

Transmission of Japanese Encephalitis (JE) Virus in Gia Luong District, Ha Bac Province, Vietnam, After Je Vaccination, 1993–1994

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Abstract: A total of 15,183 children under 10 years old (37% of target population) was immunized with JE vaccine in Gia Luong District, Ha Bac Province, Vietnam, in 1993 to 1994. JE virus transmission was investigated by swine antibody survey and virus isolation from field mosquitoes. By the hemagglutination inhibition (HI) test, 73–90% of swine were antibody positive all year round, with high geometric mean titer (GMT) of 92.67–95.14 in May and June. By suckling mouse brain inoculation, 6 JE virus strains were isolated from *Culex tritaeniorhynchus* and *Cx. vishuni*, as well as laboratory reared F1 from field-caught *Cx. tritaeniorhynchus*. Serodiagnostics by IgM-capture ELISA (MAC-ELISA) carried out on 60 of 85 clinical encephalitis cases detected 43 positives (71.66%). All these serologically confirmed JE cases had not been vaccinated. The results supported the vaccine efficacy to prevent overt JE.

Key words: Japanese encephalitis, vaccine, Vietnam

INTRODUCTION

In Vietnam, JE has been recognized as one of the most important public health problems (Tien and Lien, 1991; Igarashi, 1992). Annually occurring JE outbreaks have been serious problems feared because of their high case fatality rate and grave sequelae accompanied by permanent neurological and/or psychiatric disorders. JE virus is a typical arbovirus transmitted in nature between susceptible vertebrates by hematophagous mosquito vectors (World Health Organization, 1988; Chen, et al., 1990; Igarashi, 1992). Because of the difficulty to control vectors and amplifying vertebrate hosts, human immunization by JE vaccine is required for the prevention of JE in Vietnam (Tien and Lien, 1991; Tien et al., 1991). In Vietnam, JE vaccine of international standard has been produced at the National Institute of Hygiene and Epidemiology (NIHE). This vaccine was used to prevent human disease in Gia Luong District, Ha Bac Province, where JE virus was isolated from patients and mosquitoes (Ha and Muou, 1965; Nga et al., 1988; Nga et al., 1992). In 1993–1994, a total 15,183 children under 10 years old (37% of target population) in Gia Luong District, was immunized with JE vaccine. In order to show the vaccine

efficacy, we carried out surveillance on JE virus transmission in this study area after JE vaccination.

MATERIALS AND METHODS

Study area: Gia Luong District, Ha Bac Province, has been a JE-endemic area in north Vietnam. Every year JE cases confirmed by MAC-ELISA have been reported in hot season from May to August, and JE virus was isolated in NIHE from patients and mosquitoes. The climate in Gia Luong is subtropical, and majority of its inhabitants are farmers cultivating rice in watered paddy fields, while swine raising was commonly practiced.

JE vaccine and vaccination: Highly purified JE vaccine produced in NIHE from infected mouse brains was used to immunize children between 1 to 10 years old in Gia Luong District from February 1993. Two shots of vaccine were given with 1 week interval as primary vaccination, followed by a booster immunization in the next year.

Swine antibody survey: In 1993–1994, blood specimens were collected from slaughtered swine of 6–8 months old, once a month. Sera were treated with acetone and examined at NIHE by the standard HI test (Clarke and Casals, 1958).

Mosquito collection: Mosquitoes were collected at Gia Luong District by Nam V. S. et al, using glass-suction tubes, from 18:00 to 22:00 o'clock. Collection was performed once a month from June 1993 to December 1994. Mosquitoes were identified and females were pooled and sent to JE Laboratory, NIHE, for virus isolation. Besides, some *Cx. tritaeniorhynchus* females were kept in the Entomology Laboratory for oviposition, and their F1 mosquitoes were used for virus isolation.

Virus isolation and identification: Each pool of mosquitoes was homogenized in a motor-driven glass homogenizer using 2ml of phosphate buffered saline containing 0.4% bovine plasma albumin Fraction V (Sigma), and was centrifuged at 2,500 rpm for 15 min at 4°C. The resulting supernatant was filtrated through Millipore HA filter (25mm diameter), and the filtrate was inoculated into brains of suckling mice (0.01ml/brain). Presence of the virus antigen in the mouse brains was screened by using standard anti-JE and anti-dengue sera. The specimens showing positive reaction was identified by the sandwich ELISA.

Serodiagnosis: Sera were collected from JE suspected cases using the diagnostic criteria of World Health Organization (1988), and examined at NIHE by MAC-ELISA (Bundo and Igarashi, 1985).

RESULTS

Antibody survey on swine population

Monthly surveillance on JE antibodies in swine sera was summarized in Table 1. Antibody positive rate was high (73–95%) all year round, with average 86%. Monthly change of the GMT of HI antibody is shown in Fig. 1. The titer was high in May and June (92–95), lowest in April (19), and around 30–62 in the rest of the months.

Table 1. Monthly surveillance on anti-JE HI antibodies among slaughtered swine in Gia Luong District, Habac–Vietnam

Year	Month	No. of sera tested	No. of sera positive	% positive	GMT
1993	Nov	20	18	90.00	40.00
	Dec	48	46	95.83	30.98
1994	Jan	48	41	85.40	34.95
	Feb	51	50	98.00	62.39
	Mar	53	45	84.90	40.64
	Apr	45	33	73.30	19.59
	May	44	33	75.00	92.67
	Jun	52	44	84.60	95.14
	Jul	43	38	88.30	37.86
	Aug	46	39	84.70	46.94
	Sep	56	46	82.10	56.57
	Oct	51	48	94.10	56.57
	Nov	54	45	83.30	45.25
Total		611	526	86.08	

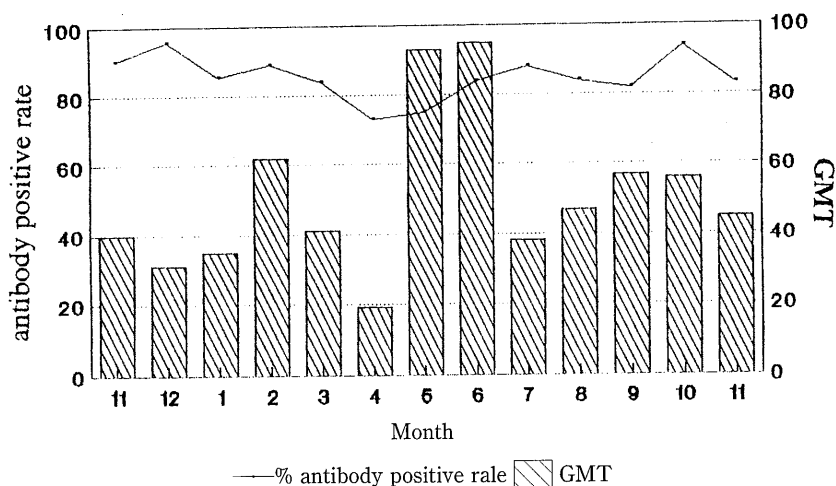


Fig. 1. Monthly change of anti-JE antibody positive rate and GMT among swine population in Gia Luong District, Habac, Vietnam 1993–1994.

Surveillance on mosquitoes

From June 1993 to December 1994, a total 1,292 mosquitoes in 37 pools were processed for virus isolation. The species composition is shown in Table 2.

Table 2. Composition of mosquito species collected in Gia Luong District, Habac Vietnam 1993–1994

Species of mosquitoes	Subtotal rate
<i>C. tritaeniorhynchus</i>	49.8%
<i>C. vishnui</i>	37.1%
<i>C. quinquefasciatus</i>	8.8%
<i>C. bitaeniorhynchus</i>	2.7%
<i>C. gelidus</i>	1.2%

The most abundant species was *Cx. tritaeniorhynchus* (49.8%), followed by *Cx. vishnui* (37.1%), and other species were less than 9% of total population. *Cx. tritaeniorhynchus* and *Cx. vishnui* were found in pigsty and human dwellings all year round and their density indices increased from April to September with a peak in July.

Result of virus isolation from these mosquitoes is shown in Table 3. In both years of 1993 and 1994, JE virus was isolated from *Cx. tritaeniorhynchus* and *Cx. vishnui*. Peculiar finding is the isolation of a JE virus strain from a pool of 7 F1 *Cx. tritaeniorhynchus* which were reared in the Entomology Laboratory of NIHE in July 1993.

Table 3. Isolation of virus from mosquitoes collected at Gia Luong District, Habac – Vietnam 1993–1994

Date of collection	Species	Pool size	Virus isolated	Code of virus strain
Jun 15. 1993	<i>C. vishnui</i>	40	JE	VN93. 117
Jun 15. 1993	<i>C. tritae</i>	31	JE	VN93. 118
July 16. 1993	<i>C. tritae</i>	35	JE	VN93. 119
July 16. 1993	<i>C. vishnui</i>	35	JE	VN93. 120
Jun 15. 1994	<i>C. tritae</i>	54	Not yet identify	VN94. 140
Jun 15. 1994	<i>C. vishnui</i>	34	JE	VN94. 141
July 1993	<i>C. tritae</i> *	7	JE	VN93. 121

Note: *C. tritae** F1 of mosquito collected in the field rearing in the Lab. Entomology.

Table 4. Number of JE patients after JE vaccination in Gia Luong District Habac— Vietnam

Year	No. of JE suspected case	No. of sera tested	No. of sera positive (%)
1993	42	19	13 (68.42)
1994	43	41	30 (73.17)
Total	85	60	43 (71.66)

Serodignosis by MAC-ELISA

Serodiagnosis was performed on 60 of the 83 clinically diagnosed encephalitis cases, and 43 of them (71.66%) showed positive reaction. All these 43 serologically confirmed JE cases were found among unvaccinated, and occurred in May, June and July.

DISCUSSION

Our results showed that in Gia Luong District JE virus has been transmitted between mosquito vectors and vertebrate hosts: swine as amplifier hosts and humans as clinical patients. Both *Cx. tritaeniorhynchus* and *Cx. vishnui* were found all year round but their density indices increased in April, when the GMT of swine antibody showed its lowest value. The low antibody level could possibly have favoured swine infection with JE virus, followed by increased GMT in May and June. The event was then accompanied by the virus isolation from vector mosquitoes in June and July. The number of human JE cases began to appear in May, increased in June, but started to decrease in July and became less in August. It is probable that when all swine population have been infected with JE virus and seroconverted, they can no longer act as amplifiers (Yamada et al., 1971). Although virus isolation was carried out from mosquitoes collected all year round, JE virus was isolated only from *Cx. tritaeniorhynchus* and *Cx. vishnui* collected in June and July. The result agrees with the report of Ha et al. (1965). The isolation of JE virus from F1 *Cx. tritaeniorhynchus* reared from field-collected females indicates transovarial transmission of JE virus in nature as postulated by Rosen (1986), and Takashima et al. (1988). Presence of antibody-positive swine all year round in the study area does not necessarily mean year round virus transmission, because the HI test can detect both IgG and IgM antibodies. Further studies are required to examine anti-JE IgM antibodies among swine, in order to correlate swine viremia with the occurrence of human JE cases.

Our demonstration of JE virus transmission in the study area after JE vaccination, and occurrence of all serologically confirmed JE cases among unvaccinated, provided supportive evidence for the efficacy of JE vaccination to prevent overt JE, confirming our previous report in Dong Anh District, Hanoi (Tien et al., 1991).

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