RESEARCH NOTE

AEDES ALBOLATERALIS, A POTENTIAL VECTOR OF NOCTURNALLY SUBPERIODIC *WUCHERERIA BANCROFTI* AND DENGUE TYPE 2 VIRUS

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Abstract. The susceptibility of *Aedes albolateralis* to nocturnally subperiodic *Wuchereria bancrofti* and dengue type 2 virus was investigated by using artificial membrane feeding and intrathoracic inoculation techniques, respectively. The results indicated that *Ae. albolateralis* was susceptible to nocturnally subperiodic *W. bancrofti* (susceptibility rate = 9.43%) and dengue type 2 virus (susceptibility rate = 100%), suggesting the potential vector of the two pathogens.

Filariasis 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 (Harinasuta *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 mosquitos. 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 mosquitos 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. albolateralis*, which is a species member of the *Ae. niveus* group, to nsp *W. bancrofti* and dengue virus.

Fully engorged female *Ae. albolateralis* 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

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reared in the insectary (12 hours illumination, 27±2ºC, 70-80% RH) using standard rearing procedures, as described by Choochote *et al* (1993). The clean-colony adult females of F_1-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 mm^3) 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 ± 1ºC 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 ($\chi^2 = 0.56$, $p > 0.05$). The infective rates were

Table 1
Susceptibility of *Ae. albolateralis* to nsp *W. bancrofti* and DEN-2 virus.

Experiments	Mosquito species		
	Ae. albolateralis	*Ae. harinasutai*	*Ae. aegypti*
W. bancrofti			
Infective rate (No.)	9.43 (5/53)[a]	16.07 (9/56)[a]	
Average No. L3 per infected mosquito (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)

[a] $\chi^2 = 0.56$; $p > 0.05$

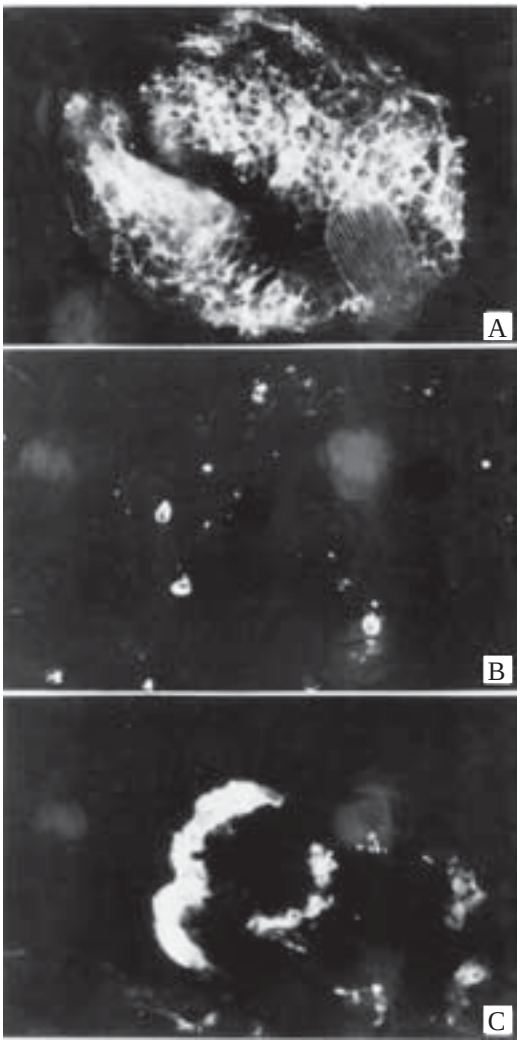

Fig 1–The brain smear (A), brain cells (B), and section of salivary gland (C) of *Ae. albolateralis* showing the positive fluorescence for the DEN-2 virus after 14 days post-inoculation.

9.43% and 16.07% in *Ae. albolateralis* and *Ae. harinasutai*, 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 mosquitos), 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.

ACKNOWLEDGEMENTS

We thank Associated Professor Dr Piya Netrawichien, Dean of the Faculty of Medicine, Chiang Mai University, for his interest in this research, and the Faculty of Medicine

Endowment Fund for Research Publication for financial support in publishing this paper.

REFERENCES

Chomcharn Y, Surathin K, Bunnag D, Sucharit S, Harinasuta T. Effect of a single dose of primaquine on a Thai strain of *Plasmodium falciparum*. *Southeast Asian J Trop Med Public Health* 1980; 11: 408-9.

Choochote W, Sukontason K, Tookyang B, Likitvong K, Pakdicharoen A. A note on laboratory colonization of *Aedes harinasutai* (Diptera: Culicidae). *J Trop Med Parasitol* 1993; 16: 25-9.

Division of Filariasis, Department of Communicable Disease Control, Ministry of Public Health. 1998: 1-33.

Dulbecco R, Vogt M. Plaque formation and isolation of pure lines with poliomyelitis viruses. *J Exp Med* 1954; 99: 167-82.

Gould DJ, Bailey CL, Vonpradist S. Implication of forest mosquitos in the transmission of *Wuchereria bancrofti* in Thailand. *Mosq News* 1982; 42: 560-4.

Gould DJ, Yuill TM, Moussa AM, Simasathien P, Rutledge LC. An insular outbreak of dengue hemorrhagic fever. III. Identification of vectors and observations on vector ecology. *Am J Trop Med Hyg* 1968; 17: 609-18.

Gubler DJ, Nalim S, Tan R, Saipan H, Saroso JS. Variation in susceptibility to oral infection with dengue viruses among geographic strains of *Aedes aegypti*. *Am J Trop Med Hyg* 1979; 28: 1045-52.

Gubler DJ, Rosen L. Variation among geographic strains of *Aedes albopictus* in susceptibility to infection with dengue viruses. *Am J Trop Med Hyg* 1976; 25: 318-25.

Harinasuta C, Sucharit S, Deesin T, Surathin K, Vutikes S. Bancroftian filariasis in Thailand, a new endemic area. *Southeast Asian J Trop Med Public Health* 1970; 1: 233-45.

Kuberski TT. Fluorescent antibody studies on the development of DEN-2 virus in *Aedes albopictus* (Diptera: Culicidae). *J Med Entomol* 1979; 16: 343-9.

Kuberski TT, Rosen L. A simple technique for the detection of dengue antigen in mosquitos by immunofluorescence. *Am J Trop Med Hyg* 1977; 26: 533-7.

Nairn RC. Fluorescent protein tracing, 4th ed. Edinburgh: Churchill Livingstone, 1976.

Pant CP, Jatanasen S, Yasuno M. Prevalence of *Aedes aegypti* and *Aedes albopictus* and observations on the ecology of dengue hemorrhagic fever in several areas of Thailand. *Southeast Asian J Trop Med Public Health* 1973; 4: 113-21.

Rosen L, Gubler DJ. The use of mosquitos to detect and propagate dengue viruses. *Am J Trop Med Hyg* 1974; 23: 1153-60.

Rudnick A. Ecology of dengue virus. *Asian J Infect Dis* 1978; 2: 156-60.

Sasa M. Human filariasis: a global survey of epidemiology and control. Tokyo: University of Tokyo Press, 1976.

Self LS. Vectors of dengue fever/dengue hemorrhagic fever with advances in their control. *Asian J Infect Dis* 1979; 3: 77-9.

Whitehead RH, Yuill TM, Gould DJ, Simasathien P. Experimental infection of *Aedes aegypti* and *Aedes albopictus* with dengue viruses. *Trans R Soc Trop Med Hyg* 1971; 65: 661-7.