CHAPTER V

RESULTS

1. Prevalence of gastroenteritis viruses

A total of 160 fecal specimens collected during January to December 2007 from pediatric patients were screened for the presence of rotaviruses (RVs), noroviruses (NoVs), sapoviruses (SaVs), astroviruses (AstVs) and adenoviruses (AdVs) by reverse transcription (RT) reaction and multiplex PCR. An example of agarose gel electrophoresis demonstrating the PCR product sizes of each virus generated by multiplex RT-PCR is shown in Figure 17. The expected PCR product sizes of group A rotavirus, adenovirus, norovirus genogroup I (NoV GI), norovirus genogroup II (NoV GII), sapovirus, and astrovirus were 395 bp, 462 bp, 330 bp, 387 bp, 434 bp, and 719 bp, respectively. As shown in Table 11, the prevalence of gastroenteritis viruses detected in the present study was 48.8% (78 of 160). Of these, group A rotavirus was found to be the most predominant virus with the prevalent rate of 27.5% (44 of 160) and none of group B or C rotaviruses was found in this study. Noroviruses was detected at 14.4% (23 of 160) which were identified as NoV GI and NoV GII. Of these, NoV GII was detected more frequently than NoV GI with the prevalent rate of 13.8% (22 of 160) and 0.6 % (1 of 160), respectively. In addition, sapoviruses, adenoviruses, and astroviruses were detected at 3.1%, 1.3%, and 0.6%, respectively. Double infection was detected at 1.9% (3 of 160), each of which was a co-infection of rotavirus with NoV GII, astrovirus, and adenovirus, respectively.

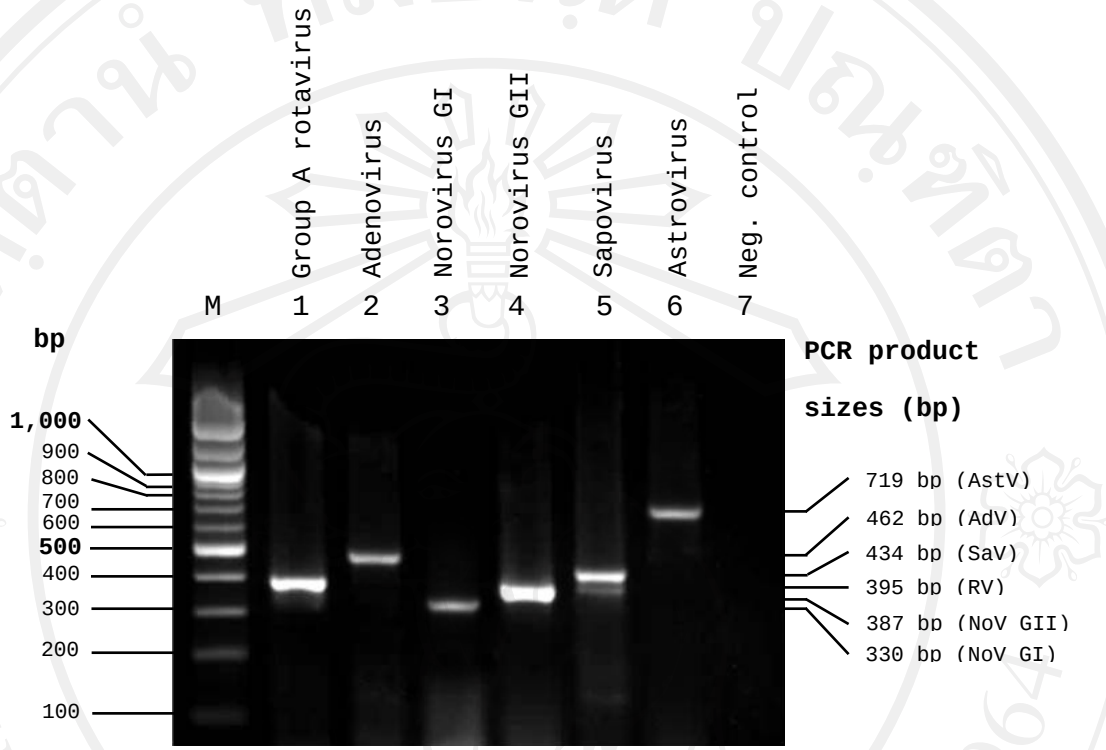

Figure 17 Detection of gastroenteritis viruses in fecal specimens by multiplex RT-PCR. Agarose gel demonstrates the PCR product sizes of group A rotavirus (395 bp; lane 1), adenovirus (462 bp; lane 2), norovirus GI (330 bp; lane 3), norovirus GII (387 bp; lane 4), sapovirus (434 bp; lane 5), and astrovirus (719 bp; lane 6). Lane M is 100 bp DNA ladder plus markers. Lane 7 is a negative control reaction in which the viral cDNA template was omitted. The molecular sizes of marker are indicated on the left and the expected PCR product sizes of each virus are indicated on the right of the gel.

Table 11 Prevalence of gastroenteritis viruses in children hospitalized with acute gastroenteritis in Chiang Mai, Thailand in 2007 as determined by multiplex RT-PCR

Number of fecal samples tested	Gastroenteritis viruses (%)											
	Rotaviruses			Noroviruses		Sapoviruses	Astroviruses	Adenoviruses	Mixed infection between Group A rotaviruses and			Total
	A	B	C	GI	GII				NoV GII	Astroviruses	Adenoviruses	
160	44	0	0	1	22	5	1	2	1	1	1	78
	(27.5)	(0)	(0)	(0.6)	(13.8)	(3.1)	(0.6)	(1.3)	(0.6)	(0.6)	(0.6)	(48.8)

2. Group A rotaviruses

2.1 G- and P-genotypes of group A rotavirus as determined by multiplex RT-PCR

A total of 47 strains of group A rotavirus were further characterized to determine for their G-and P-genotypes by RT-PCR using specific primer for amplifications of VP7 and VP4 genes and using a pool of primers specific for each G- and P- genotypes in multiplex PCR method. The genotypes were assigned based on the sizes of PCR products by comparing with those of the reference strains. Examples of agarose gel electrophoresis of G and P genotyping are shown in Figure 18 and Figure 19. The data from this study revealed that 3 different G-genotypes, G1, G2, G3, and 2 different P-genotypes, P[4], and P[8] of rotaviruses were co-circulating in the year 2007. Group A rotavirus G1 was the most predominant genotype with the prevalent rate of 66% (31 of 47) while G2 and G3 were detected with lower frequency of 17% (8 of 47), and 8.5% (4 of 47), respectively. However, G4 and G9, which used to be the most common genotypes, were not detected in this study. Determination of their P-genotypes revealed that the majority of group A rotaviruses were P[8] which represented 44.7% (21 of 47) and 14.9% (7 of 47) were found to be P[4]. Nonetheless, there were some isolates of which their G-genotype or P-genotype could not be identified by multiplex RT-PCR using type-specific primers that have been reported previously in the literature. Of these, G-nontypeable isolates were 8.5% (4 of 47) and P-nontypeable were 40.4% (19 of 47). The G- and P-genotypes of group A rotaviruses determined by multiplex RT-PCR are summarized in Table 12.

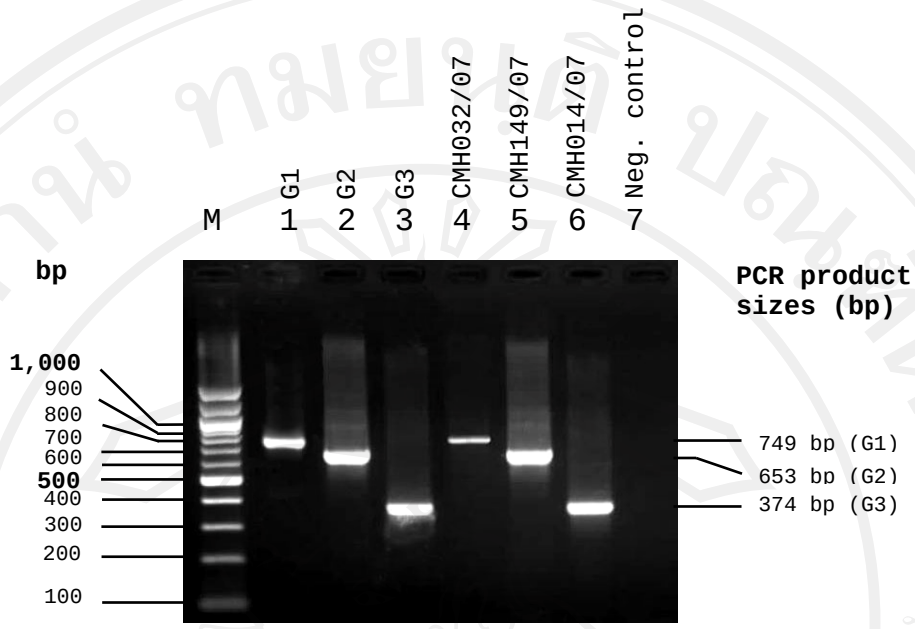

Figure 18 Agarose gel electrophoresis demonstrates the PCR product sizes of G1, G2, and G3 genotypes in comparison with the reference strains. Lane M is 100 bp DNA ladder plus markers. Lanes 1-3, reference strains of G1, G2, and G3. Lanes 4-6, test samples of rotavirus that were positive for G1, G2, and G3 with the PCR product sizes of 749 bp, 653 bp, and 374 bp, respectively. Lane 7 is a negative control reaction in which the DNA template was omitted. The molecular sizes of marker are indicated on the left and the expected PCR product sizes of each G-genotype are indicated on the right of the gel.

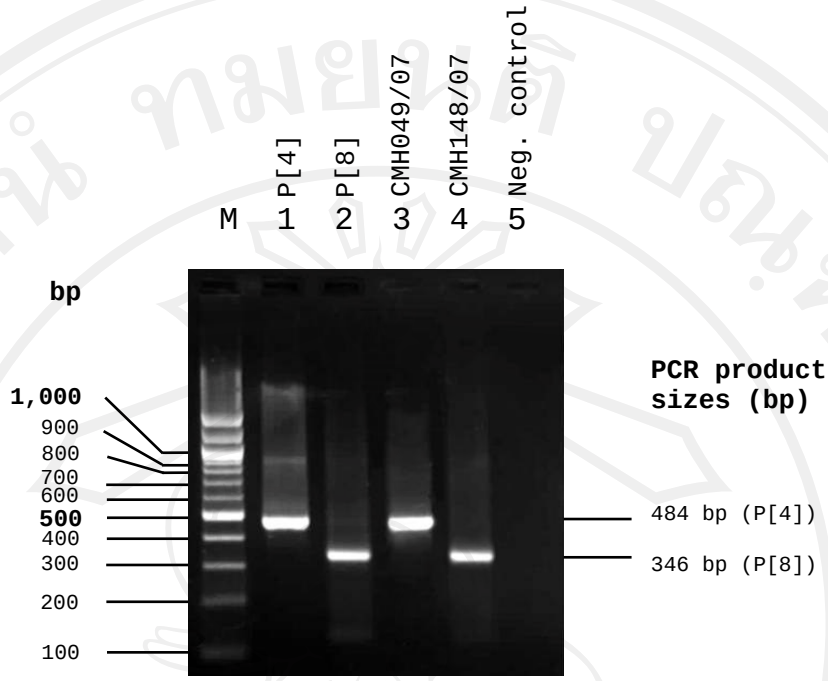

Figure 19 Agarose gel electrophoresis demonstrates the PCR products of P[4] and P[8] genotypes in comparison with the reference strains. Lane M is 100 bp DNA ladder plus markers. Lanes 1-2, reference strains of P[4] and P[8]. Lanes 3-4, test samples of rotavirus that were positive for P[4] and P[8] with the PCR product sizes of 484 bp and 346 bp, respectively. Lane 7 is a negative control reaction in which the DNA template was omitted. The molecular sizes of marker are indicated on the left and the expected PCR product sizes of each P-genotype are indicated on the right of the gel.

Table 12 The G- and P-genotypes of group A rotaviruses co-circulating in children hospitalized with acute gastroenteritis in Chiang Mai, Thailand in 2007 as determined by multiplex RT-PCR

Number of rotavirus positive samples	G-genotypes (%)				P-genotypes (%)		
	G1	G2	G3	NT [a]	P[4]	P[8]	NT [b]
47	31 (66)	8 (17)	4 (8.5)	4 (8.5)	7 (14.9)	21 (44.7)	19 (40.4)

[a] NT : G-genotype could not be identified by multiplex RT-PCR

[b] NT : P-genotype could not be identified by multiplex RT-PCR

2.2 Identification of G- and P-genotypes of rotavirus nontypeable strains by nucleotide sequence analysis

In order to identify the G- and P-genotypes of nontypeable strains, VP7 and VP4 genes of those nontypeable strains were amplified by using consensus or alternative primers specific for each gene. The full-length of VP7 gene (1,062 bp) of G-nontypeable strains were amplified by the consensus Beg9 or sBeg9 forward primers in combination with reverse primer End9 whereas the partial VP4 gene (877 bp) of P-nontypeable strains were amplified by the forward primers Con3 or HumCom5 with reverse primer Con2. The PCR products of VP7 and VP4 genes were then purified and subjected to nucleotide sequencing. The G- and P-genotypes were assigned based on the nucleotide sequences obtained by comparing with those of the reference strains available in GenBank database using NCBI BLAST program.

Among 4 strains of G-nontypeable, 3 turned out to be G1, and the other one was G2, as shown in Table 13. All G1 isolates shared a great homology of VP7 nucleotide sequence identity (96-98%) with those of the published G1 reference strains and G2 isolate shared 98% identity with those of G2 reference strains. Similarly, among 19 P-nontypeable strains, as many as 16 of 19 isolates were P[8] and the remaining 3 isolates were P[4] genotype, as shown in Table 14.

Table 13 G-genotype of nontypeable rotavirus strains as identified by nucleotide sequence analysis of VP7 gene

Human rotavirus strains (n=4)	G-genotypes
CMH002/07	G1
CMH050/07	G1
CMH056/07	G1
CMH070/07	G2

Table 14 P-genotype of nontypeable rotavirus strains as identified by nucleotide sequence analysis of VP4 gene

Human rotavirus strains (n=19)	P-genotypes
CMH002/07	P[8]
CMH007/07	P[8]
CMH014/07	P[8]
CMH015/07	P[8]
CMH016/07	P[8]
CMH017/07	P[8]
CMH019/07	P[8]
CMH026/07	P[8]
CMH029/07	P[8]
CMH032/07	P[8]
CMH060/07	P[8]
CMH066/07	P[8]
CMH069/07	P[8]
CMH078/07	P[8]
CMH082/07	P[8]
CMH105/07	P[8]
CMH028/07	P[4]
CMH030/07	P[4]
CMH031/07	P[4]

These 16 isolates of P[8] genotype shared 98% to 99% VP4 nucleotide sequences identity with those of the published P[8] reference strains. Of note, most of them were highly homologous with the Thailand P[8] strain CMH146/05 (GU288635) which was previously reported from Chiang Mai in the year 2005 (Khamrin et al.,

2010). For P[4] isolates, their VP4 nucleotide sequences exhibited a greater homology with those of P[4] reference strains, ranging from 99% to 100%. Interestingly, one P[4] isolate, CMH030/07, shared highest nucleotide sequence identity at 100% with the Russia strain 13209 (FJ409996) (Podkolzin et al., 2009).

2.3 Overall distribution of G- and P-genotypes of rotaviruses

A total of 47 strains of group A rotavirus were completely identified for their G- and P-genotypes by multiplex RT-PCR and nucleotide sequencing. Of these, G1 was the most predominant genotype, follow by G2, and G3 with the prevalent rate of 72.3% (34 of 47), 19.2% (9 of 47), and 8.5% (4 of 47), respectively (Table 15). For P-genotypes, P[8] was the most predominant genotype which accounted for 78.7% (37 of 47) whereas P[4] was detected at lower prevalent rate of 21.3% (10 of 47). Considering the G- and P-genotype combination, almost all of G1 strains (33 of 34) were found to associate with P[8] excepted for 1 strain was found to associate with P[4]. In contrast, G2 and G3 were found to associate exclusively with P[4] and P[8], respectively. Altogether, G1P[8] was the most predominant strain represented 70.2% (33 of 47), followed by G2P[4], G3P[8], and G1P[4] which accounted for 19.2% (9 of 47), 8.5% (4 of 47), and 2.1% (1 of 47), respectively.

Table 15 Relative frequency of G- and P-genotype combination of group A rotaviruses

G-genotypes	P-genotypes		Total (%)
	P[4]	P[8]	
G1	1 (2.1)	33 (70.2)	34 (72.3)
G2	9 (19.2)	0	9 (19.2)
G3	0	4 (8.5)	4 (8.5)
Total (%)	10 (21.3)	37 (78.7)	47 (100)

2.4 VP6 genogroup and NSP4 genetic group of rotaviruses

2.4.1 VP6 genogroup

In this study, the VP6 genogroup of group A rotavirus was identified by multiplex RT-PCR method recently developed by Thongprachum et al. (2010). The VP6 genogroups were assigned based on the sizes of PCR products by comparing with the reference strains. An example of agarose gel electrophoresis is illustrated in Figure 20 and the PCR products of 480 bp and 351 bp were identified as VP6 genogroup I (GI) and VP6 GII, respectively. All these 47 rotavirus isolates, 38 out of 47 (80.9%) isolates were identified as VP6 GII and the remaining 9 samples (19.1%) were identified as VP6 GI.

2.4.2 NSP4 genetic group

In general, NSP4 gene of human and animal rotaviruses can be classified into six genetic groups, including NSP4 genetic group KUN (A), Wa (B), RRV (C), EW (D), Avian (E), and Porcine (F). Only NSP4 genetic groups A, B, and C rotaviruses are known to infect humans. Therefore, genetic grouping of NSP4 gene by multiplex RT-PCR in this study using a pool of primers specific only for NSP4 genetic groups A, B, and C. As shown in Figure 21, NSP4 genetic groups were assigned based on the sizes of PCR products by comparing with the reference strains KUN (A) and Wa (B). The data revealed that the majority of rotavirus strains (38 of 47; 80.9%) detected in the present study belonged to NSP4 genetic group Wa (B) and the remaining isolates (9 of 47; 19.1%) belonged to NSP4 genetic group KUN (A).

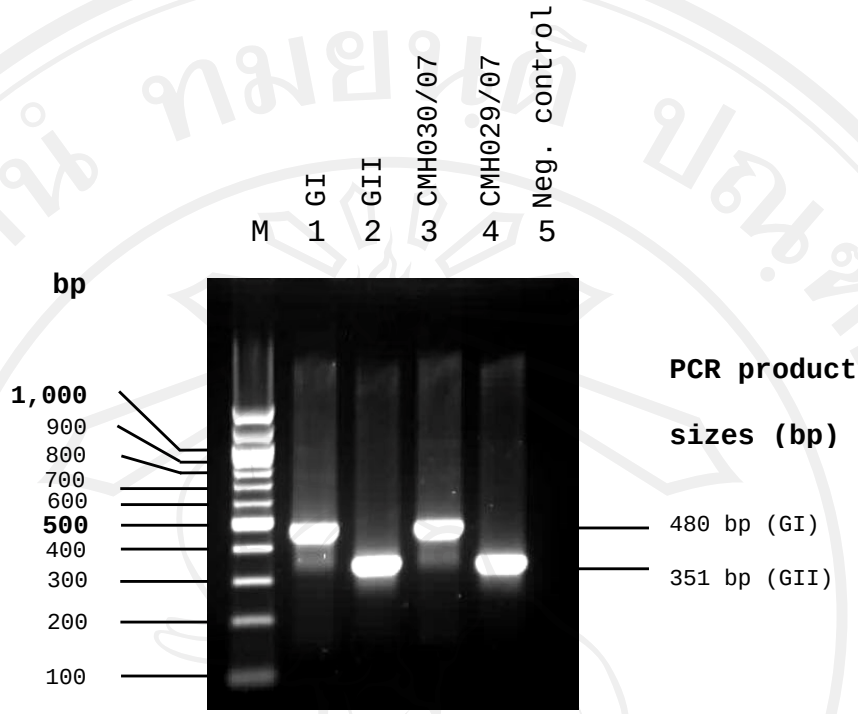

Figure 20 Agarose gel electrophoresis illustrating the VP6 genogrouping of group A rotavirus by multiplex RT-PCR. The VP6 genogroups were assigned based on the comparison of PCR product sizes with those of VP6 GI and GII of the reference strains. Lane M is a 100 bp DNA marker. Lanes 1-2, reference strains of VP6 GI and GII. Lanes 3-4, test samples that were positive for VP6 GI and GII with the product sizes of 480 bp and 351 bp, respectively. Lane 5 is a negative control reaction in which the DNA template was omitted. The molecular sizes of marker are indicated on the left and the expected PCR product sizes of each VP6 genogroup are indicated on the right of the gel.

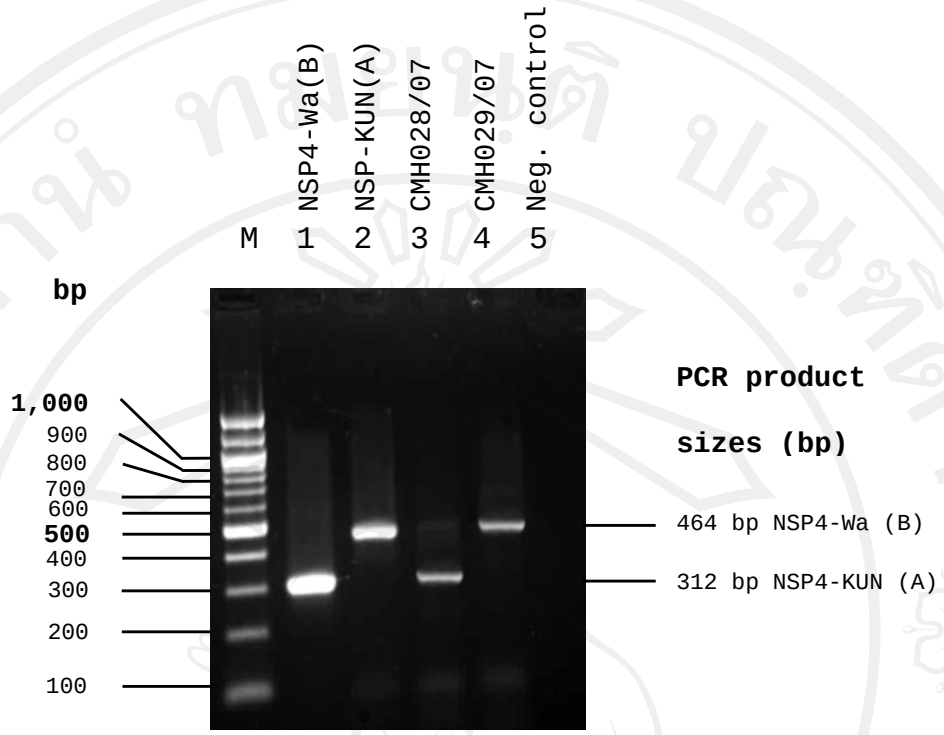

Figure 21 Agarose gel electrophoresis illustrating the NSP4 genetic grouping of group A rotavirus by multiplex RT-PCR. The NSP4 genetic groups were assigned based on the comparison of PCR product sizes with those of NSP4 genetic group KUN (A) and Wa (B) of the reference strains. Lane M is a 100 bp DNA marker. Lanes 1-2, reference strains of KUN (A) and Wa (B). Lanes 3-4, test samples that were positive for NSP4 genetic group KUN (A) and Wa (B) with the product sizes of 312 bp and 464 bp, respectively. Lane 5 is a negative control reaction in which the DNA template was omitted. The molecular sizes of marker are indicated on the left and the expected PCR product sizes of each NSP4 genetic group are indicated on the right of the gel.

2.4.3 Genetic linkage between VP6 and NSP4 genes

In human, the linkage between VP6 and NSP4 genes has been reported previously (Iturriza-Gomara et al., 2003). The VP6 GI was found to be linked to NSP4 genetic group KUN (A) and VP6 GII linked to NSP4 genetic group Wa (B). It was interesting to investigate whether this relationship could be demonstrated in the viruses from our surveillance. The results (Table 16) demonstrated that all strains of VP6 GI had NSP4 genetic group KUN (A) whereas VP6 GII had NSP4 genetic group Wa (B).

Table 16 Genetic linkage of VP6 genogroup and NSP4 genetic group of rotaviruses

Number of Rotavirus analyzed	VP6 genogroup	NSP4 genetic group		
		KUN (A)	Wa (B)	RRV (C)
9	GI	9	0	0
38	GII	0	38	0

3. Noroviruses and Sapoviruses

3.1 Genogroups of norovirus as determined by multiplex RT-PCR

For the detection of norovirus GI and GII, two pairs of primers, G1-SKF/G1-SKR and COG2F/G2-SKR were used for amplification of partial capsid gene of NoV GI and GII which generated 330 bp and 387 bp PCR products, respectively. NoV was detected in 24 of 160 of the fecal specimens tested. Almost all of NoV detected (23 of 24) were NoV GII and only 1 of 24 was NoV GI, as shown in Table 17.

Table 17 Genogroups and genotypes of noroviruses detected in children hospitalized with acute gastroenteritis in Chiang Mai, Thailand 2007

Gastroenteritis viruses [a]	**Genogroups** [a]	**Genotypes** [b]	**Number of positive samples**
Norovirus	GI	GI/14	1
	GII	GII/2	1
		GII/3	1
		GII/4	14
		GII/6	1
		GII/13	3
		GII/16	1
		GII/17	1
		GII/new	1
		Total	24

[a] Determined by multiplex RT-PCR

[b] Determined by nucleotide sequence and phylogenetic analyses

3.2 Genotypes of noroviruses as determined by nucleotide sequence and phylogenetic analyses of capsid gene

The NoV GI and GII were further characterized for their genotypes by nucleotide sequencing and phylogenetic analysis. One isolate of NoV GI and 23 of NoV GII were subjected to direct sequencing of their partial capsid gene using the consensus primers G1-SKF (for NoV GI) and COG2F (for NoV GII) as the sequencing primers. The nucleotide sequences obtained were compared to those of NoV strains available in the GenBank database using the BLAST program and the genotypes were tentatively assigned based on the BLAST results. The definite

genotype were assigned based on the phylogenetic analysis of their partial nucleotide sequences of capsid gene.

Based on the phylogenetic relationships and the classification scheme in the phylogenetic tree (Figure 22), NoV detected in the present study could be divided into two large clades, NoV GI and GII. For NoV GI, the nucleotide sequence of this strain (CMH099/07) shared a great homology (99%) with those of Hu/NoV/24/2006/SE, Hu/GI/28/JPN, and Taipei-93GI/04/TW strains while sharing about 97% identity with Saitama T7GI (AB112127), the GI/14 prototype strain. Therefore, CMH099/07 was identified as GI/14 genotype.

For NoV GII, 8 distinct genotypes, GII/2, GII/3, GII/4, GII/6, GII/7, GII/13, GII/16, and GII/new genotype, were detected in this study. More than half (14 of 23) of NoV GII isolates belonged to GII/4, 3 belonged to GII/13, and one each was identified as GII/2, GII/3, GII/6, GII/7, GII/16, and GII/new. All 14 GII/4 strains were grouped together with the prototype NoV GII/4 Bristol (X76716) strain.

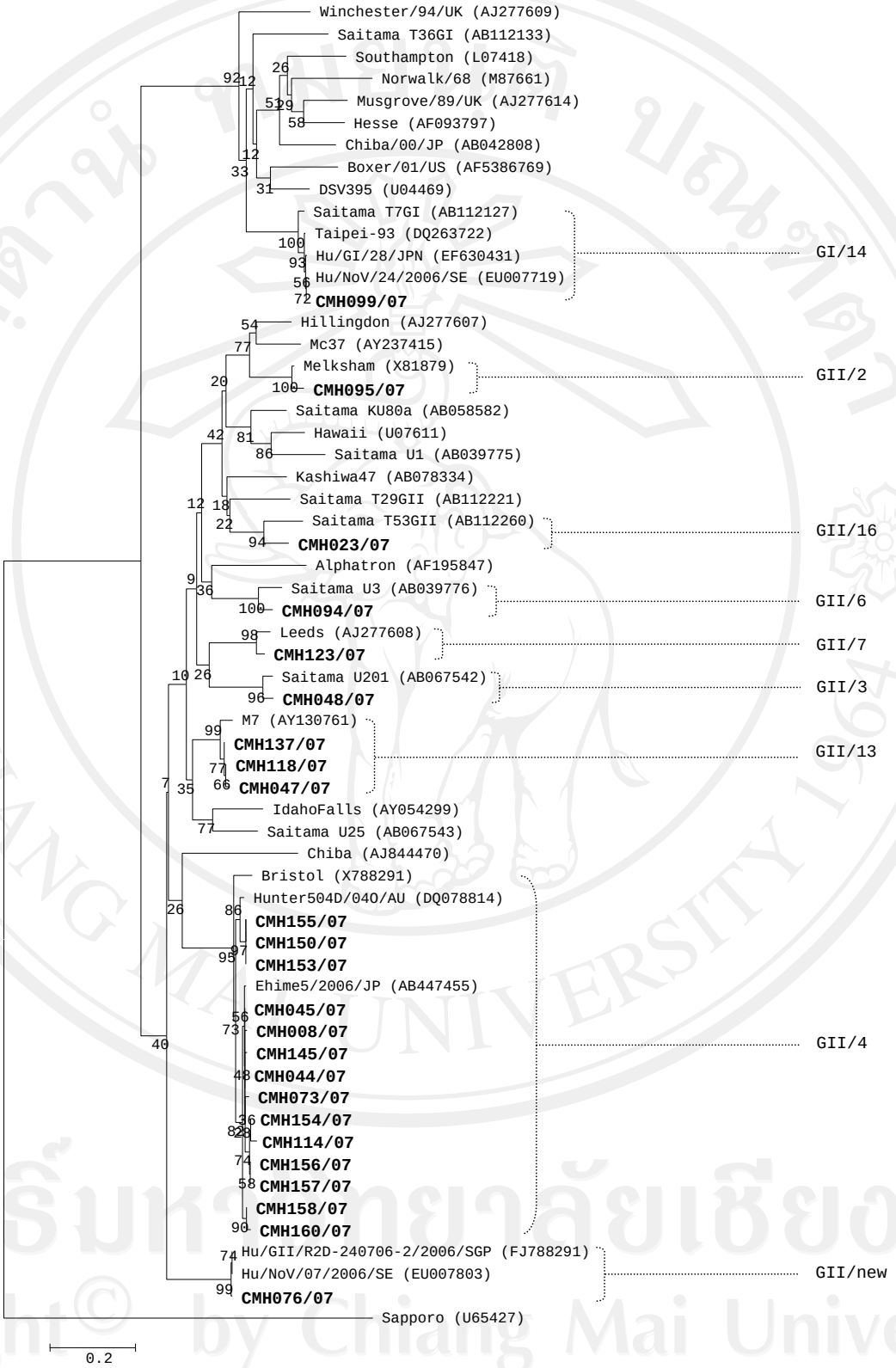

Figure 22 Phylogenetic analysis of partial capsid nucleotide sequences of noroviruses genogroup I (GI) and genogroup II (GII) strains.

Figure 22 (continue)
The tree was constructed according to the neighbor-joining method and the numbers on each branch indicate the bootstrap values. The NoV strains detected in this study are presented in boldface and the GenBank accession numbers of reference strains are given in parentheses.

Of these, 11 isolates of GII/4 (CMH045/07, CMH008/07, CMH145/07, CMH044/07, CMH073/07, CMH154/07, CMH114/07, CMH156/07, CMH157/07, CMH158/07, and CMH160/07) were clustered closely with GII/4 variant Ehime 5/2006/JP (AB447455) with 96-97% nucleotide sequence identity, whereas the other 3 isolates (CMH155/07, CMH150/07, and CMH153/07) were clustered with the GII/4 variant Hunter 504D/04O/AU (DQ078814) and shared 96-97% nucleotide sequence identity. Three isolates of the second most prevalent genotype detected in this study, NoV GII/13 (CMH137/07, CMH118/07, and CMH047/07) were clustered with M7 (AY130761) reference strain and shared 95-96% nucleotide sequence identity. For other NoV strains circulating in Chiang Mai area, GII/2 (CMH099/07) was closely related with Melksham (X81879) with 96% nucleotide sequence identity; GII/16 (CMH023/07) was closely related with Saitama T53GII (AB112260) with 89% nucleotide sequence identity; GII/6 (CMH094/07) was closely related to Saitama U3 (AB039776) with 92 % nucleotide sequence identity; GII/7 (CMH123/07) was closely related to Leeds (AJ277608) with 94% nucleotide sequence identity; and GII/3 (CMH048/07) was closely related with Saitama U201 (AB067542) with 95% nucleotide sequence identity. Most interestingly, one isolate of NoV GII detected in this study could not be assigned its genotype and referred to as GII/new. On the basis of BLAST research, its nucleotide sequence was closely related to Hu/NoV/07/2006/SE (EU007803) strain from Sweden and Hu/GII/R2G-240706/SGP (FJ788291) from

Singapore with nucleotide sequence identity of 99%. However, the genotype of these two reference strains have not been assigned but reported as unclassified genotypes or GIIx (Lysen et al., 2009, Aw et al., 2009). Accordingly, CMH076/07 strain detected in the present study was assigned as a new genotype according to two reference strains, Hu/NoV/07/2006/SE and Hu/GII/R2G-240706/SGP.

3.3 Genogroups and genotypes of sapoviruses as determined by partial nucleotide sequence and phylogenetic analyses of capsid gene

Of 160 fecal specimens tested, 5 were positive for SaV, as determined by multiplex RT-PCR. Partial capsid gene of all 5 SaV were amplified and direct sequenced using the consensus SLV5371 as the amplifying and sequencing primer. The sequences obtained were compared to those of SaV reference strains available in the GenBank database by using the BLAST program. The genogroups and genotypes of SaV were tentatively assigned based on the BLAST results. However, the definite genogroups and genotypes were assigned based on phylogenetic analysis of their partial nucleotide sequences of capsid gene.

As shown in Figure 23, phylogenetic analysis of partial capsid sequences 5 SaV strains, (CMH125/07, CMH034/07, CMH040/07, CMH131/07, and CMH025/07) revealed that all were clustered closely together with GI/1 reference strains Sapporo (U65427) and Manchester (X86560). The nucleotide sequences of these GI/1 were most closely related (99% identity) to Chanthaburi-74 (AY646854) which was GI/1 reported from Chanthaburi province, Thailand in 2004 while sharing lesser degree of identity (96-97%) to Mc114 (AY237422) which was detected previously in the same epidemic area (Chiang Mai) in 2001. In addition, those SaV GI/1 shared nucleotide sequence identity with other Thai GI/1 strains, Tak-69/Thai (AY646864), Songkhla-6/Thai (AY646857), and Nongkhai-74/Thai (AY646853)

at 95-98% identities. The SaV GII, GIV, and GV, which used to be the common genogroups in human, were not detected in the present study.

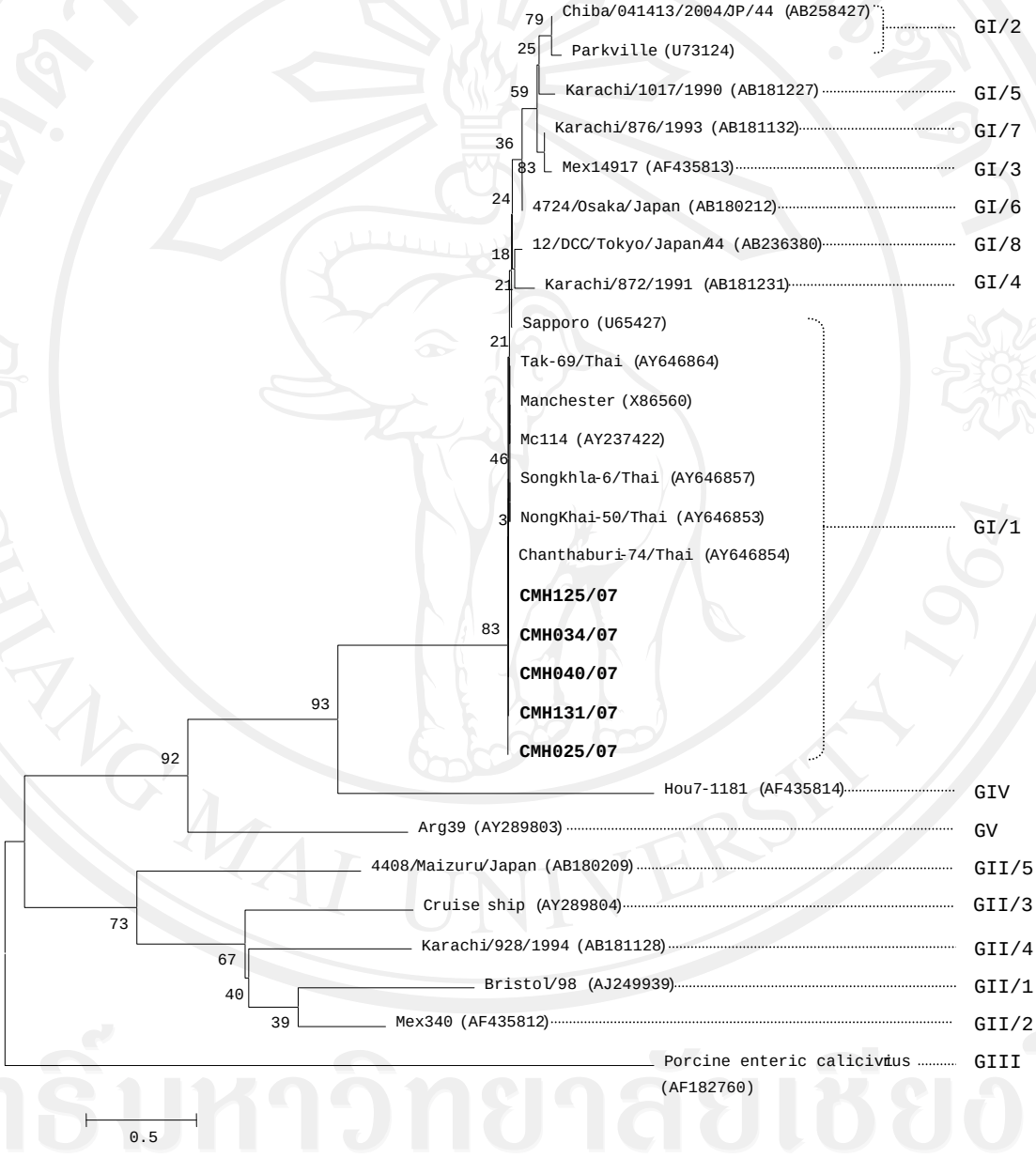

Figure 23 The phylogenetic analysis of partial capsid nucleotide sequences of SaV. The tree was constructed according to neighbor-joining method and the numbers on each branch indicate the bootstrap values. The SaV strains detected in this study are presented in boldface and the GenBank accession numbers of reference strains are given in parentheses.

4. Astroviruses and Adenoviruses

4.1 Genotypes of astrovirus as determined by nucleotide sequence and phylogenetic analyses

Two strains of AstV were detected in this study by multiplex RT-PCR. Partial capsid genes of these two AstV were amplified and direct sequenced using the consensus PreCAP1 as the amplifying and sequencing primer. The sequences obtained were compared to those of AstV strains available in the GenBank database by using the BLAST program. The genotypes of AstV were tentatively assigned based on the BLAST results. Nevertheless, the definite genotypes were assigned based on phylogenetic analysis of partial nucleotide sequences of their capsid gene. Based on the phylogenetic analysis of capsid sequences (Figure 24), AstV could be divided into 7 genotypes, AstV1 to AstV8 (except AstV7). Moreover, AstV1 could be further divided into four small clusters (called subtypes), including subtypes AstV1a, AstV1b, AstV1c, and AstV1d. In the present study, one AstV strain (CMH053/07) belonged to AstV1d and its capsid nucleotide sequence shared closest identity at 99% with France strain, 944/FRA (FJ905421), and shared 97-98% with the other strains of the AstV lineage 1d, including Vietnamese strains and other strains isolated from Spain, Germany, Brazil, and China [Bcn1.1/97-00 (AF348753), Bcn1.3/97-00 (AF348755), Dresden/04 (AY720892), BrG1-5 (DQ139825), RJ8479/BR/04 (DQ381498), and WH2009/04-05 (DQ788612), respectively]. Another AstV strain detected in the present study (CMH036/07) belonged to AstV2. The nucleotide sequence of its capsid gene shared highest homology (98%) with CMH259/01/2001/THA (EU363888) which was AstV2 reported from Chiang Mai in 2001, and 97% to Norway strain, NORAS 1128 (AB000290), while sharing only 93%

nucleotide identity to the prototype strain, Human astrovirus type 2 (L13745).

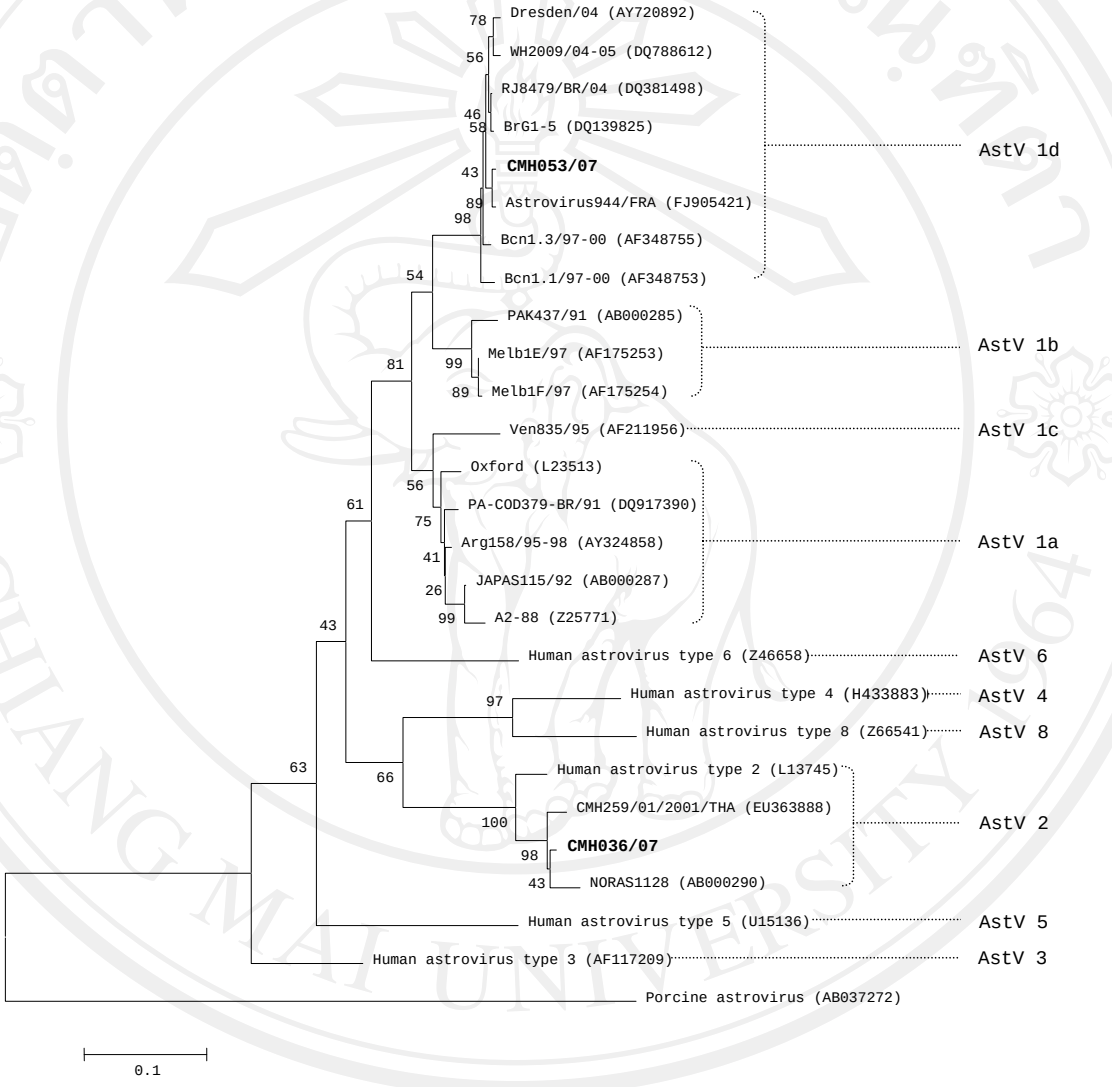

Figure 24 The phylogenetic analysis of partial capsid nucleotide sequences of AstV. The tree was constructed according to neighbor-joining method and the numbers on each branch indicate the bootstrap values. The AstV strains detected in this study are presented in boldface and the GenBank accession numbers of reference strains are given in parentheses.

4.2 Genotypes of adenoviruses as determined by nucleotide sequence and phylogenetic analyses

In the present study, 3 AdV strains were detected by multiplex RT-PCR. The partial hexon gene of these 3 AdV strains were amplified and direct sequenced using the consensus Ad1 primer as the amplifying and sequencing primer. The sequences obtained were compared to those of AdV strains available in the GenBank database by using the BLAST program. The genotypes of AdV were tentatively assigned based on the BLAST results. However, the definite genotypes were assigned based on phylogenetic analysis of partial nucleotide sequences of their hexon gene.

The phylogenetic analysis of partial hexon sequences of AdV demonstrated that AdV detected in this study were classified into three distinct genotypes, Ad1, Ad3, and Ad41, as shown in Figure 25. The Ad41 strain (CMH115/07) shared a great homology (98-99%) with other human Ad41 reference strains within Human adenovirus type 41 prototype strain (X51783) cluster. The Ad1 strain (CMH039/07) shared nucleotide sequence with those of Ad1 reference strains at 97% to 99% within Human adenovirus type 1 (AF534906) cluster. The Ad3 strain (CMH116/07) shared nucleotide sequence most closely related (100%) to Guangzhou01 (DQ099432) and 04TW5919/TW (DQ677325) which were the Ad3 strains reported from China and Taiwan, respectively, while sharing lesser degree of identity (98-99%) to Ad3 strain from Korea [Ad00/7KNIH/KOR (AF542129)], US [NHRC126/USA (AY599836)], and Human adenovirus type 3 (X76549) prototype.

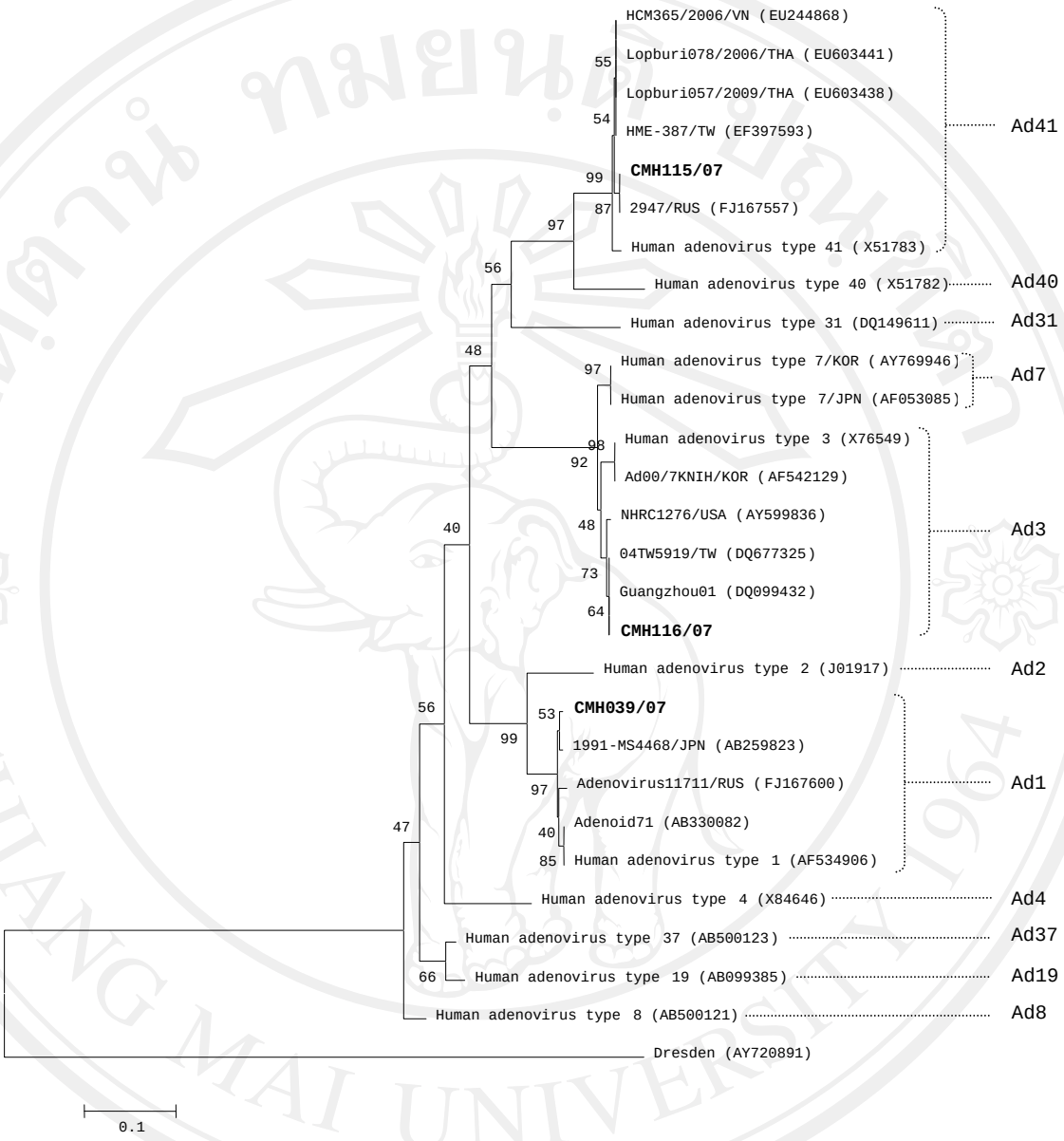

Figure 25 The phylogenetic analysis of partial hexon nucleotide sequences of AdV. The tree was constructed according to neighbor-joining method and the numbers on each branch indicate the bootstrap values. The AdV strains detected in this study are presented in boldface and the GenBank accession numbers of reference strains are given in parentheses.