

# AN ECOLOGICAL SURVEY OF DENGUE VECTOR MOSQUITOS IN CENTRAL LAO PDR

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**Abstract.** An ecological survey of dengue vector mosquitos was carried out in June 2000 in central Lao PDR. Two areas in Khammouane Province, Nongbok and Thakhek, were selected for the survey. Of the 7 mosquito species identified, *Aedes aegypti* was dominant in both study areas. The container index for *Ae. aegypti* in Nongbok was 51.8% and was significantly higher than that of Thakhek (40.2%); moreover, significant differences between the study areas were found with records to containers and to the conditions surrounding the houses. The key containers in Nongbok were water jars, whereas drums or small or discarded containers had the highest occurrence rate of *Ae. aegypti* in Thakhek. *Mesocyclops aspericornis* was found in large water jars and cement water tanks; no *Aedes* larvae were found at these sites. Strategy to control dengue vectors in the study areas was discussed.

## INTRODUCTION

Dengue and dengue hemorrhagic fever have become a serious threat to public health throughout Southeast Asia (Gubler, 1997). There have, however, been few studies of the ecology of dengue vector mosquitos in Lao PDR (Jennings *et al*, 1995). Since vector control is the most effective measure currently used to reduce transmission of the disease (Reiter and Gubler, 1997), more studies of the ecology of the dengue vector mosquitos, *Aedes aegypti* and *Ae. albopictus*, are needed in order to enhance the control strategy in Lao PDR.

Although a system for reporting cases of dengue fever in Lao PDR has yet to be properly established, an outbreak of dengue fever was reported in Khammouane Province, central Lao PDR, in 1998. Two areas in this province were selected for the present study,

and breeding sites and conditions surrounding houses were examined in each study area to clarify area differences in vectors in relation to the difference in environmental conditions. During the larval survey, the presence of other insects and microorganisms, especially copepods, was checked to make a list of natural enemies of mosquito larvae.

## MATERIALS AND METHODS

### Study area

Two areas were selected for the survey of dengue vector mosquito larvae: Ban Sibaubang, Nongbok, and Thakhek City. Nongbok is situated some 38 km south of Thakhek City; the population of our study village (Ban Sibaubang) in 1999 was 921 and was composed of 188 families living in 174 houses. The population in 1999 of Thakhek City was 45,660 and was composed of 5,966 families living in 6,240 houses. Five different blocks in Thakhek City were examined during the survey.

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### House survey

A larval survey was conducted in each study area to determine the main breeding sites of dengue vector mosquitos and to examine the relationship between the ecological conditions surrounding the houses and the variation in larval density among houses. Larvae were collected using a pipette and small hand net (20 cm diameter) and taken to the laboratory for identification. The kind, location (indoor/outdoor) and size of each container and the presence/absence of larvae were recorded. There were seven kinds of container : cement water tanks; large water jars (>60 cm in diameter); medium-sized water jars (35 - 55 cm); small water jars (<30 cm); used tyres; drums; and other small or discarded containers (ant-traps, tin cans, bottles, coconut shells etc).

### Natural enemies and conditions around the houses

During the larval survey, the presence of copepods and other insects was recorded for water containers with or without a very low density of *Aedes* larvae. The situation of each house was described in terms of the following: the total number of trees of each of 5 different height classes (<1 m, 1-2 m, 2-3 m, 3-5 m, >5 m); the percentage of the soil surface covered with vegetation; the distance from the nearest neighbor.

### Ovitrap survey

A dark plastic container (15 cm diameter; 12 cm depth) was used as an ovitrap. In each study area, 8 houses were selected at random; four traps were set for each house: two indoors and two outdoors. The inside wall of the ovitrap was covered with a piece of cloth (8 x 28 cm); the cloth was changed weekly and the number of eggs was recorded. The larvae that hatched were reared to adulthood and identified to estimate the proportion of *Ae. aegypti* and *Ae. albopictus* in the eggs.

## RESULTS AND DISCUSSION

### House survey

A total of 655 containers from 136 houses in Nongbok and 547 containers from 125 houses in Thakhek were examined and 17,484 mosquito larvae were collected.

Seven mosquito species were identified (Table 1); *Ae. aegypti* was dominant in both areas. The container and house indices for *Ae. aegypti* were 51.8% and 94.9% respectively in Nongbok and 40.2% and 75.2% respectively in Thakhek. The container index was significantly higher for Nongbok than for Thakhek ( $\chi^2=16.6$ ;  $p<0.001$ ). The difference in the house index between the two areas was not signifi-

Table 1  
Mosquito species and number of containers and houses positive for each mosquito species found in central Lao PDR, June 2000.

| Species                      | Nongbok    |            | Thakhek    |           |
|------------------------------|------------|------------|------------|-----------|
|                              | Containers | Houses     | Containers | Houses    |
| <i>Aedes aegypti</i>         | 339 (51.8) | 129 (94.9) | 220 (40.2) | 94 (75.2) |
| <i>Aedes albopictus</i>      | 80 (12.2)  | 60 (44.1)  | 60 (11.0)  | 40 (32)   |
| <i>Anopheles (Cellia) sp</i> | 4 (0.61)   | 4 (2.94)   | 0 (0)      | 0 (0)     |
| <i>Armigeres sp</i>          | 3 (0.46)   | 2 (1.47)   | 36 (6.6)   | 28 (22.4) |
| <i>Culex (Lutia) sp</i>      | 1 (0.15)   | 1 (0.74)   | 0 (0)      | 0 (0)     |
| <i>Culex sp</i>              | 98 (15)    | 63 (46.3)  | 40 (7.3)   | 31 (24.8) |
| <i>Toxorynchites sp</i>      | 9 (1.37)   | 8 (5.88)   | 3 (0.6)    | 3 (2.4)   |

Figures in parentheses show the percentage.

cant ( $\chi^2=1.6$ ;  $p=0.21$ ). For *Ae. albopictus*, there was no significant difference in the container and house indices between the study areas ( $\chi^2=0.36$ ,  $p=0.55$  and  $\chi^2=1.81$ ;  $p=0.18$ ). The incidence of *Armigeres* sp in Thakhek was very high.

Table 2

A list of natural enemies of mosquito larvae and the number of containers positive for each natural enemy found in the larval survey in central Lao PDR, June 2000.

| Natural enemy                   | Area    |         |
|---------------------------------|---------|---------|
|                                 | Nongbok | Thakhek |
| <b>Insects</b>                  |         |         |
| Hemipterous bug (adult)         | 2       | 0       |
| Coleopterous beetle (larva)     | 1       | 0       |
| <i>Toxorynchites</i> sp         | 9       | 3       |
| <i>Culex (Lutia)</i> sp         | 1       | 0       |
| <b>Copepods</b>                 |         |         |
| <i>Mesocyclops aspericornis</i> | 4       | 2       |

Five natural enemies of mosquito larvae were found during the house survey (Table 2). The most interesting of these was the copepod *Mesocyclops aspericornis*, collected from large water jars (70 cm diameter; 1 m depth). The ability of copepods to control mosquito larvae, especially *Aedes* mosquitos, has been evaluated both in the laboratory and in field experiments since the 1980s (Marten *et al*, 1994). Field trials of dengue vector control conducted in Vietnam suggested that *Mesocyclops* was a potential biological measure for the control of *Ae. aegypti* (Nam *et al*, 1998), although limited efficiency was observed in Vientiane, Lao PDR (Jennings, 1995). Experimental studies are required to establish the viability of the biological control of *Aedes* larvae by using *Mesocyclops* in Lao PDR. Other than *Mesocyclops aspericornis*, only *Toxorynchites* sp was found in both study areas.

The composition of the 7 kinds of containers and the occurrence of the main mosquito species are shown in Table 3. The results indicate that the availability of the containers

Table 3

Container type and total numbers and occurrence of dominant mosquitos observed in central Laos, June 2000.

| Container type      | Total | Number of containers with |                       |              |                  |
|---------------------|-------|---------------------------|-----------------------|--------------|------------------|
|                     |       | <i>Ae. aegypti</i>        | <i>Ae. albopictus</i> | <i>Culex</i> | <i>Armigeres</i> |
| <b>Nongbok</b>      |       |                           |                       |              |                  |
| Cement tanks        | 24    | 12 (50.0)                 | 0 (0)                 | 5 (20.8)     |                  |
| Water jars (large)  | 252   | 153 (60.7)                | 36 (14.3)             | 38 (15.1)    |                  |
| Water jars (medium) | 129   | 65 (50.4)                 | 13 (10.1)             | 15 (11.6)    |                  |
| Water jars (small)  | 91    | 51 (56.0)                 | 10 (11.0)             | 17 (18.7)    |                  |
| Used tyres          | 10    | 3 (30.0)                  | 3 (30.0)              | 0 (0)        |                  |
| Drums               | 96    | 34 (35.4)                 | 7 (7.29)              | 12 (12.5)    |                  |
| Others              | 53    | 21 (39.6)                 | 11 (20.8)             | 11 (20.8)    |                  |
| <b>Thakhek</b>      |       |                           |                       |              |                  |
| Cement tanks        | 66    | 24 (36.4)                 | 2 (3.0)               | 5 (7.6)      | 0 (0)            |
| Water jars (large)  | 8     | 2 (25.0)                  | 0 (0)                 | 0 (0)        | 1 (13.0)         |
| Water jars (medium) | 67    | 32 (47.8)                 | 5 (7.5)               | 3 (4.5)      | 2 (3.0)          |
| Water jars (small)  | 101   | 28 (27.7)                 | 4 (4.0)               | 5 (5.0)      | 4 (4.0)          |
| Used tyres          | 39    | 14 (35.9)                 | 9 (23.0)              | 13 (33.3)    | 5 (13.0)         |
| Drums               | 131   | 51 (38.9)                 | 4 (3.1)               | 6 (4.6)      | 3 (2.3)          |
| Others              | 135   | 69 (51.1)                 | 36 (27.0)             | 8 (5.9)      | 21 (16.0)        |

Figures in parentheses show the percentage.

Table 4

Average number of eggs (per week per trap) collected from ovitraps placed at Nongbok and Thakhek, central Lao PDR during 8-22 June 2000.

| Area    | Indoors | Outdoors | In and outdoors | % <i>Ae. aegypti</i> |
|---------|---------|----------|-----------------|----------------------|
| Nongbok | 78.0    | 51.3     | 65.0            | 73.01 <sup>a</sup>   |
| Thakhek | 70.0    | 51.5     | 61.2            | 92.37 <sup>a</sup>   |
| Total   | 74.0    | 51.4     | 63.2            | 16.87                |

<sup>a</sup>Significantly different between the areas ( $\chi^2=43.58$ ,  $p<0.001$ ).

Table 5

Conditions surrounding the houses at the 2 study areas in central Lao PDR, June 2000.

|   | Area    |         |
|---|---------|---------|
|   | Nongbok | Thakhek |
| Height of trees <sup>a</sup>                      |         |         |
| < 1m  | 85      | 66      |
| 1-2m  | 125     | 159     |
| 2-3m  | 243     | 202     |
| 3-5m  | 377     | 388     |
| > 5m  | 194     | 607     |
| Trees / house <sup>b</sup>                        | 7.6c    | 11.4d   |
| Vegetation coverage (%) <sup>b</sup>              | 59.3c   | 28.0d   |
| Distance to the nearest neighbor (m) <sup>b</sup> | 4.3c    | 3.9d    |

<sup>a</sup>Frequency distribution of tree height was significantly different between the areas ( $\chi^2=162.9$ ,  $p<0.001$ ).

<sup>b</sup>Average in the same row followed by the same letter was not significantly different ( $p>0.05$ ).

for mosquito breeding and the utilization of containers by dengue vectors differed between the two study areas. The composition of the containers was significantly different between Nongbok and Thakhek ( $\chi^2=319.9$ ,  $p<0.001$ ). In Nongbok, about 72% of containers were water jars, whereas water jars accounted for 32.2% of container in Thakhek. In Thakhek, nearly half of the containers (48.6%) were drums or small or discarded containers. In Nongbok, more than 50% of water jars were infested with *Ae. aegypti*. Compared with Nongbok, the *Ae. aegypti* population in Thakhek utilized a higher proportion of used tyres, drums a small or discarded containers.

Our results clearly showed that the key

containers for the breeding of *Ae. aegypti* differed between the 2 study areas. Water jars, as opposed to drums or small or discarded containers, were the favored containers in Thakhek. The removal of discarded containers from households must be the first act of dengue vector control in Thakhek. The water jars in Nongbok, however, were a necessary part of daily life. Besides larvicide treatment, the introduction of *Mesocyclops aspericornis* into water jars might work as a control measure in Nongbok.

#### Ovitraps survey

The average number of eggs per ovitrap was calculated and the values are compared in Table 4. The difference in egg numbers between areas and between indoors and outdoors was not significant ( $F=0.06$ ;  $p=0.81$  and  $F=2.01$ ;  $p=0.16$  respectively). The percentages of *Ae. albopictus* that appeared from the collected eggs were 7.63% and 27.0% for Nongbok and Thakhek respectively and were significantly different ( $\chi^2=43.58$ ;  $p<0.001$ ).

#### Surrounding conditions

The frequency distribution of tree height (Table 5) was significantly different between Nongbok and Thakhek ( $\chi^2=162.9$ ;  $p<0.001$ ): about 42.7% of trees were >5 m tall in Thakhek compared with only 18.9% in Nongbok. The number of trees per house was significantly greater in Thakhek than in Nongbok ( $t=4.2$ ;  $p<0.001$ ) and the percentage of the soil surface covered with vegetation was significantly smaller in Thakhek than in Nongbok ( $t=8.0$ ;  $p<0.001$ ). The distance to the nearest neighbor did not differ significantly between the areas ( $t=1.1$ ;

p=0.27). Because the height of each house (from the ground to the roof) was about 5 m in most cases, the houses in Thakhek were below the tree canopy and a large part of the soil surface did not receive direct sunlight. On the other hand, the houses in Nongbok had trees (7.6 / house) most of which were <5 m in height. As a result, a large part of the soil surface had direct sunlight and therefore grasses grew well. These differences in the environment surrounding the houses probably affect the breeding of mosquitos (Tun-Lin *et al*, 1995) and their natural enemies.

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#### REFERENCES

- Gubler DJ. Dengue and dengue hemorrhagic fever: its history and resurgence as a global public health problem. In: Gubler DJ, Kuno G, eds. *Dengue and dengue hemorrhagic fever*. New York: CAB International, 1997: 1-22.
- Jennings CD, Phommasack, B Sourignadeth, B, Kay BH. *Aedes aegypti* control in the Lao People's Democratic Republic, with reference to copepods. *Am J Trop Med Hyg* 1995; 53: 324-30.
- Marten, GG, Bordes ES, Nguyen M. Use of cyclopoid copepods for mosquito control. *Hydrobiologia* 1994; 292/293: 491-6.
- Nam VS, Yen NT, Kay B, Marten GG, Reid HW. Eradication of *Aedes aegypti* from a village in Vietnam, using copepods and community participation. *Am J Trop Med Hyg* 1998; 59: 657-60.
- Reiter P, Gubler DJ. Surveillance and control of urban dengue vectors. In: Gubler DJ, Kuno G, eds. *Dengue and dengue hemorrhagic fever*. New York: CAB International, 1997: 425-62.
- Tun-Lin W, Kay BH, Barnes A. The premise condition index: a tool for streamlining surveys of *Aedes aegypti*. *Am J Trop Med Hyg* 1995; 53: 591-4.