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Seroepidemiological study of arbovirus infections in Khammouane Province, Lao PDR

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ABSTRACT

Seroepidemiological survey of three arbovirus infections were conducted at Khammouane Province, Lao P.D.R. A total of 141 human sera were collected at the laboratory of Khammouane provincial hospital and examined for the antibodies to dengue (DEN), Japanese encephalitis (JE) and chikungunya (CHIK) viruses by neutralization test. Positive rates of antibodies to DEN-1, 2 and 3 were high in the age groups older than 11 - 15 years old, while that of antibody to DEN-4 increased rather slowly with age. JE antibody-positive rate also increased with age and reached over 50% in 31 - 40 age group. CHIK antibody-positive rate increased with age and reached 50% in the age group over 51 years old. The results indicate that DEN viruses were widely circulating in this area. Although JE does not appear to be a big problem at the moment in this area, the circumstances indicate the possibility of becoming a JE epidemic area in future. Epidemiological surveillance must be continued. *Ryukyu Med. J., 15(1)19~22, 1995*

Key words: seroepidemiology, dengue, Japanese encephalitis, chikungunya, Lao, PDR

INTRODUCTION

The Lao People's Democratic Republic (Lao PDR), a landlocked country, located in Indochina. The lifestyle as well as climate in Lao PDR is similar to those of the neighboring countries, such as Thailand, Vietnam and Cambodia. In 1987, a large epidemic of dengue (DEN) fever (DF)/dengue hemorrhagic fever (DHF) occurred during the rainy season (May-September) in Vientiane, Lao PDR¹. In the same year, large outbreaks of DF/DHF were also reported in Thailand, Vietnam and Myanmar². Since then, small outbreaks of DEN have been reported^{1,3}. *Aedes aegypti* play a major role in the transmission of this disease. Chikungunya (CHIK) virus is also transmitted by *Aedes aegypti*. The disease induced by this virus shows DEN-like symptom⁴. Although there were reports of CHIK infection in south-east Asia, the situation in Lao PDR remained unknown⁵. On the other hand, in 1989, five Japanese encephalitis (JE) cases were confirmed in Vientiane areas. However, few virological and serological survey has been done. *Culex tritaeniorhynchus* is the main vector of JE virus transmission⁶. The main breeding site of the mosquito is irrigated rice field. Swine is the most susceptible animal, thus serving as the most important animal reservoir⁷. We first conducted seroepidemiological studies on DEN and JE

virus infections in Vientiane area in 1990⁸. However, the situation in other places in Lao PDR was not known.

In this study, we made a serological survey of arbovirus infection in Khammouane province under the Japan International Cooperation Agency-World Health Organization-Lao PDR trilateral collaborating Primary Health Care (PHC) Project. In this paper we describe the results of serological tests of DEN, JE and CHIK infections in Khammouane province.

MATERIALS AND METHODS

Study area

Khammouane province is one of the seventeen provinces in Lao PDR and located at about 350km south-east from Vientiane (Fig. 1). The estimated population is about 210,000. Majority of the inhabitants are farmers. Khammouane Provincial Hospital is in Thakhek, the only city in the province.

Serum specimens

Human sera were obtained at the laboratory of Khammouane Provincial Hospital during the period of January to June, 1993. The sera were diluted 1:20 with Eagle's minimum essential medium (MEM) containing 2% fetal calf

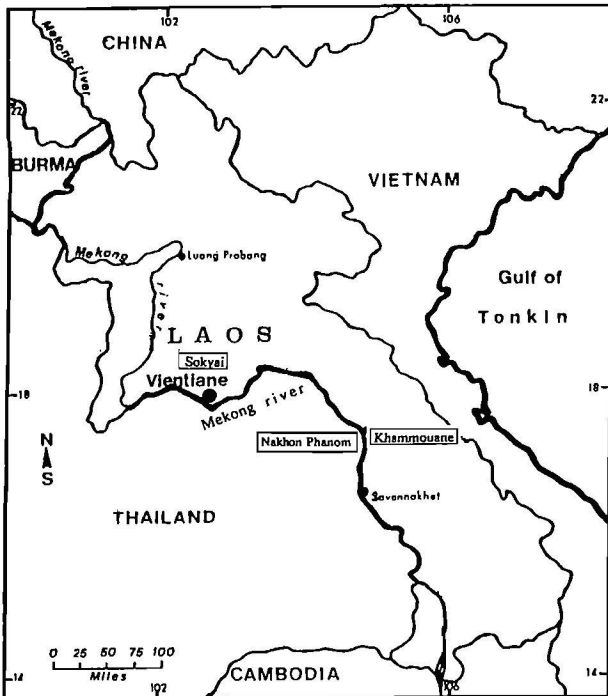


Fig. 1 Map of Lao PDR

serum (FCS) and antibiotics, heat-inactivated at 56 °C for 30 min and stored at -20 °C until used.

Cells and viruses

BHK-21 and Vero cells were grown in Eagle's MEM containing 5% FCS. In the maintenance medium, FCS was reduced to 2%. Stock viruses of DEN-1 (Hawaiian), DEN-2 (New Guinea B), DEN-3 (H087), DEN-4 (H-241), and JE (Nakayama) were prepared as 10% suckling mouse brain suspensions and CHIK (B3) was prepared from infected Vero cell culture.

Neutralization (N) test

For the assay of DEN and JE N antibody titers, fifty percent focus-reduction N test on BHK-21 cells in 96-well microplate using peroxidase-anti-peroxidase staining method was used. For the assay of CHIK antibody, fifty percent plaque reduction N test on Vero cells was used. The sera were first screened for N antibody at a serum dilution of 1:20. The sera with a focus reduction rate of 75% or more were serially diluted and reacted with the virus as above. The reciprocals of serum dilutions giving 50% focus reduction was defined as the N antibody titer^{9,10}. The N titers less than 20 was defined negative.

RESULTS AND DISCUSSION

A total of 141 sera were collected at the laboratory of Khammouane Provincial Hospital. They were classified according to the age groups (Table 1). The number of the youngest age group was not enough for evaluation, and is enclosed in parenthesis.

Table 1 Number of serum specimens tested

Age group	No. of specimens
0 - 5	(2)
6 - 10	11
11 - 15	10
16 - 20	21
21 - 30	46
31 - 40	22
41 - 50	12
51 - yrs	17
Total	141

() indicates the number of specimens less than 10.

Fig. 2 shows the percent incidences of N antibodies to DEN type 1 - 4 and JE viruses in different age groups. Positive rates of N antibodies to DEN-1, 2 and 3 increased with age, rapidly reaching over 90% by 11 - 15 age group and kept thereafter, while that of JE antibody increased rather slowly with age, reaching over 50% by 31 - 40 years old and increased thereafter. The positive rate of DEN-4 antibody also increased with age. However, there was no age group that showed over 50%. Fig. 3 shows the geometric means of N antibody titers (GMT) to four serotypes of DEN and JE viruses in different age groups. The GMT's of N antibody to DEN-1 and DEN-2 was high by 11 - 15 years old and maintained similar (N titer of about 300) thereafter, indicating that these DEN serotypes were dominant in this area. N antibodies to DEN-4 and JE viruses were relatively low. However, there were five cases in which JE antibody titers showed over 1,000. This may indicate that JE virus exists in this area, although it has not become epidemic. Antibody level to DEN-3 was intermediate, with the mean titers between 10 - 200.

Fig. 4 shows the percentages of serum specimens with no N antibody, monotype, two-type, and three- or four-type antibodies to DEN viruses. The percentage of the groups with multi-type antibodies rose to about 90% by the age of 11 - 15 years old, suggesting that different serotypes of DEN viruses are widely distributed in this area. The main type of the monotypic group was DEN-2 virus.

Fig. 5 shows the relative incidences of N antibodies to DEN and JE viruses. Serum specimens were classified into 4 groups with respect to the presence or absence of N antibodies to DEN and JE viruses. The proportion of the specimens with positive DEN antibodies and negative JE antibody was between 50 - 80% in younger age groups (i.e., from 0 - 5 to 31 - 40 age groups). The proportion of the specimens positive for both JE and DEN antibodies increased with age and reached 83.3% in 41 - 50 age group. The group with positive JE and negative DEN antibodies was not seen. This indicates that compared with DEN viruses, JE

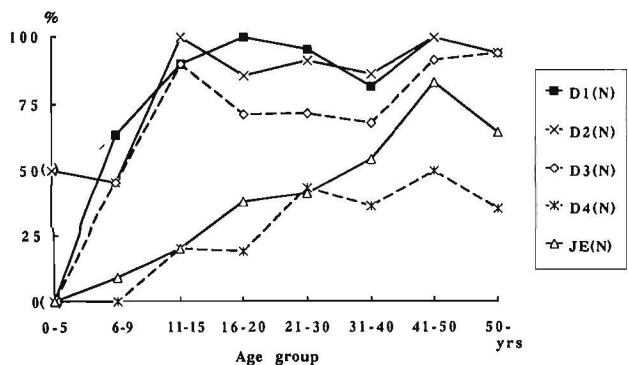


Fig. 2 Positive rate of N antibodies to DEN and JE viruses in different age groups. Parenthesis indicates the number of specimens less than 10.

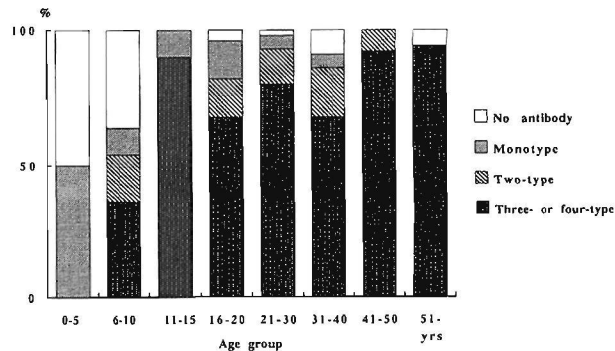


Fig. 4 Relative frequency of specimens possessing no antibody, monotype antibody, and multitype antibodies to DEN viruses in different age groups.

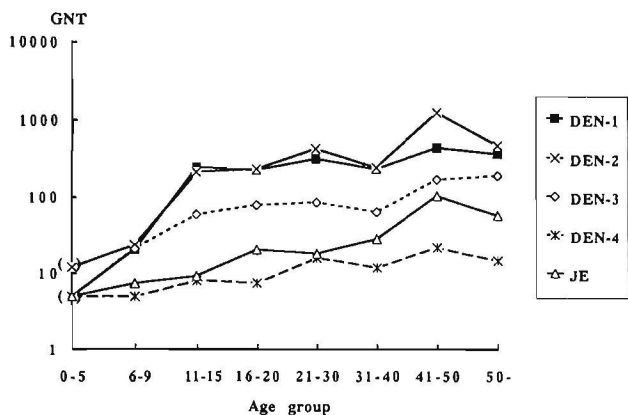


Fig. 3 Geometric mean titers of N antibodies to DEN and JE viruses in different age groups. Parenthesis indicates the number of specimens less than 10.

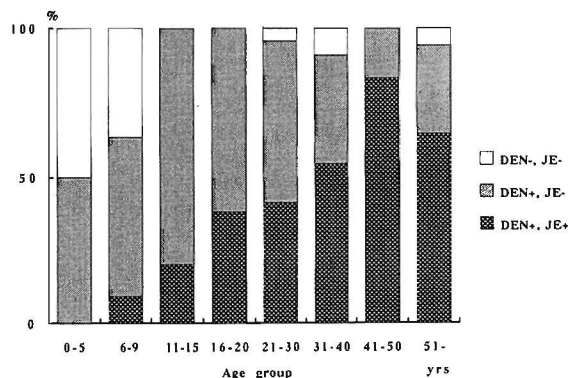


Fig. 5 Relative frequency of specimens possessing antibodies to JE and/or DEN viruses in different age groups. J : JE virus antibody; D : DEN virus antibody; + : antibody positive; - : antibody negative.

virus is still less widely spread in this area.

Fig. 6 shows the incidences of N antibody to CHIK virus in different age groups. The positive rate reached over 50% in senior age group (over 51 years). The incidence was lower than those of DEN and JE antibodies. It is evident that this virus is present in this area. To our knowledge, this is the first report of the presence of CHIK in this country. The symptom of CHIK virus infection is reported to be similar to that of DEN virus infection⁴⁾. Therefore, most of the CHIK infection must have been misdiagnosed clinically as DEN infection.

According to the five-year plan issued by the Lao PDR Government, the irrigation field in Khammouane Province would be expanded over 400% during 1990 through 1995. Since 1960's, the outbreaks of acute encephalitic syndrome or viral encephalitis have been reported in the surrounding countries, such as China, Vietnam and Thailand^{7, 11, 12)}. There were reports that about 30% of acute encephalitic syndrome or viral encephalitis showed anti-JE HI antibody-positive⁷⁾. Expansion of irrigation and increased swine raising may provide increased JE transmission, leading to out-

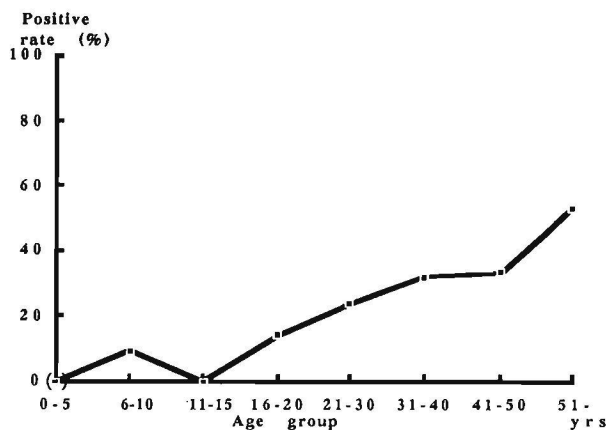


Fig. 6 Positive rate of CHIK virus antibody in different age groups. Parenthesis indicates the number of specimens less than 10.

breaks of JE infection. Mass vaccination against JE has not yet started. Thus, most children in this area are susceptible to JE virus. We are planning to continue JE survey by patient survey and swine serum screening.

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