Filaria due to Wuchereria bancrofti and dengue hemorrhagic fever (DHF) are important mosquito-borne human diseases in Thailand. With respect to W. bancrofti, an endemic area of this nocturnally subperiodic (nsp) type is distributed throughout the Thai-Myanmar border. The area is rural, hilly, semi-forested and Aedes harinasutai, Ae. desmotes, Ae. annandalei, Ae. imitator and Mansonia dives are the important vectors (Harinasut et al., 1970; Gould et al., 1982; Division of Filariasis, 1998). DHF (DEN-1,2,3 and 4), epidemics recur every year in the rainy season, and Ae. aegypti is the important vector in urban areas, whereas, Ae. albopictus is the vector in some suburban and rural areas (Gould et al., 1968; Whitehead et al., 1971; Pant et al., 1973; Gubler and Rosen, 1976; Gubler et al., 1979). Rudnick (1978) and Self (1979) demonstrated that there is zoonotic DHF in Malaysia and Vietnam that is being maintained by basic forest cycles involving wild monkeys and jungle mosquitoes. The important jungle vector is a species of mosquito found commonly in the high canopy that prefers to feed on monkeys and man, and spreads the infection further by Ae. albopictus and Ae. aegypti. Interestingly, the virus has been isolated from both rainforest mosquitoes in the Ae. niveus group, and from monkey-blood. Kanchanaburi Province is an endemic area of nsp W. bancrofti and Ae. harinasutai, which is a member of the Ae. niveus group, has been suspected of being important vector. The Ae. niveus group is a proven jungle vector of DHF in other countries and it is also an important vector of bancroftian filariasis in Kanchanaburi Province. Therefore, it becomes increasingly desirable to determine whether the susceptibilities of other Ae. niveus group members for nsp W. bancrofti and dengue virus are similar to the principle vectors, Ae. harinasutai and Ae. aegypti. This study, therefore, reports experiments on the susceptibility of Ae. albopictus, which is a species member of the Ae. niveus group, to nsp W. bancrofti and dengue virus.

Fully engorged female Ae. albopictus were wild-caught and collected by using a human-baited trap from Sangkhla Buri district, Kanchanaburi Province. They were then allowed to oviposit, and the hatched larvae were
reared in the insectary (12 hours illumination, 27±2°C, 70-80% RH) using standard rearing procedures, as described by Choochote et al (1993). The clean-colony adult females of F₁-progeny were used for the infection of nsp W. bancrofti and DEN-2 virus.

Five-day-old adult female Ae. albolateralis and an Ae. harinasutai control mosquitos which had fasted for 12 hours, were allowed to feed on the heparinized blood of a carrier infected with nsp W. bancrofti (microfilarial density = 0.2 mf per mm³) using the artificial membrane feeding technique described by Chomcharn et al (1980). Fourteen days after feeding, all mosquitos were dissected in normal saline solution and examined under a dissecting microscope. The number of mosquitos with one or more infective larvae in any part of the body (head, thorax, abdomen) was recorded.

The prototype of the DEN-2 virus (New Guinea C strain) was obtained from the Department of Virology, USA Medical Component, Armed Forces Institute of Medical Sciences (AFRIMS), Bangkok. The virus was passed 32 times in suckling mice and 4 times in LLC-MK 2 cell lines for plaque purification by using the method of Dulbecco and Vogt (1954) before preparation of the stock virus. The fluorescein conjugated DEN-2 virus antibody was prepared by conjugating the defibrinated anti-DEN-2 virus hyperimmune mouse ascitic fluid with fluorescein isothiocyanate (FITC) dye (Sigma®) according to the method described by Nairn (1976). Five-day-old adult female Ae. albolateralis and Ae. aegypti control mosquitos which had fasted for 12 hours, were inoculated intrathoracically with 0.38 μl of DEN-2 virus (0.38 μl = 417 plaque-forming units) using a fine glass capillary needle (Rosen and Gubler, 1974). The inoculated mosquitos were maintained at 32 ± 1°C for 14 days. Detection of the viral antigen in the brain and salivary gland of Ae. albolateralis and Ae. aegypti was carried out by using the direct immunofluorescent technique, as described by Kuberski and Rosen (1977) and Kuberski (1979).

Details of the susceptibility of Ae. albolateralis to nsp W. bancrofti and the DEN-2 virus are shown in Table 1. Pictures of a brain smear, brain cells, and a section of the salivary gland of the Ae. albolateralis, which was found to be positive for the DEN-2 virus by using direct immunofluorescent staining, are illustrated in Fig 1.

The results of the dissection of all infected mosquitos on day 14 revealed that Ae. harinasutai was more susceptible to nsp W. bancrofti than Ae. albolateralis, although there was no statistically significant difference (χ² = 0.56, p > 0.05). The infective rates were

| Table 1 | Susceptibility of Ae. albolateralis to nsp W. bancrofti and DEN-2 virus. |
|---------|------------------|------------------|------------------|
|         | Ae. albolateralis | Ae. harinasutai  | Ae. aegypti      |
| W. bancrofti | Infection rate (No.) | 9.43 (5/53)⁺     | 16.07 (9/56)⁺    |
|           | Average No. L3 per infected mosquitos (range) | 1.20 (1-2) | 2.55 (1-6) |
|           | L3-distribution | % Head (No.) | 16.67 (1)       | 21.74 (5) |
|           |                  | % Thorax (No.) | 66.66 (4)       | 65.22 (15) |
|           |                  | % Abdomen (No.) | 16.67 (1)       | 13.04 (3) |
| DEN-2 virus | Infection rate (No.) | 100 (51/51) | 100 (51/51) |

⁺χ² = 0.56; p > 0.05
9.43% and 16.07% in *Ae. albolateralis* and *Ae. aegypti*, respectively. The infective larvae obtained from both mosquito species were very active and found to be distributed in all regions of the head, thorax and abdomen. They also behaved similarly. More than 16% of infective larvae could migrate from the thorax to the head and proboscis.

Observation on the replication of the DEN-2 virus, which was intrathoracically inoculated into 51 female *Ae. albolateralis* and 51 female *Ae. aegypti* using the direct immunofluorescent technique, demonstrated that after 14 days post-inoculation the DEN-2 virus antigen was prominent in the salivary gland (Fig 1A) and cortical cells of the brain (Fig 1B,C). It was also found occasionally in fat body cells, but rarely in the ovary (observed in only 3 mosquitoes), and never in the gut epithelium and alimentary canal. The susceptibility rates in both mosquito species were 100% identical. In fact, the virus antigen in the cortical cells of the brain of *Ae. aegypti* appeared to be more prominent than in *Ae. albolateralis*, as determined by the intensity of the fluorescence.

In order to determine a mosquito vector in an endemic area of mosquito-borne human diseases, it is necessary to confirm the susceptibility rate in a laboratory bred, clean mosquito colony that has been fed on carrier blood containing pathogens (Sasa, 1976). Therefore, by using this criterion, the susceptibility test in an experimental laboratory is a useful parameter when suspecting the potential vector of a certain mosquito species. Nonetheless, susceptibility alone does not imply an important role in the transmission of disease in nature, whereas a refractory result can entirely rule out its significance. The potential laboratory vector, *Ae. albolateralis* in the present study, suggested the possibility that this mosquito species could transmit nsp *W. bancrofti* and DHF. Further investigations on *Ae. albolateralis* as a potentially natural vector of nsp *W. bancrofti* and DHF in rural areas of Sangkla Buri district, Kanchanaburi Province, and/or other associated endemic areas, should be performed. An additional academic conclusion is that the satisfactory susceptibility (16.07%) of indigenous *Ae. harinasutai* to indigenous nsp *W. bancrofti* has confirmed its role as a naturally transmissive vector.

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