CHAPTER V

RESULTS

1. Prevalence and distribution of Norovirus, Sapovirus, and Astrovirus infections

A total of 296 fecal specimens collected from infants and young children hospitalized with acute gastroenteritis were tested for the presence of Norovirus genogroup I (NVGI), Norovirus genogroup II (NVGII), Sapovirus (SV), and Human Astrovirus (HAstV) by RT-PCR using specific primers as shown in Figures 4 and 5.

In total, NV, SV, and HAstV were detected in 40 of 296 (13.5%) fecal specimens collected from children with acute gastroenteritis. Of these, NV was detected in 24 (8.1%) of fecal specimens tested. Seven of these (2.4%) were identified as GI and 17 (5.7%) as GII. Among NVs detected, NVGII was more predominant (70.8%) than NVGI (29.2%). In addition, SV was detected in 10 (3.4%), while HAstV was found in 7 (2.4%) of specimens tested. One of the specimens tested was positive for both NVGI and SV (Table 3). NVGI, NVGII, SV, and HAstV were detected in 27 of 187 (14.4%) in the first period (May 2000 – April 2001) and 14 of 109 (12.8%) in the following period (May 2001 – March 2002) (Table 3). This finding demonstrated that no significant difference of detection rate between these two periods.

The monthly distribution of NVGI, NVGII, SV, and HAstV infections in children hospitalized with diarrhea were shown in Figure 6. In the year 2000, NVGII infection was detected with high peak (~25%) in November. In addition, infections

associated with NVGI, SV, and HAstV as well as NVGI/SV mixed infection were also identified with lower detection rate than that of NVGII. In 2001, infections of almost all NVGI, NVGII, SV, and HAstV tended to occur in the first seven months of the year, even though NVGII remained detectable in October. Infection of NVGI and/or GII was detected continuously from January to July with a peak of NVGII (18.2%) in April. SV infection was detected in 5 months with a fluctuation in detection rates. HAstV infection had occurred in the first 2 months, disappeared in the following 3 months, then appeared again in the last 2 months with an increasing rates. During January to March 2002, NVGI/GII, NVGII, and HAstV infections were separately detected in each month in which HAstV detection rate (~22%) was higher than those of the other two viruses. However, SV infection was not observed in this period.

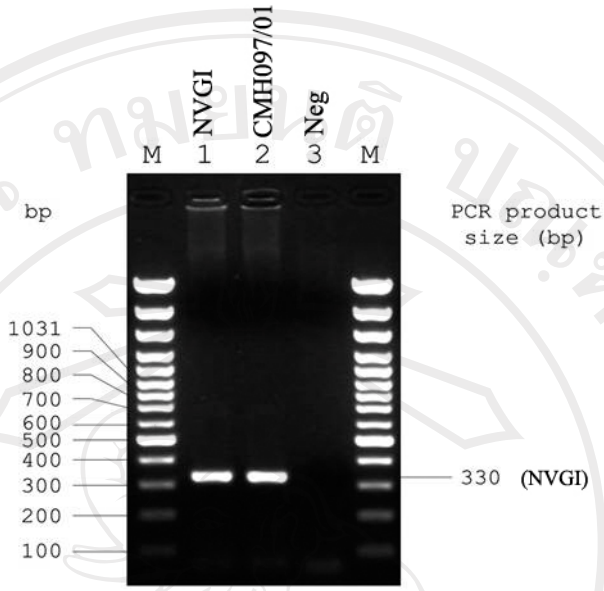

Figure 4. Agarose gel electrophoresis demonstrating NVGI. Lane 1, reference strain of NVGI (Mc100). Lane 2, a representative of tested sample (CMH097/01) that positive for NVGI. Lane 3, a negative control was included along with the tested samples. Lane M, 100 bp DNA Ladder marker. The molecular sizes of marker are indicated on the left and the expected PCR product size of NVGI on the right of the gel.

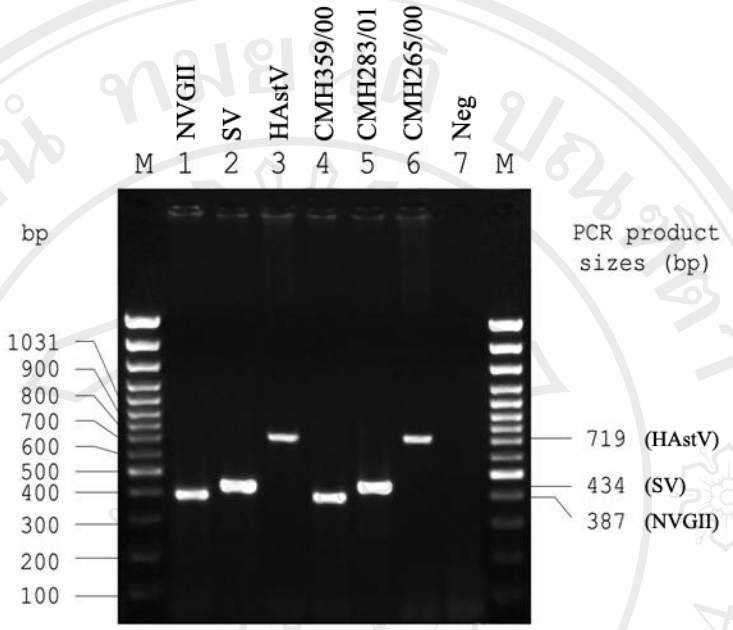

Figure 5. Agarose gel electrophoresis demonstrating NVGII, SV, and HAstV. Lane 1-3, reference strains of NVGII (Mc11/02), SV (Mc37/03), and HAstV (146/04), respectively. Lane 4-6, the representatives of tested samples that positive for NVGII (CMH359/00), SV (CMH283/01), and HAstV (CMH265/00), respectively. Lane 7, a negative control was included along with the tested samples. Lane M, 100 bp DNA Ladder marker. The molecular sizes of marker are indicated on the left and the expected PCR product sizes of NVGII, SV, and HAstV on the right of the gel.

Table 3. The prevalence of NVGI, NVGII, SV, and HAstV detected in children hospitalized with acute gastroenteritis in Chiang Mai, Thailand, during May 2000 to March 2002 period

Date of specimen collection	Number of specimen tested	Number of positive (%)				Total (%)
		NVGI	NVGII	SV	HAstV	
May 2000 - Apr 2001	187	5 (2.7)	11 (5.9)	8 (4.3)	3 (1.6)	27 (14.4)
May 2001 – Mar 2002	109	2 (1.8)	6 (5.5)	2 (1.8)	4 (3.7)	14 (12.8)
Total (%)	296	7* (2.4)	17 (5.7)	10* (3.4)	7 (2.4)	41* (13.9)

* One specimen was positive for both NVGI and SV

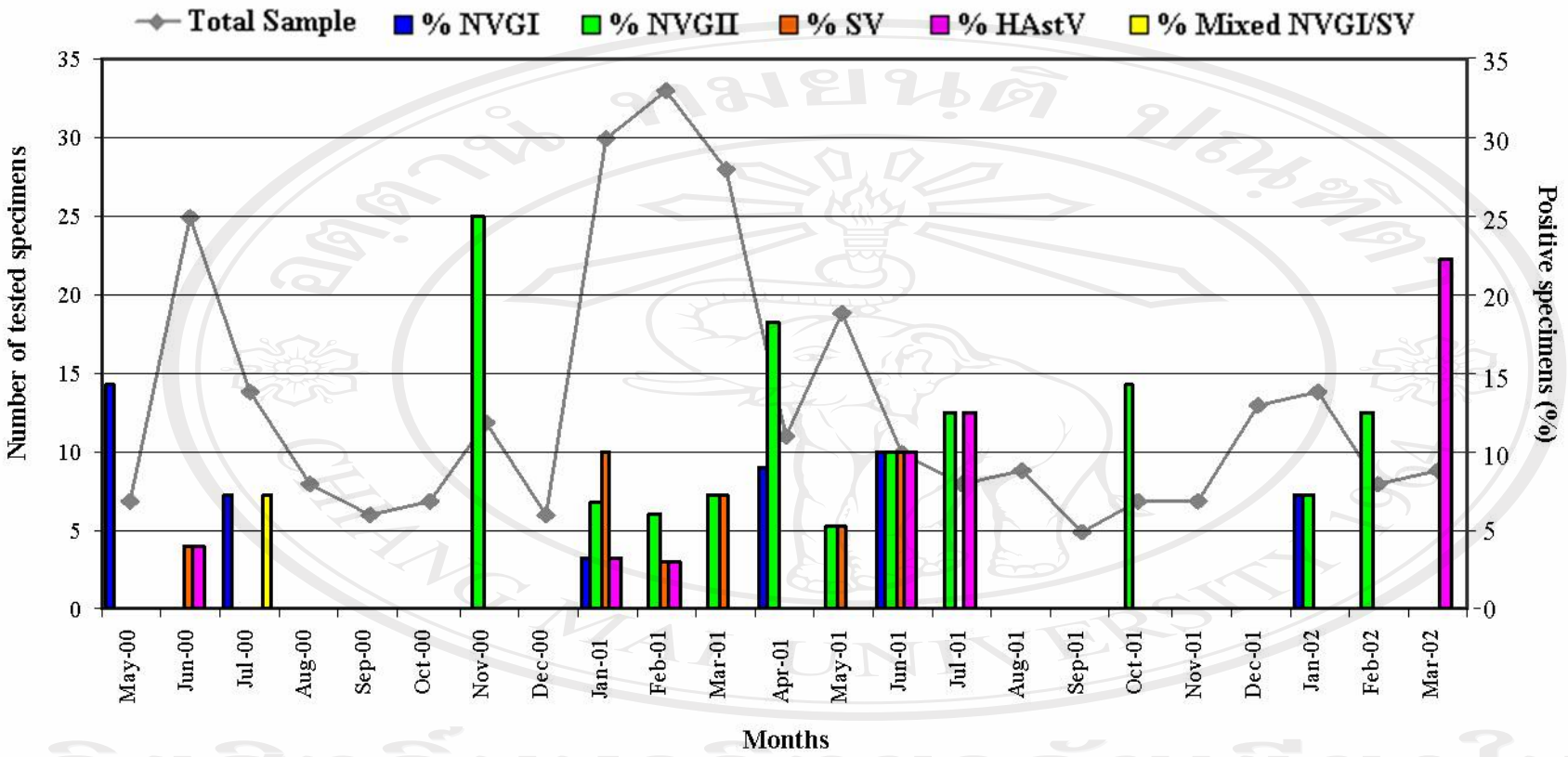

Figure 6. Monthly distribution of NVGI, NVGII, SV, and HAstV infections among children hospitalized with diarrhea in Chiang Mai, Thailand, during May 2000 to March 2002 period as detected by RT-PCR [Number of tested specimens, —◆—; Positive specimens (%), □]

The rates of NVGI, NVGII, SV, and HAstV infections among children in different age groups (from newborn up to 5 years) were also analyzed (Table 4). There was no difference in infection rates of all viruses detected among children in each age groups.

2. Nucleotide sequences and phylogenetic analyses of partial capsid genes of NV, SV, and HAstV

All PCR products (cDNA) positive for NVGI, NVGII, SV, and HAstV were purified and sequenced. The total of 41 positive nucleotide sequences including 7 NVGI, 17 NVGII, 10 SV, and 7 HAstV nucleotide sequences were translated into amino acid sequences by GeneDoc program. NVGI and NVGII were grouped by phylogenetic analysis of their sequences based on the recent NV capsid region classification scheme of Zheng et al., 2006 and Okada et al., 2005. SV was also classified by phylogenetic analysis of its sequences based on the recent SV capsid region classification scheme of Akihara et al., 2005.

2.1 Analysis of partial capsid gene of NV

The total of 24 specimens tested, 7 of NVGI and 17 of NVGII were direct sequenced for the partial capsid gene using the consensus G1-SKF (for NVGI) and COG2F (for NVGII) as the sequencing primers which priming at 3′ end of the cDNA strand. The partial nucleotide sequences and sequencing trace profile of capsid genes of the representative NVGI (CMH097/01) and NVGII (CMH359/00) strains were depicted in Figure 7a and 7b, respectively.

Table 4. Age distribution of Norovirus, Sapovirus, and Human astrovirus infections among children hospitalized with acute gastroenteritis in Chiang Mai, Thailand, during May 2000 to March 2002 period

Age	Number of Specimen tested	Number of positive				Total (%)
		NVGI	NVGII	SV	HAstV	
≤ 5 months	51	1	4	1	-	6 (11.8)
6-11 months	79	3*	5	2*	-	10* (12.7)
1-2 years	122	2	7	4	5	18 (14.8)
> 2-5 years	44	1	1	3	2	7 (15.9)
Total	296	7*	17	10*	7	41*

* One specimen was positive for both NVGI and SV

Comparison of nucleotide and deduced amino acid sequences among 7 NVGI strains exhibited 72.0 to 99.3% and 80.2 to 100% identities, respectively (Table 5). For 17 NVGII strains, they shared 70.9 to 99.7% nucleotide and 60.2 to 100% deduced amino acid identities among themselves (Table 7). The deduced amino acid sequences of 7 NVGI and 17 NVGII strains were used to construct the phylogenetic tree in comparison with the corresponding sequences of the representative NVGI and NVGII reference strains and analyzed by using the recent NV capsid region classification scheme of Zheng et al., 2006. The 7 NVGI sequences were grouped into 5 distinct GI genotypes as the following : 2 NV strains were NVGI genotype 4 (NVGI/4) (typified by Chiba virus cluster) with the percent deduced amino acid sequence identity of 98.7 to 100%, 2 were NVGI genotype 6 (NVGI/6) (known as the Hesse virus cluster) with the percent deduced amino acid sequence identity of 96.2 to 100%, 1 was NVGI/3 (DSV395 virus cluster) with the percent deduced amino acid sequence identity of 98.7 to 100%, and the other one was NVGI/7 (Winchester virus cluster) with the percent deduced amino acid sequence identity of 97.5 to 100%. Only 1 NVGI strain, CMH308/01, could not be grouped into any genotype, however, it belonged to genotype 13 (NVGI/13) with the percent deduced amino acid sequence identity of 100% according to the classification scheme of Okada et al., 2005 (Table 6 and Figure 8a).

All 17 NVGII sequences were grouped into 7 distinct GII genotypes. Approximately half of NVGII strains (9 isolates) were classified into NVGII genotype 4 (NVGII/4) (known as the Lordsdale virus cluster) with the percent deduced amino acid sequence identity of 96.2 to 100%. Two NV strains were NVGII genotype 3 (NVGII/3) (Toronto virus cluster) with the percent deduced amino acid sequence

identity of 94.9 to 100%. Another two NV strains were grouped into NVGII genotype 10 (NVGII/10) (Erfurt virus cluster) with the percent deduced amino acid sequence identity of 98.7 to 100%. The remaining four NV strains, one of each belonged to NVGII/1 (Hawaii virus cluster) (98.7 to 100% identity), NVGII/6 (Seacroft virus cluster) (97.4 to 100% identity), NVGII/8 (Amsterdam virus cluster) (100% identity), and NVGII/15 (J23 virus cluster) (100% identity), respectively (Table 8 and Figure 8b).

2.2 Analysis of partial capsid gene of SV

All 10 SV amplicons obtained were direct sequenced for partial capsid gene using the consensus SLV5317 as the sequencing primer which priming at 3′ end of the cDNA strand. The partial nucleotide sequence and sequencing trace profile of capsid gene of a representative SV (CMH283/01) was depicted in Figure 9.

The percent identity of nucleotide and deduced amino acid sequence of the partial capsid gene among 10 SV strains were 53.1 to 99.7% and 50.5 to 97.8%, respectively (Table 9). The deduced amino acid sequences of these 10 SV strains were analyzed and classified into 2 distinct genogroups, GI and GII, according to the recent SV capsid region classification scheme described by Akihara et al., 2005. SVGI (typified by the Manchester virus) was a more common genogroup (80%) than SVGII (20%) (known as London virus). Eight strains of SVGI were further classified into 3 genotypes as the following : 4 strains were genotype 1 (GI/1) with the percent deduced amino acid sequence identity of 98.8 to 100%, 3 were genotype 4 (GI/4) with the percent deduced amino acid sequence identity of 97.6 to 100%, and 1 was genotype 5 (GI/5) with the percent deduced amino acid sequence identity of 100%, respectively. Two strains of SVGII, one of each belonged to genotype 1 (GII/1)

(100% identity), and genotype 2 (GII/2) (100% identity), respectively (Table 10 and Figure 10).

2.3 Analysis of partial capsid gene of HAstV

All 7 HAstV amplicons obtained were direct sequenced for partial capsid gene using the consensus PreCAP1 as the sequencing primer which priming at 3′ end of the cDNA strand. The partial nucleotide sequence and sequencing trace profile of capsid gene of the representative HAstV (CMH265/00) was depicted in Figure 11.

Seven HAstV sequences obtained in this study were analyzed and classified by multiple alignment with the corresponding sequences of 18 representative reference strains from GenBank nucleotide sequence databases. The percent identity of the partial capsid nucleotide and deduced amino acid sequences of these HAstV strains were 81.4 to 99.7% and 89.4 to 99.5%, respectively (Table 11). Phylogenetic analysis of the partial capsid deduced amino acid sequences of 7 HAstV strains demonstrated that all of them were classified into 4 distinct genotypes or serotypes. Two strains belonged to serotype 1 with the percent deduced amino acid sequence identity of 95.8 to 98.4%, two belonged to serotype 2 with the percent deduced amino acid sequence identity of 98.4 to 98.9%, one belonged to serotype 3 with the percent deduced amino acid sequence identity of 99.4%, and two belonged to serotype 5 with the percent deduced amino acid sequence identity of 98.9 to 100%, respectively (Table 12 and Figure 12).

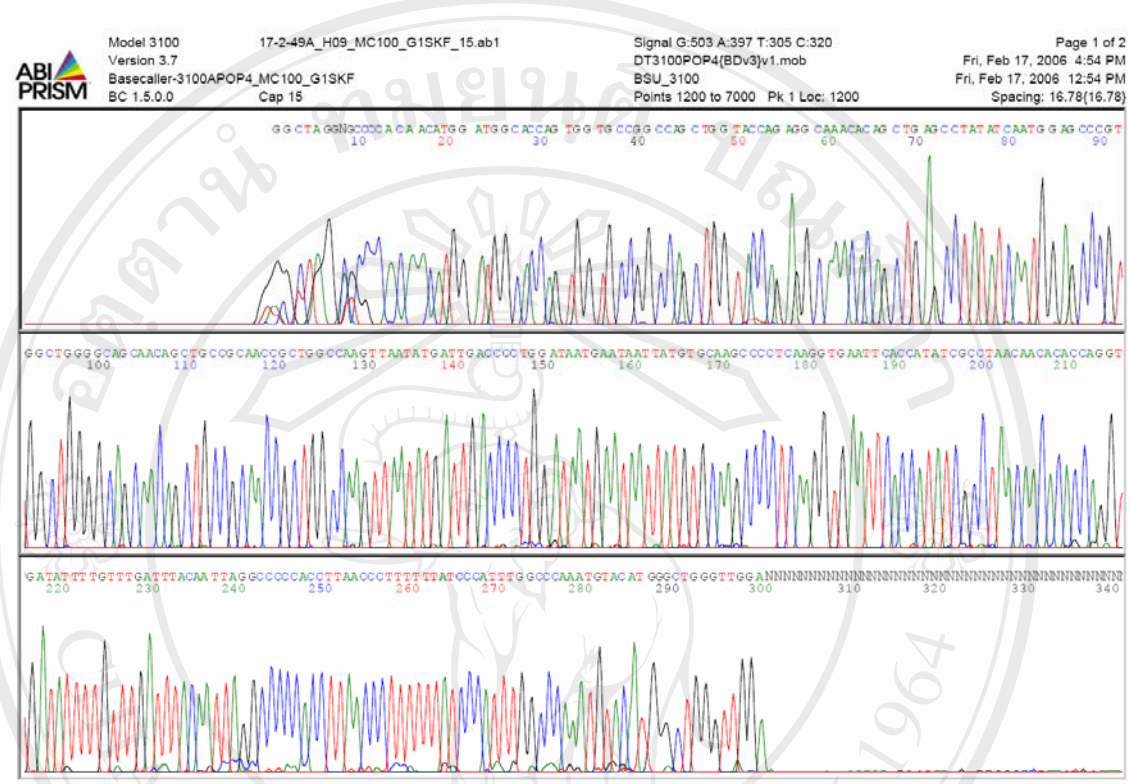

Figure 7a. An example of nucleotide sequencing trace profile of partial capsid gene amplicon of NVGI (CMH097/01)

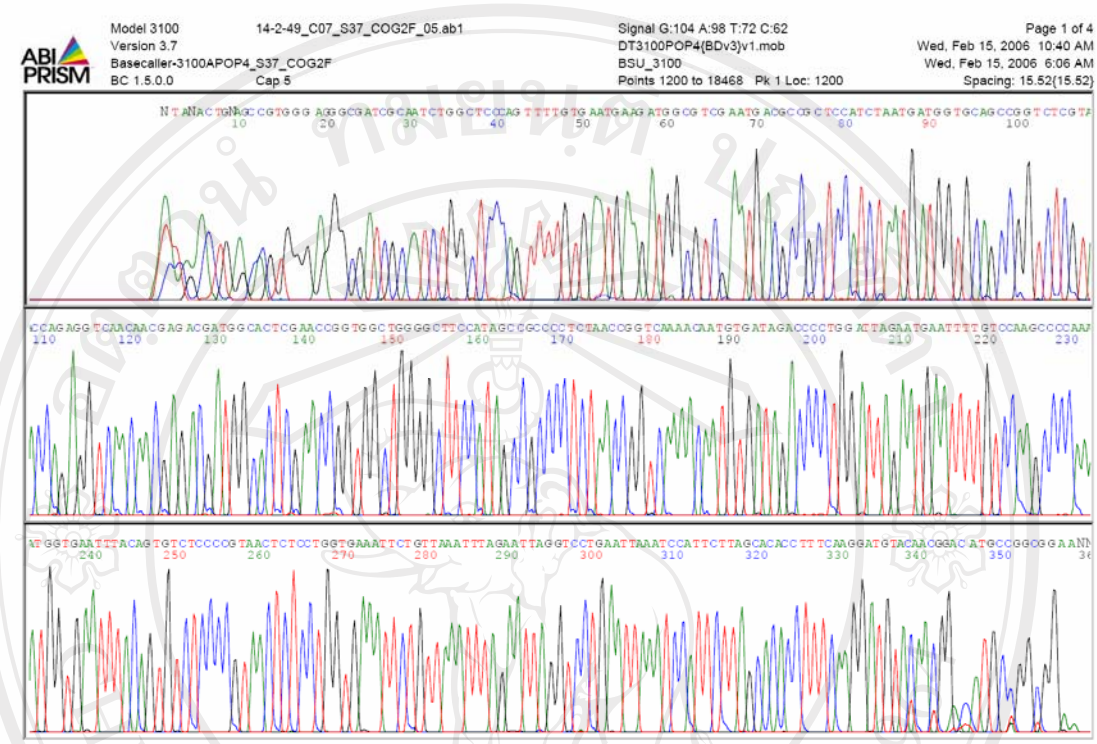

Figure 7b. An example of nucleotide sequencing trace profile of partial capsid gene amplicon of NVGII (CMH359/00)

Table 5. Nucleotide and amino acid {} sequence identity (%) between 7 NVGI and reference strains in the partial capsid region

	NVGI/3			NVGI/4			NVGI/6		NVGI/7		NVGI/13	
Strains	DSV395	Stav/95/Nor	Chiba/000520/00/JP	Chiba/00/JP	Koblenz/433/00/DE	Valetta/95/Malta	Hesse	WUG1	Winchester/94/UK	Chiba/000782/00/JP	Saitama T36GI/01/JP	Saitama T53aGI/02/JP
CMH097/01	88.8 {98.7}	95.8 {98.7}	99.1 {100}									
CMH251/01				96.2 {100}	99.1 {98.7}	100 {100}						
CMH329/02				95.0 {100}	97.9 {98.7}	98.7 {100}						
CMH010/00							85.9 {96.2}	99.1 {98.7}				
CMH183/00							86.7 {97.5}	95.8 {100}				
CMH120/01									90.9 {97.5}	98.3 {100}		
CMH308/01											99.1 {100}	99.1 {100}

Table 6. Nucleotide and amino acid {} sequence identity (%) among 7 NVGI strains in the partial capsid region

	NVGI/3	**NVGI/4**		**NVGI/6**		**NVGI/7**	**NVGI/13**
Strains	CMH097/01	CMH251/01	CMH329/02	CMH010/00	CMH183/00	CMH120/01	CMH308/01
CMH097/01	100 {100}	77.6 {82.5}	77.6 {82.5}	82.2 {92.5}	81.4 {91.2}	78.9 {88.7}	76.3 {93.8}
CMH251/01		100 {100}	98.7 {100}	77.6 {85.0}	78.5 {86.2}	77.6 {82.5}	71.0 {80.2}
CMH329/02			100 {100}	76.8 {85.0}	77.6 {86.2}	76.4 {82.5}	71.8 {80.2}
CMH010/00				100 {100}	99.1 {98.7}	77.6 {83.7}	72.2 {86.4}
CMH183/00					100 {100}	76.8 {82.5}	71.4 {86.4}
CMH120/01						100 {100}	73.8 {85.1}
CMH308/01							100 {100}

Table 7. Nucleotide and amino acid {} sequence identity (%) between 17 NVGII and reference strains in the partial capsid region

	NVGII/1			NVGII/3				NVGII/4					NVGII/6			NVGII/8		NVGII/10			NVGII/15	
Strains	Hawaii	Westover/ 302/94/US	NongKhai-22/Thai	Toronto	Arg320	Oberhausen 455/01/DE	Tak-62/Thai	Lordsdale /93	Bristol	416/97003 156/96/LA	Chiba/021 050/02/JP	Sakaeo-14/Thai	Seacroft/ 90/UK	Saitama U3	NongKhai-51/Thai	Amsterdam/9 8-18/98/NET	Saitama U25	Erfurt/546/ 00/DE	Chiba/000 325/00/JP	Vietnam 026	J23/99/ US	Mex7076/ 99
CMH359/00	94.4 {100}	97.8 {98.7}	97.8 {100}																			
CMH126/01				93.6 {96.2}	99.1 {98.7}	98.3 {100}	100 {100}															
CMH344/02				93.2 {94.9}	97.4 {97.4}	99.1 {98.4}	98.3 {98.7}															
CMH052/01								94.5 {98.7}	94.5 {98.7}	97.4 {100}	95.3 {97.4}	96.6 {100}										
CMH068/01								94.5 {98.7}	94.5 {98.7}	97.4 {100}	95.3 {97.4}	96.6 {100}										
CMH091/01								94.5 {98.7}	94.5 {98.7}	97.4 {100}	95.3 {97.4}	96.6 {100}										
CMH096/01								94.1 {96.2}	94.1 {96.2}	97.0 {97.4}	93.2 {94.9}	99.1 {97.4}										
CMH112/01								94.2 {98.7}	92.4 {98.7}	96.2 {100}	93.2 {97.4}	95.7 {100}										
CMH241/01								94.5 {97.4}	94.5 {97.4}	97.4 {98.7}	93.6 {96.2}	99.5 {98.7}										
CMH262/01								93.6 {98.7}	93.6 {98.7}	97.4 {100}	93.6 {97.4}	97.0 {100}										
CMH298/01								94.1 {98.7}	94.1 {98.7}	97.0 {100}	94.9 {97.4}	96.2 {100}										
CMH309/01								93.6 {97.4}	93.6 {97.4}	96.6 {98.7}	93.6 {96.2}	95.7 {98.7}										
CMH323/02													96.2 {98.7}	97.0 {100}	89.9 {97.4}							
CMH357/00																96.6 {100}	97.8 {100}					
CMH037/00																		99.5 {100}	99.5 {98.7}	99.1 {98.7}		
CMH247/01																		99.1 {100}	99.1 {98.7}	98.7 {98.7}		
CMH148/01																					95.4 {100}	95.8 {100}

Table 8. Nucleotide and amino acid {} sequence identity (%) among 17 NVGII strains in the partial capsid region

	NVGII/1	NVGII/3		NVGII/4									NVGII/6	NVGII/8	NVGII/10		NVGII/15
Strains	CMH359/00	CMH126/01	CMH344/02	CMH052/01	CMH068/01	CMH091/01	CMH096/01	CMH112/01	CMH241/01	CMH262/01	CMH298/01	CMH309/01	CMH323/02	CMH357/00	CMH037/00	CMH247/01	CMH148/01
CMH359/00	100 {100}	75.2 {84.8}	74.7 {83.5}	69.7 {74.6}	69.7 {74.6}	69.7 {74.6}	70.1 {75.9}	68.9 {74.6}	69.7 {74.6}	68.9 {74.6}	70.1 {74.6}	69.3 {74.6}	72.6 {84.8}	73.1 {81.0}	77.7 {88.6}	78.1 {88.6}	69.0 {73.7}
CMH126/01		100 {100}	98.3 {98.7}	73.1 {73.4}	73.1 {73.4}	73.1 {73.4}	73.1 {74.6}	72.2 {73.4}	72.6 {73.4}	72.6 {73.4}	73.1 {73.4}	71.8 {72.1}	77.7 {83.5}	75.6 {81.0}	76.8 {84.8}	76.8 {84.8}	72.7 {72.5}
CMH344/02			100 {100}	72.2 {73.4}	72.2 {73.4}	72.2 {73.4}	73.5 {73.4}	71.8 {73.4}	73.1 {73.4}	72.2 {73.4}	72.2 {73.4}	71.0 {72.1}	76.8 {82.2}	74.7 {79.7}	76.0 {83.5}	76.0 {83.5}	71.4 {71.2}
CMH052/01				100 {100}	100 {100}	100 {100}	95.7 {97.4}	95.7 {100}	96.2 {98.7}	97.0 {100}	99.5 {100}	98.3 {98.7}	72.6 {78.4}	73.5 {73.4}	69.7 {73.4}	69.7 {73.4}	66.9 {68.7}
CMH068/01					100 {100}	100 {100}	95.7 {97.4}	95.7 {100}	96.2 {98.7}	97.0 {100}	99.5 {100}	98.3 {98.7}	72.6 {78.4}	73.5 {73.4}	69.7 {73.4}	69.7 {73.4}	66.9 {68.7}
CMH091/01						100 {100}	95.7 {97.4}	95.7 {100}	96.2 {98.7}	97.0 {100}	99.5 {100}	98.3 {98.7}	72.6 {78.4}	73.5 {73.4}	69.7 {73.4}	69.7 {73.4}	66.9 {68.7}
CMH096/01							100 {100}	94.9 {97.4}	99.5 {98.7}	96.2 {97.4}	95.3 {97.4}	94.9 {96.2}	71.4 {79.7}	75.2 {74.6}	70.1 {74.6}	70.1 {74.6}	66.1 {70.0}
CMH112/01								100 {100}	95.3 {98.7}	98.7 {100}	95.3 {100}	94.9 {98.7}	72.2 {78.4}	74.3 {73.4}	68.9 {73.4}	68.9 {73.4}	68.1 {68.7}
CMH241/01									100 {100}	96.6 {98.7}	95.7 {98.7}	95.3 {97.4}	71.0 {78.4}	74.7 {73.4}	69.7 {73.4}	69.7 {73.4}	65.7 {68.7}
CMH262/01										100 {100}	96.6 {100}	96.2 {98.7}	71.8 {78.4}	74.3 {73.4}	69.7 {73.4}	69.7 {73.4}	66.9 {68.7}
CMH298/01											100 {100}	97.8 {98.7}	72.2 {78.4}	73.1 {73.4}	69.7 {73.4}	69.7 {73.4}	67.3 {68.7}
CMH309/01												100 {100}	72.2 {78.4}	72.6 {72.1}	70.1 {73.4}	70.1 {73.4}	66.5 {68.7}
CMH323/02													100 {100}	75.2 {84.8}	74.3 {87.3}	74.7 {87.3}	69.4 {75.0}
CMH357/00														100 {100}	76.8 {86.0}	76.8 {86.0}	70.2 {81.2}
CMH037/00															100 {100}	99.5 {100}	70.2 {82.5}
CMH247/01																100 {100}	70.2 {82.5}
CMH148/01																	100 {100}

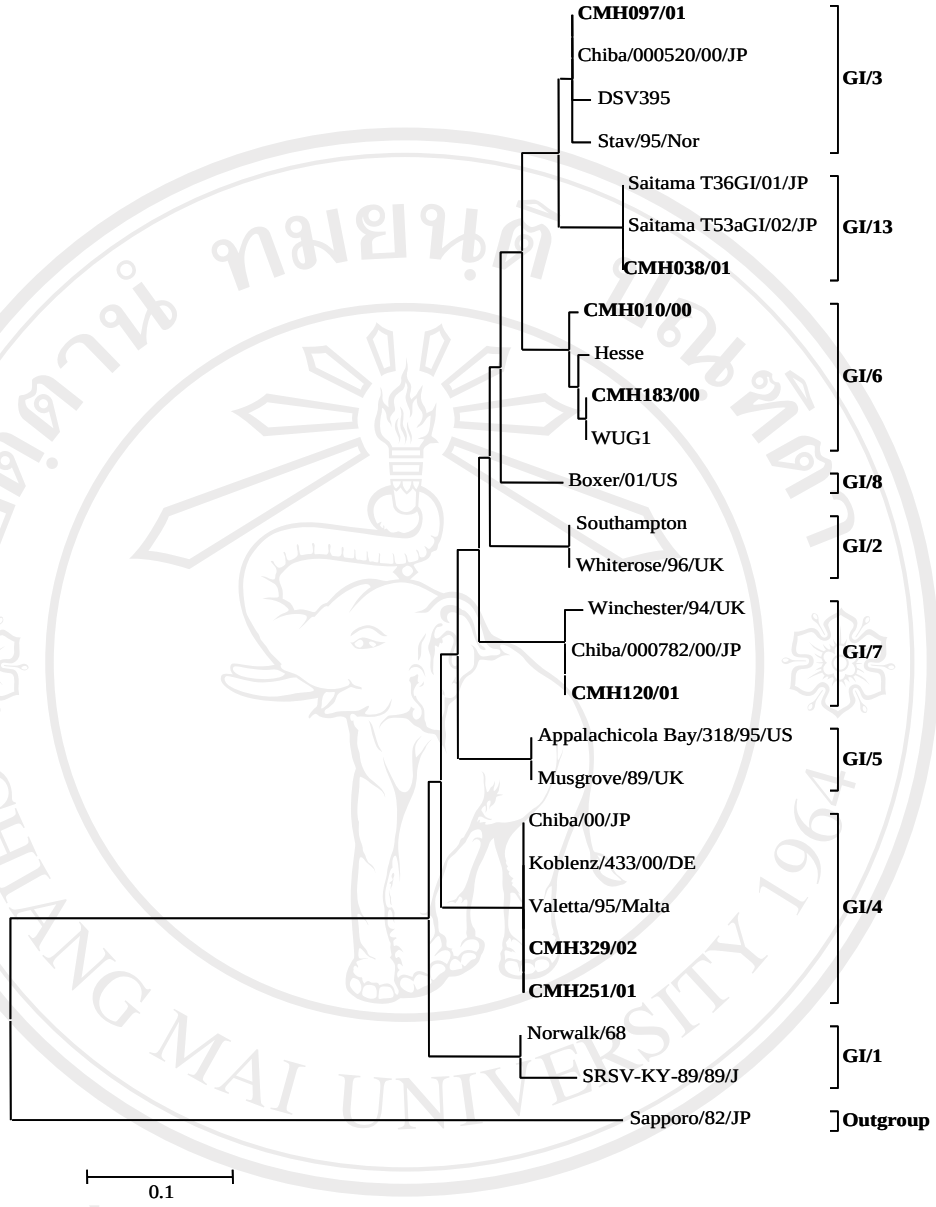

Figure 8a. Phylogenetic analysis of partial capsid deduced amino acid sequences of NVGI strains detected between the year 2000 to 2002 in Chiang Mai, Thailand. The tree was constructed by multiple alignment of 7 NVGI positive sequences (indicated in boldface), 19 reference sequences, and 1 outgroup sequence. In the phylogenetic tree, NVGI strains were classified into eight distinct genotypes from 1 to 8. Sapporo/82/JP was used as an outgroup strain for phylogenetic analysis. Bootstrap values are 1,000 replicates based on neighbor-joining and distance methods. Tree is unrooted. Genotypes or genetic clusters are divided by brackets.

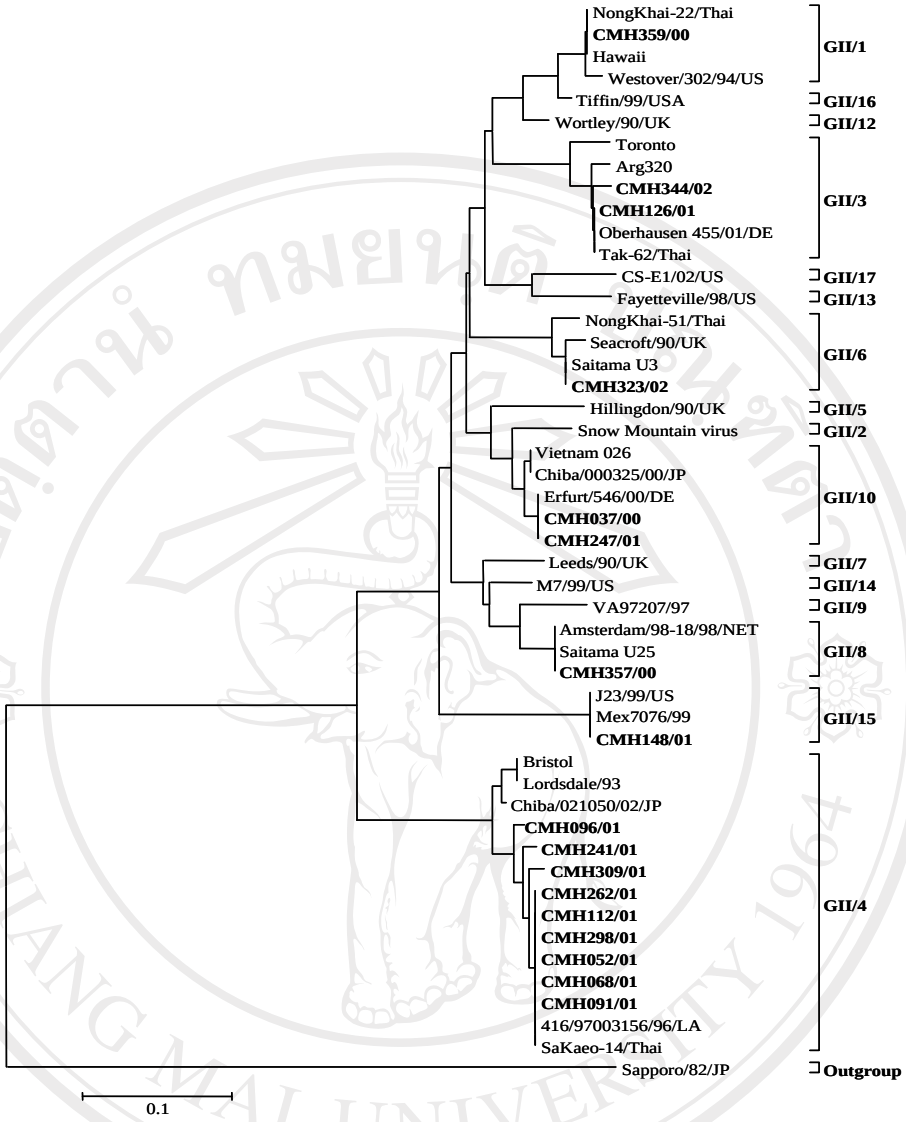

Figure 8b. Phylogenetic analysis of partial capsid deduced amino acid sequences of NVGII strains detected between the year 2000 to 2002 in Chiang Mai, Thailand. The tree was constructed by multiple alignment of 17 NVGII positive sequences (indicated in boldface), 31 reference sequences, and 1 outgroup sequence. In the phylogenetic tree, NVGII strains were classified into sixteen distinct genotypes from 1 to 10 and 12 to 17 (except genotype 11 of porcine NVGII). Sapporo/82/JP was used as an outgroup strain for phylogenetic analysis. Bootstrap values are 1,000 replicates based on neighbor-joining and distance methods. Tree is unrooted. Genotypes or genetic clusters are divided by brackets.

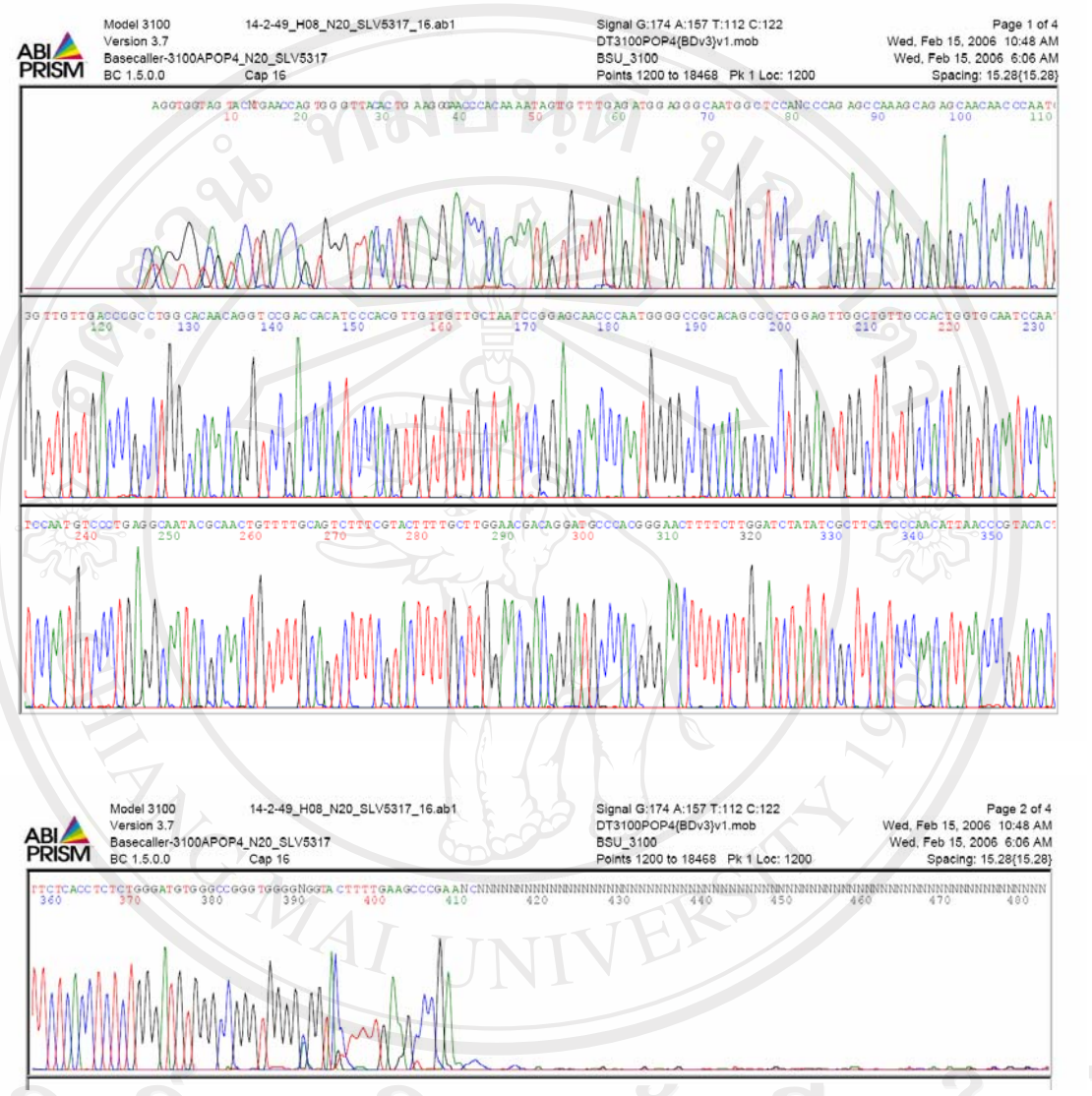

Figure 9. An example of nucleotide sequencing trace profile of partial capsid gene amplicon of SV (CMH283/01)

Table 9. Nucleotide and amino acid {} sequence identity (%) between 10 SV and reference strains in the partial capsid region

	SVGI/1							SVGI/4		SVGI/5		SVGII/1		SVGII/2	
Strains	Sapporo/ 82/JP	Manchester/ 93	NongKhai- 50/Thai	Chanthaburi /74/Thai	Tak- 69/Thai	Songkhla- 6/Thai	Karachi/938 /94	Karachi/102 1/92	Moscow/45 36/02/RF	Karachi/ 877/90	Karachi/ 1026/92	London/ 92	Lyon/598/ 97/F	Mex340/90	Moscow/21 96/02/RF
CMH077/01	98.4 {100}	100 {100}	99.6 {99.8}	99.2 {98.8}	99.6 {98.8}	100 {100}	99.6 {100}								
CMH111/01	98.4 {100}	100 {100}	99.6 {99.8}	99.2 {98.8}	99.6 {98.8}	100 {100}	99.6 {100}								
CMH283/01	98.0 {100}	99.6 {100}	99.2 {98.8}	98.9 {98.8}	99.2 {98.8}	99.6 {100}	99.2 {100}								
CMH284/01	98.0 {100}	99.6 {100}	99.2 {98.8}	98.9 {98.8}	99.2 {98.8}	99.6 {100}	99.2 {100}								
CMH217/01								97.2 {97.6}	96.8 {97.6}						
CMH218/01								97.2 {97.6}	96.8 {97.6}						
CMH257/01								98.8 {100}	100 {100}						
CMH311/01										99.6 {100}	99.6 {100}				
CMH002/00												96.4 {100}	98.0 {100}		
CMH010/00														82.8 {100}	98.8 {100}

Table 10. Nucleotide and amino acid {} sequence identity (%) among 10 SV strains in the partial capsid region

	SVGI/1				SVGI/4			SVGI/5	SVGII/1	SVGII/2
Strains	CMH077/01	CMH111/01	CMH283/01	CMH284/01	CMH217/01	CMH218/01	CMH257/01	CMH311/01	CMH002/00	CMH010/00
CMH077/01	100 {100}	100 {100}	99.6 {100}	99.6 {100}	87.8 {88.2}	87.8 {88.2}	88.6 {88.2}	84.3 {87.0}	53.9 {55.2}	56.6 {56.4}
CMH111/01		100 {100}	99.6 {100}	99.6 {100}	87.8 {88.2}	87.8 {88.2}	88.6 {88.2}	84.3 {87.0}	53.9 {55.2}	56.6 {56.4}
CMH283/01			100 {100}	100 {100}	87.5 {88.2}	87.5 {88.2}	88.2 {88.2}	83.9 {87.0}	54.2 {55.2}	57.0 {56.4}
CMH284/01				100 {100}	87.5 {88.2}	87.5 {88.2}	88.2 {88.2}	83.9 {87.0}	54.2 {55.2}	57.0 {56.4}
CMH217/01					100 {100}	100 {100}	96.8 {97.6}	81.6 {84.7}	51.9 {51.7}	56.6 {52.9}
CMH218/01						100 {100}	96.8 {97.6}	81.6 {84.7}	51.9 {51.7}	56.6 {52.9}
CMH257/01							100 {100}	80.8 {84.7}	51.9 {51.7}	57.9 {52.9}
CMH311/01								100 {100}	50.7 {50.5}	53.5 {51.7}
CMH002/00									100 {100}	76.9 {95.2}
CMH010/00										100 {100}

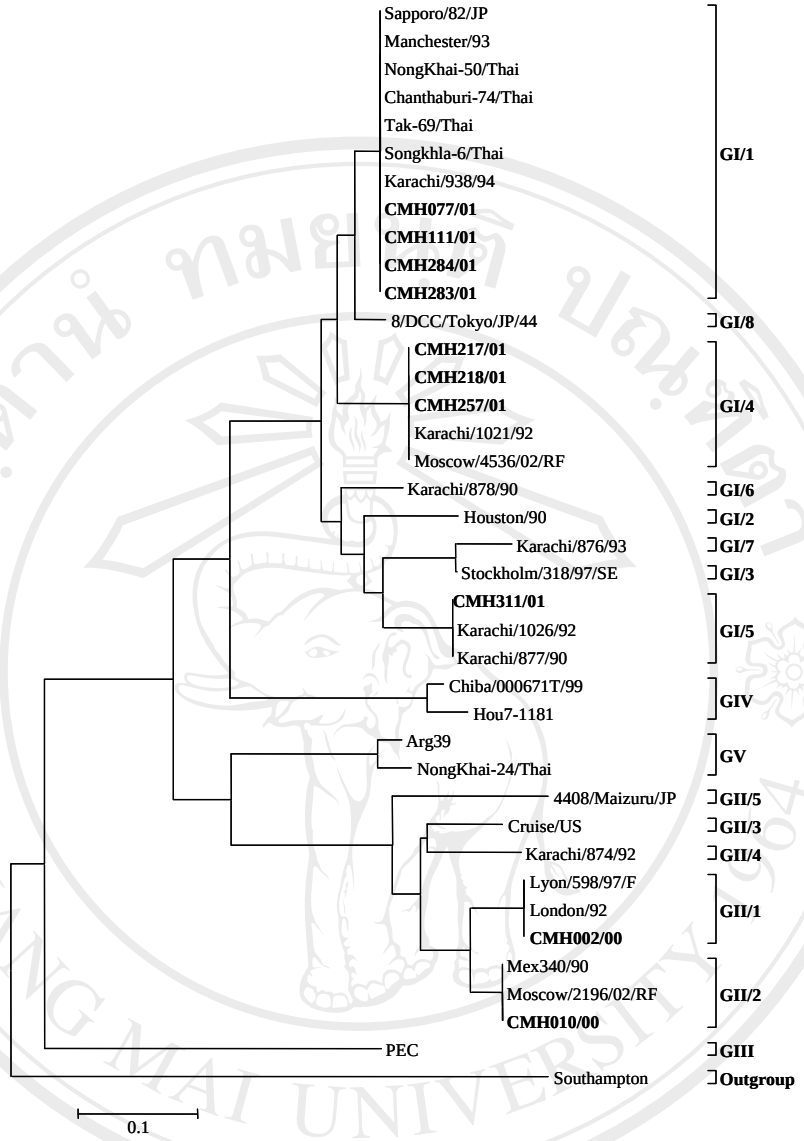

Figure 10. Phylogenetic analysis of partial capsid deduced amino acid sequences of SV strains detected between the year 2000 to 2002 in Chiang Mai, Thailand. The tree was constructed by multiple alignment of 10 SV positive sequences (indicated in boldface), 27 reference sequences, and 1 outgroup. In the phylogenetic tree, SV strains were classified into genogroup I, II, IV, and V. SVGI was further classified into 8 genotypes, while SVGII into 5 genotypes, respectively. SVGIII (Porcine enteric calicivirus, PEC) was also analyzed. Southampton was used as an outgroup strain for phylogenetic analysis. Bootstrap values are 1,000 replicates based on neighbor-joining and distance methods. Tree is unrooted. Genotypes or genetic clusters are divided by brackets.

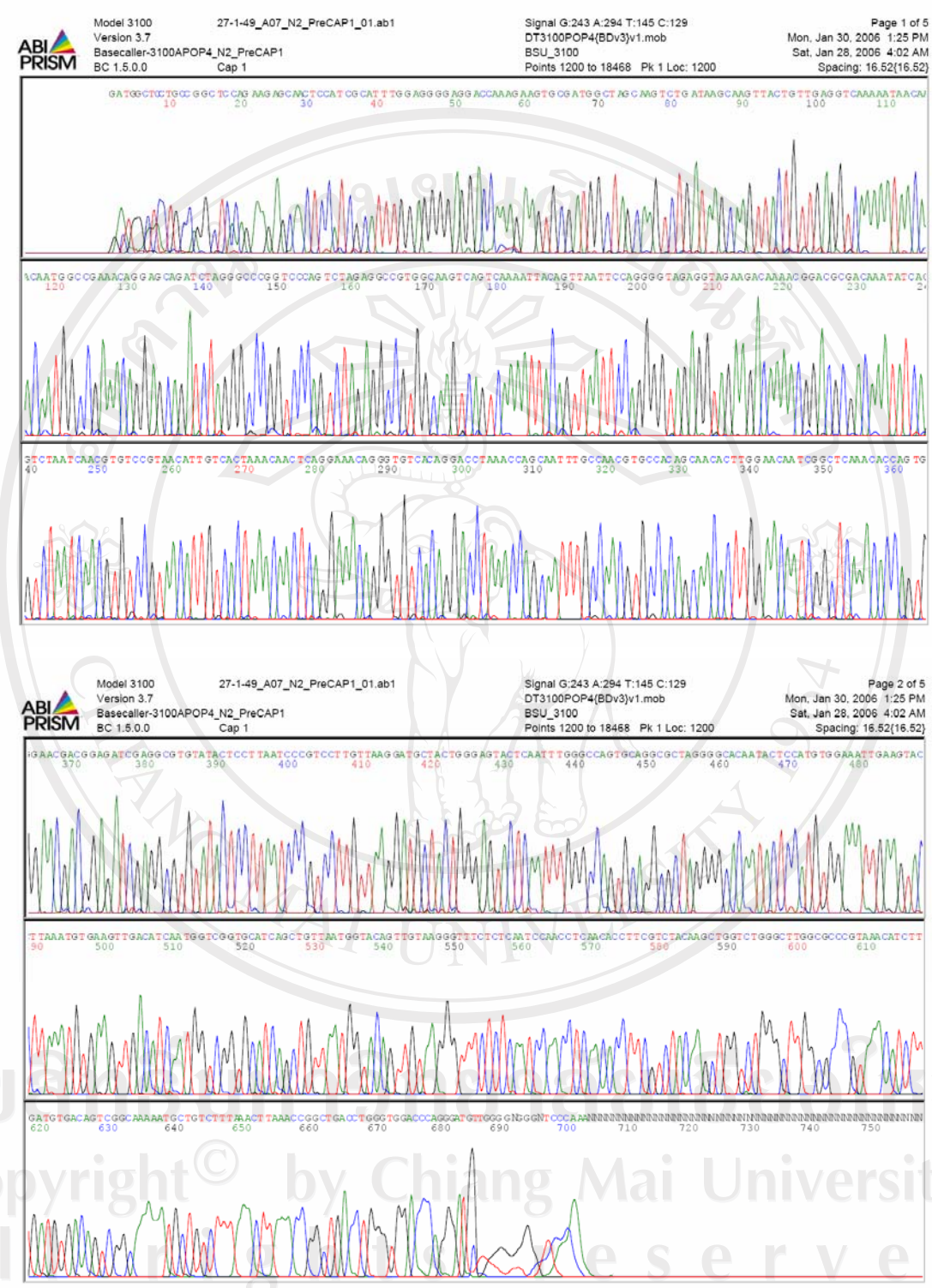

Figure 11. An example of nucleotide sequencing trace profile of partial capsid gene amplicon of HAstV (CMH265/00)

Table 11. Nucleotide and amino acid {} sequence identity (%) between 7 HAstV and reference strains in the partial capsid region

	HAstV-1			**HAstV-2**		**HAstV-3**		**HAstV-5**		
Strains	Oxford	P437/91	816/93	L06802	L13745	AF117209	AF292074	Goiania/GO/ 12/94/Brazil	CHN198	AF292072
CMH350/02	92.6 {95.8}	97.9 {97.3}	98.2 {97.9}							
CMH352/02	92.9 {96.3}	97.9 {97.9}	98.2 {98.4}							
CMH259/01				93.6 {98.4}	93.6 {98.9}					
CMH362/01				93.2 {98.4}	93.2 {98.9}					
CMH265/00						96.2 {99.4}	96.2 {99.4}			
CMH053/01								95.1 {100}	94.6 {98.9}	95.3 {100}
CMH074/01								95.1 {100}	94.6 {98.9}	95.3 {100}

Table 12. Nucleotide and amino acid {} sequence identity (%) among 7 HAstV strains in the partial capsid region

	HAstV-1		**HAstV-2**		**HAstV-3**	**HAstV-5**	
Strains	CMH350/02	CMH352/02	CMH259/01	CMH362/01	CMH265/00	CMH053/01	CMH074/01
CMH350/02	100 {100}	99.6 {99.4}	79.7 {89.5}	79.7 {89.5}	79.8 {90.6}	81.7 {92.1}	81.6 {92.1}
CMH352/02		100 {100}	79.7 {90.1}	79.7 {90.1}	80.2 {91.1}	81.7 {92.7}	81.6 {92.7}
CMH259/01			100 {100}	99.6 {100}	81.9 {91.1}	80.7 {90.1}	80.5 {90.1}
CMH362/01				100 {100}	82.3 {91.1}	80.7 {90.1}	80.5 {90.1}
CMH265/00					100 {100}	83.5 {94.7}	83.3 {94.7}
CMH053/01						100 {100}	99.6 {100}
CMH074/01							100 {100}

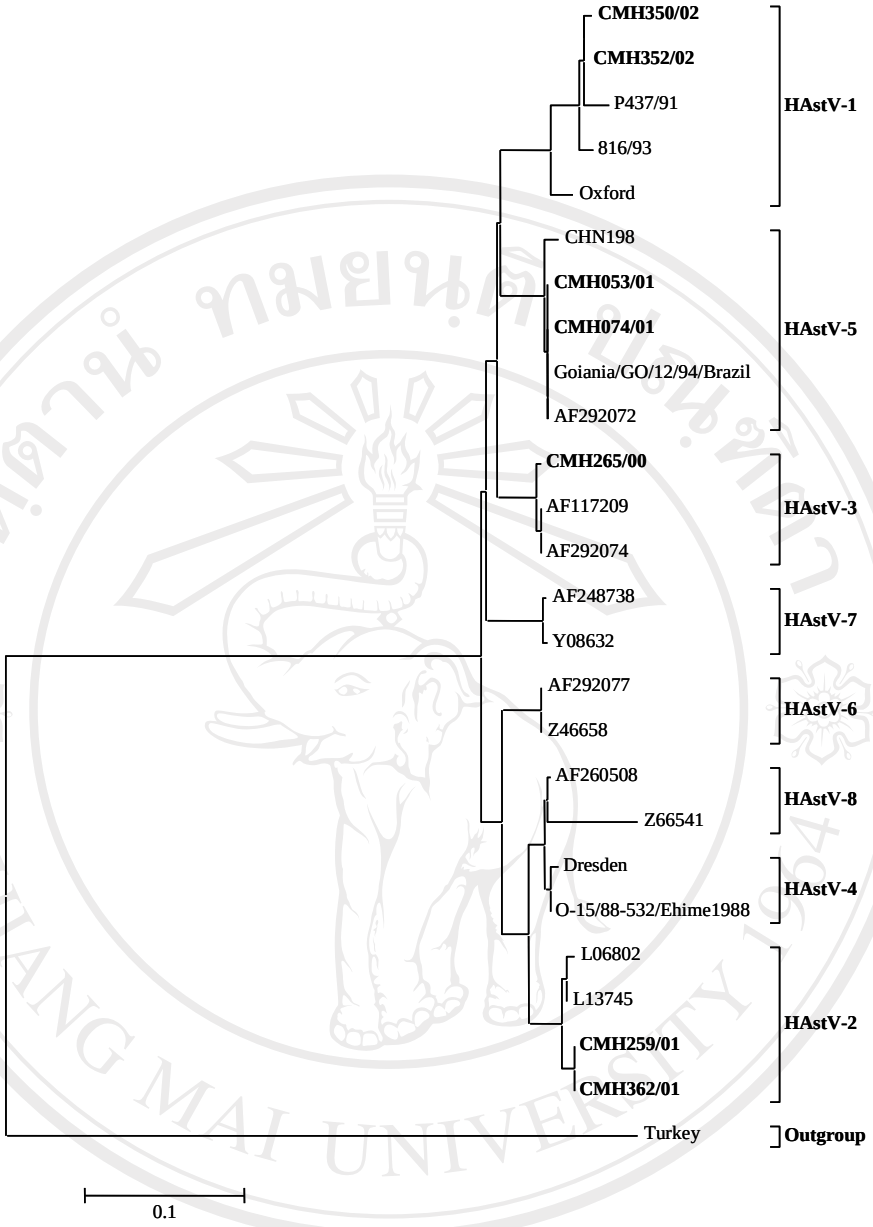

Figure 12. Phylogenetic analysis of partial capsid deduced amino acid sequences of HAstV strains detected between the year 2000 to 2002 in Chiang Mai, Thailand. The tree was constructed by multiple alignment of 7 HAstV positive sequences (indicated in boldface), 18 reference sequences, and 1 outgroup sequence. In the phylogenetic tree, HAstV strains were classified into eight serotypes, 1 to 8. Turkey AstV was used as an outgroup strain for phylogenetic analysis. Bootstrap values are 1,000 replicates based on neighbor-joining and distance methods. Tree is unrooted. Genotypes or genetic clusters are divided by brackets.