CMH-S123-15, and CMH-S161-15). The GII.Pe/GII.4 recombinant was identified initially in Australia in 2007-2008 and the capsid of this recombinant was identified as 2006b variant (Eden et al., 2010). In 2012, the emergence of the new pandemic NoV GII.4 variant Sydney 2012 as a recombinant of GII.Pe and GII.4 was reported and found to be associated with epidemic acute gastroenteritis in several countries, including Australia, New Zealand, France, Spain, Japan, and China (Arana et al., 2014; Chan et al., 2013; Eden et al., 2013; van Beek et al., 2013; Wu et al., 2015). Comparison of the RdRp nt sequence of the GII.Pe/GII.4 strains detected in this study with other reference strains, it was found that our strains were closely related to the strains from Taiwan (KJ451059_Hu/GII.Pe/2012/TW) and Australia (JX459908_Hu/GII.Pe/Sydney/2012/AU). In addition, their VP1 sequences were closely related to the GII.4 variant Sydney 2012 reported from Australia (JX459908_Hu/GII.4/Sydney/2012/AU), Canada (KF509947_Hu/GII.4/AlbertaEI337/2011/CA), and Russia (KT224474_Hu/GII.4/Novosibirsk/2014/RU).

The GII.P7/GII.6 recombinant strain was initially reported from Senegal in 1976 (Epifanova, 2015) and later this recombinant strain was reported from several countries

worldwide, including Uruguay (Fajardo et al., 2014), Brazil (Hernandez et al., 2016), South Africa (Mans et al., 2014), China (Wu et al., 2015), and Australia (Bruggink et al., 2016b). The GII.P7/GII.6 recombinant strains were detected in our study in 2007, 2013, and 2014. The RdRp gene of two strains (CMH-N115-13 and CMH-S101-14) were clustered together and were closely related to the strain identified in Taiwan in 2014 (KM267741_Hu/GII.P7/Yilan/2014/TW), whereas another strain (CMH-N094-07) detected in 2007 was closely related to the strain from Taiwan in 2013 (KM267743_ Hu/GII.P7/Kaohsiung/2013/TW). Analysis of VP1 sequences of CMH-N115-13 and CMH-S101-14 revealed that their nt sequences were closely related to the strains from China (KR706448_Hu/GII.6/BZ16/2014/CN) and Taiwan (KM267742_Hu/GII.6/ Kaohs/2013/TW), while CMH-N094-07 was closely related to the Vietnamese strain (EU137733_Hu/GII.6/HCMC311/2006/VN). These data revealed that the GII.P7/GII.6 recombinants in our study were closely related with other recombinants circulating in Asia.

Another 5 minor recombination patterns detected in the present study were GII.P12/GII.4, GII.P7/GII.14, GII.Pg/GII.12, GII.Pg/GII.1, and GII.P16/GII.2 which were detected in 2005, 2007, 2011, 2012, and 2013, respectively. The RdRp and VP1 genes of GII.P7/GII.14 (CMH-047-07) strain were closely related to those of the strains reported from Japan (KJ196297_Hu/GII.P7/Fukuoka/2007/JP) and China (JQ751042_Hu/ GII.14/Wuhan/2008/CN). The RdRp gene of GII.Pg/GII.12 (CMH-N082-11) strain was also closely related to the strain from China (JQ889815_Hu/GII.Pg/F128/2009/CN) and the VP1 gene was identical to those of the strain from Japan (LC089410_Hu/ GII.12/Aichi417-11/2011/JP). This recombinant pattern has been reported previously from Australia (Bruggink et al., 2016a; Eden et al., 2010), China (Zeng, 2011; Sang et al., 2014), United states (Takanashi et al., 2011), and Spain (Arana et al., 2014). The GII.Pg/GII.1 (CMH-N009-12) strain was identical to the Thai recombinant strain reported previously (Phumpholsup et al., 2015) in both RdRp and VP1 genes (KR007960_Hu/ GII.Pg/GII.1/B1341/2014/TH). The GII.Pg/GII.1 recombinant strain has been reported in many countries worldwide such as Belgium (Mathijs et al., 2011), Germany (Hoffmann et al., 2013), France (Loury et al., 2015), and Australia (Bruggink et al., 2016a). The GII.P16/GII.2 (CMH-S099-13) was closely related to the strain from China (JQ751039_Hu/GII.P16/Wuhan/2010/CN) in the RdRp gene and was identical to the

strain from Australia (KT239612_Hu/GII.2/NSW8680/2013/AU) in the VP1 gene. This recombinant pattern has been reported as the causes of sporadic and outbreak cases in many countries worldwide such as Germany (Niendorf et al., 2017), France (Bidalot et al., 2017), China (Ao et al., 2017; Lu et al., 2017), and Japan (Thongprachum et al., 2017). The GII.P12/GII.4 (CMH-076-05) strain was closely related to the strain from China (HM802555_Hu/ GII.12/2005/CN) in the RdRp gene and was also closely related to the strain from Japan (EF630464_Hu/GII.4/57/JN) and Korea (HQ213829_Hu/GII.4/ 205/2004/KR) in the VP1 gene. The GII.P12/GII.4 recombinant pattern has been reported previously only from sporadic cases of acute gastroenteritis in China (Han et al., 2015). Most of NoV recombinant strains in our study were detected in dry and cool seasons, which is in good agreement with a report from Italy (Martella et al., 2013). In this study, unfortunately, analysis and comparison of the clinical significance could not be performed due to the lack of overall clinical data of the patients. In order to clarify this point, further studies on NoV surveillances as well as its clinical significance need to be performed.