CHAPTER VI

DISCUSSION

This study, which covered a period from May 2000 to March 2002, was conducted in Chiang Mai to estimate the prevalence of NV, SV, and HAstV in association with acute gastroenteritis among hospitalized children under 5 years of age. We detected 8.1% of NV and 3.4% of SV single infections, and 0.3% of NV/SV mixed infections. The prevalence of these viruses were rather low as compared to the study of Guntapong and colleagues (2004) who reported 14% of NV and 11% of SV single infections, and 4% of NV/SV mixed infections among hospitalized children in the other four different regions of Thailand. However, this finding is in good agreement with the study performed previously in Chiang Mai among hospitalized infants and children under 5 years of age which reported 7.6% and 3.8% detection rates of NV and SV single infections and 0.95% NV/SV mixed infections (Hansman et al., 2004b). Our results are consistent with those reported from several other countries. A report from Spain (Madrid), 12.9% and 1.3% of fecal specimens were positive for NV and SV, respectively (Buesa et al., 2002). Another report from Spain (Asturias) demonstrated that NV was responsible for 8.6% of acute gastroenteritis occurred among hospitalized children (Boga et al., 2004). Also, a report from Australia, the prevalent rate of NV and SV infection in hospitalized young children was 8.7% and 0.4%, respectively (Kirkwood et al., 2005). Another report from Pakistan showed that 9.9% NV and 3.2% SV were detected among hospitalized children (Phan et al., 2004).

Epidemiological data of HAstV as a causative agent of gastroenteritis in Thailand is limited. In 1991, Herrmann et al. first detected HAstV serotype 2, by ELISA, in 8.6% of children hospitalized with diarrhea in Bangkok. Later, a second report demonstrated that 14% of children hospitalized with diarrhea in Ratchburi province in the central part of Thailand were infected with HAstV (Echeverria et al., 1994). In 2004, Sirinavin et al (2006) detected HAstV, by ELISA, in 30.7% of diarrheal neonates in nursery of a maternity ward of Ramathibodi Hospital, Bangkok. The present study, we first reported a simultaneus cocirculation of HAstV with NV and SV in the same geographical area of Chiang Mai, even though only 2.4% were detected. However, our results are similar to those of the studies reported globally, i.e., 4.6% in U.S.A (Mendez-Toss et al., 2004), 2.7% in Spain (Boga et al., 2004), 1.6% in Hungary (Jakab et al., 2004), 3.0-4.3% in Australia (Mustafa et al., 2000; Schnagl et al., 2002), 8.5% in China (Qiao et al., 1999), 0.3% in Japan (Sakamoto et al., 2000), and 7.5% in Korea (Kang et al., 2002).

In the present study, 58.5% of viral strains identified among hospitalized children belonged to NV, while the remaining 24.3% and 17.0% belonged to SV and HAstV, respectively. The findings were not unexpected since SV and HAstV are usually associated with clinically milder diarrheal disease (Pang et al., 2000; Sakai et al., 2001; Dalton et al., 2002; Marie-Cardine et al., 2002), and uncommon in children hospitalized with sporadic acute gastroenteritis (Wofaardt et al., 1997; Buesa et al., 2001; Kirkwood et al., 2001). In addition, one infant was infected with both NV and SV strains without the significant difference in clinical severity from those with single infection. Similarly, several reports described the high rate of mixed infection as detected by RT-PCR method (Pang et al., 2000; Bereciartu et al., 2002; Oh et al.,

2003). The findings of two or more viruses in stools may be explained by virus from an earlier episode still being excreted and then superinfecting with another virus that cause an acute infection. However, it may be difficult to judge which of the two viruses, detected by highly sensitive RT-PCR, is the etiologic agent of particular episode. It is possible that the infection with more than one virus may increase the clinical outcomes of diarrheal disease. In our study, the clinical outcome was not significantly different between patients infected single and multiple virus infections. The results are in good agreement with a study that applying a clinical severity score indicate that the severity of diarrheal illness is not reflected in the proportion of mixed infections (Pang et al., 2000).

In many countries, NV, SV, and HAstV infections are prevalent in the winter months (Qiao et al., 1999; Mounts et al., 2000; Phan et al., 2005), though several studies show no seasonal distribution (Lewis et al., 1997; Nakata et al., 1998; Wright et al., 1998; O'Ryan et al., 2000; Akihara et al., 2005). In our study, the distribution patterns of NV, SV, and HAstV infections were also investigated during May 2000 to March 2002. Overall, infections of almost all viruses tend to occur in the first seven months of the year (January to July), which are winter, summer, and rainy seasons, in Chiang Mai, except for those of NV still occurred in November 2000 and October 2001. In one year round of 2001, NV was detected continuously from January to July with a peak in April, which is the summer month. Similar observations were also reported from the United Kingdom (Lopman et al., 2003), Southeastern Australia (Wright et al., 1998; Marshall et al., 1999), New Zealand (Greening et al., 1999; 2001), and Spain (Boga et al., 2004), in which NV infection tend to occur more

commonly in spring/summer. In the same period, SV and HAstV were detected only in 5 and 4 months, respectively, so the seasonal patterns of these viruses are unclear.

Our results demonstrated no significant difference of detection rate in these age groups. This finding, however, is in agreement with that of the previous study done in Vietnam (Hansman et al., 2004a).

Our investigations revealed a predominance of NVGII strains, which corresponds with the majority of the studies reported previously, suggesting that GII strains are more prevalent than GI strains in sporadic and epidemic gastroenteritis (Schreier et al., 2000; Buesa et al., 2002; Oh et al., 2003; Boga et al., 2004; Hansman et al., 2004b). However, a report from Brazil demonstrated that NVGI strains (from five distinct clusters) were predominant in a family based cohort study (Parks et al., 1999). In addition, NVGI strains were also reported as predominant type in children hospitalized with acute gastroenteritis in Central Australia (Schnagl et al., 2000). Moreover, strains of NV genogroup II, genotype 4 (NVGII/4) belonging to the Lordsdale cluster were identified in approximately half (53%) of NVGII strains detected among children with sporadic gastroenteritis in Chiang Mai, Thailand. The results showed that the NVGII/4 strains were predominant not only in this region but also circulating throughout Thailand since they were detected in the other regions of this country during 2002-2003 (Guntapong et al., 2004). Furthermore, the findings are consistent with other studies that NVGII/4 is associated with sporadic gastroenteritis in children conducted during the same period in many countries, including Spain (Roman et al., 2002), Hong Kong (Lau et al., 2004), Vietnam (Hansman et al., 2004a), Japan (Okada et al., 2005), Australia (Kirkwood et al., 2005), and France (Bon et al., 2005).

Sequence analysis of all nine NVGII/4 strains (Lordsdale virus cluster) revealed highest homology (96.2-98.7% nucleotide and 97.4-100% amino acid identities) with the NVGII/4 strains (Lordsdale virus cluster) identified in Chiba prefecture, Japan during September 1999 to June 2004 (Okada et al., 2005). In addition, all of our NVGII/4 strains were also closely related (95.7-97.4% nucleotide and 96.1-100% amino acid identities) to the NVGII/4 strains (Lordsdale virus cluster) detected in Ho Chi Minh City, Vietnam during December 1999 to November 2000 (Hansman et al., 2004a). This is an interesting finding since NVGII/4 strains detected in different geographical regions were closely related to each other.

NV genogroup II, genotype 4 (NVGII/4) known as the 95/96-US strain has been recognized as the major cause of gastroenteritis outbreaks (55%) in the United States during the 1995-1996 season. Later, the 95/96-US subset strains accounted for 26% of gastroenteritis outbreaks that reported to the Centers for Disease Control (CDC) during the 1997-1998 season (Noel et al., 1999). The 95/96-US subset strains decreased significantly since 1998 in the US and Europe (Fankhauser et al., 1998; Lopman et al., 2004) but continued to cause outbreaks and sporadic cases of gastroenteritis in several countries (Foley et al., 2001; White et al., 2002). Interestingly, all of our 9 NVGII/4 strains were closely related to the 95/96-US strain at 94.7-96% nucleotide and 97.4-100% amino acid identities (Figure 8b). Similarly, a study among cases from outbreaks and sporadic gastroenteritis conducted in New South Wales, Australia between July 1997 and September 2000 reported that the majority of NVGII strains (83%) were the members of 95/96-US subset (White et al., 2002). In addition, a similar study of sporadic gastroenteritis performed in hospitalized children in Vietnam between December 1999 and November 2000

revealed that 25% of NVGII strains were closely related to the 95/96-US strain (Hansman et al., 2004a). Furthermore, a study conducted in Hong Kong during July 2001-June 2002 demonstrated that most of the predominant NVGII/4 strains (Bristol virus cluster) detected in gastroenteritis outbreaks were closely related to the 95/96-US subset strain (Lau et al., 2004). These findings suggest that the 95/96-US-like strain is still an important cause of gastroenteritis in both the sporadic cases and outbreaks worldwide.

Two NVGII/3 strains detected in this study were also closely related to the NVGII/3 Japanese strains (Okada et al., 2005) and the NVGII/3 Vietnamese strains (Hansman et al., 2004a) with a high identity at the nucleotide and the amino acid levels, ranging from 93.6-100% and 98.7-100%, respectively. Recently, NVGII/3 known as the Arg320-like strain was reported as the predominant genotype that cause gastroenteritis among hospitalized children and suddenly appeared between October 1999 and February 2000 in Osaka, Japan (Iritani et al., 2003). Later, the Arg320-like strains were also associated with sporadic gastroenteritis occurred between January to March 2000 in Vietnam (Hansman et al., 2004a). Sequence analysis of our two NVGII/3 strains showed high homology with the Arg320 strain at 96-96.7% nucleotide and 97.4-98.7% amino acid identities.

Additionally, our NV strains of other genotypes including GII/1, GII/6, GII/8, GII/10, GI/3, GI/4, GI/6, GI/7, and GI/13 were also closely related with the corresponding genotypes identified in Japan (Okada et al., 2005) and Vietnam (Hansman et al., 2004a) with a high identity at the nucleotide and the amino acid levels ranging from 91.5-100% and 98.7-100%, respectively. It should be noted that NVGII/15 (CMH148/01) is distantly related (66.8% nucleotide and 73.0% amino acid

identities) to NVGII/15 strains described by Okada et al. (2005). However, the CMH148/01 strain had high identity (95.4-95.8% nucleotide and 100% amino acid) with two strains (J23/99/US and Mex7076/99) which were used as GII/15 reference strains in the classification scheme of Zheng et al. (2006).

Furthermore, NVGII/1, NVGII/3, NVGII/4, and NVGII/6 identified in this study had high identity (90.1-99.6% nucleotide and 96.9-100% amino acid) with the corresponding genotypes detected in another four different regions of Thailand from November 2002 to April 2003 (Guntapong et al., 2004). In contrast, NVGI, NVGII/8, NVGII/10, and NVGII/15 strains detected in this study were not detected by Guntapong's study (2004).

Taken together, these findings possibly suggest that NV strains of several genotypes currently circulating in this region and other regions of Thailand, and other countries during the same period might have descended from the same ancestor.

SVs belonging to genogroup I (SVGI) have been reported as the predominant srains associated with gastroenteritis worldwide (Okada et al., 2002; Phan et al., 2004; Akihara et al., 2005; Phan et al., 2005; 2006). In our study, SVGI was identified in 80% (8 of 10) of all SV strains. Moreover, SV genotype 1 of genogroup I (SVGI/1) strains (typified by the Manchester virus) were detected in 50% of SVGI strains. The SVGI/1 strains were also reported as the major genotype circulating in the other four different geographical regions of Thailand (Guntapong et al., 2004), Sweden (Vinjé et al., 2000), France (Bon et al., 2005), Australia (Wright et al., 1998), Japan (Okada et al., 2002; Akihara et al., 2005), and Pakistan (Phan et al., 2005).

Based on the analysis of partial capsid sequences, four SVGI/1 strains detected in this study had high identity (96.7-98.9% nucleotide and 95.6-97.8% amino acid)

with SVGI/1 strains detected in patients with sporadic gastroenteritis in the Chiba prefecture from June 1998 to May 2001 (Okada et al., 2002). In addition, our SVGII/1 and SVGII/2 strains showed high identity (94.5-96.5% nucleotide and 86.2-97.4% amino acid) with the corresponding genotypes identified by Okada et al. (2002). In contrast, our three SVGI/4 strains were distantly related (80.4-81.2% nucleotide and 78.2-79.1% amino acid) to SVGI/4 strains described by Okada et al. (2002). However, our SVGI/4 strains were closely related (97.0-97.9% nucleotide and 97.3-99.1% amino acid) to the SVGI/4 reference strain (Karachi/1021/92) in the classification scheme of Akihara et al. (2005).

Similarly, SVGI/1 strains had high identity (97.4-98.5% nucleotide and 96.7-97.8% amino acid) with the SVGI/1 representative strains detected in other four different regions of Thailand (Figure 10) from November 2002 to April 2003 (Guntapong et al., 2004). In contrast, SVGI/4, SVGI/5, SVGII/1, and SVGII/2 strains detected in our study were not detected by Guntapong et al. (2004).

In this study, HAstV seems to play a minor role (2.4%) in gastroenteritis in children compared to NV (8.1%) and SV (3.4%). The HAstV genotype/serotype 1 (HAstV-1), HAstV-2, and HAstV-5 were detected at the same rate (28.6%), while HAstV-3 was relatively less common (14.3%). However, the finding is similar to those reported from other regions of the world, i.e. Australia (Mustafa et al., 2000), Germany (Oh and Schreier, 2001), and Spain (Guix et al., 2002). The HAstV-4, even though it is a common serotype, it was not detected in our study during this period, which consistent with the report from South Africa (Nadan et al., 2003). The absence of HAstV-6, HAstV-7, and HAstV-8 in our study was not unexpected since these serotypes are seldom detected (Glass et al., 1996; Mustafa et al., 2000; Guix et al.,

2002). However, HAstV-8 strains appear to be more common in the African continent, France, Mexico, and Spain (Naficy et al., 2000; Taylor et al., 2001; Chikhi-Brachet et al., 2002; Cunliffe et al., 2002; Guix et al., 2002; Nadan et al., 2003; Mendez-Toss et al., 2004), suggesting that these strains might be more epidemiologically relevant than previously recognized. The HAstV-1 strains detected in this study are closely related to HAstV-1 strains detected in infants and children hospitalized with gastroenteritis in Barcelona, Spain between May 1997 and April 2000 (Guix et al., 2002) with a high identity at the nucleotide and amino acid levels, ranging from 91.6-99.1% and 99.1-100%, respectively. Additionally, strains of HAstV-2 and HAstV-3 are also closely related to the corresponding serotypes detected in Spain with 85.3-95.1% nucleotide and 100% amino acid identities (Guix et al., 2002).