# INTENSE TRANSMISSION OF JAPANESE ENCEPHALITIS VIRUS TO PIGS IN A REGION FREE OF EPIDEMIC ENCEPHALITIS

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## INTRODUCTION

Japanese encephalitis virus (JEV), an arthropod-borne flavivirus transmitted by rice-field breeding mosquitoes of the genus Culex, is a cause of major epidemics of encephalitis throughout most of Asia from Japan to India (Shope, 1980). However, the geographic distribution of JEV is not limited to the epidemic region; JEV has also been isolated from mosquitoes in Malaysia, Indonesia, and the Philippines (Simpson *et al.*, 1970; Van Peenan *et al.*, 1974; Trosper *et al.*, 1980), all countries where epidemic Japanese encephalitis has not been reported (Okuno, 1978). In Thailand, encephalitis epidemics are confined to the northern region of the country (Hoke *et al.*, 1985), where a dramatic increase of hospital admissions for acute encephalitis occurs annually during the months of June, July, and August. No such increase is observed in the south. There are no obvious reasons for this difference.

Pigs are thought to be the main amplifying host of JEV in Thailand. Most adult swine raised in northern Thailand have serum antibodies to JEV, and JEV-seronegative sentinel pigs set out during the epidemic season rapidly seroconvert (Johnson *et al.*, 1974). However, no published information is available on JEV transmission to pigs in the "encephalitis-silent" southern region. We describe here a study showing that JEV transmission to pigs in southern Thailand is remarkably intense.

## MATERIALS AND METHODS

Abattoir blood collections : Abattoirs in the three southern provinces of Chumphon, Surat Thani, and Prachuab Khiri Khan were visited during mid-May 1983. Five milliliter jugular or anterior vena caval blood specimens (Carle and Dewhirst, 1942) were collected from approximately 30 pigs at each abattoir. An attempt was made to select only young pigs (age 4 to 12 months) from several locations around the province. Pig ages were estimated by animal weight and features. Specimens were transported on wet ice to Bangkok.

Sentinel pigs: Young pigs were purchased in Bangkok, bled, and kept in screened rooms. Sera were tested for JEV antibodies by the plaque reduction neutralization method, and seronegative animals were selected as sentinels. On 11 July 1983, ten pigs were transported by truck to Chumphon Province for placement. Two pigs were stationed at each of five locations, all of which were 3 to 10

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kilometers apart, in areas near the provincial capital. All sentinels were penned near locally raised pigs. Five milliliters of venous blood was obtained from each sentinel every two to three days. Serum was immediately separated and stored frozen in dry ice.

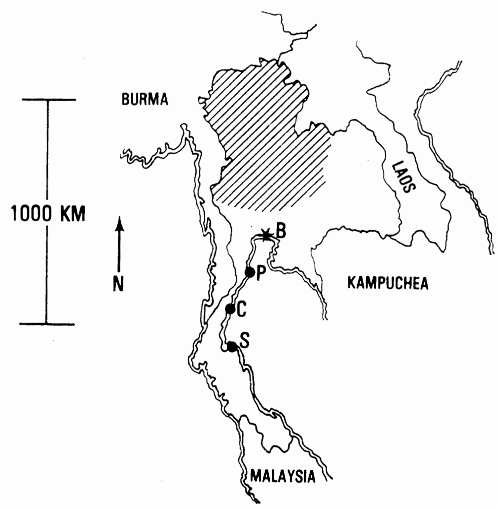


Fig. 1—Map of Thailand. The star represents Bangkok. P = Prachuap Khiri Khan; C = Chumphon; S = Surat Thani. Cross-hatching denotes the region of recurrent epidemics of Japanese encephalitis.

Hemagglutination inhibition (HAI) serology: Sera were absorbed with goose red blood cells, extracted with acetone, and tested for HAI activity against 8 HA units of prototype JEV-infected or Tembusu-infected suckling mouse brain antigens in a microtiter adaptation of the method of Clarke and Casals (1958). Sera producing complete inhibition of hemagglutination at a 1:10 dilution were deemed positive.

Assay for JEV immunoglobulin M (IgM) antibodies: Untreated sera were tested at a dilution of 1:100 in an antibody capture solid phase enzyme linked immunoassay for porcine JEV IgM antibodies (Burke *et al.*, 1985).

Virus isolation: Sera which had been obtained during the week before a sentinel pig seroconverted were cultured for JEV in mosquito cell cultures. Initial isolation attempts using C6/36 *Aedes albopictus* cells (Igarashi, 1978) were abortive due to toxic effects of pig sera, even when diluted. Subsequently, serum specimens were diluted 1:4 in L-15 media supplemented with 10% tryptose phosphate broth and 2% fetal calf serum, and 0.25 milliliters were inoculated onto monolayers of *Aedes pseudoscutellaris* (LSTM-AP-61) mosquito cells (Pudney *et al.*, 1982) in 2 ounce glass bottles. After 30 min adsorption at room temperature, 5 ml of the same media was added, and the flasks were incubated for 10-14 days at 28°C. Virus growth was detected and identified by indirect immunofluorescent staining with monoclonal antibodies (Gould, E., per. comm.; Henchal *et al.*, 1982). Isolates were confirmed as JEV by the plaque reduction neutralization method (Russell and Nisalak, 1967) on monolayers of LLC-MK2 cells with antisera raised in monkeys to the prototype Nakayama strain. Triturated mosquito pools were similarly processed by inoculation onto monolayers of C6/36.

Mosquito collections: Light traps (CDC type) were positioned near sentinel pig pens without carbon dioxide bait. Traps were set at dusk and retrieved at dawn. A target sample size of 300 mosquitoes per location was set; when met, collections at that site were discontinued. Specimens were frozen, transported to Bangkok, and sorted according to species. Pools of 10 to 100 mosquitos of known JEV vector species (Chamberlain, 1980) were processed for virus isolation.

## RESULTS

Abattoir serum collections: Seventy-four of 100 abattoir-collected swine sera collected in the three southern provinces contained

Table 1

JEV HAI antibodies in sera from pigs at abattoirs in three provinces in southern Thailand.

| Location | Pig age (est.) | | Total |
|---|---|---|---|
| | 4-6 months | 7-12 months | |
| Chumphon | 7/13 | 14/16 | 21/29 |
| Surat Thani | 18/21 | 16/19 | 34/40 |
| Prachuap Khiri Khan | 19/31 | 0/0 | 19/31 |
| Total | 44/65 | 30/35 | 74/100 |

JEV HAI antibodies (Table 1). The distribution of positive titers was 1:10 in 3; 1:20 in 4; 1:40 in 16; 1:80 in 21; 1:160 in 20; 1:320 in 9; and 1:640 in one. Titers were 2 to 8 fold higher to JEV antigen than to Tembusu virus antigen in 70 of the 74 positive sera. The antibody prevalence in pigs estimated to be older than six months (30/35, 89% was greater than that among younger pigs (44/65, 68%; by Fisher's exact test $p = .04$). Although the overall antibody prevalences were not significantly different between the three provinces, the antibody prevalence among young pigs was higher ($p < .05$) at Surat Thani than at the other two provinces.

Table 2

JEV infections in sentinel pigs in Chumphon province.

| Date | Pig No. A1-1 | A1-2 | A2-1 | A2-2 | A3-1 | A3-2 | B1-1 | B1-2 | B2-1 | B2-2 |
|---|---|---|---|---|---|---|---|---|---|---|
| July | | | | | | | | | | |
| 13 | 0 | 0 | 0 | 0 | 0 | 0 | - | - | - | - |
| 14 | - | - | - | - | - | - | 0 | 0 | 0* | 0 |
| 15 | 0 | 0 | 0 | 0 | 0 | 0 | - | - | - | - |
| 16 | - | - | - | - | - | - | 0 | 0 | 0 | 0* |
| 18 | 0 | 0 | 0* | 0 | 0 | 0 | - | - | - | - |
| 19 | - | - | - | - | - | - | 0* | 0 | 80 | 0 |
| 20 | 0 | 0 | 0 | 0* | 0* | 0 | - | - | - | - |
| 21 | - | - | - | - | - | - | 160 | 0 | 320 | 160 |
| 22 | 0 | 0 | 80 | 20 | 0 | 0* | 320 | 0 | 320 | 320 |
| 25 | 0 | 0 | 320 | 160 | 160 | 80 | - | - | - | - |
| 26 | - | - | - | - | - | - | 320 | 0 | 160 | 640 |
| 27 | 0 | 0 | 320 | 160 | 160 | 160 | - | - | - | - |
| 28 | - | - | - | - | - | - | 320 | 0 | 160 | 320 |
| 29 | 0 | 0 | 320 | 160 | 160 | 160 | - | - | - | - |
| 30 | - | - | - | - | - | - | 320 | 0 | 640 | 160 |
| August | | | | | | | | | | |
| 1 | - | - | 320 | 160 | 160 | 160 | - | - | - | - |
| 2 | - | - | - | - | - | - | - | 0 | - | - |
| 3 | 0 | 0 | - | - | 80 | 160 | - | - | - | - |
| 5 | 0 | 0 | - | - | 80 | 160 | - | 0 | - | - |

Numbers shown are JEV HAI titers of sentinel pig sera. Pigs were set out in the afternoon of 11 July 1983.
* Denote specimens from which JE virus was isolated. 0 = titer of less than 1:10.

Table 3

Mosquito light-trap collections in Chumphon province.

| Site | Date | Total No. collected | CT* | CG | CV | CF | Others |
|---|---|---|---|---|---|---|---|
| A1 | 12 July | 19 | 5 | 2 | 0 | 0 | 12 |
| | 14 July | 80 | 12 | 10 | 0 | 2 | 56 |
| | 20 July | 121 | 39 | 20 | 4 | 0 | 58 |
| | 21 July | 134 | 42 | 8 | 2 | 0 | 82 |
| A2 | 12 July | 25 | 3 | 5 | 1 | 1 | 15 |
| | 16 July | 55 | 20 | 10 | 1 | 2 | 22 |
| | 22 July | 232 | 138 | 11 | 1 | 1 | 81 |
| A3 | 14 July | 66 | 26 | 20 | 4 | 1 | 15 |
| | 16 July | 139 | 59 | 17 | 0 | 1 | 62 |
| | 24 July | 129 | 73 | 6 | 9 | 1 | 40 |
| | 2 Aug | 378 | 250 | 106 | 0 | 20 | 2 |
| B1 | 13 July | 41 | 0 | 2 | 2 | 4 | 33 |
| | 15 July | 244 | 30 | 32 | 11 | 27 | 144 |
| | 18 July | 219 | 31 | 31 | 1 | 3 | 153 |
| B2 | 13 July | 2,871 | 1,447 | 45 | 132 | 61 | 1,186 |
| | Totals | 4,753 | 2,175 | 325 | 168 | 124 | 1,961 |

*CT = *Cx. tritaeniorhynchus;* CG = *Cx.gelidus;* CV = *Cx.vishnui* group; CF = *Cx. fuscocephalus;* Others = 33 other species.

Seven sera (five young pigs and two older pigs) contained levels of JEV IgM greater than 100 units, suggestive of infection within the preceding two weeks.

Sentinel pigs. Seven of the ten sentinel pigs stationed at five locations in Chumphon province developed JEV HAI antibodies and high levels of JEV IgM antibodies ($\geq 400$ units) during the 4 weeks of monitoring (Table 2). All seven animals seroconverted within 15 days of placement. At three locations both pigs became infected, at one location one did, and at one location neither did. JEV was isolated from all seven seroconverting pigs from blood specimens drawn on days 3, 5, 7, 8, 9, 10, and 11 days after placement, respectively.

Mosquito collections: Fifteen light-trap collections were made. Trap yields varied from 19 to 2871, with a mode of 129 mosquitoes (Table 3). Thirty-seven species were identified. Known JEV vector species were found in all fifteen collections. *Culex tritaeniorhynchus* accounted for 46% of all specimens; 10 or more *Cx. tritaeniorhynchus* were present in 12 of the 15 traps. A single collection at location B2, where both sentinel pigs developed JEV viremia in 5 days or less, contained the greatest number of *Cx. tritaeniorhnchus* (1,447 mosquitoes); the lowest mean trap yields of *Cx. tritaeniorhynchus* (24 and 20 mosquitoes per night) occurred at locations A1 and B1 where two and one pigs, respectively, remained uninfected. JEV was not isolated from any of the 2,792 mosquitoes of known vector species.

Human encephalitis in Chumphon Province: The human population of Chumphon Province in 1980 was 330,000. From 1974 through 1983 an average of 2 cases of encephalitis per year in Chumphon were reported to the Royal Thai Government Ministry of Public Health. During 1983 only one case of unspecified viral encephalitis was reported from Chumphon Province (attack rate 0.3 per 100,000), while for the year 1983 a total of 1,179 cases were reported from the 17 northern Thai provinces (overall attack rate 12.2 per 100,000; S. Jatanesen, pers. comm.).

## DISCUSSION

Epidemic Japanese encephalitis recurs every year in northern Thailand during the early rainy season. Epidemics are abrupt and dramatic, quickly filling provincial hospital pediatric wards. Seasonal attack rates often exceed 100 per 100,000 among children under the age of 15 years. Epidemic Japanese encephalitis also occurs in northeastern and central Thailand with lower attack rates than in the north. In contrast, epidemic Japanese encephalitis has never been reported in the southern region. Sporadic cases of encephalitis are reported from southern provinces, but total yearly attack rates are only one tenth to one fifth of those in the north, and there is no seasonal peak (Hoke *et al.*, 1985). Serologic evidence of JEV infection has been obtained in a few cases from southern provinces (Gunakasem *et al.*, 1981), but there has never been a systematic study in the region because encephalitis has not been perceived as a significant problem. The epidemiology of human Japanese encephalitis in southern Thailand appears similar to that in Malaysia and the Philippines, where infrequent sporadic cases of Japanese encephalitis have been documented but where epidemic encephalitis is unknown (Okuno, 1978). The geographic zone of distribution of JEV extends well beyond the epidemic regions in Asia and Southeast Asia, for JEV has been isolated from mosquitoes in Java (Van Peenen *et al.*, 1974), Luzon (Trosper *et al.*, 1980), and Sarawak (Simpson *et al.*, 1970), and JE neutralizing antibodies have been detected in a high proportion of animal and human sera in these tropical Asian regions (Simpson *et al.*, 1970; Smith *et al.*, 1974; Kanamitsu *et al.*, 1979).

Given the complex natural transmission cycle of JEV, any attempt to identify the critical missing factor (s) in these "encephalitis silent" areas is a formidable task. Nonetheless, information gleaned in a study of JEV transmission in a silent area might lead to application of more rational control efforts in the epidemic regions. There are five key elements to be considered in an analysis of transmission of a vector-borne zoonosis: (1) viruses, (2) vectors, (3) vertebrate hosts, (4) climate, and (5) humans. None of the known differences between North and South Thailand regarding any of these elements can fully account for the different patterns of JEV transmission. Human population, rice field, and pig densities are comparable, and annual rainfall and temperature patterns in the upper isthmus provinces of the south are quite similar to some of the severely affected provinces in the north.

As a first step, we sampled pigs at abattoirs for serologic evidence of previous JEV infection. In the north, JEV transmission to pigs is lowest during January through March, and remains low until June (Johnson *et al.*, 1974). We sampled during May, and found that 68% of pigs born in the south within the preceding six months had already developed serum JEV HAI antibodies. Eight per cent of all young pigs had high serum levels of JEV IgM, consistent with JEV infection within the preceding two weeks. We then selected the province of Chumphon for more detailed study. The rate of seroconversion of sentinel pigs in Chumphon, 7 of 10 within two weeks, was actually greater than the rate we had observed during the peak of the previous epidemic season in the northern province of Kamphaengphet, in which 9 of 17 pigs seroconverted within three weeks (Burke *et al.*, 1985 b). In one of the sentinel pigs in Chumphon, viremia occurred within three days; transmission to this animal must have occurred within hours of placement. Virus strains recovered from all seven animals were clearly identified as JEV by both plaque reduction neutralization with polyvalent antisera and by immunofluorescent staining of infected cells with monoclonal antibodies. Despite this evidence of intense transmission of JEV to pigs, only one case of human encephalitis was reported from Chumphon Province. Other cases may have occurred and gone unreported, but certainly no epidemic occurred.

One possible explanation for our findings is that the Southern Thai JE strains are avirulent for man. Significant variation in virulence among strains of St. Louis encephalitis virus (Monath *et al.*, 1980), West Nile virus (Unrigar and Pavri, 1977), and JEV (Huang and Wong, 1963) have previously been documented in animal challenge studies. Laboratory studies are currently underway to compare the Chumphon JEV isolates with strains collected in northern Thailand. Another possible explanation is that the mosquitoes which transmit JEV to pigs in the south are unable to transmit the virus to man. Although mosquito light trap collections in Chumphon included ample numbers of species known to transmit JEV, it is possible that the vector competence of the strains of these species in southern Thailand is inferior to that of northern strains of the same species. Lastly, the immune status of the Chumphon human population with respect to dengue (Hammon, 1969) may be important. Studies of the prevalence of dengue and JEV neutralizing antibodies in human in southern Thailand should be conducted.

## SUMMARY

Epidemic Japanese encephalitis recurs annually in the northern provinces of Thailand, but in the southern provinces cases of human encephalitis are rare. We investigated transmission of Japanese encephalitis virus (JEV) to pigs in southern Thailand. Blood specimens from one hundred young pigs at abattoirs in three southern provinces were tested for JEV hemagglutination inhibiting (HAI) antibodies. Seventy-four percent were positive. Ten seronegative sentinel pigs were placed at five locations in one southern province. Seven of the ten pigs developed JEV HAI and JEV IgM ELISA antibodies within two weeks of placement. JEV was isolated from all seven seroconverting sentinel pigs from blood specimens collected 3 to 11 days after placement. Fifteen light-trap mosquito collections at the five locations all included known JEV vectors, some in large numbers. We conclude that there is intense transmission of JEV to pigs in southern Thailand despite the rare occurrence of human encephalitis in the same region.

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