

## Arbovirus Infections in Pilot Areas in Laos

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**Abstract:** Since 1993, virological and seroepidemiological survey of arbovirus infections was conducted in pilot areas in Laos under the World Health Organization–Japan International Cooperation Agency–Laos trilateral Primary Health Care Project. Human sera were obtained at the laboratory in Khammouane Provincial Hospital, and at Sok Yai village in Vientiane Municipality, and antibodies to Japanese encephalitis (JE), dengue (DEN) and chikungunya (CHIK) viruses were assayed by neutralization test. The sera were classified according to the age groups. In Khammouane area, antibody–positive rates to DEN–1, 2, and 3 reached high (over 90%) by 11–15 years old and kept high thereafter. Antibody–positive rates to DEN–4 and JE increased with age and reached over 50% by 21–30 and 31–40 years old, respectively. In Sok Yai area, antibody–positive rates to DEN–2 and 3 were over 50% in all age groups. The positive rate of DEN–1 antibody reached 50% by 11–15 years old and increased with age. Although DEN–4 and JE antibody–positive rates fluctuated among age groups, they tended to increase with age. JE antibody survey in swine sera indicated that the JE virus was active during the rainy season. Positive rate of CHIK antibody tended to increase with age and reached over 50% in older age groups. These results indicate that DEN–1, 2, and 3 viruses are circulating more frequently in these areas. DEN–4, JE and CHIK viruses also exist with less activity.

In terms of the primary health care for the prevention and control of arbovirus infections, the project team conducted health education and environmental sanitation in Sok Yai village. They also organized several training courses on the diagnosis and treatment of JE and DEN for junior doctors, health workers, and laboratory technicians.

*Key words:* Dengue, Japanese encephalitis, seroepidemiology, primary health care.

## INTRODUCTION

Japanese encephalitis (JE) is one of the life-threatening diseases in the south-east Asian countries, transmitted by mosquitoes, mainly *Culex tritaeniorhynchus* (Shope, 1980, Igarashi, 1992a). Although there is no report of the isolation of JE virus in Laos, our previous serological survey in Vientiane suggested the existence of JE virus in this country (Bunlu *et al.*, 1992). On the other hand, in 1987, a large epidemic of dengue fever (DF)/dengue hemorrhagic fever (DHF) occurred during the rainy season in Vientiane. A total of more than 6,000 hospitalized cases with high morbidity rate was reported to the Ministry of Public Health (Bunlu *et al.*, 1992; Fukunaga *et al.*, 1993). In the same year, large outbreaks of DF/DHF were also reported in Thailand, Vietnam and Myanmar (Halstead, 1992). Since then, small outbreaks of dengue have been reported in Laos. *Aedes aegypti* may play a major role in the transmission of this disease in this area (Fukunaga *et al.*, 1993). However, few virological and serological studies were conducted. Chikungunya (CHIK) virus infection, also transmitted by *Aedes aegypti*, causes dengue-like symptoms (Casals and Clarke, 1965). Although there were reports of CHIK infection in South-east Asia and Africa (Hammon *et al.*, 1960), the situation in Laos remained unknown.

Since 1993, virological survey and control of mosquito-borne viral diseases had started at the pilot areas in Laos as a part of World Health Organization-Japan International Cooperation Agency-Laos collaborating Primary Health Care Project. As the first step of the Project, seroepidemiological survey of JE, DEN and CHIK virus infections were conducted to understand the situation of the infections due to these viruses. In this report, we show the results of seroepidemiological studies of JE, DEN and CHIK in two pilot areas and the preventive measures on the primary health care basis.

## MATERIALS AND METHODS

*Pilot areas:*

One pilot area was called Sok Yai village, located at about 11km north-east from the center of Vientiane. It belongs to the Vientiane Municipality (Fig. 1) Population of the village is about 1,100 inhabitants with about 200 families. Majority of the family are farmers. There are large rice fields around the village. Some areas are irrigated and two-crop fields, while other areas are one-crop fields. It was said that in 1989, five children living in and around this village were afflicted with JE infection. Anti-JE IgM was detected by IgM-capture ELISA. Another pilot area was Thakhek, Khammouane province, about 350km south-east from Vientiane (Fig. 1). Majority of the inhabitants in this area are also farmers.

*Serum specimen:*

Total of 148 human sera were collected from the inhabitants of Sok Yai village, and 141 human sera were collected at the laboratory of Khammouane Provincial Hospital, Thakhek.

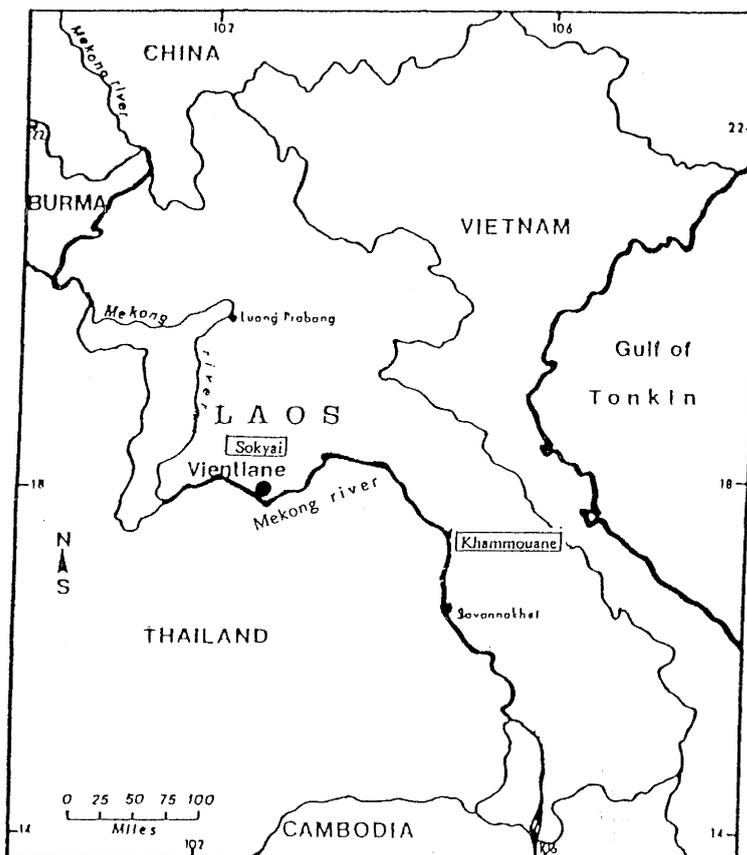


Fig.1 Map of Lao PDR. The pilot areas are indicated with square

Swine sera were collected at a slaughterhouse from January 1993 to July 1994 from those born and grown in Vientiane Municipality (average 27 sera per month). The sera were also collected from two baited newborn swine regularly from April 1993 to November 1993. All the sera were heat inactivated at 56C for 30 min before use.

*Neutralization (N) test:*

Fifty percent focus-reduction N test on BHK-21 cells in 96-well microplate using peroxidase-anti-peroxidase (PAP) staining method was employed for the DEN- and JE-antibody titrations (Okuno *et al.*, 1985; Ishimine *et al.*, 1987), and 50% plaque reduction N test on Vero cells was employed for CHIK antibody titration.

*Enzyme-linked immunosorbent assay (ELISA):*

JE antibody in swine sera were assayed by ELISA (Bundo *et al.*, 1982).

## RESULTS AND DISCUSSION

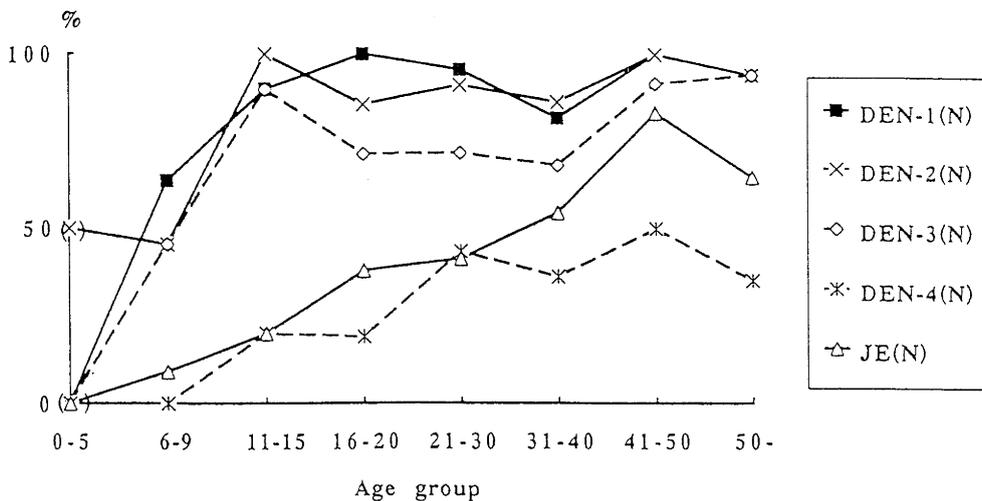
The human sera were classified according to the age groups (Table 1). The number of sera in some age groups were not enough. The numbers of sera less than 10 were shown with parenthesis.

**Table 1.** Number of specimens tested

Age group	Sokyai	Khammouane
0-5	(9)	(2)
6-10	60	11
11-15	20	10
16-20	(9)	21
21-30	20	46
31-40	19	22
41-50	11	12
51-yrs	0	17
Total	148	141

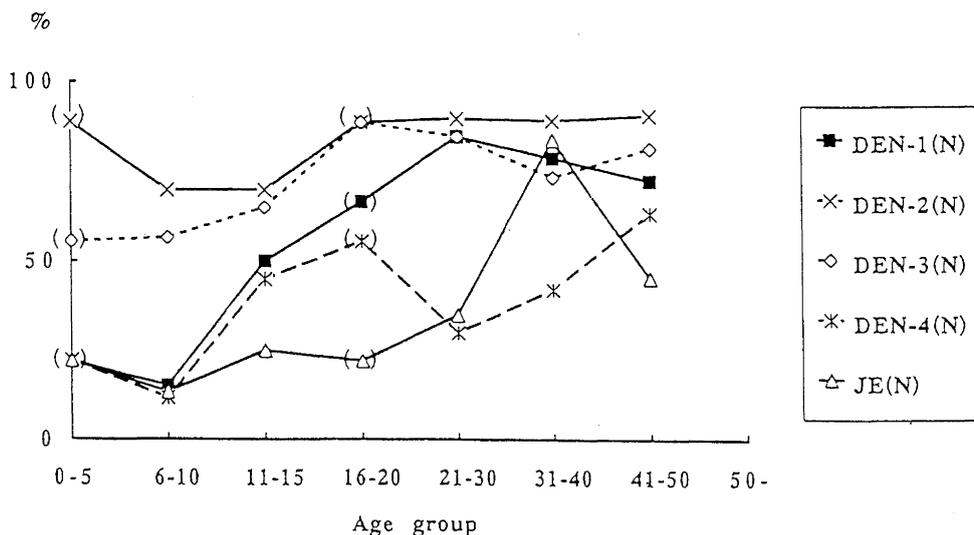
( ) indicates the number of specimens less than 10.

Fig. 2 shows the positive rates of antibodies to DEN and JE viruses in Khammouane province. The positive rates to DEN type 1, 2, and 3 show similar patterns and reach high (over 90%) by 11-15 years old and keep high thereafter. JE and DEN type 4 antibody-positive rates increase rather slowly. JE antibody is detected in over 50% of the adults older than 31 years.



**Fig. 2.** Positive rates of N antibodies to DEN and JE viruses in different age groups in Khammouane  
 Parenthesis indicates the number of specimens less than 10.

In Sok Yai, the patterns are somewhat complicated (Fig. 3). Positive rates of DEN type 2 and 3 antibodies seem to be high. The positive rates to other DEN serotypes and JE tend to increase with age, but they are not as clear as those of Khammouane province. There were several sera from younger age groups (6–10 and 11–15 years old) whose JE antibody titers showed over 1,000, which suggested inapparent infection with JE virus.



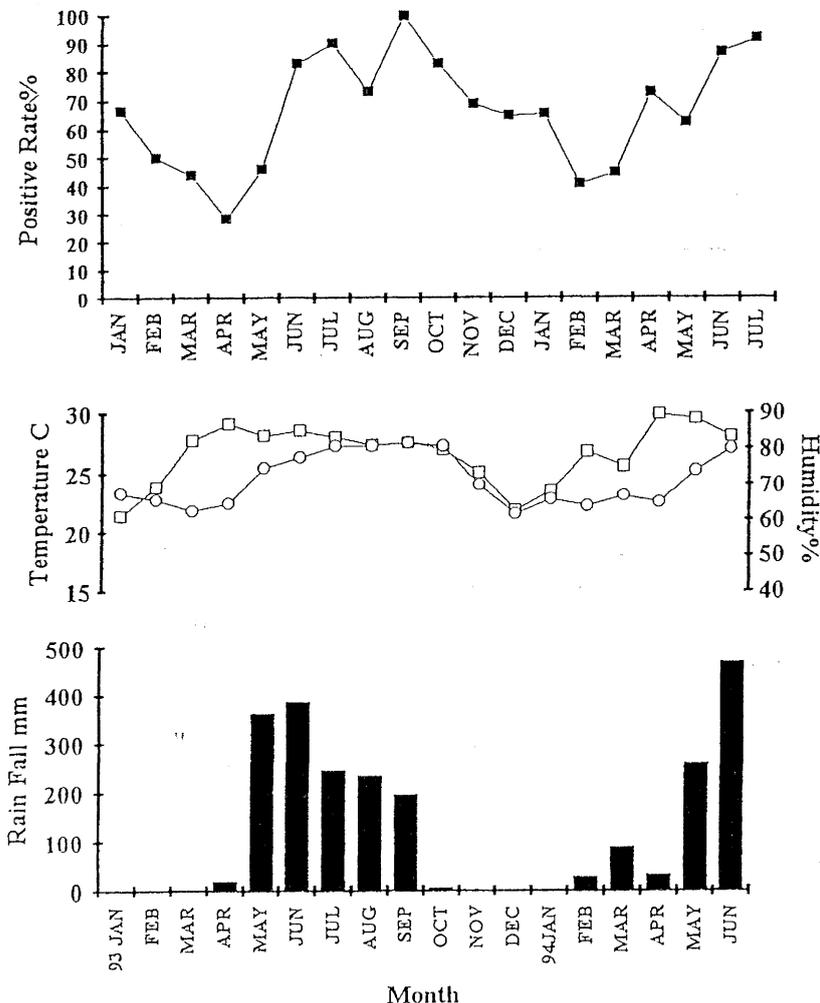
**Fig. 3.** Positive rates of N antibodies to DEN and JE viruses in different age groups in Sok Yai  
Parenthesis indicates the number of specimens less than 10.

The main amplifier of JE virus is swine (Igarashi, 1992a). JE virus dissemination in nature has been monitored by antibody survey of slaughtered swine blood in Japan. Therefore, in order to study the activity of JE virus, we collected swine sera at the slaughterhouse every month and tested for JE antibody by IgG-ELISA. Fig. 4 shows the monthly fluctuations of JE antibody-positive rate in swine sera together with meteorological data. The rainy season started late in April in 1993. JE antibody-positive rate was over 50% during June 1993 through January 1994, and April through July. Positive rate was low during dry season. The rises in JE antibody titer in two baited newborn swine were observed in July and August (Fig. 5). These results may indicate the existence of JE virus in these areas and its activity during rainy season. Virus isolation from the swine sera was not successful. Surveillance of vector mosquito (*Culex tritaeniorhynchus*) in Sok Yai village is now under way.

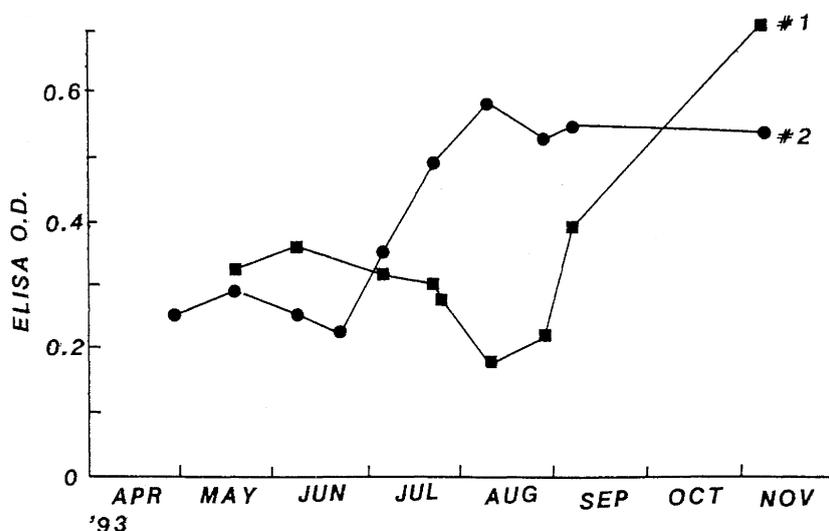
Although JE virus seemed to exist in these areas, the activity of JE virus seemed to be rather low and many children didn't have JE antibody. According to the Ministry of Agriculture, the Government has plan to develop irrigation field. According to the five-year plan (1990–1995), it would expand over 1.5-fold in Vientiane area, and 4-fold in Kham-

moane area. Since 1960's, the outbreaks of acute encephalitic syndrome or viral encephalitis were reported in the surrounding countries, such as China, Vietnam and Thailand (Igarashi, 1992a, b). There were reports that about 30% of acute encephalitic syndrome or viral encephalitis showed anti-JE HI antibody-positive (Igarashi, 1992a). Expanding of irrigation and increased swine raising may provide increased JE transmission, leading to outbreaks of JE infection. We are planning to continue JE survey by patient survey and swine serum screening.

Positive rate of DEN antibody was high since childhood. Antibodies to DEN type 1, 2 and 3 were high. In 1994, DEN epidemic started since May. We had isolated several DEN



**Fig. 4.** Monthly fluctuations of JE antibody-positive rate in swine sera and meteorological data  
The sera were collected at a slaughterhouse in Vientiane and JE antibody was assayed by ELISA.

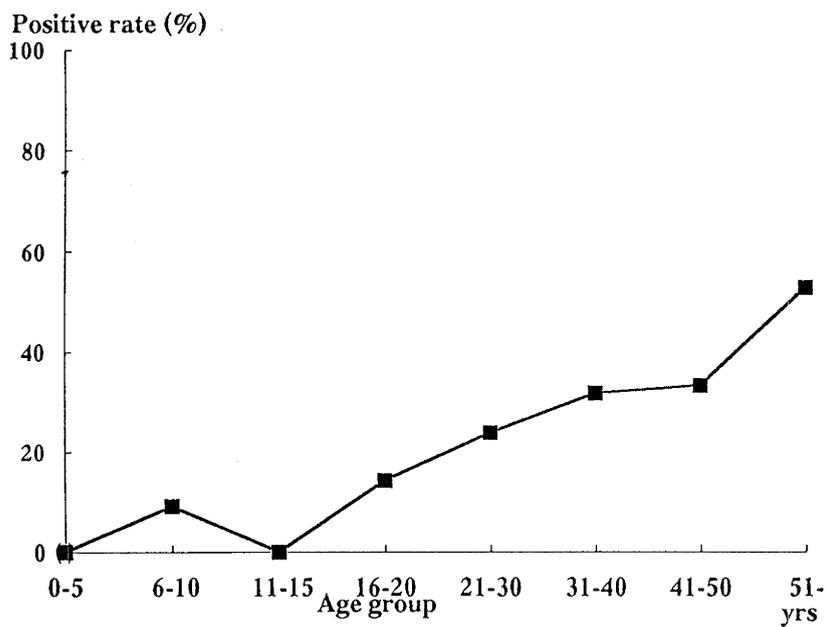


**Fig. 5.** Temporal changes of JE antibody titers in baited swine sera. The sera were collected from two newborn swine every month and JE antibody was assayed by ELISA.

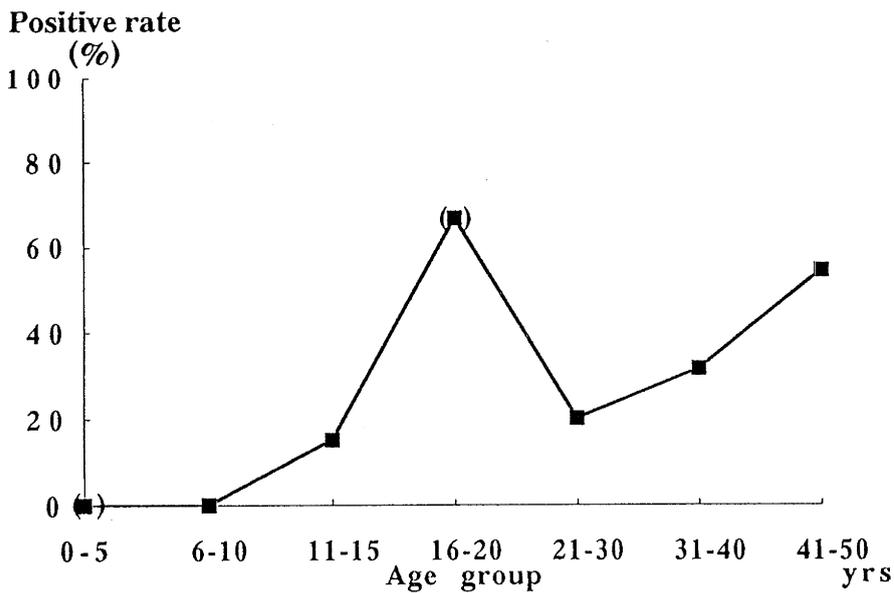
type 1 and type 2 viruses from the patient with clinical DEN and patients with high fever of unknown origin, using mosquito cells (C6/36).

Figs. 6 and 7 show the positive rates of N antibody to CHIK virus in Khammouane province and Sok Yai village, respectively. CHIK antibody—positive rates increased with age and reached 50% in older age groups. Although the positive rates were not as high as DEN antibody in both areas, this virus also seemed to exist in these areas. The vector of this virus is probably *Aedes aegypti*, the same vector as DEN virus. Since the symptom of CHIK virus infection is similar to those of DEN virus (Casals and Clarke, 1965), infection with this virus might be diagnosed as DEN infection. Detailed survey is now under way.

On a Primary Health Care basis, the project team, with the cooperation of the village chief, teachers, health staffs, and others, conducted health education. They held a so-called "health festival" and explained the role of mosquitoes on JE and DEN infections by playing drama, and singing molam, the traditional Lao song. They encouraged the farmers to keep their livestock apart from their houses, in the evening before having dinner, to fill a house with smoke to let mosquitoes away, and to use repellent soap. Now, it was said that all the families use mosquito net when sleep. The team also prepared booklets in Lao language and organized several training courses for junior doctors and laboratory staffs of main provincial hospitals, and trained how to diagnose and treat DEN and JE patients. This project will continue for 5 years.



**Fig. 6.** Positive rate of N antibody to CHIK virus in different age groups in Khammouane  
 Parenthesis indicates the number of specimens less than 10.



**Fig. 7.** Positive rate of N antibody to CHIK virus in different age groups in Sok Yai  
 Parenthesis indicates the number of specimens less than 10.

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