## **CHAPTER I**

## INTRODUCTION

Acute gastroenteritis or acute diarrhea is one of the most common illness throughout the world, especially in developing countries, and presents the major cause of morbidity and mortality (Wilhelmi et al., 2003). Children under 5 years of age are particularly affected more than 700 million cases every year (Snyder et al., 1982). Furthermore, annual mortality associated with gastroenteritis has been estimated to be 5 to 8 million cases (Kasper et al., 2005). Viral gastroenteritis is an infection causes by a variety of viruses. The viruses that cause diarrhea are mostly RNA viruses, including rotaviruses, noroviruses, sapoviruses, astroviruses, and, only one is DNA virus; adenoviruses (Lyman et al., 2009). These viruses are responsible for numerous outbreaks of nonbacterial gastroenteritis in several settings, including hospitals, day care centers, nursing homes, and schools (Dey et al., 2007). It is, therefore, necessary to comprehensively study the prevalence and distribution of gastroenteritis virus strains in different part of the world.

Among different types of gastroenteritis viruses, rotaviruses are the most common and are the major cause of severe diarrhea in infants and young children worldwide (Parashar et al., 2003, 2006). It has been estimated recently that 527,000 children aged under 5 years die from rotavirus diarrhea each year, with more than 85% of deaths occur in developing countries in Africa and Asia (Parashar et al., 2009). Rotavirus is classified into 7 groups, group A to G. All 7 groups have been reported in variety of animal species. However, only groups A, B, and C are associated with infection in humans, of which group A is the most common cause of acute gastroenteritis. Group A rotavirus strain nomenclature has been described by dual classification system with G-genotypes and P-genotypes (Estes et al., 2001). Up to date, 23G-genotypes and 31P-genotypes have been reported (Matthijnsens et al., 2008; Schumann et al., 2009; Ursu et al., 2009), with various G-P combinations. Epidemiological studies have demonstrated that rotavirus G-genotypes G1, G3, G4, and G9 in combination with P-genotypes P[8] and G2 with P[4] are the most frequently associated with human rotavirus infection globally (Santos and Hoshino, 2005). According to the studies from seven European countries (Belgium, France, Germany, Italy, Spain, Sweden and UK), the prevalence of rotaviruses were as high as 40.6% and G1 to G4 and G9 were the common genotypes and, G4 and G9 were found in all study areas (Van damme et al., 2007). In Asia (especially Bangladesh, China, India, Japan, Korea, Thailand, and Vietnam), G1 was found to be a predominant genotype in most published reports, however, in some studies G2 or G4 was the most predominant. The G9 was the predominant strain in Bangladesh and India during 1996-2000 and also outstandingly spread in Japan (Okitsu-Nekishi et al., 2004) and Thailand (Khamrin et al., 2006a). In contrast, the percentage of G3 seemed to be low except for China in some years (Okitsu-Nekishi et al., 2004). The P[4] and P[8] were the most prevalent P-genotypes found over the years in both surveillances in Europe and Asia.

During 2000-2001 in Chiang Mai, Thailand, the surveillance information revealed that group A rotavirus was detected at the prevalence rate of 34% in children hospitalized with acute gastroenteritis. Of these, G9P[8] was the most predominant genotype (Khamrin et al., 2006a). Later, the prevalence of G9 reached a peak of 100% in 2002 and decreased rapidly in the next three consecutive years. In addition, G2 strain reemerged in the epidemic season of 2003, whereas G1 became the most predominant strain in 2004 (Khamrin et al., 2007b).

Nowadays, the nontypeable strains of which their G- and P-genotypes could not be identified by the specific primers reported previously have been described throughout the world. Additionally, the increasing data of new strains and unusual strains in human population have also been reported, for example, G3P[3] (Khamrin et al., 2006b), G8P[6] (Martini et al., 2008), and G3P[10] (Khamrin et al., 2009a) which were rarely reported to associate with human infection.

In addition, group A rotavirus can be classified into VP6 genogroup and NSP4 genetic group. The VP6 genogroups GI and GII, and NSP4 genetic groups KUN (A), Wa (B), and RRV (C) have been reported in human infection. Epidemiological studies reveal that most of human viruses belong to VP6 GII and NSP4 genetic group Wa (B) (Iturriza-Gòmara et al., 2003). According to the surveillance in Thailand, during the year 2000-2002, 98% of group A rotavirus belonged to VP6 GII, 2% belonged to GI, whereas all of them belonged to NSP4 genetic group Wa (B) (Khamrin et al., 2006a).

Recently, however, human caliciviruses, including noroviruses (NoVs) and sapoviruses (SaVs), have also been considered as the major cause of acute gastroenteritis in all age groups, particularly in young children and in the elderly (Estes et al., 2006).

Although, five distinct genogroups (GI-GV) of NoVs have been reported to co-circulating in natural worldwide, the stains related to human disease mostly

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belonged to genogroup I (GI) and GII (Patel et al., 2009). Based on epidemiological data, the detection rate of NoV appeared to be diverge among geographical regions. Overall, infection with NoV GII occurred more frequently than GI strains, and GII/4 (genogroup II, genotype 4) appeared to be the most predominant genotype of the outbreak worldwide (Papaventsis et al., 2007). It is interesting to note that GII/3 was sometime found to be the predominant genotype in some counties, for instance, in Tokyo, GII/3 was detected to as high as 76.9% whereas in the same study GII/4 was detected at only 23.1% (Okame et al., 2006). In Thailand, the data from many studies were consistent with the reports from worldwide that the major NoV strains belonged to GII/4. Other strains that found to co-circulating in Thailand belonged to GII/1, GII/2, GII/3, GII/6, GII/16, and GII/17 (Guntapong et al., 2004; Khamrin et al., 2007c; Malasao et al., 2008; Kittigul et al., 2010) and the genetic diversity seemed to rely on the time period and geographic regions of investigation. The epidemiological data of NoV GI revealed that GI/1, GI/2, GI/3, GI/4, GI/6, GI/7, and GI/13 were circulated in Thailand (Malasao et al., 2008; Kittigul et al., 2010).

Among the caliciviruses, SaVs have also been reported as a causative agent of acute gastroenteritis with less frequency than NoV (Yan et al., 2003). The virus appeared in sporadic cases and spread more frequently in young children than in adults. Most SaV detected in pediatric patients throughout the world belonged to genogroup I (GI) and GII, whereas GIV and GV were sometimes reported to associate with human infection. None of GIII has been reported in human and it is known to infect swine (Hansman et al., 2007). The GI/1 (genogroup I, genotype 1) has been recognized as the most common SaV strain circulating worldwide. However, the first report of SaV outbreak in Taiwan demonstrated that GI/2 was a predominant strain in

the year 2007 (Wu et al., 2008). Interestingly, two SaV genogroups, GII/2 and GI/1, were detected in one clinical sample from oyster-associated diarrhea outbreak in Japan during 2002-2006 (Nakagawa-Okamoto et al., 2009). In Thailand, a wide variety of SaV strains have been reported. The most prevalent strain was GI/1, whereas other strains and genotypes, GI/2, GI/4, GI/5, GII/1, GII/2, GII/3, and GIV, were also found to be co-circulating in this country and the rate of detection varied from time to time (Khamrin et al., 2007c; Malasao et al., 2008; Kittigul et al., 2009).

Beside rotaviruses and caliciviruses, astroviruses (AstVs) are the fourth most common cause of acute gastroenteritis which affected mainly young children and the elderly. Epidemiological studies revealed that AstVs are mix-infected frequently with rotaviruses and caliciviruses (Vasickova et al., 2005). The viruses have been classified into eight genotypes (AstV1 to AstV8) and, in general, AstV1 is the most prevalent genotype whereas others are rare (Wang et al., 2001). According to a previous study in Vietnam by Nguyen et al. (2008), all of the detected AstV belonged to genotype 1. However, several other genotypes such as AstV2, AstV3, and AstV5 were reported in Thailand and China (Malasao et al., 2008; Shan et al., 2009).

Adenoviruses (AdVs) are the only DNA virus among several RNA viruses that cause diarrhea. The enteric adenovirus that associated most frequently with acute gastroenteritis in human are AdV genotypes 40 (Ad40) and Ad41 of subgenera F (Fabiana et al., 2007). However, Ad12, Ad18, and Ad31 of subgenera A, and Ad1, Ad2, Ad5, and Ad6 of subgenera C have been reported rarely to involve in sporadic cases of acute gastroenteritis (Wilhelmi et al., 2003)

In order to draw the overall picture and to update the prevalence of viral gastroenteritis in Thailand, it is essential to carry out the epidemiological surveillance

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of gastroenteritis virus infections. The purpose of the present study is to investigate the prevalence and genotypic distributions of rotaviruses, noroviruses, sapoviruses, astroviruses and adenoviruses in children hospitalized with acute gastroenteritis by using multiplex RT-PCR and nucleotide sequencing for the detection and identification of the genotypes of the viruses.



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