CHAPTER 1

Introduction

Human norovirus (NoV) and sapovirus (SaV) are recognized as one of the leading cause of epidemic acute gastroenteritis in human of all age groups worldwide (Ahmed et al., 2013; Estes et al., 2006). Both viruses are classified into the family *Caliciviridae.* The viruses spread by several modes of transmission. The predominant modes of transmission are person to person contact and food borne spread. These viruses are associated with outbreaks in several setting including hospitals, nursing home, cruise ships, and the military (Knipe et al., 2013; Robilotti et al., 2015; Widdowson et al., 2005). The incubation periods of NoV and SaV infections are usually less than 24 to 48 hours, the clinical symptoms include acute vomiting, non-bloody diarrhea, abdominal cramps, nausea, chills, myalgia, headache, and low-grade fever (Robilotti et al., 2015; Vinjé, 2015). The clinical symptoms cause by both viruses are indistinguishable from those by other diarrheal viruses, therefore, laboratory diagnosis for identification of these pathogens is essential (Oka et al., 2015). NoV infection is responsible for 18% of all cases of gastroenteritis worldwide, while the SaV infection is less common (Ahmed et al., 2014; Oka et al., 2015). These infections relate to an estimated 200,000 deaths in children under 5 years of age in developing countries (Patel et al., 2008). The treatment after NoV infection is supportive, involving oral fluid and electrolyte replacement therapies. In some patients, antiemetics and antimotility agents are given (Green, 2013; Robilotti et al., 2015). Up to date, for control of NoV and SaV gastroenteritis diseases, vaccines are not yet available. However, most promising efficacy NoV vaccine candidate is now focusing on the use of VLPs as immunogen (Green, 2013; Oka et al., 2015; Richardson et al., 2013; Robilotti et al., 2015). Until an effective and sustainable vaccine is developed, the management of viral infection will depend on the infection control efforts (Robilotti et al., 2015).

NoV is classified, based on the major capsid amino acid sequence (VP1) into seven

genogroups (G), GI to GVII (Robilotti et al., 2015; Vinjé, 2015). Each genogroup is subdivided into several genotypes. NoV GI and GII are the most predominant genogroups of NoV infection in human worldwide (Vega et al., 2014; Vinjé, 2015). To date, GI is subdivided into nine genotypes (GI.1 to GI.9) and GII into twenty-two genotypes (GII.1 to GII.22) based on VP1 sequence. NoV GII.4 is the most predominant genotype that associated with up to 70-80% of the outbreaks worldwide (Kroneman et al., 2008; Patel et al., 2009; Ramani et al., 2014; Siebenga et al., 2009; Vinjé, 2015). Moreover, NoV GII.4 can be further classified into several variants, including US95/96 (1995), European variant (2002), Farmington Hills (2002), Hunter (2004), Minerva (2006a), Den Haag (2006b), New Orleans (2009), and Sydney (2012) (Bull et al., 2006; Vinjé, 2015). During 2014 to 2015, an emergence of NoV GII.17 variant Kawasaki has been reported in many countries in Asia (Chan et al., 2015; Dang Thanh et al., 2016; Khamrin et al., 2016; Lee et al., 2015; Lu et al., 2015; Matsushima et al., 2015). Recently in 2016-2017, the predominant genotype has been changed from GII.17 to GII.2 in several countries (Ao et al., 2017; Bidalot et al., 2017; Lu et al., 2017; Luke Tzu-Chi et al., 2017; Niendorf et al., 2017; Thongprachum et al., 2017).

Globally, based on the epidemiological studies in various countries, the NoV infection has been reported at about 10.7-64.9% (Chan-It et al., 2012; Chhabra et al., 2009, 2010; Lindell et al., 2005; Mladenova et al., 2015; Osborne et al., 2015; Page et al., 2017; Tan et al., 2015; Thongprachum et al., 2016; Timurkan et al., 2017; Zhang et al., 2016b; Zhirakovskaia et al., 2015). In Thailand, the prevalence of NoV infection in children under 5 years old hospitalized with acute gastroenteritis ranged from 8.6-23.8%. A wide variety of different NoV genotypes have been reported depending on the location of the study and surveillance period (Bodhidatta et al., 2015; Chaimongkol et al., 2012, 2014; Guntapong et al., 2004; Hansman et al., 2004; Khamrin et al., 2007, 2010; Malasao et al., 2008; Neesanant et al., 2013; Thongprachum et al., 2013). The most prevalent genotype was GII.4, followed by GII.3, whereas other genotypes were GII.1, GII.2, GII.6, GII.7, GII.11, GII.12, GII.13, GII.15, GII.16, GII.17, GII.20 and GII.21. Recently, NoV GII.17 has been reported as an etiologic agent of an outbreak of acute gastroenteritis at a daycare center in Bangkok during October 2014 (Phumpholsup et al., 2015b). The distribution of NoV in Chiang Mai, Thailand was detected throughout the year with the peak in June and December (Chaimongkol et al., 2014).

Genome recombination has been frequently observed in several RNA viruses. Recombination of NoV genome is one of a driving force of the evolution of new strains by exchanging sequence between two related viruses during co-infection of a host cell (van Regenmortel et al., 2010). The site of NoV recombination has frequently observed in the region located at the junction of ORF1/ORF2 (RdRp/ capsid VP1) (Ambert-Balay et al., 2005).

The prototype Snow Mountain virus, the first natural recombinant NoV strain, was detected in 1997 (Bull et al., 2005). This virus carried RdRp genotype of GII.Pc and capsid genotype of GII.2. In 2000 and 2001, outbreaks of NoV across Europe, Australia, and Asia were from recombinant strain GII.Pb/GII.3 (Ambert-Balay et al., 2005; Bon et al., 2005; Bull et al., 2005; Phan et al., 2006a, 2006c; Reuter et al., 2005). Recently, several NoV recombinant strains were also found in South Korea (Truong et al., 2014), China (Sang et al., 2014) and Japan (Motomura et al., 2016). Of these, the recombinant strains found in Japan belonged to GII.P2/GII.4 and GII.Pg/GII.12. The GII.P12/GII.3 were reported in China. However, there was only one study described the NoV recombinant strains detected during 2009-2014 in Thailand and three recombination patterns (GII.P21/GII.3, GII.P12/GII.3, and GII.P12/GII.1) have been reported (Phumpholsup et al., 2015a). In Thailand, few molecular epidemiological studies of NoV have been conducted and various detection rates were reported in different epidemiological setting. Therefore, continue comprehensive screening and molecular characterization of NoV strains need to be investigated.

For SaV, this virus is also known to cause outbreaks and sporadic cases of acute gastroenteritis in children worldwide. The virus has been genetically classified based on the complete VP1 (capsid) nucleotide sequence into five genogroups (GI to GV) (Farkas et al., 2004; Oka et al., 2012). Those genogroups are subdivided into several genotypes according to the nucleotide sequence variation in the capsid gene (Liu et al., 2015; Oka et al., 2012). To date, four genogroups including GI, GII, GIV, and GV are known to infect human (Oka et al., 2015). Each of human SaV GI and GII is subdivided into seven genotypes (GI.1 to GI.7 and GII.1 to GII.7). The GIV and GV are subdivided into one and two genotypes, respectively (Farkas et al., 2004; Hansman et al., 2007a; Oka et al., 2012; Okada et al., 2006). It should be noted that the prevalence of SaV is much lower

than those of NoV (Gao et al., 2015; Grant et al., 2017; Sisay et al., 2016; Thongprachum et al., 2015; Zhang et al., 2016b).

The data from previous studies worldwide reported the prevalence of SaV infection ranging from 0.3-18.0% (Grant et al., 2017; Iritani et al., 2014; Lasure et al., 2017; Liu et al., 2015; Matussek et al., 2015; Oka et al., 2015; Reymao et al., 2016; Shioda et al., 2016; Sisay et al., 2016; Svraka et al., 2010; Zhang et al., 2016b). The large outbreak of SaV infection associated with foodborne infection have been reported in Japan (Kobayashi et al., 2012; Oka et al., 2015). Based on the epidemiological studies in Thailand, the prevalence of SaV was reported ranging from 1.2-11%. The most predominant genotype was GI.1, whereas other genotypes, GI.2, GI.4, GI.5, GII.1, GII.2, GII.3, and GIV have also been reported (Chaimongkol et al., 2012, 2014; Guntapong et al., 2004; Hansman et al., 2004; Khamrin et al., 2007, 2010).

In order to obtain the current prevalence of NoV and SaV that cause acute gastroenteritis in hospitalized children, it is essential to continue to conduct the molecular epidemiology of these viruses in Chiang Mai, Thailand. Because of the predominant circulation of NoV GII in this area, it is interesting to investigate the recombination events and evolution of NoV GII infection in this area during the past decade (2005 to 2015). Thus, the purposes of this study are to determine the molecular epidemiology of NoV and SaV infections in children hospitalized with diarrhea in Chiang Mai, Thailand during 2015 to 2016 and to investigate the genetic recombination of NoV which was circulated in Chiang Mai, Thailand during the past decade.