

## Epidemiological Situation of Dengue Infection in Lao P. D. R.

Toshihiko FUKUNAGA<sup>1,2</sup>, Bounlay PHOMMASACK<sup>4</sup>, Khanthong BOUNLU<sup>5</sup>,  
Mika SAITO<sup>1</sup>, Masayuki TADANO<sup>1</sup>, Yoshihiro MAKINO<sup>1,2</sup>,  
Kazumi KANEMURA<sup>1</sup>, Sakae ARAKAKI<sup>1</sup>, Masaki SHINJO<sup>3</sup>  
and Sithat INSISIENGMAY<sup>5</sup>

<sup>1</sup> *Department of Virology,*

<sup>2</sup> *Research Center of Comprehensive Medicine,*

<sup>3</sup> *Department of Preventive Medicine, Faculty of Medicine, University of the Ryukyus, 207, Uehara, Nishihara, Okinawa, 903-01, Japan*

<sup>4</sup> *Aedes Control Unit, Vientiane Municipality Health Service, Lao P. D. R.*

<sup>5</sup> *National Institute of Hygiene and Epidemiology, Km 3 Thadeua Road, Vientiane, Lao P. D. R.*

**Abstract :** In 1979, 37 cases of dengue hemorrhagic fever (DHF) were reported to the Ministry of Health for the first time in Laos. In 1985, a total of 1,759 cases of DHF was reported as the first epidemic. In 1987, larger epidemics of DHF occurred with a total of 6,567 cases in the Vientiane Municipality and a total of 3,098 cases in four provinces. The morbidity rate of the outbreak in Vientiane was as high as 1,530/100,000.

In the end of 1988, an *Aedes* Control Unit (ACU) was set up in the Vientiane Municipality to reduce the *Aedes* larva density and strengthen health education to the residents. Since 1989, DHF epidemic such as seen in 1985 or 1987 has not occurred in Vientiane so far. Activities of the ACU seem to be efficacious in reducing the larva density, judged by the Breteau and House indices obtained. However, another approach to show the reduction of dengue virus transmission has not been tried yet.

In 1990, a seroepidemiological study on inhabitants in the Vientiane Municipality was performed. The results indicated followings : (1) The percentage prevalences of neutralizing (N) antibodies to dengue (DEN) 1-4 viruses increased with age, but the prevalences of N antibodies to DEN 3 and DEN 4 were lower than those to DEN 1 and DEN2 viruses. (2) The geometric mean titer of N antibody to DEN 2 was highest among 4 serotypes in every age group. (3) By the age of 15 years, the majority (90%) of the inhabitants were infected with two or more serotypes of DEN virus.

*Key words :* Dengue hemorrhagic fever (DHF), Epidemiology, *Aedes* vector control, Laos

### INTRODUCTION

Lao People's Democratic Republic (PDR) is a landlocked country with a total

population of approximate 4.2 millions and borders on China, Myanmar, Thailand, Cambodia and Vietnam. Plain areas along the Mekong River, facing Thailand, are suited to agriculture. More than 50% of the total population are living in these areas, such as Vientiane Municipality, Khammouane, Savannakhet and Champassak Provinces, where large epidemics of dengue hemorrhagic fever (DHF) occurred in 1987. In Vientiane Municipality, people had experienced DHF epidemic on a smaller scale in 1985 but the outbreak of DHF in 1987 was more than five times as many as the number of cases in 1985 and about 4,500 cases of children with DHF were hospitalized in June and July concentrically.

In Lao PDR, there are difficulties in collecting information on infectious diseases, particularly outside Vientiane Municipality, since reporting system of the Health Administration is not working well and a laboratory in the National Institute of Hygiene and Epidemiology (NIHE), which is the only one laboratory for virology in Lao PDR, is not functioning well due to serious shortage of budget and poor equipment.

This paper reports the epidemiological situation of DHF in Lao PDR with the epidemics occurred, activity of the vector mosquito control and some data of a sero-epidemiological study.

#### MATERIALS AND METHODS

**Data of dengue hemorrhagic fever (DHF) outbreak occurred in Laos :** Numbers of DHF cases and other epidemiological data were obtained from the National Institute of Hygiene and Epidemiology (NIHE), Institute of Malariology, Parasitology and Entomology (IMPE), Ministry of Health, and Vientiane Municipality Health Service.

**Vector mosquito control in Vientiane Municipality :** In order to reduce density of the vector mosquito (*Aedes aegypti*) larva, an *Aedes* Control Unit (ACU) was set up in Vientiane Municipality in the end of 1988. The main activity of ACU is to survey density of the *Aedes* larva and monitor and follow up it in houses, together with advice and encouragement to make efforts. Vientiane Municipality has an approximate population of 430 thousands and is divided administratively into eight, four urban and four rural, districts. The activity of ACU has been concentrated more intensively on the four urban districts (Chanthabury, Sikhottabone, Xaysettha and Sisattanak), which are considered high risk areas of DHF. Works have been conducted by ACU staffs, but school children, health workers and Red Cross volunteers have been trained for *Aedes* larva survey to supplement shortage of ACU staffs. In each district, several hundreds of houses have been usually surveyed for the larva per month, except October, November and December in some years. The density of *Aedes* larva was evaluated by the Breteau and House Indices.

Breteau Index (BI) :      Number of water containers with *Aedes* larva per 100 houses surveyed.

House Index (HI) :      (Number of houses with *Aedes* larva positive-water container/Number of houses surveyed) × 100

**Table 1.** Number of serum specimens tested.

Age group (years old)	Number of specimens
0 - 5	12
6 - 10	18
11 - 15	17
16 - 20	17
21 - 30	20
31 - 40	20
41 - 50	18
51 -	19
Total	141

In comparison of the average BI and HI in January to June with those in July to December, 1987, in each district, the significant differences were examined by *t*-test and chi-square-test for BI and HI, respectively. In comparison of the average BI and HI in January to June, 1987, with those in 1989 to 1992, examination of significant differences was performed for both indices by analysis of variance first and then by Tukey's multiple comparisons method (Tukey, 1953 ; Kramer, 1956), using computer program of the Statistical Analysis System (SAS ; SAS Institute Inc., NC, USA).

**Serological study on residents in Vientiane Municipality :** A total of 141 serum specimens were collected by age groups at the Mahosot Hospital, NIHE and Blood Bank in Vientiane during February to March, 1990. Number of specimens tested is shown by age groups in Table 1. The specimens were examined for the neutralization (N) antibody titers to dengue (DEN) type 1 (Hawaiian strain), type 2 (New Guinea B strain), type 3 (H-87 strain) and type 4 (H-241 strain) viruses by focus reduction N tests, using peroxidase-anti-peroxidase (PAP) staining technique, as described elsewhere (Okuno *et al.*, 1985 ; Ishimine *et al.*, 1987)

## RESULTS

**Outbreak of dengue hemorrhagic fever (DHF) in Laos :** Since 1979, when 37 DHF cases were reported for the first time to the Ministry of Health, two large epidemics have occurred with a total of 1,774 cases (15 deaths) in 1985 and a total of 9,699 cases (295 deaths) in 1987, so far (Table 2). In the epidemics in 1987, the highest morbidity rate of DHF was observed in Vientiane Municipality and the highest mortality rate was in Champasak Province, as shown in Table 3. Numbers of DHF reported cases and deaths of residents in Vientiane Municipality by month and year are shown in Table 4. In the DHF outbreak

**Table 2.** DHF reported cases in Laos, 1983-1987.

	1983	1984	1985	1986	1987
Cases	404	22	1,774	365	9,699
(Deaths)	(5)	(14)	(15)	(43)	(295)

Source : IMPE

**Table 3.** Number of DHF reported cases in Laos in 1987.

Province/municipality	DHF inpatient	Deaths
Vientiane Municipality	6,567	(91)
Vientiane Province	34	(1)
Champassak Province	1,844	(178)
Savannakhet Province	1,042	(23)
Luang Prabang Province	113	(0)
Khammouane Province	99	(2)
Total	9,699	(295)

Source : IMPE

**Table 4.** DHF inpatient cases of residents in Vientiane Municipality by month and year.

Year Month	1985	1986	1987	1988	1989	1990	1991	1992	1993
Jan.	0	2(0)	7(1)	3(0)	3(1)	0	0	0	0
Feb.	0	3(0)	16(0)	2(0)	9(0)	1(0)	2(1)	2(0)	0
Mar.	0	5(0)	9(0)	6(0)	3(0)	0	4(0)	0	0
Apr.	0	12(0)	13(2)	11(0)	13(0)	0	1(0)	1(0)	0
May.	5(0)	16(0)	361(10)	19(0)	19(0)	0	2(0)	0	0
Jun.	12(0)	17(0)	2,052(27)	9(1)	14(0)	3(0)	4(0)	1(0)	8(0)
Jul.	37(3)	29(0)	2,405(37)	3(0)	11(0)	6(0)	17(0)	3(0)	9(0)
Aug.	95(5)	16(0)	221(4)	2(0)	6(0)	30(0)	37(0)	3(0)	11(0)
Sep.	532(4)	14(0)	131(6)	12(0)	6(0)	35(0)	24(0)	3(0)	
Oct.	395(2)	7(0)	17(0)	2(0)	2(0)	5(0)	2(0)	0	
Nov.	NA	NA	NA	NA	NA	NA	0	0	
Dec.	NA	NA	NA	NA	NA	NA	0	0	
Total	1,076(14)	121(0)	5,232(87)	69(1)	86(1)	80(0)	93(1)	13(0)	28(0)

Source : Vientiane Municipality Health Service.

( ): deaths

NA : not available

in 1985, the largest number of cases was reported in September and the second was in October, while in the outbreak in 1987, the largest number was recorded in July and the second was in June. The rainy season usually begins in May and ends in October in Laos. Since 1988, no large outbreak has been reported in Vientiane Municipality (Table 4). Table 5 shows the age distribution of DHF cases reported from hospitals in Vientiane Municipality during 1990 to 1992. The majority (86.2%) of DHF cases were under 15 years old, with high distributions in the age groups of 5-9 and 10-14 years old.

**Efficaciousness of vector control activity in Vientiane Municipality:** In 1987, a total of 23,970 houses were surveyed in the four urban districts in Vientiane Municipality. The average number of houses surveyed per month in each district was 499. Breteau Index (BI) and House Index (HI) by month in four districts in 1987 are shown in Table 6. Marked decreases of both indices were observed after July in all four districts, particularly in Xaysettha and Sisattanak. This seems to be explained as follows: A large outbreak of DHF took place in Vientiane in June and July concentrically in 1987 (Table 4), and people were afraid of the outbreak very much and began to make possible efforts to reduce the

**Table 5.** Age distribution of DHF cases in Vientiane Municipality

Age group (yr.)	1990	1991	1992 (-Sep.)	Total
0 - 1	6	7	8	21 ( 5.0%)
2 - 4	6	20	44	70 (16.8%)
5 - 9	15	68	54	137 (32.9%)
10 - 14	26	92	13	131 (31.4%)
15 - 24	4	42	2	48 (11.5%)
25 -	2	3	4	9 ( 2.1%)
Total	59	232	125	416

Source: NIHE

**Table 6.** BI and HI by month in four districts in 1987

District	Index	Jan.	Feb.	Mar.	Apr.	May.	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
Chanthabury	BI	37	70	113	63	106	46	23	22	44	48	25	40
	HI	23	36	66	36	63	36	16	17	32	44	20	35
Sikhottabone	BI	60	50	56	90	130	46	46	20	8	8	20	35
	HI	39	36	43	44	80	40	34	20	8	16	15	40
Xaysettha	BI	53	20	47	60	50	80	2	0	12	13	4	44
	HI	46	16	36	47	30	64	2	0	8	12	8	23
Sisattanak	BI	88	76	77	56	60	100	0	0	80	65	30	23
	HI	46	46	53	56	40	53	0	0	36	50	20	20

*Aedes* larva density according to the advice and encouragement of the Municipality Health Service. On the assumption that 100 houses had been surveyed per month in each district, average BI and HI in January to June were compared with those in July to December, 1987 (Table 7). The significant differences between them were confirmed in each district ( $P < 0.05$ ). The average BI and HI in January to June, 1987, were regarded as the standard level of *Aedes* larva density without the vector control activity and were compared with those in January to June in 1989 to 1992 in each district (Table 8). Significant differences were demonstrated for both BI and HI in each district in these years ( $P < 0.05$ ), except the HI in Sikhottabone district in 1992. Results indicate that the ACU activity has been efficacious in reducing density of *Aedes* larva in the four urban districts in Vientiane Municipality.

**A seroepidemiological study on residents in Vientiane Municipality :** The prevalences of neutralizing (N) antibodies to DEN 1-4 viruses increased with age as shown in Fig. 1. The N antibody prevalences to DEN-1 and DEN-2 rose to 82% and 88%, respectively, by the age of 11-15 years old and reached 100% by the age of 21-30 years old, while those to DEN-3 and DEN-4 appeared to be lower and did not reach 100% in any age group.

Fig. 2 shows the geometric means of N antibody titers (GMTs) to four serotypes of

**Table 7.** Comparison of average BI and HI in Jan. to Jun. with those in Jul. to Dec., 1987

District	Index	Jan.-Jun.	Jul.-Dec.
Chanthabury	BI	72.5	33.7
	HI	44.0	27.3
Sikhottabone	BI	72.3	22.8
	HI	47.3	22.2
Xaysettha	BI	51.7	12.3
	HI	40.2	8.8
Sisattanak	BI	76.2	33.0
	HI	49.0	21.3

**Table 8.** Comparison of average BI and HI in Jan. to Jun., 1987, with those in 1989 to 1992.

Year	Chanthabury		Sikhottabone		Xaysettha		Sisattanak	
	BI	HI	BI	HI	BI	HI	BI	H
1987	72.5	44.0	72.3	47.3	51.7	40.2	76.2	49.0
1989	11.2	8.0	20.5	12.0	13.3	10.2	9.8	7.5
1990	20.0	12.3	9.7	8.0	13.2	13.9	5.5	5.7
1991	13.6	11.6	11.8	10.8	10.5	9.3	5.5	5.3
1992	10.6	10.4	21.4	28.2	11.4	8.0	6.5	5.3

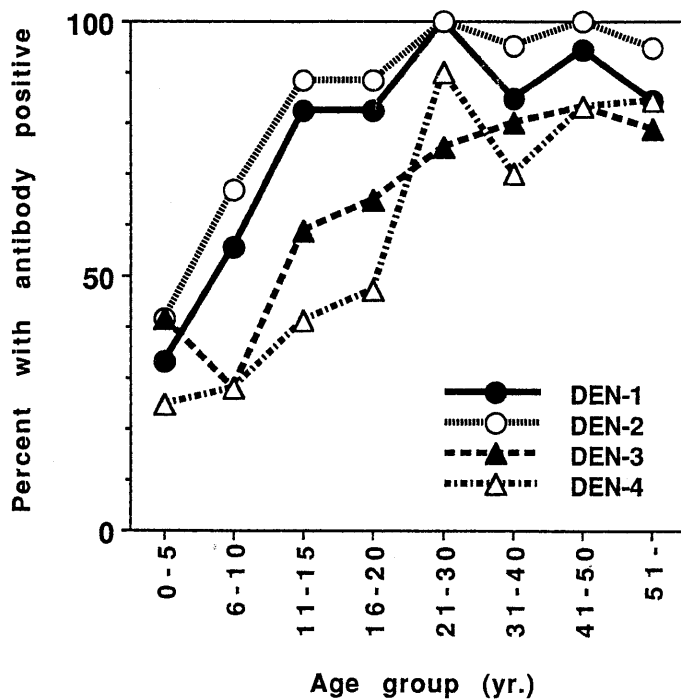


Figure 1. Percentage prevalences of N antibodies to DEN viruses.

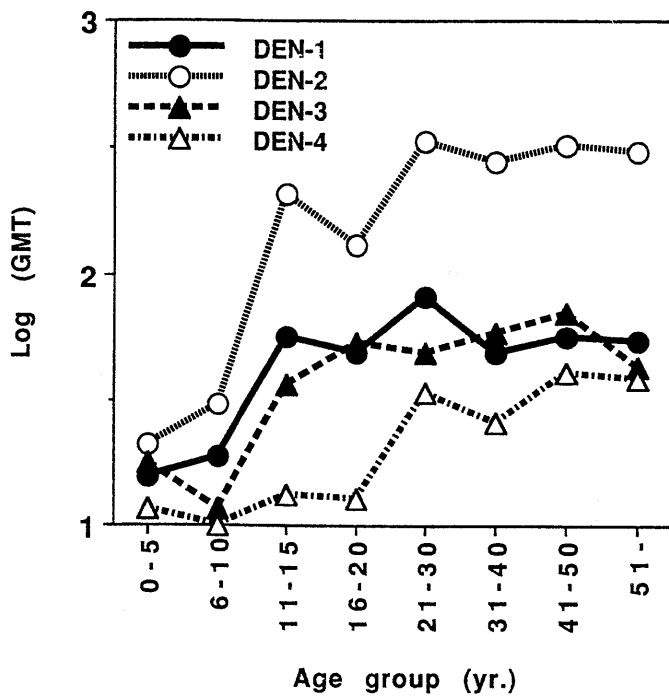
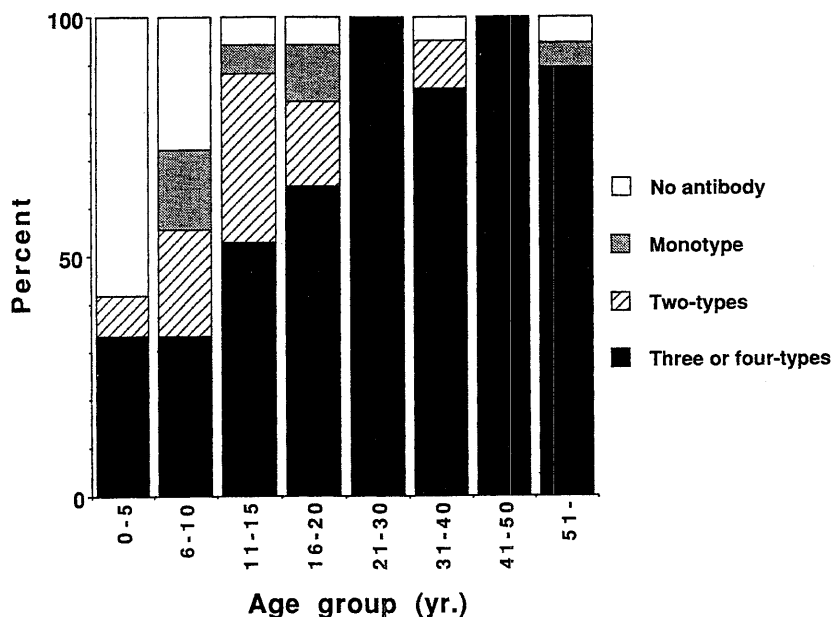


Figure 2. Geometric mean titers of N antibodies to DEN viruses. Negative samples were supposed to have titers equal to 1 : 5.



**Figure 3.** Relative frequency of specimens possessing no N antibody, monotype, two-, and three- or four-types of N antibodies to DEN viruses.

DEN virus in age groups. In each age group, the GMT to DEN-2 virus was highest and rose up to more than 1 : 300 by the age of 21-30 years old, remaining at a similar level till the age group of 51-years old.

Multiple DEN serotype infections in individuals were examined by N antibody frequency and results are shown in Fig. 3. The percentage frequency of groups with multitype N antibodies (group 3 and group 4) to DEN virus rose to about 90% by the age of 11-15 years old, suggesting that majority (90%) of children in Vientiane had been infected with two or more serotypes of DEN virus by the age of 15 years old.

#### DISCUSSION

During the DHF epidemic of 1987, number of hospitalized cases exceeded the admission capacity of pediatrics wards in two main hospitals in Vientiane and many extra beds were added in the corridors. This experience induced to set up the *Aedes* Control Unit (ACU) in Vientiane Municipality Health Service in the end of 1988. The activities were supervised by the ACU staffs and carried out with the community health participation, including school children. In order to evaluate the activities to reduce density of the vector larva, the Breteau and House indices were employed. In these indices, high and low risks of virus transmission are considered to be as follows:

Breteau Index (BI) > 50 : high risk ; < 5 : low risk

House Index (HI) > 10 : high risk ; < 1 : low risk



Although the average BI and HI in January to June, 1989 to 1992 in the urban four districts had been lower significantly than those in January to June in 1987 in any district, both indices did not give the low risk values. In particular, the HI gave many high risk values ( $>10$ ), except Sisattanak district (Table 8). It is, however, a fact that no large epidemic has occurred in Vientiane Municipality since 1988 (Table 4). A water container with the larva is an indicator in both indices, but the size of water container with the larva is not involved; that is, a small water container such as ant guard and a large water container such as drum of 200 liters give the same value in the indices, even if there is a big difference between the numbers of vector larva in the two water containers.

Since dengue vaccine is not available at present, the vector control seems to be a major means to prevent DHF epidemic. If the vector control were performed intensively and BI and HI were kept at the low risk levels in an area, most of children would remain to be free of antibody to DEN virus. Such a child would be easily infected with DEN virus outside the area and bring the virus into the area, but the epidemic would not occur in the area due to low density of the vector.

A seroepidemiological study on serum specimens collected in February to March, 1990, demonstrated that, of the four serotypes, the highest prevalence and geometric mean titer of N antibody were against DEN-2 virus. The findings suggest that the large DHF outbreak in Vientiane in 1987 might be caused by DEN-2 virus.

#### ACKNOWLEDGEMENTS

The authors wish to express thanks to Dr. Khamlien Pholsena, Director, Institute of Malariology, Parasitology and Entomology, for precious information on DHF epidemics in Laos. Some data were obtained through the Primary Health Care Project in Laos, supported by the Japan International Cooperation Agency (JICA).

#### REFERENCES

- 1) Ishimine, T., Tadano, M., Fukunaga, T. and Okuno, Y. (1987): An improved micromethod for infectivity assays and neutralization tests of dengue viruses. *Biken J.*, 30, 39–44.
- 2) Kramer, C. Y. (1956): Extension of multiple range tests to group means with unequal numbers of replications. *Biometric*, 12, 307–310
- 3) Okuno, Y., Fukunaga, T., Tadano, M., Okamoto, Y., Ohnishi, T. and Takagi, M. (1985): Rapid focus reduction neutralization test of Japanese encephalitis virus in microtiter system. *Arch. Virol.*, 86, 129–135
- 4) Tukey, J. W. (1953): The problem of multiple comparisons. [Unpublished]