Chapter 4

Results

Part 1 Restriction fragment length polymorphism (RFLP) of ORF5 of the Thai PRRSV.

Five Thai isolates of the US genotype were amplified by RT-PCR and cut with *Mlu*I, *Hinc*II, *Sac*II and *Hae*III as describe by Cheon and Chae (2000). The RFLP patterns were showed in Fig 5. Each isolate had a numeric code for its ORF5 RFLP pattern after being treated with the selected enzymes, *Mlu*I, *Hinc*II, *Sac*II and *Hae*III, respectively. The RFLP patterns were show in Fig.5 *Mlu*I cut (code II) only for the vaccine virus. *Hinc*II cut all samples tested in this study with 2 diffferent cutting patterns. Code I yielded the products at approximately 320 and 400 bp, while code II yielded the products at approximately 250 and 500 bp. *Sac*II had no cut pattern (code I). *Hae*III had 2 cut patterns. The RFLP cutting patterns of the Thai isolates of the US genotype were showed in Table 2. The cutting sites and some sequence of field isolates (02SP2, 01NP2, 02PB1) were displayed in the appendix B.

Similar to the US geneotype, 5 Thai isolates of the EU genotypes were amplified by RT-PCR as describe in Pirzadeh et al (1998) and the ORF5 products were cut by *Pst*I, *Hae*II, *Cla*I and the enzymes used were decided from firstmarket webcutter program. The RFLP patterns were showed in Fig 6. Similar to the US isolates, the results were given a numeric code as the following: *Pst*I and *Cla*I have 2 cut patterns, no cut (code 1) and cut (code 2). *Hae*II had 3 different cutting patterns, code 1 no cut, code 2 yielded the products at approximately 300 bp while code 3 yielded the products

at approximately at 300, 400 bp. The RFLP patterns were showed in Table 2. The cutting sites of some sequences from the field isolates (01RB1, 01CB1, 03RB1, 02BR1) were displayed in the appendix B.

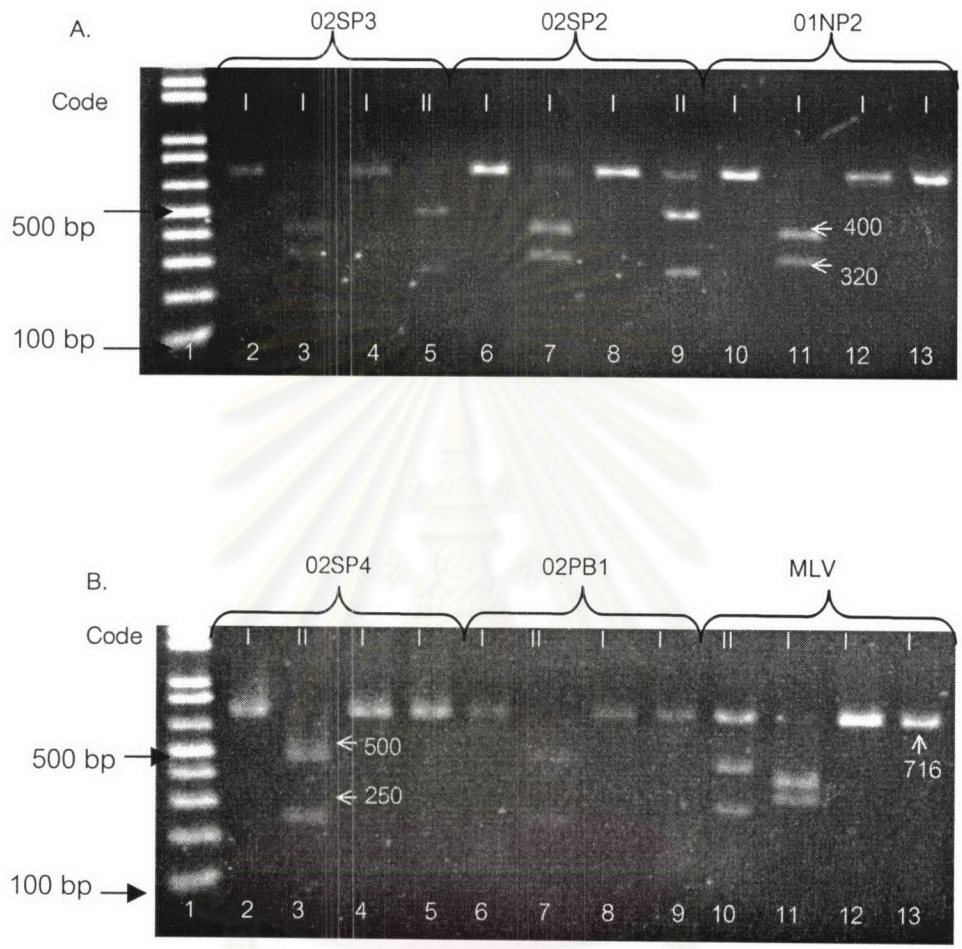


Fig 5 The RFLP patterns of ORF5 of Thai isolates (US genotypes) and the US-MLV vaccine. ORF5-PCR products were treated with 4 restriction endonuclease enzymes. 1A) Lane 1 100 bp ladder; Lane 2, 6, 10 (*Mlu*I); Lane 3, 7, 11 (*Hinc*II); Lane 4, 8, 12 (*Sac*II); Lane 5, 9, 13 (*Hae*III). 1B) Lane 1 100 bp ladder; Lane 2, 6, 10 (*Mlu*I); Lane 3, 7, 11 (*Hinc*II); Lane 4, 8, 12 (*Sac*II); Lane 5, 9, 13 (*Hae*III).

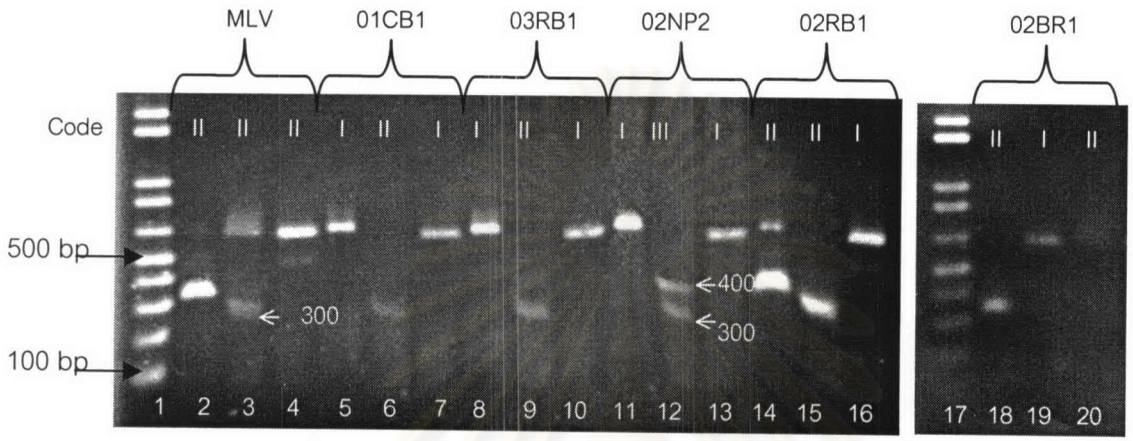


Fig 6 The RFLP patterns of ORF5 of 1 PRRSV isolates. ORF5-PCR products are treated with 3 restriction enzyme. Lane 1,17 100 bp ladder; Lane 2, 5, 8, 11, 14 and 18 were treated with *Pst*I; Lane 3, 6, 9, 12, 15 and 19 were treated with *Hae*II and Lane 4, 7, 10, 13, 16 and 20 were treated with *Cla*I.

Table 2 Sources and RFLP patterns of PRRSV isolates in Thailand.

Genotypes	samples	Sources	provinces	year	Cutting pattern[a]
US	02SP2	Serum	Suphan Buri	2002	1-1-1-2
	02SP3	Serum	Suphan Buri	2002	1-1-1-2
	02SP4	Serum	Suphan Buri	2002	1-2-1-1
	01NP2	Serum	Nakorn Pathom	2001	1-1-1-1
	02PB1	Serum	Pracheen Buri	2001	1-2-1-1
	MLV-vacc	Resp PRRS®	-	-	2-1-1-1
EU	02RB1	Serum	Ratchaburi	2002	2-2-1
	01CB1	Serum	Chonburi	2001	1-2-1
	03RB1	Serum	Ratchaburi	2003	1-2-1
	02NP2	Serum	Nakorn Pathom	2002	1-3-1
	02BR1	Serum	Burirum	2001	2-1-2
	MLV-vacc	Porcilis®	-	-	2-2-2

[a] The cutting pattern were cut by *Mlu*I, *Hinc*II, *Sac*II, *Hae*III in US genotypes and *Pst*I, *Hae*II, *Cla*I in EU genotypes, respectively.

Part 2 Pathological study of the selected Thai isolates of PRRSV.

1. Clinical signs

The clinical signs of PRRSV inoculated pigs are observed and noted everyday including the respiratory score, rectal temperature and other abnormal behavioral changes. The experimental room condition, relative humidity and room temperature were noted in each room. The data were showed in Table 3,4.

2. Gross and microscopic findings

The respiratory system contained the most remarkable lesions. Grossly, pneumonia was characterizes by multifocal, tan-mottled areas with irregular and indistinct borders (Fig 7). Secondary bacterial infection was evident in 2 pigs of the US group. Pleuritis was seen in one pigs and pericarditis and peritonitis were seen in another pig necropsied. Lung scores were showed in Table 5. Scores were evaluated from both gross or microscopic finding independently. Pigs infected with 01NP1 had higher lung scores than those infected with 02SB3, in both gross and microscopic lesions. PRRSV antigen was confirmed using immunohistochemistry (IHC) (Fig 8). All pigs infected with PRRSV were positive for PRRSV staining in the lungs.

Table 3 Respiratory scores of PRRSV inoculated pigs in each room.

dpi	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Pig no.	Respiratory score[c]														
EU /1	2	1	4	1	1	1[b]	1[b]	1[b]	1	2	-	-	-	-	-
EU /2	0	1	4[b]	2	1	1	-	-	-	-	-	-	-	-	-
EU /3	2	1	4[b]	2	1	1	1[b]	1[b]	2[b]	2	-	-	-	-	-
EU /4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
EU /5	0	1	3	1	1	1	1	1[b]	2[b]	2	2[b]	2[b]	2	2	2
EU /6	0[b]	1	3[b]	1	1	1	-	-	-	-	-	-	-	-	-
EU /7	0	1	4	1	1	1[b]	1[b]	1	2	-	-	-	-	-	-
EU /8	0	1	3	1	1	1	1[b]	1[b]	2[b]	2	2[b]	2	2	2	2
EU /9	0	1	3	1	1	1	1	1[b]	2[b]	2	2[b]	2	2	2[b]	2
US /1	0	0[b]	0	0	0[b]	0[b]	-	-	-		-	-	-	-	-
US /2	0	0[b]	0	0	0[b]	0[b]	-	-	-		-	-	-	-	-
US /3	0	0	0	0	0[b]	0[b]	0	0	0[b]	0[b]	-	-	-	-	-
US /4	0	1[b]	0	0	0[b]	0[b]	0[b]	1[b]	1[b]	0[b]	1[b]	1[b]	2[b]	2[b]	4
US /5	0	1	0	0	0[b]	0[b]	-	-	-	-	-	-	-	-	-
US /6	0	0[b]	0	0	0[b]	0[b]	0[b]	0[b]	0[b]	2[b]	-	-	-	-	-
US /7	0	1[b]	0	0	0	0	0[b]	0[b]	0[b]	0[b]	0[b]	0[b]	1	1	1
US /8	0	0[b]	0	0	0[b]	0[b]	0[b]	0	1[b]	1[b]	-	-	-	-	-
US /9	0	0	0	0	0	0[b]	0	0	0	0	0[b]	0[b]	0	0	1
Con /1	0	1	0	0	0	0	-	-	-	-	-	-	-	-	-
Con /2	0	1	0[b]	0	0	0[b]	1[b]	0[b]	0	0	-	-	-	-	-
Con /3	0	1	1	1	0[b]	0	0	0	0	0	0	0	0[b]	0	0

[b] The rectal temperature over 40 °c

[c] Respiratory score : 0= normal, 1 = dyspnea and/or mild tachypnea when stress, 2= dyspnea and/or mild tachypnea when rest, 3= dyspnea and/or moderate tachypnea when stress, 4= dyspnea and/or moderate tachypnea when rest, 5= dyspnea and/or severe tachypnea when stress, 6= dyspnea and/or severe tachypnea when rest (Halbur et al., 1995b).

Table 4 Relative humidity.

dpi	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Gr.	Relative humidity[a] (%)														
EU	83	83	83	91	91	83	91	91	87	91	91	91	91	91	83
US	76	75	69	76	79	76	80	69	76	79	80	79	76	75	76
Con	79	79	83	68	72	72	75	79	83	91	83	79	79	72	75

[a] Relative humidity, at average room temperature 30 - 31 °c

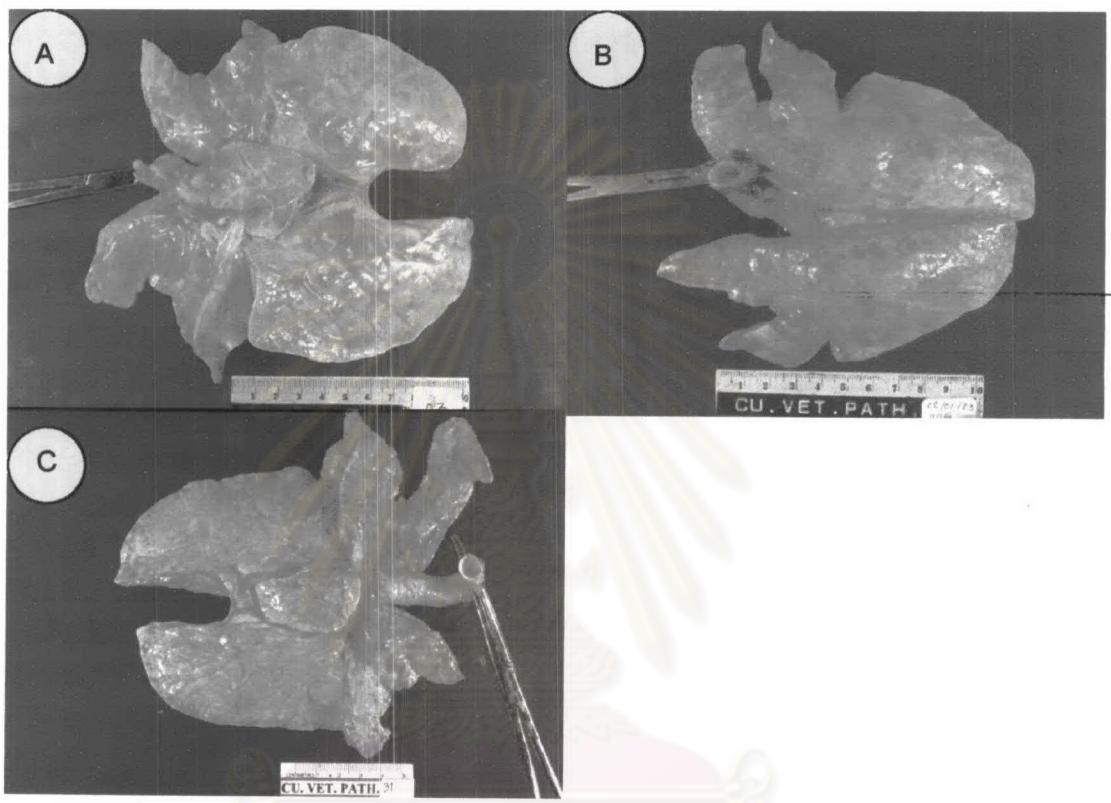


Fig 7 Lungs from 02SB3 - infected (A), 01NP1 - infected (B) and control (C) pigs necropsied at 9 dpi. Diffuse pneumonia was seen more in the US group (B). Negative control pig (C) had no remarkable lesion.

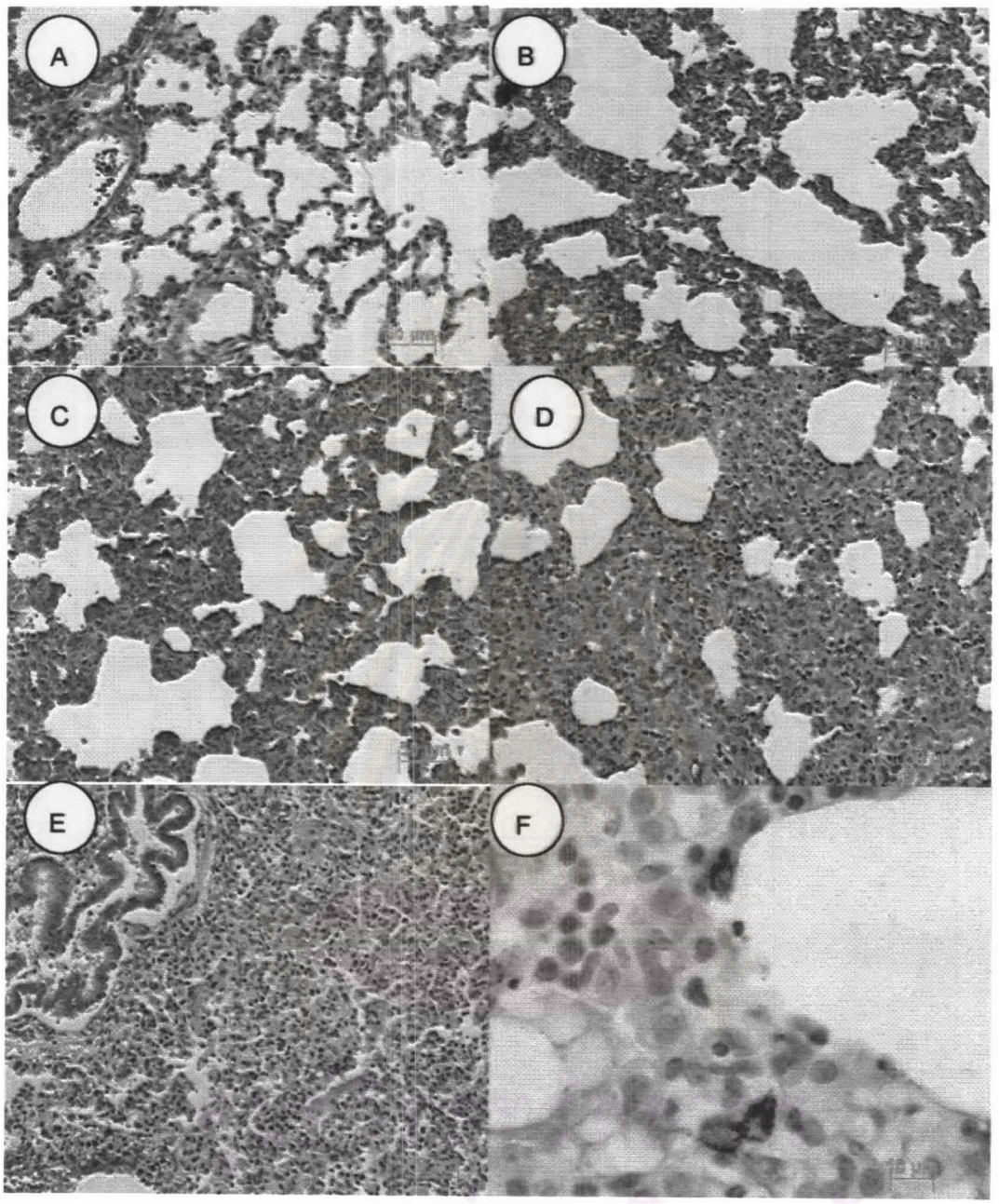


Fig 8 Lungs from PRRSV-infected pigs showing multifocal interstitial pneumonia characterizes by grade 0 = no lesion (A), grade 1 = mild interstitial pneumonia (B), grade 2 = moderate multifocal interstitial pneumonia (C), grade 3 = moderate diffuse interstitial pneumonia (D), grade 4 = severe interstitial pneumonia (E) (HE staining). PRRSV-positive cells were stained with SDOW17 showing brown granular staining (F).

Table 5 Gross and microscopic lung scores and immunohistochemistry results.

Dpi	Pig #	Gross[a]		Microscopic[b]		IHC
		Score	X ± SD	Score	X ± SD	
5	EU /2	1.5	1.5	0.7	1.5 ± 1.1	+
	EU /6	1.5		2.3		+
	US /1	11	7.7 ± 6.7	1.3	1.7 ± 0.4	+
	US /2	0		1.7		+
	US /5	12		2		+
	Con /1	0	0	0	0	-
9	EU /1	16	27.7 ± 23.8	3.7	3.1 ± 0.6	+
	EU /3	55		2.7		+
	EU /7	12		2.7		+
	US /3	35	54 ± 19	2.7	2.6 ± 0.1	+
	US /6	54		2.7		+
	US /8	73		2.5		+
	Con /2	0	0	1.3	1.3	-
15	EU /5	44	32.7 ± 11	2	2.7 ± 0.6	+
	EU /8	22		3		+
	EU /9	32		3		+
	US /4	59	60.7 ± 8.6	3.7	3.1 ± 0.5	+
	US /7	70		3		+
	US /9	53		2.7		+
	Con /3	0	0	0	3	-

[a] Total 100 points.

[b] 0 = no remarkable lesion, 1 = mild interstitial pneumonia, 2 = moderate multifocal interstitial pneumonia, 3 = moderate diffuse interstitial pneumonia, 4 = severe interstitial pneumonia.

In the lymphoid organs, enlarged (2x) and edematous of lymph nodes with tan in colored were obviously seen especially in the inguinal area, as early as 5 dpi. In both groups, the size was greater at 15 dpi (3-4x) with firm and whitish-tan colored (Fig. 11). Microscopically, mild to severe lymphoid necrosis of the lymph nodes was seen at 5-9 dpi (Fig. 9). Later, at 15 dpi, follicular hyperplasia was seen. The germinal centers were necrotic, depleted, edematous with cell debries. The cortical contained small cystic spaces variably lined by epithelium containing proteinaceous fluid or cell debries. Although, there were no remarkable gross lesions in other lymphoid tissues, microscopically, there were mild lymphoid necrosis, depletion and hyperplasia in the thymus, spleen and lymphoid follicles in tonsils. One pig in the 01NP1-infected group had diphteritic tonsilitis.

In addition, mild to moderate lymphocytic rhinitis was also seen in both infected groups as early as 5 dpi, but mild suppurative rhinitis occurred in one 02SB3-infected pig at 9 dpi (Fig 10). Both of inoculated groups had lesions in the kidneys characterized by diffuse petechial hemorrhage of the renal cortex as early as 5 dpi (Fig.12) but milder at 9 and 15 dpi. No remarkable lesion was shown microscopically in the kidney. However, no evidence of swine fever virus was detected using both virus isolation and PCR.

Only in the 01NP1-infected pigs had perivascular cuffing by lymphocytes and macrophages and multifocal gliosis in the cerebrum (Fig.13) and mild to moderate

mutifocal lymphohistiocytic perivascular myocarditis (Fig.14) when necropsied at 15 dpi. Other groups had no microscopic lesions in either the brain or the heart.

3. Serology

All pigs were free from PRRSV based on serological results before inoculation. The control pigs remained free from PRRSV until the last day of necropsy. PRRSV antibody titer was first detected in both - infected group as early as 9 dpi, 83.3% and 100 % seropositive in 02SB3 and 01NP1, respectively. Until 15 dpi, the antibody titer was 100% positive in both infected group (Fig. 15B, 16B). Virus isolation and titration were performed from serum. The presence of PRRS viremia was found at 5 dpi in both infected groups. The virus titers were peaked at 9 dpi in both - infected pigs with the titer a high as $10^{3.5}$ and $10^{3.25}$ $TCID_{50}$/50 µl in 01NP1 and 02SB3, respectively (Fig. 15A, 16A).

No crossed contamination among groups was demonstrated. In addition, other common infectious diseases of pigs in Thailand such as Aujeszky's Disease virus using serum neutralization, Swine Fever virus using virus isolation, NPLA, PCR and Porcine Circo virus using PCR were ruled out. None yielded any positive result.

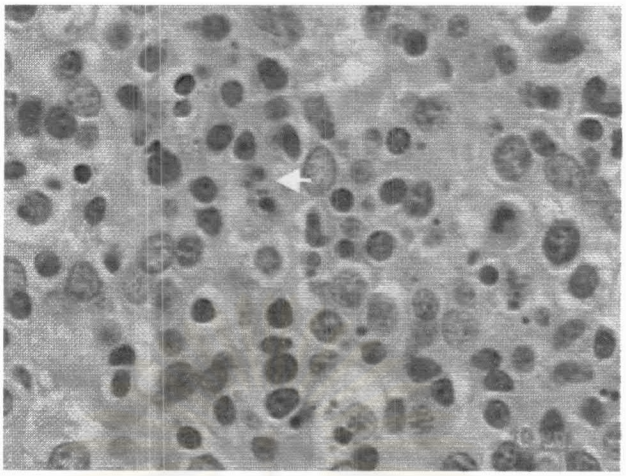


Fig 9 Lymphoid necrosis (arrow) in tracheobronchial lymph node of pig no. US/8 at 9 dpi (HE staining).

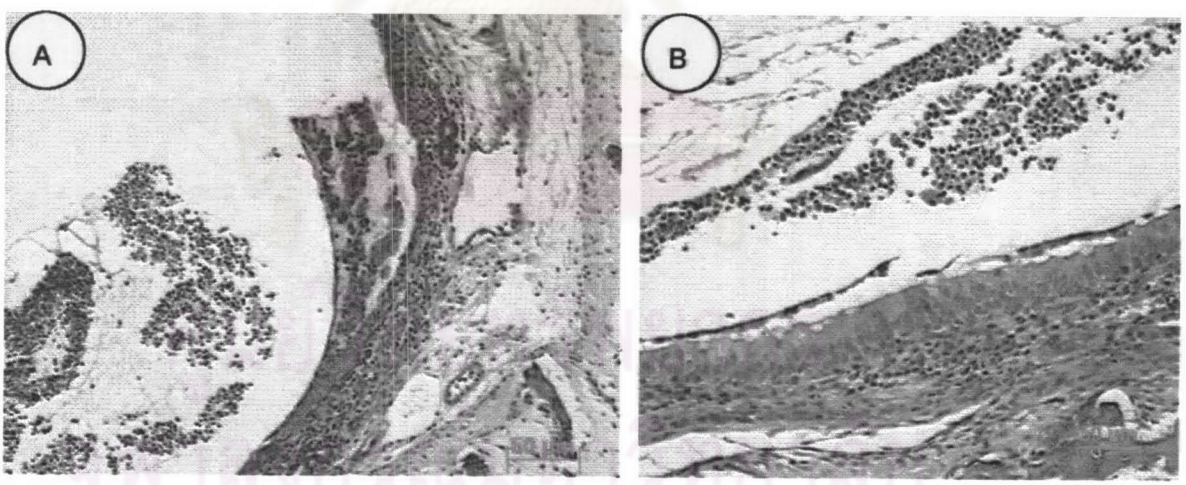


Fig 10 Mild lymphoplasmacytic rhinitis (arrow) (A) and desquamation of nasal epithelial and suppurative exudate (arrow head) (B) of 02SB3-infected pig at 9 dpi (HE staining).

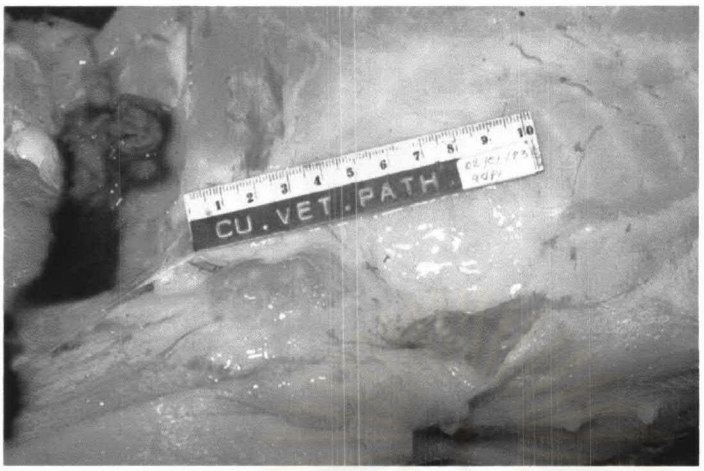


Fig 11 Enlargement of inguinal lymph node (2x) of pig no. US/3 at 9 dpi.

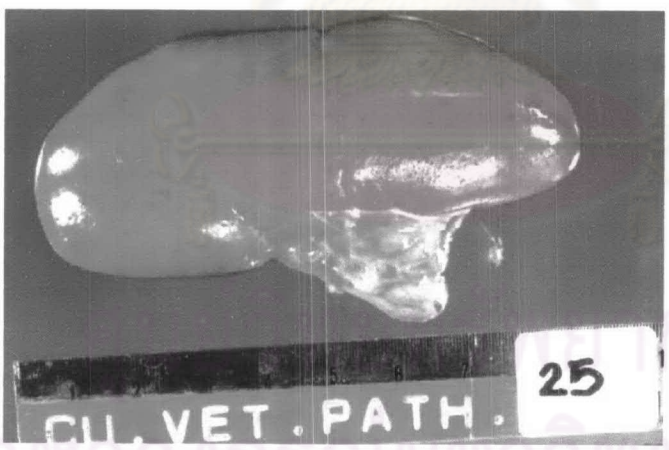


Fig 12 Diffuse petechial hemorrhage on the renal cortex from 01NP1 - infected pig at 5 dpi.

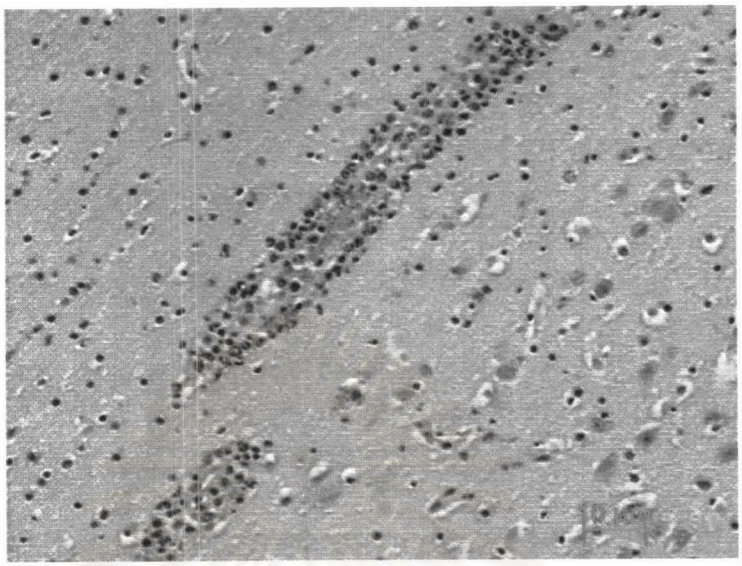


Fig 13 Mild to moderate non-suppurative encephalitis with multifocal gliosis in 01NP1 - infected pig at 15 dpi.

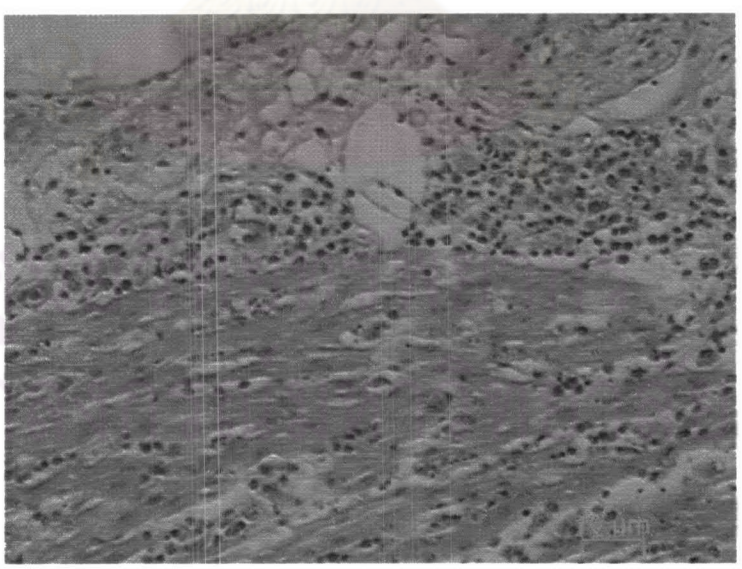
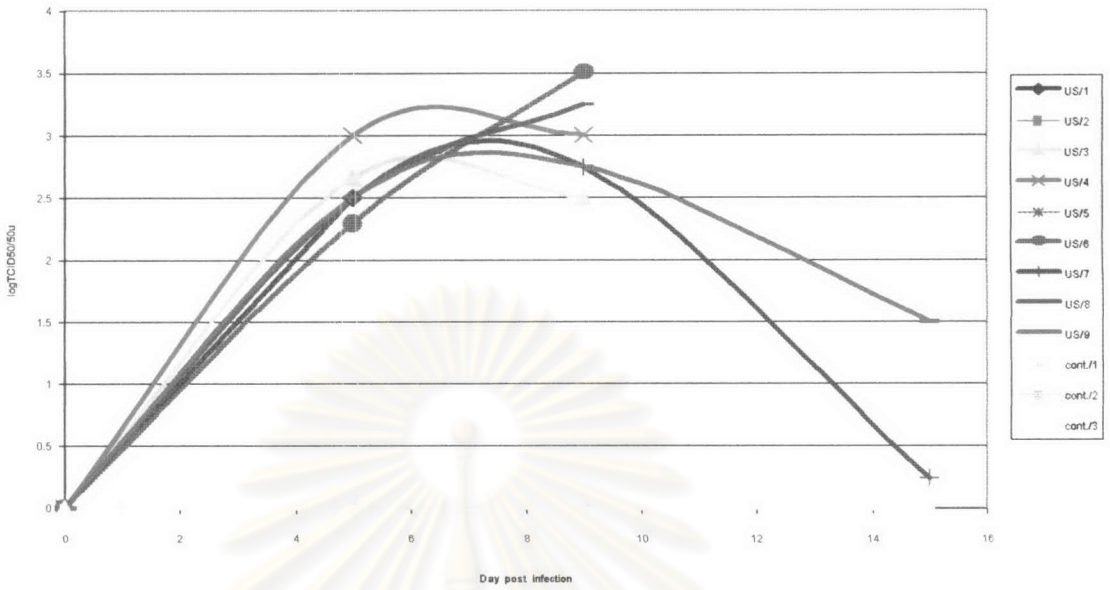


Fig 14 Nonsuppurative myocarditis in 01NP1 - infected pig at 15 dpi.

A.



B.

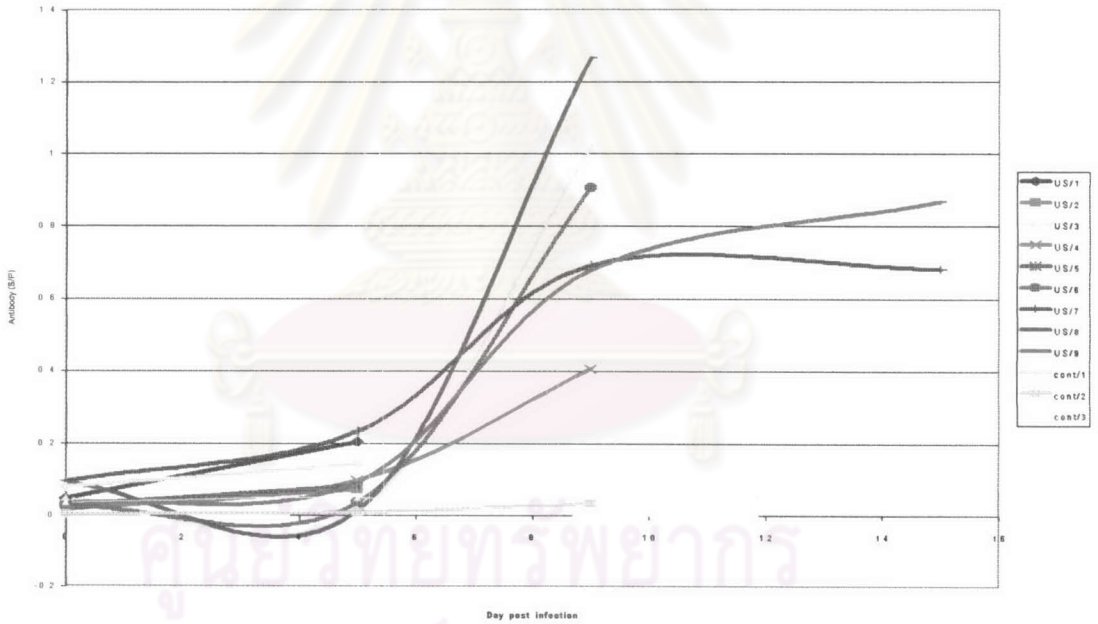


Fig 15 Viral titer (log_{10} $TCID_{50}$/50 µl) (A) and ELISA titer (S/P ratio) (B) of infected pigs, 01NP1 (US/1-US/9) at 0, 5, 9 and 15 dpi.

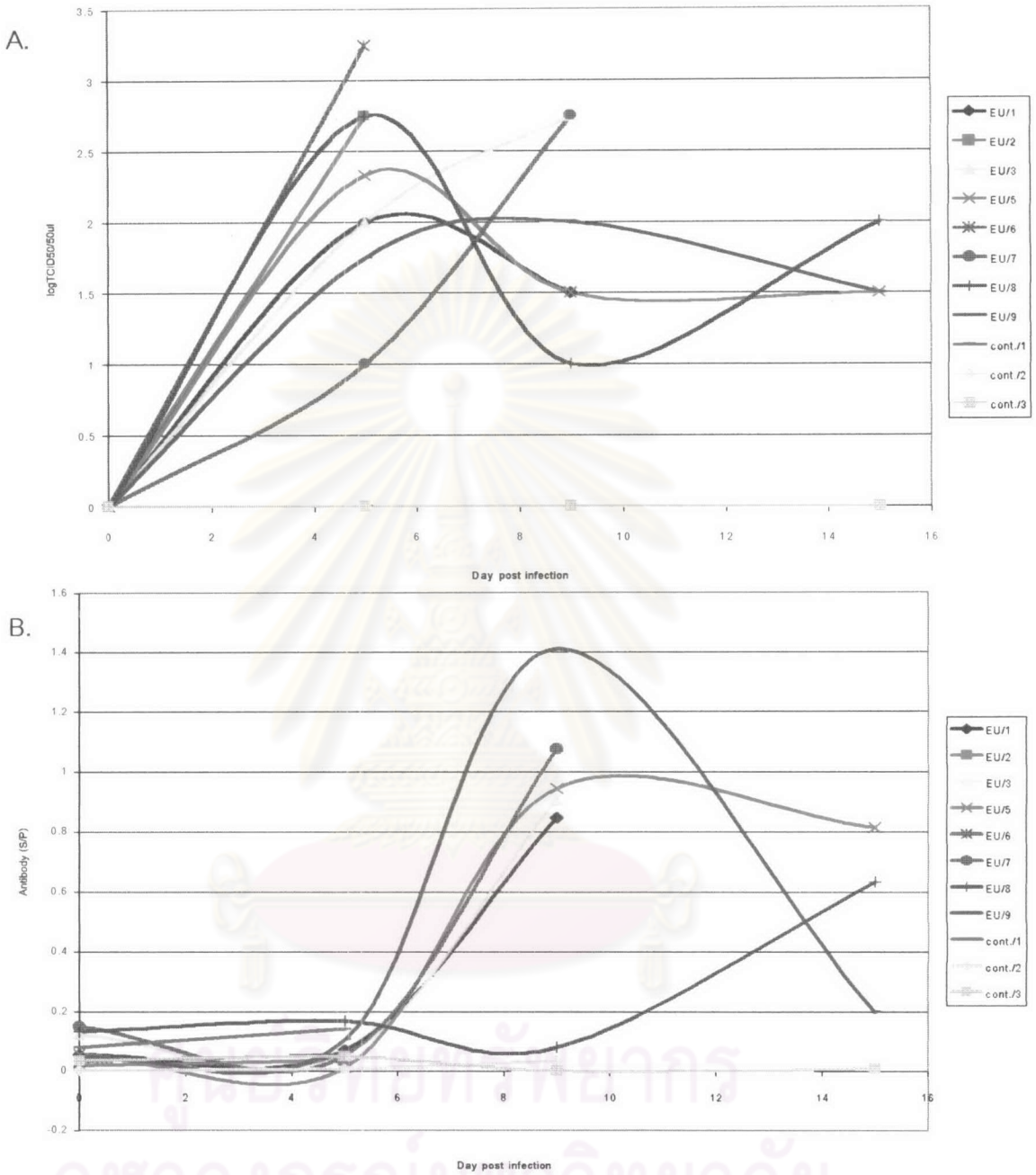


Fig 16 Viral titer (log_{10} $TCID_{50}$/50 µl) (A) and ELISA titer (S/P ratio) (B) of infected pigs, 02SB3 EU/1-EU/9) at 0, 5, 9 and 15 dpi.