CHAPTER V

RESULTS

1. Detection of diarrheal viruses by RT- multiplex PCR

A total of 332 stool specimens collected in one year round in 2008 were screened for 10 types of diarrheal viruses. Before conducting the molecular detection of 10 types of diarrheal viruses, the positive controls which are known to be positive for SaV, AiV, RCV, HPeV, NoV GI, NoV GII, EV, AdV, RAV, AstV from the previous studies were used as positive control in this novel RT-multiplex PCR method. The expected PCR product sizes of SaV, AiV, RCV, HPeV, NoV GI, NoV GII, EV, AdV, RAV, and AstV were 100 bp, 158 bp, 205 bp, 270 bp, 330 bp, 387 bp, 440 bp, 482 bp, 569 bp, and 719 bp, respectively. All the positive controls showed the correct PCR product sizes as shown in Figure 1.

By screening with multiplex-PCR, 4.2% were positive for 5 types of diarrheal viruses. Among these, AdV, EV, AiV, NoV GII, and HPeV were detected (Figures 2, 3, 4, 5, and 6, respectively), while SaV, RCV, NoV GI, RAV, and AstV were not found in this study. AdV and EV were detected as the most prevalent viruses in this study (1.2%, 4 out of 332), followed by AiV (0.9%, 3 out of 332) and NoV GII (0.6%, 2 out of 332), respectively. In addition, mixed infection of 2 viruses between NoV GII and HPeV was also detected in one fecal specimen (0.3%), as shown in Table 8.

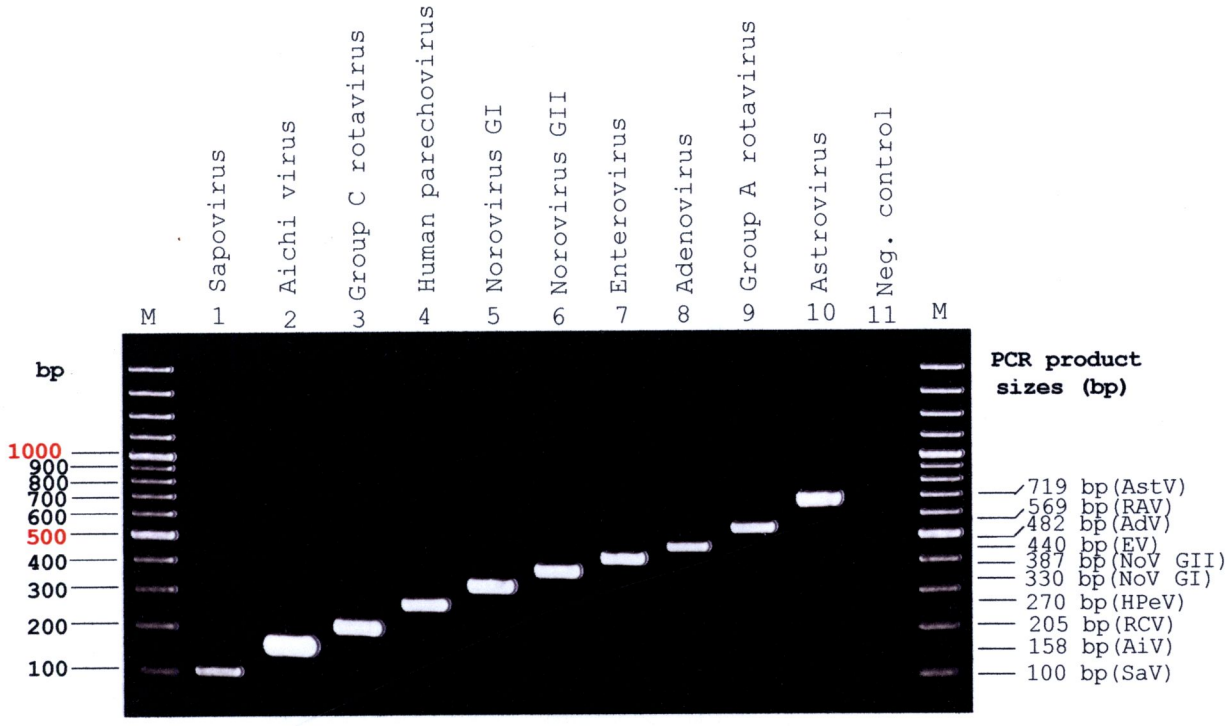

Figure 1 Agarose gel electrophoresis demonstrating the expected PCR product size of 10 viruses. Lane 1, SaV (100 bp); lane 2, AiV (158 bp); lane 3, RCV (205 bp); lane 4, HPeV (270 bp); lane 5, NoV GI (330 bp); lane 6, NoV GII (382 bp); lane 7, EV (440 bp); lane 8, AdV (487 bp); lane 9, RAV (569 bp); lane 10, AstV (719 bp); lane 11, a negative control. Lane M, 100 bp DNA ladder.

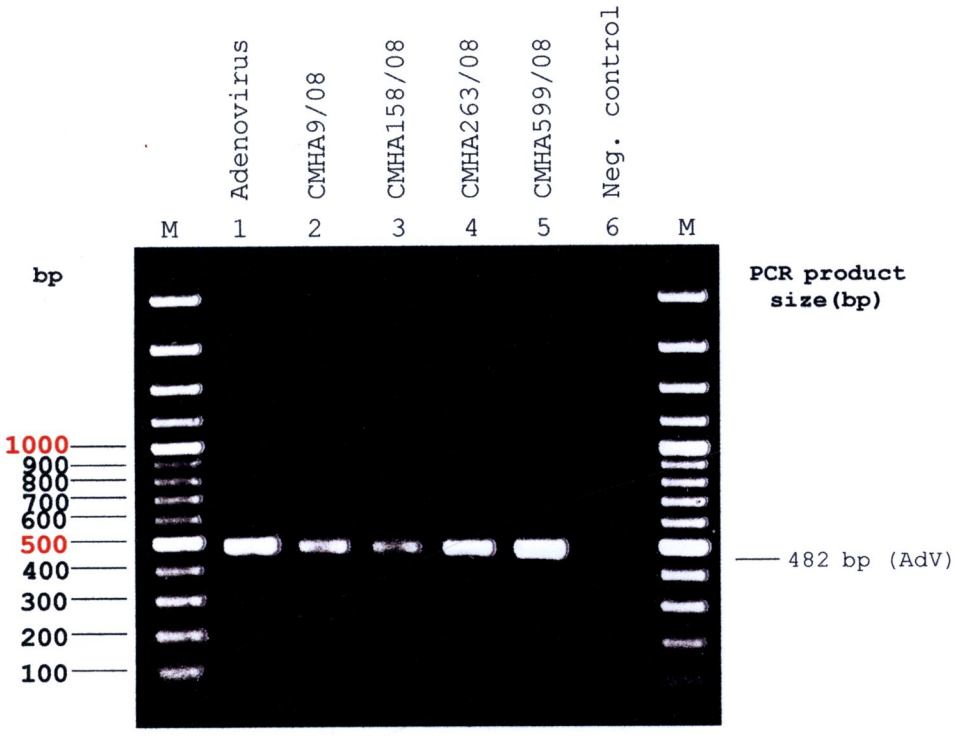

Figure 2 Agarose gel electrophoresis demonstrating the expected PCR product size of adenoviruses. Lane 1, reference strain of AdV; lanes 2-5, tested samples (CMHA9/08, CMHA158/08, CMHA263/08, and CMHA599/08) that were positive for AdV, and lane 6, a negative control. Lane M, 100 bp DNA ladder.

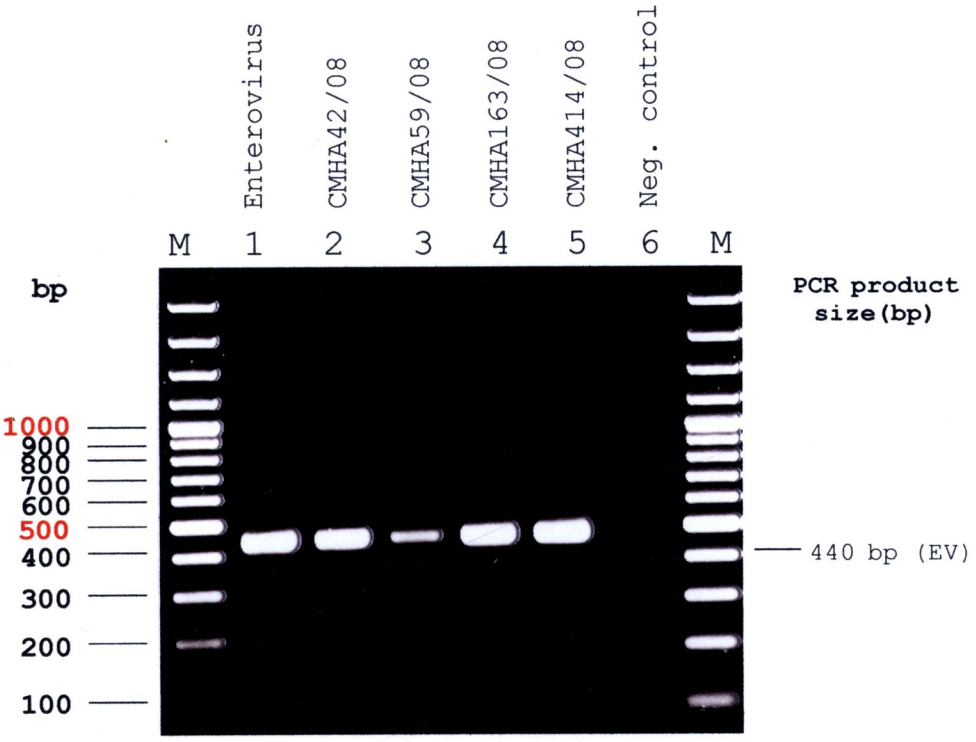

Figure 3 Agarose gel electrophoresis demonstrating the expected PCR product size of enteroviruses. Lane 1, reference strain of EV; lanes 2-5, tested samples (CMHA42/08, CMHA59/08, CMHA136/08, and CMHA414/08) that were positive for EV, and lane 6, a negative control. Lane M, 100 bp DNA ladder.

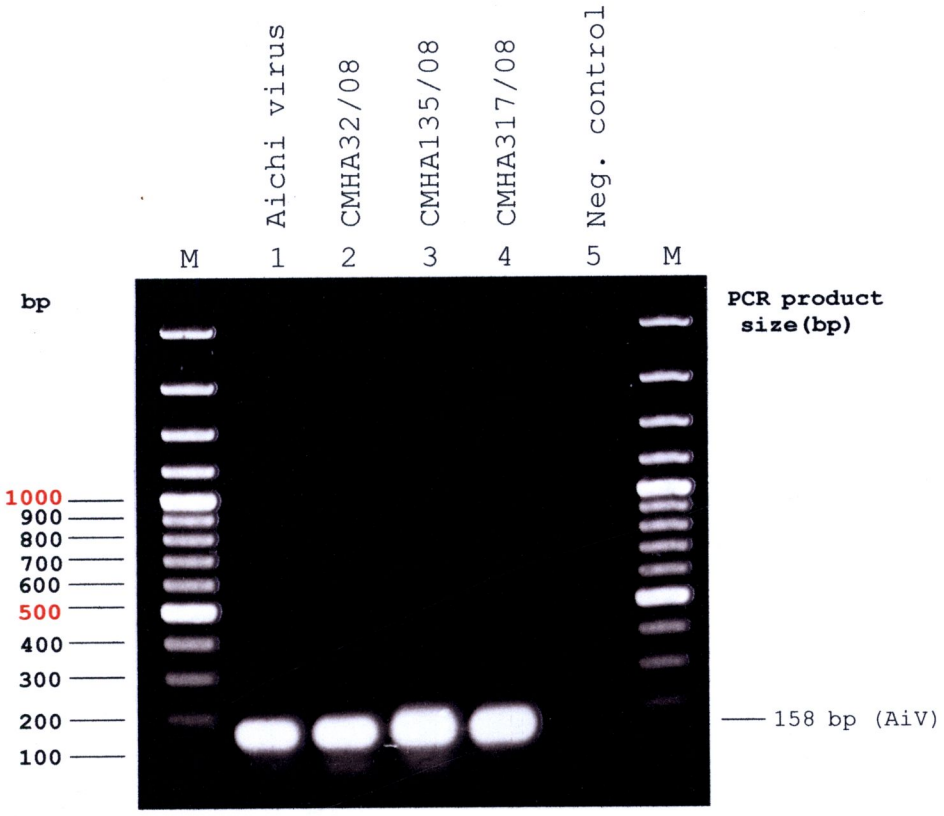

Figure 4 Agarose gel electrophoresis demonstrating the expected PCR product size of Aichi viruses. Lane 1, reference strain of AiV; lanes 2-4, tested samples (CMHA32/08, CMHA135/08, and CMHA317/08) that were positive for AiV, and lane 5, a negative control. Lane M, 100 bp DNA ladder.

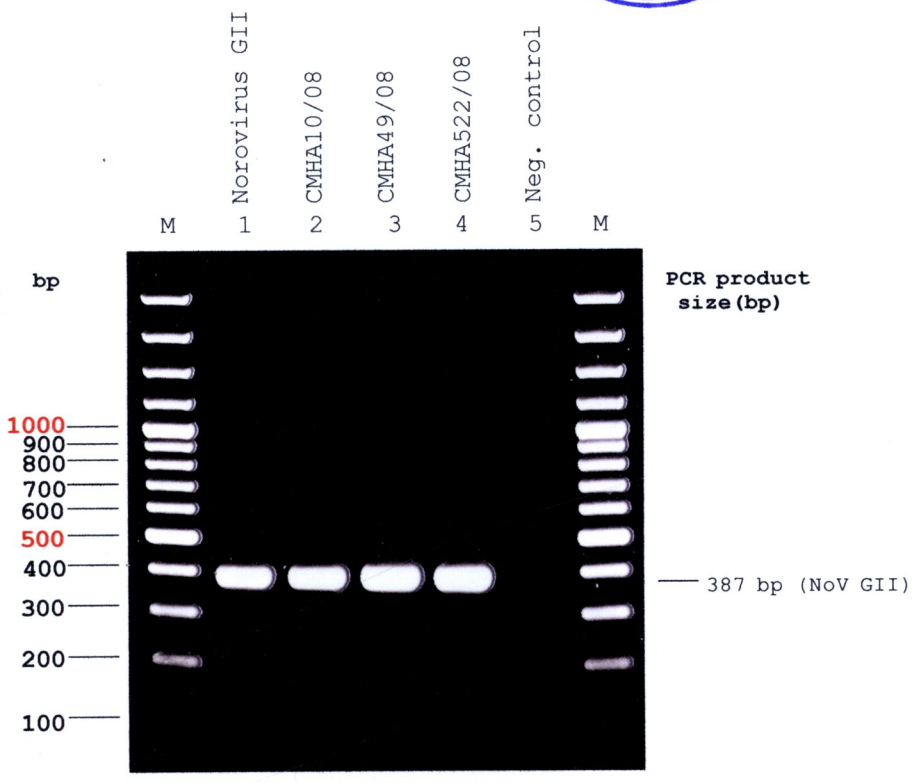

Figure 5 Agarose gel electrophoresis demonstrating the expected PCR product size of noroviruses GII. Lane 1, reference strain of NoV GII; lanes 2-4, tested samples (CMHA10/08, CMHA49/08, and CMHA522/08) that were positive for NoV GII, and lane 5, a negative control. Lane M, 100 bp DNA ladder.

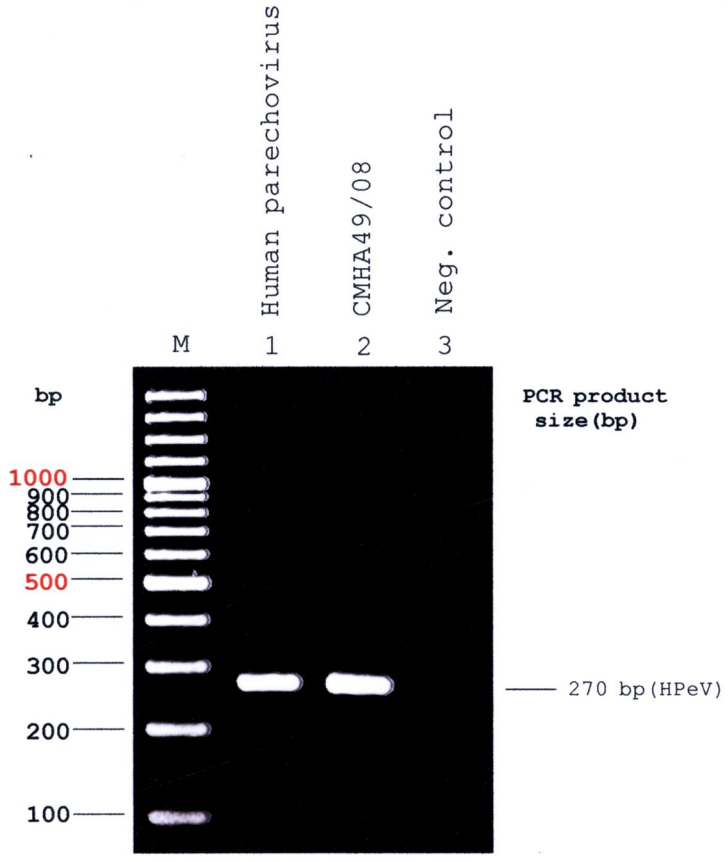

Figure 6 Agarose gel electrophoresis demonstrating the expected PCR product size of human parechoviruses. Lane 1, reference strain of HPeV; lane 2, tested samples (CMHA49/08) that were positive for HPeV, and lane 3, a negative control. Lane M, 100 bp DNA ladder.

Table 8 Prevalence of diarrheal viruses in adults with diarrhea in Chiang Mai, Thailand in 2008 determined by RT-multiplex PCR

Number of fecal specimens tested	Number of diarrheal viruses positive (%)											Total (%)
	Sapovirus	Aichi virus	Group C rotavirus	Human parechovirus	Norovirus GI	Norovirus GII	Enterovirus	Adenovirus	Group A rotavirus	Astrovirus	Mixed infection	
332	0/332 (0)	3/332 (0.9%)	0/332 (0)	0/332 (0)	0/332 (0)	2/332 (0.6%)	4/332 (1.2%)	4/332 (1.2%)	0/332 (0)	0/332 (0)	1*/332 (0.3%)	14 (4.2%)

* Mixed-infection of 2 viruses between norovirus GII and human parechovirus.

2. Nucleotide sequencing and phylogenetic analyses of adenoviruses, enterovirus, Aichi viruses, noroviruses GII and human parechoviruses

The PCR products obtained from the specimens that positive for diarrheal viruses were purified, and sequenced by using Big-Dye Terminator Cycle Sequencing kit (ABI PRISM 3100, Carlsbad, USA). Among these 14 specimens, AdV and EV each was found in 4 samples. AiV and NoV GII each was found in 3 and 2 samples, respectively. HPeV was detected in 1 sample. The obtained sequences were compared with the reference sequences by searching for the close by related reference sequences using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) server from NCBI database (http://www.ncbi.nlm.nih.gov/nuccore). Those reference sequences obtained from BLAST search, the viruses of each genotypes/ genogroups/ subgroups/ species, as well as our study sequences were aligned using ClustalX software, and further classified for their genotypes/ genogroups/ subgroups/ species by phylogenetic analysis using MEGA 4 software (Tamura et al., 2007).

2.1 Analysis of partial capsid gene of adenoviruses

The partial hexon gene (482 bp) of AdV capsid was sequenced by using Ad2 specific primer. From BLAST search and clustalX alignment, 4 AdVs found in this study (CMHA9/08, CMHA158/08, CMHA263/08, and CMHA599/08) were genetically variable. Two AdV sequences (CMHA263/08 and CMHA599/08) were most closely related to the prototype strain of AdV40 (X51782) at 99% nucleotide sequence identity. The CMHA263/08 strain was most closely related to HME562 strain (EF570136), while the CMHA 599/08 was most closely related to Dugan strain (AB330121), 6643 strain (FJ167565), HME562 strain (EF570136) at 99% nucleotide

sequence identity. In addition, the CMHA9/08 strain showed nucleotide sequence identity at 99% with AdV25 prototype strain (DQ149623) and BP-1 strain (AB330106) while CMHA158/08 was closely related (99% identity) with AV-3153 strain (AB330105) and prototype strain of AdV24 (DQ149622), of subgroup D.

The phylogenetic analysis of partial hexon gene sequence of AdV demonstrated clearly that 4 AdV strains detected in this study were classified into 3 genotypes of 2 distinct subgroups. CMHA9/08 and CMHA158/08 belonged to genotypes AdV25 and AdV24 of subgroup D. The CMHA263/08 and CMHA599/08 belonged to AdV40 and both strains were in subgroup F, as shown in Figure 7. It is interesting to note that, AdV strain detected in this study were quite difference from the AdVs isolated previously in children in Chiang Mai in 2007, which belonged to AdV1, AdV3, and AdV41 genotypes. Comparing 4 AdV strains identified in this study with those of the AdV strains isolated previously in children in Chiang Mai (CMHA9/07, CMHA25/07, and CMHA16/07) revealed the nucleotide sequence identity ranging only from 77-89%.

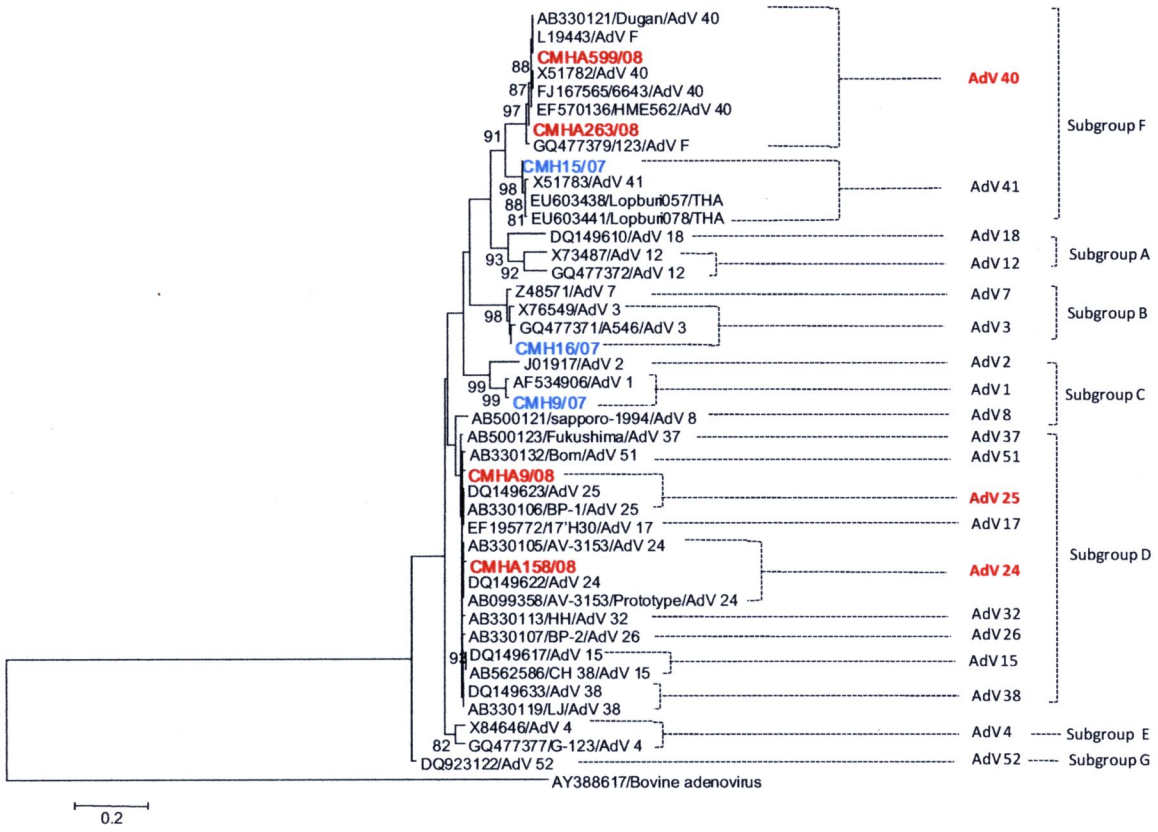

Figure 7 Phylogenetic analysis of the partial nucleotide sequences (482 bp) of the hexon gene of adenoviruses. The GenBank accession numbers of reference strains were indicated proximal to the strain name. The AdV strains detected in this study and previous study in children were indicated in red and blue, respectively. The tree was constructed according to neighbor-joining method. The number on each branch indicates the bootstrap value.

2.2 Analysis of partial 5' untranslated region (5'UTR) of enteroviruses

The 4 EV strains (CMHA42/08, CMHA59/08, CMHA136/08, and CMHA414/08) found in this study were genetically variable. The CMH59/08 strain showed highly nucleotide sequence identity (96%) with echovirus 30 (AM237034) reference strain and belonged to EV of species B. Comparing the nucleotide sequence of CMHA59/08 with those of EVs in species B isolated previously in children in Chiang Mai (CMH86/07, CMH134/07, and CMH135/07), it was observed that the sequence identities were ranging from 93-99%. The CMHA42/08 strain was identical (100%) to poliovirus genotype 3, MF1 strain (AJ783739), 31974 strain (FJ460227) and HeB strain (FJ859192). It is interesting to note that CMHA136/08 strain is most closely related to CMH116/07 strain which was isolated previously form a child in Chiang Mai at 95% nucleotide sequence identity, and also similar to MOR83 reference strain (EF015020). All of these strains were coxsackievirus A20. Moreover, the CMHA414/08 strain shared nucleotide sequence identity with BAN04-1067 reference strain (EF015010) of enterovirus 99 at 90% and with enterovirus 99 prototype strain (EF555644) at 83%.

Based on the phylogenetic analysis of EV 5'UTR sequence, 4 strains of EV found in this study belonged to 4 genotypes of 2 species (B and C) of EV (Figure 8). The CMHA42/08 was the poliovirus 3, CMHA136/08 was coxsackie virus A20, CMHA414/08 was enterovirus 99, and all 3 strains were clusted within EV species C. The CMHA59/08 was the only EV strain clusted in EV species B and was identified as Echovirus 30. Moreover, phylogenetic tree clearly demonstrated that CMHA136/08 belonged to coxsackie virus A20 and was the only strain that showed nucleotide sequence most closely related to EV (CMH116/07) which was isolated

previously in Chiang Mai in 2007. The data confirmed that coxsackievirus A20 was circulated in Chiang Mai and caused diarrhea both in children and adults.

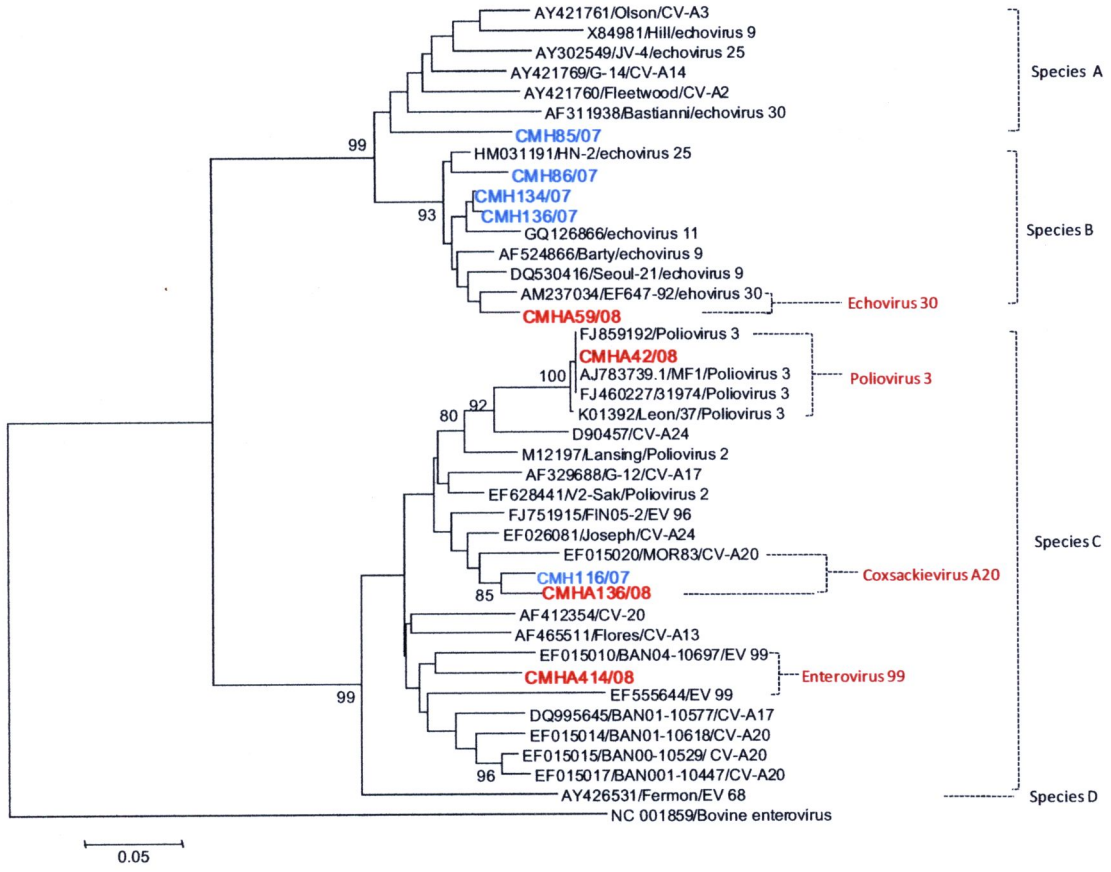

Figure 8 Phylogenetic analysis of the partial nucleotide sequence (440 bp) of the 5' untranslated region (5' UTR) of enteroviruses. The GenBank accession numbers of reference strains were indicated proximal to the strain name. EV detected in adults in this study and previous studies in children were indicated in red and blue, respectively. The tree was constructed according to neighbor-joining method. The number on each branch indicates the bootstrap value.

2.3 Analysis of the 3C and the N terminus of 3D regions (3CD) of Aichi viruses

In the present study, 3 AiVs strains (CMHA32/08, CMHA135/08, and CMHA317/08) were detected by RT-multiplex PCR screening method. For characterization of their genotypes, the partial 3CD region was amplified and the PCR product size was 519 bp as shown in Figure 9. The PCR amplicons of 519 bp were subjected to direct sequencing. The obtained sequences were compared to those of AiV strains available in the GenBank database by BLAST program and clustalX alignment. Sequence analysis revealed that 2 AiV strains (CMHA32/08 and CMHA317/08) showed highly nucleotide sequence similarity among themselves, and also similar to these of AiV genotype B, Chsh3 (FJ890521), Chsh4 (FJ890522), and Chsh6 (FJ890517) reference strains from China at 98% nucleotide sequence identity. In addition, the nucleotide sequence of one strain of AiV found in this study (CMHA135/08) was most closely related to AiV genotype A, 494/97 (AB092828), A1471/96 (AB034650), 364/96 (AB092826), and J-482 (EF079154) reference strains from Japan at the nucleotide sequence identity of 96%. It is interesting to point out that the CMHA135/08 AiV identified in this study is not so closely related with the AiV T-132/02 strain, the only one AiV previously found in children with diarrhea in Chiang Mai area in 2002. Nucleotide sequence identities between these 2 strains were only 94%.

The phylogenetic analysis shown in Figure 10 clearly demonstrated that all 3 AiV strains from adults with diarrhea were clustered into two major genotypes, genotypes A and B. The CMHA135/08 strain belonged to genotype A with the nucleotide sequence similar to the AiV strain (EF079160) which was isolated previously from children in Chiang Mai, in 2002. The other two AiV strains

(CMHA32/08 and CMHA317/08) were clustered closely together with other AiV genotype B reference strains. The data clearly demonstrated that AiV of both genotypes A and B were circulating in this area in adults with diarrhea in Chiang Mai, Thailand.

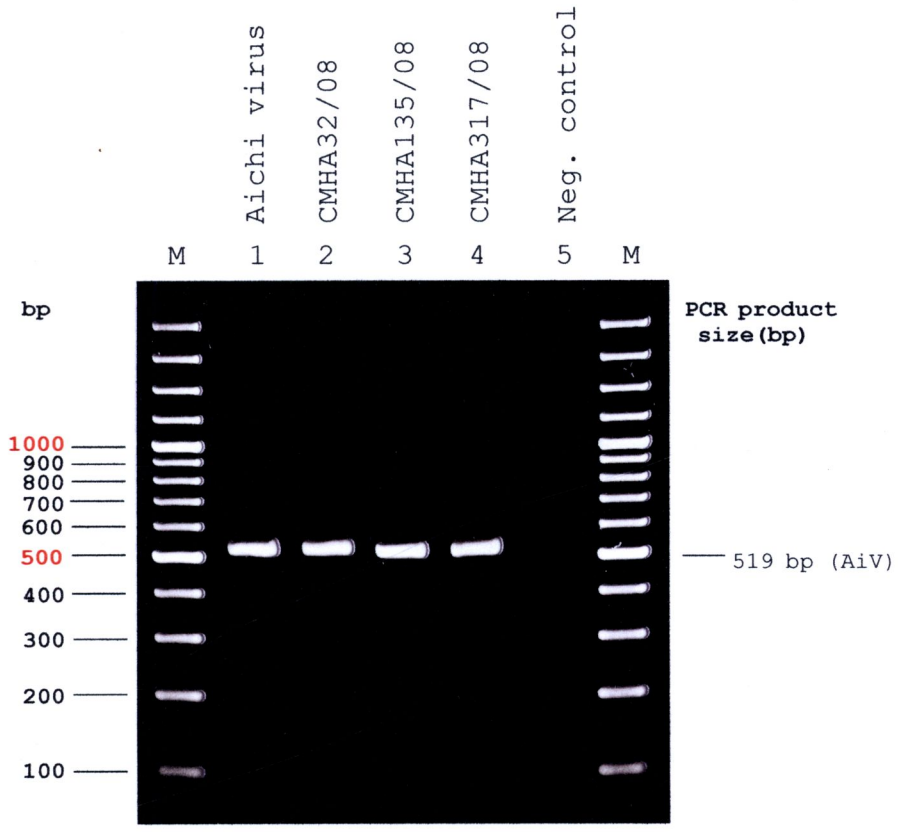

Figure 9 Agarose gel electrophoresis demonstrating the expected PCR product size of Aichi viruses at the 3CD regions. Lane 1, reference strain of AiV; lanes 2-4 test samples (CMHA32/08, CMHA135/08, and CMHA317/08) that were positive for AiV; lane 5, a negative control reaction. Lane M, 100 bp DNA ladder.

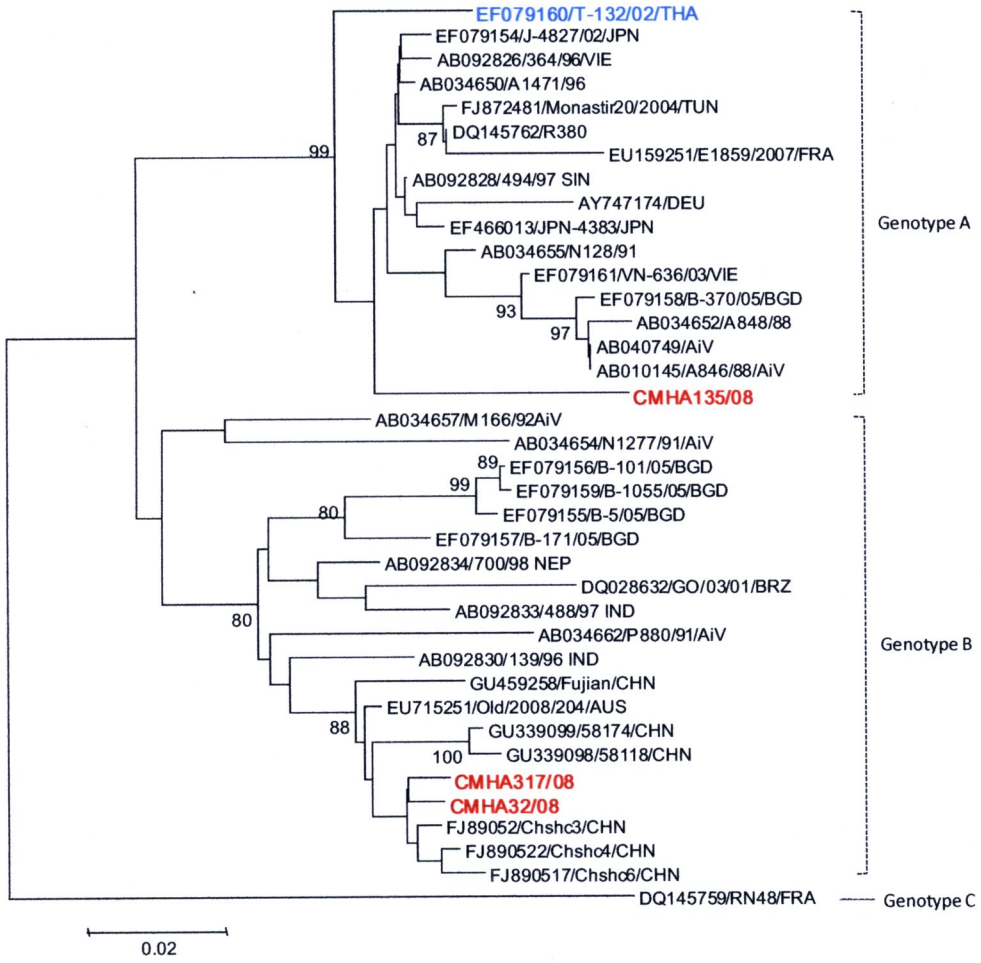

Figure 10 Phylogenetic analysis of the 3CD region nucleotide sequences (519 bp) of Aichi viruses. The GenBank accession numbers of reference strains were indicated proximal to the strain name. AiV detected in this study and previous studies in children were indicated in red and blue, respectively. The tree was constructed according to the neighbor-joining method. The number on each branch indicates the bootstrap value.

2.4 Analysis of partial capsid gene of noroviruses GII

The nucleotide sequences of partial capsid gene of NoV GII strains (CMHA10/08, CMHA49/08, and CMHA552/08) obtained from this study were compared to those of NoV strains available in the GenBank data base using the BLAST program and clustalX alignment. Two strains of NoV GII (CMHA552/08 and CMHA49/08) were identical (100% identity) to Nagano strain (AB541303) which was the GII/4 strain reported from Japan, while sharing lesser degree of identity (99%) to Beijing/74 (EU703746), Beijing/334 (EU703755), CHD-032304 (HM624049), Shanghai/SH2 (GU991353) which were reported from China, and also similar to Sakai4 (AB541344), Chiba5 (AB541234) which were reported from Japan, and Seoul/027 (HM636147) from Korea. The other NoV GII strain, CMHA10/08 shared a great homology at 98% with NZ327 (EF187497) which was reported from New Zealand. Moreover, this CMHA10/08 also showed high degree of sequence identity (97%) with other NoV GII/4 strain isolated from Japan, United Kingdom, and Thailand. Those included Hokkaido5 (AB541267), Amori2 (AB447343), B4S6 (AY587958), CMH38/02 (EF600760), CMH041/02 (EF600762), CMH43/02 (EF600764), and Sakaeo-14 (AY646868). In addition, CMHA10/08 showed less sequence identity (80%) with other 3 NoV GII/4 strains (CMH150/07, CMH153/07, and CMH155/07) isolated previously from children with diarrhea in Chiang Mai in 2007.

Based on the phylogenetic relationships and the classification scheme in Figure 11, all NoVs detected in the present study (CMHA10/08, CMHA49/08, and CMHA552/08) belonged to NoV GII/4 which carried nucleotide sequence somewhat

differed from the NoV GII/4 strains isolated previously from children in Chiang Mai area.

The NoV GII/4 were further characterized for their variant genotypes by nucleotide sequencing and phylogenetic analysis as shown in Figure 12. From sequence alignment of NoV GII/4 strains in the present study with NoV GII/4 varaint strain available in the GenBank database using ClustalX software revealed that CMHA49/08 strain was identical (100% identity) to Nagano2 strain (AB541303) which were the NoV GII/4 variant strains reported from Japan. In addition, CMHA49/08 showed high sequence identity (97-98%) with other NoV GII/4 variant strains detected in Japan including Hokkaido4 (AB541266), FUMI (AB543808), Osaka3 (AB541324), Saga1 (AB447456), and Hokkaido3 (AB447440). Moreover, the CMHA552/08 strain was most similar to Nagano2 (AB541303) at the nucleotide sequence identity of 98%, while sharing lesser degree of identity (97%) to NoV GII/4 variant strains from Japan [Osaka3 (AB541324), Saga1 (AB447456), and Hokkaido3 (AB447440)]. Comparing of two NoV GII/4 variant strains (CMHA49/08 and CMHA552/08) with the NoV GII/4 variant strains detected previously in children in Chiang Mai showed 81-84% nucleotide identity. Another NoV GII/4 variant strain (CMHA10/08) showed high degree of sequence identity (97%) with the NoV GII/4 variant strains isolated from Germany [Mannheim131 (GQ303445)], Australia [NSW892U (HM748973)], and Japan [Aichi1 (AB541202), Osaka1 (AB541320)], and showed 95-96% nucleotide identity with Niigata1 strain (AB541310), Iwate3 (AB541272) which were those reported from Japan, and NSW001P (GQ845367) from Germany. In contrast, the CMHA10/08 was compared with the NoV GII/4

variant strain from previously detected in children in Chiang Mai in 2007 revealed only 84% nucleotide sequence identity.

Based on the phylogenetic relationships and the classification of NoV GII/4 variant scheme, NoV GII/4 vairants detected in the present study could be divided into two variants, NoV GII/4 2006a variant and NoV GII/4 2006b variant (Figure 13). The CMHA10/08 belonged to NoV GII/4-2006a variant while CMHA49/08 and CMHA552/08 belonged to NoV GII/4-2006b variant. Two NoV GII/4 variant strains, CMHA49/08 and CMHA552/08 were most closely related to Osaka3 (AB541324) and Nagano2 (AB541303) which were NoV GII/4 variants reported from Japan, and shared a great sequence identity (83%) with CMH158/07 and CMH160/07 strains which were isolated previously from children in 2007 in Chiang Mai. Moreover, One strain of NoV GII/4 variant (CMHA10/08) was most similar (88%) to NoV GII/4 variant strains (CMH150/07, CMH153/07, and CMH155/07) which were isolated from children in Chiang Mai area in 2007.

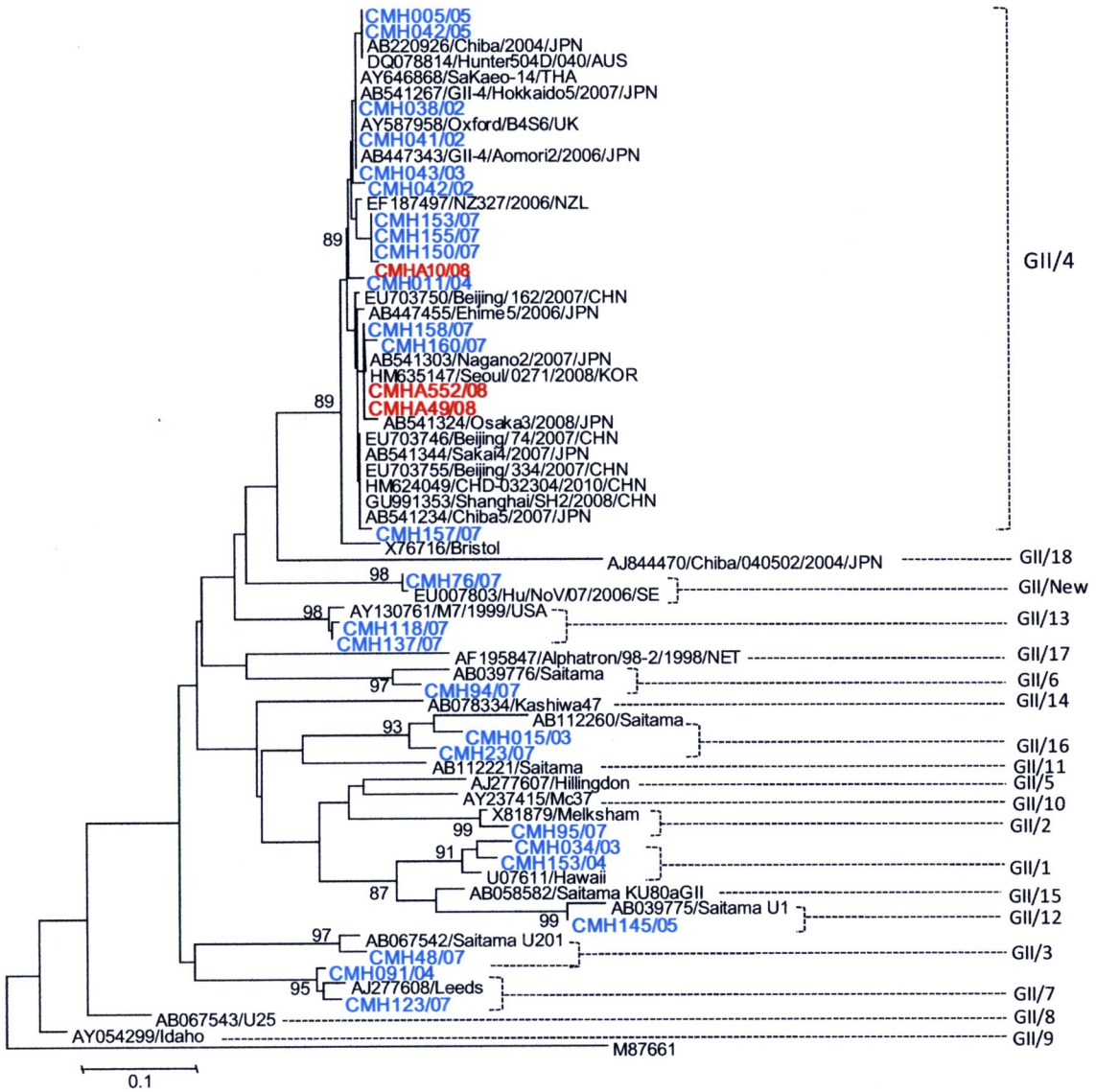

Figure 11 Phylogenetic analysis of the partial nucleotide sequences (387 bp) of capsid gene of noroviruses genogroup II. The GenBank accession numbers of reference strains were indicated proximal to the strain name. NoV GII detected in this study and in previous study in children were indicated in red and blue, respectively. The tree was constructed according to the neighbor-joining method. The number on each branch indicates the bootstrap value.

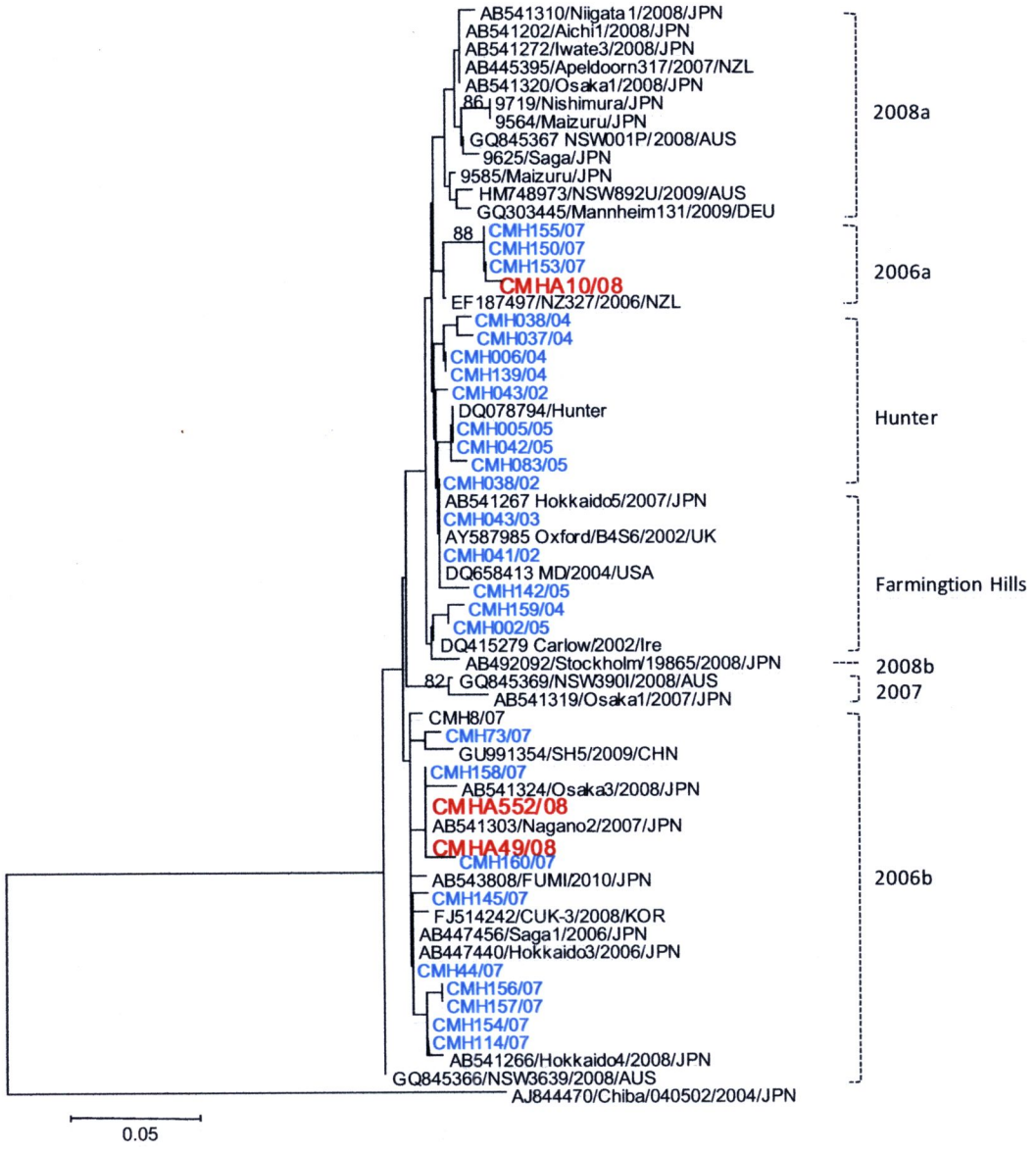

Figure 12 Phylogenetic analysis of the partial nucleotide sequences (387 bp) of capsid gene of noroviruses genogroup II/4 variants. The GenBank accession numbers of reference strains were indicated proximal to the strain name. NoV GII/4 variant detected in this study and previous study in children were indicated in red and blue, respectively. The tree was constructed according to neighbor-joining method. The number on each branch indicates the bootstrap value.

2.5 Analysis of partial capsid gene of human parechovirus

Classification of HPeV into genotype is based on the VP1 capsid gene. Partial VP1 nucleotide sequence of capsid gene sequence was amplified by specific primers which targeted to amplify the 760 bp PCR product of partial VP1 gene. If the PCR product of 760 bp was not amplified, nested-PCR was performed the PCR product of 477 bp. The gel electrophoresis demonstrating the PCR product of 477 bp is shown in Figure 13. The partial VP1 gene of 477 bp was purified and sequenced by using HPeV-VP1-R as a sequencing primer. From BLAST search and ClustalX alignment, 1 strain of HPeV (CMHA49/08) found in the present study was most closely related to T-141 strain isolated previously from a child in Chiang Mai, Thailand in 2005 (FJ648762) at 96% nucleotide sequence identity. When comparing the sequence with other Thai HPeV strains previously isolated in Chiang Mai area in 2005 [T-69 (FJ648761), T-96 (FJ648757), and T-103 (FJ648759)], the nucleotide sequence identity was only 75% although all these strains were HPeV1. Moreover, The CMHA49/08 strain was also similar to HPeV strains reported from Netherland [03-0812 (AB443809), 677008 (FJ373135), K63-94 (GQ183025), 7555312 (FM178558)], Germany [BNI-R21 (EU024634)], and Japan [A10987 (AB112487), JP-8275 (HQ163882)] with the nucleotide sequence identities ranging from 88-90%.

Phylogenetic analysis of partial VP1 gene (Figure 14) confirmed that CMHA49/08 strain was most closely related to T-141 strain (FJ648762) but quite different from the other HPeV strains isolated previously in children in Chiang Mai in 2005. The HPeV strains previously isolated in Chiang Mai were classified into 4 distinct genotypes including HPeV1 [T-69(FJ648761), T-96 (FJ648757), T-103

(FJ648759), T-141 (FJ648762)], HPeV2 [T-144 (FJ648760)], HPeV3 [T-68 (FJ648756), T-102 (FJ648758)], and HPeV4 [T-96 (FJ648755)].

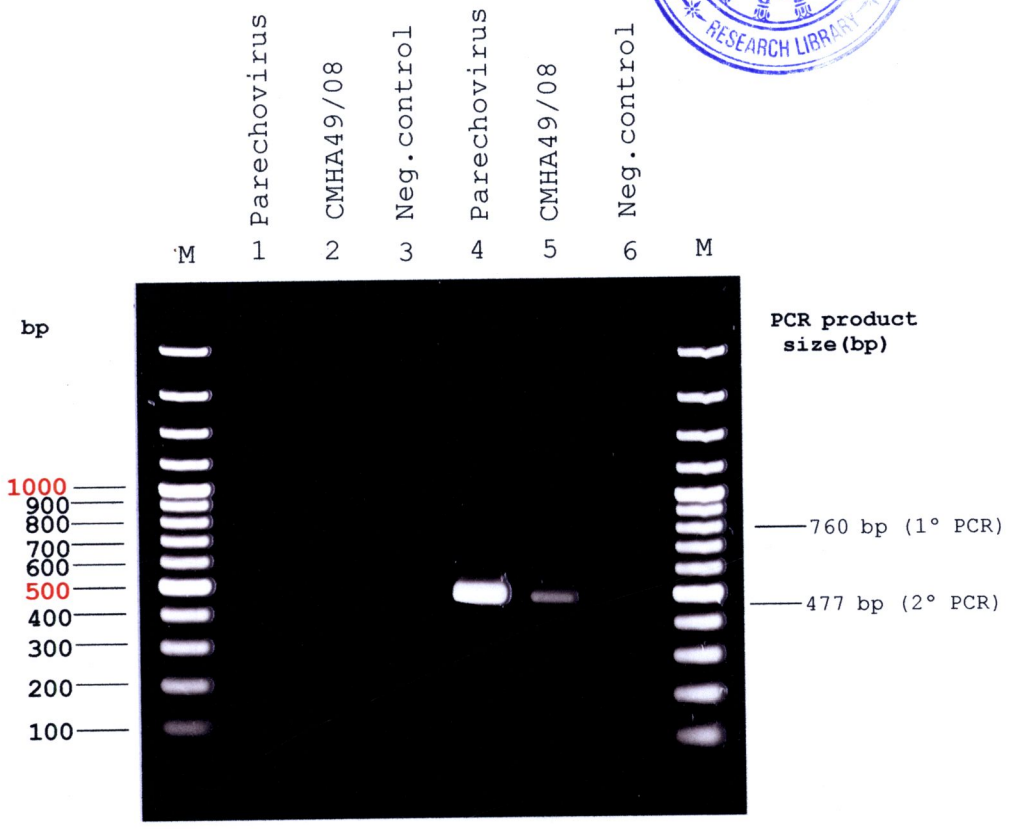

Figure 13 Agarose gel electrophoresis demonstrating the expected PCR product size of human parechoviruses using specific primer for VP1 capsid gene. Lanes 1 and 4, reference strain of HPeV; lanes 2 and 5, HPeV positive (CMHA49/08); lanes 3 and 6, negative controls. Lane M, 100 bp DNA ladder.

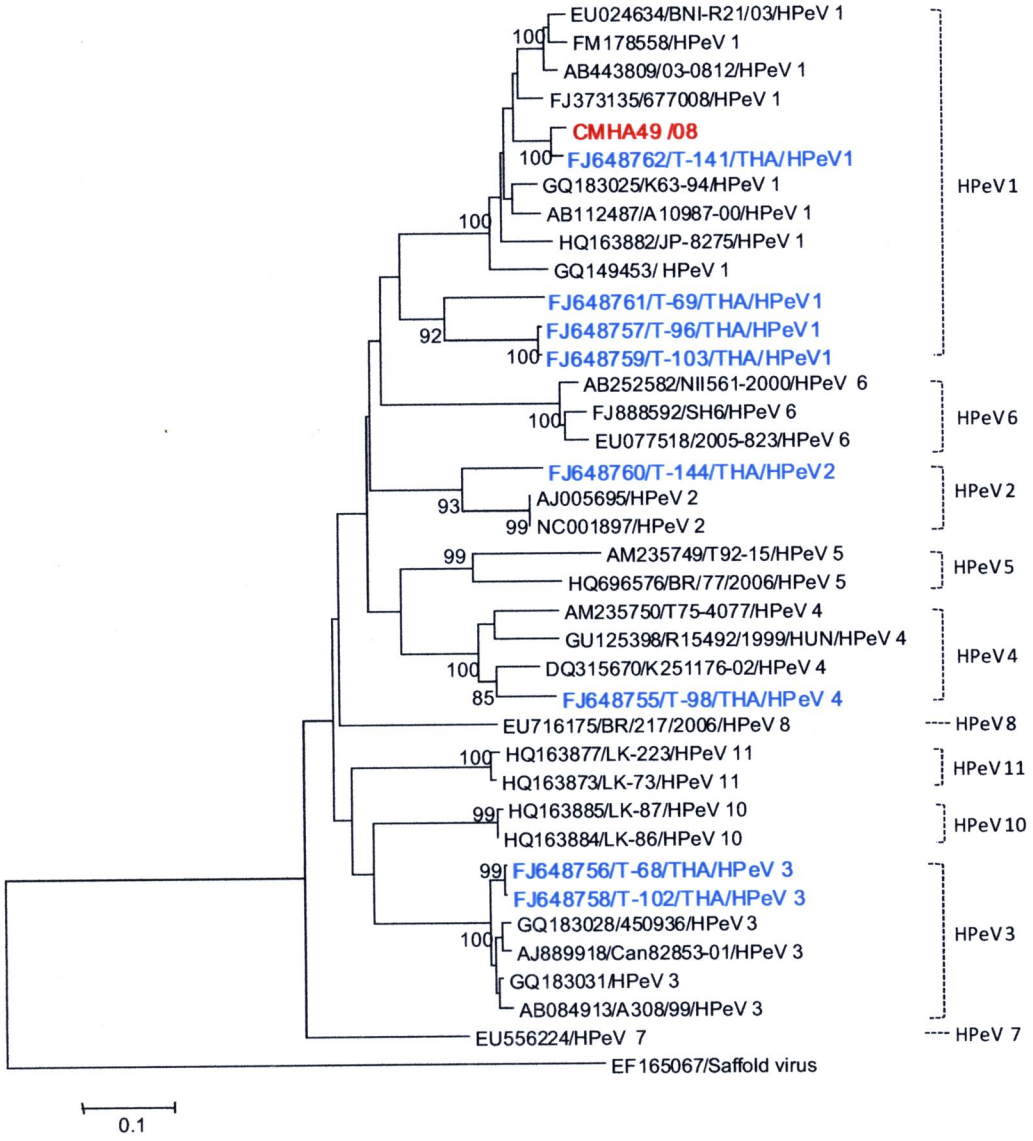

Figure 14 Phylogenetic analysis of the partial nucleotide sequences (477 bp) of capsid gene of human parechoviruses. The GenBank accession numbers of reference strains were indicated proximal to the strain name. HPeV detected in this study and previous studies in children in Chiang Mai were indicated in red and blue, respectively. The tree was constructed according to neighbor-joining method and the number on each branch indicates the bootstrap value.

CHAPTER VI

DISCUSSION

Viral gastroenteritis is one of the most important infectious diseases affecting infants and young children in Thailand (Maneekarn and Ushijima, 2000; Guntapong et al., 2004; Pham et al., 2007). However, the epidemiological data of diarrheal viruses in adults is limited. Thus far, only few studies had been conducted in adults with diarrhea (Campos et al., 2008; Podkolzin et al., 2009). In Thailand, most of the epidemiological surveillances of the viruses associated with diarrhea are conducted mainly in infants and young children. (Guntapong et al., 2004; Kittigul et al., 2009, 2010, 2011). Therefore, in the present study the epidemiological surveillance and molecular characterization of diarrheal viruses were conducted in adults with diarrhea in Chiang Mai, Thailand. It was observed that among the specimens tested, only 4.2% was positive for diarrheal viruses in adults. Five types of viruses from a total of 10 screened viruses were detected in this study. The monoinfection with one virus was found at 1.2% for each of AdV and EV, 0.9% of AiV, and 0.6% of NoV GII. In addition, mixed infection between NoV GII and HPeV was identified at 0.3%. To our knowledge, this is the first epidemiological data demonstrating the overall picture of viruses associated with diarrhea in adults in Thailand. Previous surveillance studies from Europe, America, Asia, and Australia demonstrated that the detection rates of diarrheal viruses in adults varied greatly, ranging from 0.5% - 63.3% (Sanekata et al.,

2003; Gao et al., 2009; Podkolzin et al., 2009; Wang et al., 2009; Tatte et al., 2010; Yamashita et al., 2010). Recently, the epidemiological data of diarrheal viruses in adults in China reported that the prevalent rate of diarrheal viruses during 2007-2008 was 34.8% (Podkolzin et al., 2009), which was about 8 fold higher than our study. The relatively low rate of diarrheal virus detection in adults in this study suggests that virus is an unusual cause of acute gastroenteritis in adults. Further investigation for bacterial or parasite infections may help to clarify this point.

The epidemiological data of enteric AdV (subgroup F, types 40 and 41) has been reported from several countries worldwide. However, the reports in Asian countries are quite limited. In Thailand, infection with AdV40 and 41 was first reported in 1988 in children hospitalized with diarrhea in Bangkok by Hermann et al. (1988). Then, 21 years after the first report, Kittigul et al. (2009) demonstrated that AdV infection rate in children with acute gastroenteritis in Lopburi province during 2006-2007 was 1.5%. Recently, Chaimongkol et al. (2012) had reported the prevalence of AdV in children with gastroenteritis in Chiang Mai in 2007 at 1.9%. To our knowledge, these 3 reports are the only reports of AdV infection in children hospitalized with diarrhea in Thailand. Recently, the surveillance of diarrheal viruses in adults with diarrhea in china found that NoV was the most predominant with the prevalent rate of 26.4% (Podkolzin et al., 2009). In contrast, our study demonstrated that the prevalence of AdV in adults with diarrhea was 1.2%, which is different from the reported in China. However, the prevalence of AdV reported in this study is more or less the same as those reported previously in children in Chiang Mai in 2007 (Chaimongkol et al., 2012) and in Lopburi during 2006-2007 (Kittigul et al., 2009).

Although 3 published articles were retrived from literature search, only recent reports from Chiang Mai and Lopburi had deposited the partial hexon gene sequences of AdV in NCBI GenBank database. Therefore, comparison of the AdV sequences in our study had been performed only with the AdV sequences reported recently in Chiang Mai and Lopburi provinces. When comparing the AdV strains found in this study with those of previous studies, the data clearly demonstrate that the AdV genotype identified from children and adults are relatively different. The AdV strains found in adults are AdV24 and AdV25 of subgroup D and AdV40 of subgroup F, while the strains identified previously from children are AdV1 of subgroup D, AdV3 of subgroup B, and AdV41 of subgroup F.

AdV infection occurs worldwide and may involve in the disease in several systems and organs, including upper and lower respiratory tract, gastrointestinal (GI) tract, urinary tract, and ocular infections. The AdV types that commonly infect GI tract, so called enteric adenoviruses, are AdV40 and AdV41 in subgroup F (Dey et al., 2009). The AdVs in subgroup A (types 12, 18, and 31) have also been reported to cause enteric illness (Brown et al., 1996). It is interesting to note that in the present study we detected AdV24 and AdV25, in addition to AdV40, in adult patients with diarrhea. The AdV types 24 and 25 are classified into subgroup D and commonly infect the eye and causing conjunctivitis and epidemic karatoconjunctivitis (World and Horowitz, 2007). To our knowledge, this is the first report of AdV24 and AdV25 in adult patients with diarrhea.

In Thailand, the epidemiological study of EV infection in children with acute gastroenteritis has been initially reported in Chiang Mai province in 2007. The prevalent rate of EV infection in that study was 3.8%, and the viruses detected

including echovirus 11, echovirus 25, echovirus 30, and coxsackievirus A20. (Chaimongkol et al., 2012). Most recently, the epidemiological data of EV infection in children with acute gastroenteritis during 2007-2008 in Japan have been reported with an infection rate of 16.6% (Pham et al., 2010a). In our study, the EV detection rate in adult patients is 1.2% which is lower than those of the studies recently reported in Thailand and Japan. Sequence analysis clearly demonstrates that EV strains found in our study are genetically variable. The EV found in adults in this study belonged to echovirus 30, cosackievirus A20, poliovirus 3, and enterovirus 99, which is different from the EV strains circulated in children in the same area, except for coxsackievirus A20 is detected both in children and adults.

Analysis of 5' UTR nucleotide sequences of 4 EV, CMHA42/08, CMHA59/08 , CMHA136/08, and CMHA414/08 strains detected in this study revealed that they belonged to species B and C. When comparing the nucleotide sequences of the EV strains found in this study with those of EV available in NCBI GeneBank database, the CMHA59/08 is closely related to CF647-92 strain which is the echovirus 30 detected in patients with meningitis in France in 1992 (Mirand et al., 2007). The CMHA414/08 is closely related to BAN04-1067 strain, which is the enterovirus 99 detected in patient with acute flaccid paralysis in Bangladesh in 2004 (Brown et al., 2009). The CHMA136/08 strain is closely related to MOR83 strain which is the coxsackievirus A20 that was isolated in Morocco in 1983 (Brown et al., 2009), while the CMHA42/08 strain is closely related to 3 other strains of poliovirus 3 which were homologus to MF1, 31974, and Leon/37 strains (Pavlov et al., 2005; Zhang et al., 2010). Poliovirus 3 could be found in natural resource (Pavlov et al., 2005). Therefore, Poliovirus 3 detected in patient with diarrhea in this study may be

originated from contaminated food or water. The finding of several EV genotypes in stool samples imply that the EV circulating in this area are genetically diversed and after the systemic infection, the virus can be sheded and spreaded in the environment and infect other patients in the area.

AiV has been proposed as a causative agent of gastroenteritis after detected initially in gastroenteritis outbreak in Japan (Yamashita et al., 1993, 2000) and in Germany (Oh et al., 2006). Recently, there were additional data of the detection of AiV in several countries such as Japan, Bangladesh, Thailand, Vietnam, and Hungary (Pham et al., 2007; Reuter et al., 2009b). In Thailand, epidemiological data of AiV is limited. From the literature search, there is only one report on the detection of AiV in children with diarrhea in Chiang Mai, Thailand (Pham et al., 2007). It was found that AiV was an uncommon pathogen associated with acute gastroenteritis in children, as it was detected at a very low detection rate of 0.9% (Pham et al., 2007). To our knowledge, there is no other report of AiV infection in adults with diarrhea in this country. Therefore, we had conducted the survey study of AiV infection in adult population with diarrhea. It was observed that the prevalence of AiV in adults is similar to those in children with a low detection rate only 0.9%. However, the prevalence of this virus in Japan is relatively higher than our study. The reports from Japan demonstrated that AiV was detected at 18.8% in 1993 and 20.5% in 2000 (Yamashita et al., 1993, 2000).

Molecular genetic analysis of the only one AiV strain previously found in a child with diarrhea in Chiang Mai, Thailand demonstrated that it was genotype A (Pham et al., 2007). It is interesting, however, to note that among 3 strains of AiV detected in our study, 1 belongs to genotype A and the other 2 belong to genotype B.

These data clearly demonstrate that AiV circulates in this area are genetically variable and genotypes found in adults are difference from those detected in children with diarrhea. This data establishes the baseline information of the prevalence of AiV in adults with diarrhea.

Several epidemiological reports of NoV infections have shown that NoV GII are the most predominant genotype in all parts of the world, whereas NoV GI has been widely observed with sporadic cases of acute diarrhea (Buesa et al., 2002; Malasao et al., 2008). In Thailand, NoV infection was first reported in children with acute gastroenteritis in Chiang Mai during 2000-2001 with the frequency of 8.6% to 8.9% (Hansman et al., 2004; Malasao et al., 2008). Afterward, the prevalent rate of NoV infection in children with acute gastroenteritis in Chiang Mai was investigated and found that during the year 2002-2004 the pravalence was 14.1%, 2005 was 6.8%, and 2007 was 13.8% (Khamrin et al., 2007b; Khamrin et al., 2010; Chaimongkol et al., in press). Most recently, the surveillance of NoV GII in Lopburi province in 2006-2007 revealed the prevalence of NoV GII infection in patients with acute gastroenteritis of all ages as high as 44.7% (Kittigul et al., 2010). Surprisingly, NoV GII detection rate in adults with diarrhea in the present study is as low as only 0.9%. There are several explanations for a relatively low detection rate of NoV GII in adults. One possibility is that NoV GII may not the major pathogen causing diarrhea in adults. Secondly, sensitivity of the RT-multiplex PCR used in this study may lower than the RT-multiplex PCR used in 2000-2007, and lower sensitivity than RT- nested PCR used by Kittigul et al. (2010). To clarify these points, further study may need to be conducted.

Sequence analysis of all 3 NoV strains revealed that these 3 strains belonged to NoV GII, no NoV GI was identified in this study. These data are in good agreement with the previous report as only NoV GII was found in adults in China (Podkolzin et al., 2009). The accumulated data of NoV distributions in Thailand also demonstrate that GII is the most prevalence genogroup associated with diarrhea in children population. This finding is in good agreement with the finding of previous study in children in Thailand and is also similar to those reports from other regions of the world (Khamrin et al., 2007b, 2010; Podkolzin et al., 2009; Nataraju et al., 2010; Dai et al., 2011; Chaimongkol et al., in press). Among NoV GII, several genotypes have been reported previously in Chiang Mai in 2007 including GII/2, GII/3, GII/4, GII/6, GII/7, GII/13, and GII/16 genotypes. However, it was observed that all NoV GII strains detected in the present study belonged to only NoV GII/4 genotype. This finding suggest that the GII/4 is the common genotype associated with diarrhea in both children (Guntapong et al., 2004; Hansman et al., 2004; Bull et al., 2006; Dey et al., 2007; Siebenga et al., 2007; Guo et al., 2009) and adults (Campose et al., 2008).

Sequence analysis of the partial capsid gene of NoV GII/4 also demonstrated that NoV GII/4 found in adults belonged to 2 variants, 2006a and 2006b, and showed the close genetic relationship with those of NoV GII/ strains previously reported in children in Chiang Mai, Thailand. These data imply that the viruses circulating in adults and in children may be originated from the same ancestor and NoV GII/4 genotype is the major pathogen that causing diarrhea in both adults and children in this area.

In Thailand, epidemiological surveillance of HPeV infection in children hospitalized with diarrhea has been initially performed in 2005. The HPeV infection in that study was detected at the prevalent rate of 14.6% (Pham et al., 2010b). However, from the literature search there is no other additional report of the HPeV in Thailand. Therefore, HPeV was screened in the stool specimens collected from adult patients with diarrhea collected in 2008 in Chiang Mai, Thailand. Our study clearly demonstrates that HPeV is an unusual cause of acute gastroenteritis in adults compared to other viruses. Previous study of the prevalence of HPeV infection in South Korea demonstrated that HPeV is also an unusual cause of acute gastroenteritis in children with the infection rate of 2% (Han et al., 2011). However, it is detected as high as 55% in China (Zhong et al., 2011). In the present study, only one HPeV strain (0.3%) was found in the total of 332 fecal specimens tested. The prevalence is much lower than those of the recent studies previously reported in Thailand, South Korea, and China (Pham et al., 2010b; Han et al., 2011; Zhong et al., 2011). HPeVs have been classified into fourteen genotypes (Ito et al., 2004; Benschop et al., 2006; Watanabe et al., 2007; Benschop et al., 2008; Drexler et al., 2009; Li et al., 2009). The accumulated epidemiological surveillances of HPeV in stool samples worldwide indicate that HPeV1 is the predominant genotype detected in diarrheal children (Watanabe et al., 2007; Baumgarte et al., 2008; Benschop et al., 2006, 2008, 2010; Tapia et al., 2008; Boros et al., 2010; Ito et al., 2010). Analysis of the VP1 gene of HPeV CMHA49/08 strain found in this study revealed that it is HPeV1. The CMHA49/08 is closely related to T-141 strain which was detected previously in children with acute gastroenteritis in Chiang Mai, Thailand in 2005 (Pham et al., 2010b). This finding is in good agreement with the findings of previous studies

reported from other region of the world that HPeV1 is the predominant type found in patients with diarrhea (Baumgarte et al., 2008; Benschop et al., 2008; Pham et al., 2010; Zhang et al., 2011; Pham et al., 2011b).