

Immune Response of Pigs (Sus scrofa) Inoculated with Dengue and Japanese Encephalitis Viruses

Principal Investigators : Robert Edelman, LTC, MC
Dennis O. Johnsen, MAJ, VC

Associate Investigators : Ananda Nisalak, M.D.
Suchinda Udomsakdi, M.D.

Assistant Investigators : Suwana Vithanomsat, B.S.
Aree Boriharnvanakett, B.S.
Panor Srisongkram, B.S.
Nonglak Khananurak, B.S.
Chalam Chantrasri, B.S.

PURPOSE AND DESCRIPTION: The purpose of this limited project was to determine if dengue virus can infect pigs, as determined by viremia and antibody response. We reasoned that if pigs were not infected by repeat dengue virus challenge in the laboratory, then one could interpret with greater confidence the HI serology results observed in Chiangmai sentinel pigs, which developed HI antibodies to JEV with associated cross-reactive HI antibodies to dengue (Table 1).

PROGRESS: Two Thai pigs (Sus scrofa), 2-3 months of age, were purchased by the Department of Veterinary Medicine, and pretested for JEV and dengue antibodies. Pig 54 was apparently infected in the pig farm near Nakorn Pathom shortly before it was purchased and brought to Bangkok (Table 3). During the experiment, the pigs were held in rooms screened against mosquitoes. Following three pre-infection serum collections, the pigs were inoculated subcutaneously with dengue virus that had been isolated previously from mosquitoes and passed two times in MK2 cell cultures. Pig 52 received a dengue 3 strain and pig 54 received a dengue 4 strain on two occasions about 6 weeks apart. Serum samples for antibody titrations were obtained at weekly intervals for 4 weeks and for virus isolation every day 7 days after each inoculation. Serum was inoculated into MK2 cell cultures using the immediate and delayed plaque technique. No dengue virus was recovered from either pig.

As shown in Tables 2 and 3, dengue viruses inoculated subcutaneously on two different occasions produced neither a primary HI antibody response in pig 52, nor an anamnestic response in pig 54.

Once it had become apparent that the 2 pigs were not infected by dengue virus, they were inoculated subcutaneously with JEV. The antibody response to JEV challenge is also shown in Tables 2 and 3. Although both pigs responded to JEV by a 4-fold or greater increase in HI antibody titers against JEV and dengue, JEV could not be recovered from pig blood samples obtained every day for 7 days after inoculation. It is notable that the pattern of HI titers seen in both pigs (high titered JEV and lower titered dengue antibodies) are quite similar to the HI antibody patterns typically observed in the sentinel pigs in Chiangmai Valley (Table 1).

We conclude from these results that pigs cannot be experimentally infected with dengue viruses, but they can be infected with JEV. The antibody detected in sentinel pigs in Chiangmai was probably not induced by dengue viruses.

Unfinished studies include examination of porcine PRNT & HI antibody response to Tembusu virus challenge in the laboratory, a repeat search for JE viremia in pigs, and determination of the specificity of pig serum IgM antibody for recent JEV or Tembusu virus infections.

Table 1.
 Chiangmai Sentinel Pig Serology: Positive HI Antibody
 Conversions^(a) for One Month (October 1970)

Pig No.	HI Antibody titers						Chik
	JE	D4	D3	D2	D1		
S-96	160	0 ^(b)	0	0	0	0	0
S-99	320	40	40	0	0	0	0
S-114	640	160	40	0	0	0	0
S-122	1280	80	40	20	0	0	0
S-135	1280	320	80	80	0	0	0

(a) Serum HI antibody titers of all 5 pigs were <20 to all antigens in September 1970
 (b) 0 = <20

Table 2. Immune Response of a Pig Inoculated with Dengue 3 Virus and Subsequently JEV

Pig No.	Treatment	Date	Serum HI Titer				
			JE	D4	D3	D2	D1
52	Pre-Bleed	8 June 70	0 ^{a)}	0	0	0	0
	"	7 July	0	0	0	0	0
	"	10 July	0	0	0	0	0
b) Inject D ₃ (2 × 10 ⁴ PFU)		10 July	0	0	0	0	0
	Post-Bleed	17 July	0	0	0	0	0
	"	24 July	0	0	0	0	0
	"	31 July	0	0	0	0	0
	"	7 Aug	0	0	0	0	0
	"	26 Aug	0	0	0	0	0
c) Inject D ₃ (3 × 10 ⁴ PFU)		26 Aug	0	0	0	0	0
	Post-Bleed	3 Sept	0	0	0	0	0
	"	10 Sept	0	0	0	0	0
	"	17 Sept	0	0	0	0	0
	"	24 Sept	0	0	0	0	0
d) Inject JEV (1 × 10 ⁴ PFU)		1 Oct					
	Post-Bleed	9 Oct	1280	160	160	80	40
	"	16 Oct	5120	160	160	160	40
	"	23 Oct	2550	160	160	80	40
	"	30 Oct	2560	80	80	80	40

a) 0 = > 20

b) Dengue 3 strain KS69-0128-1, isolated from A. aegypti; used at second MK2 cell passage.

c) Dengue 3 strain BK69-154-E1, isolated from A. aegypti; used at second MK2 cell passage.

d) JEV strain BKM1137-70, isolated from C. tritaeniorhynchus; used at second MK2 cell passage.

Table 3. Immune Response of a Pig Inoculated with Dengue 4 Virus and Subsequently JEV

Pig No.	Treatment	Date	Serum HI Titer				
			JE	D4	D3	D2	D1
54	Pre-Bleed	8 June 70	0 ^{a)}	0	0	0	0
	"	7 July	160	40	0	0	0
	"	10 July	160	40	0	0	0
b) Inject D ₄ (1×10 ⁴ PFU)		10 July					
	Post-Bleed	17 July	320	40	0	0	0
	"	24 July	160	20	0	0	0
	"	31 July	160	20	0	0	0
	"	7 Aug	80	0	0	0	0
c) Inject D ₄ (1.5×10 ⁵ PFU)		26 Aug					
	Post-Bleed	3 Sept	80	0	0	0	0
	"	10 Sept	40	0	0	0	0
	"	17 Sept	40	0	0	0	0
	"	24 Sept	40	0	0	0	0
d) Inject JEV (1×10 ⁴ PFU)		1 Oct					
	Post-Bleed	9 Oct	160	40	40	40	40
	"	16 Oct	160	40	40	40	40
	"	23 Oct	160	40	40	40	40
"	30 Oct	160	40	40	40	40	

a) 0 = > 20

b) Dengue 4 strain KS68-3M 89, isolated from A. aegypti; used at second MK2 cell passage.

c) Dengue 4 strain KS68-AM25, isolated from A. aegypti; used at second MK2 cell passage.

d) JEV strain BKM1137-70, isolated from C. tritaeniorhynchus; used at second MK2 cell passage.