A STUDY ON SUSCEPTIBILITY OF INDONESIA COLONIES OF AEDES AEGYPTI AND AEDES ALBOPICTUS MOSQUITOES TO EXPERIMENTAL INFECTION WITH DENGUE TYPE 3 AND CHIKUNGUNYA VIRUSES

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INDEXING WORDS

mosquito; virus multiplication; dengue; chikungunya; infection; electron microscope

SYNOPSIS

Indonesia colonies of *Aedes aegypti* and *Aedes albopictus*

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* This paper is a collection of the following articles as a thesis presented to the Faculty of Kobe University School of Medicine, in partial fulfilment of the requirements for the degree of Doctor of Philosophy: (1) Soekiman, S., Machfudz, Subagyo, Adipoetro, S., Yamanishi, H., and Matsumura, T., ICMR Annals. 1984. 4. 143/151; (2) Soekiman, S., Konishi, E., and Matsumura, T., Kobe J. Med. Sci. 1986. 32. 127/132; (3) Soekiman, S., Konishi, E., and Matsumura, T., Jpn, J. Trop. Med. Hyg. 1987. 15. (in press); (4) Soekiman, S., Matsumura, T., and Yamanishi, H., Jpn. J. Med. Sci. and Biol. 1986. 39. 207/211; (5) Soekiman, S., and Matsumura, T., Jpn. J. Trop. Med. Hyg. 1987. 15. (in press). The author received written permission from co-authors (Machfuds, Subagyo, Adipoetro, S., Yamanishi, H., Matsumura, T., and Konishi, E.) and journals (ICMR Annals, Kobe J. Med. Sci., Jpn. J. Trop. Med. Hyg., and Jpn. J. Med. Sci. and Biol.).

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mosquitoes were studied for their biological characteristics, susceptibility to oral infection with dengue type 3 and chikungunya viruses and the virus multiplication processes in the salivary glands were investigated morphologically using an electron microscope. Biological studies showed that these mosquitoes were similar with each other in egg production, larval and pupal developments and speed of blood feeding. Growth curves of dengue type 3 and chikungunya viruses in *Aedes aegypti* and *Aedes albopictus* indicated that both mosquito species were susceptible to oral infection with these viruses. Electron microscopic observations of the salivary glands of *Aedes aegypti* and *Aedes albopictus* infected with chikungunya virus showed that this organ plays an important role in producing and maintaining high virus titers in these mosquitoes. The results suggest that both *Aedes* species are potent vectors of dengue and chikungunya viruses in Indonesia.

INTRODUCTION

Aedes aegypti is a domestic mosquito usually found indoors in urban areas, while *Aedes albopictus* is widely found outdoors in rural and urban areas. The breeding sites of these mosquitoes were found in tires, drums, barrels, water-pots, metal and ceramic containers, coconut shells, snail shells, tree holes, plant axiles, cut bamboos and others.[7] *Aedes aegypti* is one of the main vectors of dengue hemorrhagic fever (DHF) virus in Indonesia. Dengue type 1 (DEN-1) and dengue type 3 (DEN-3) viruses have been isolated from this species collected in DHF endemic areas of Indonesia and all of the types of DEN viruses have been already isolated from the patients suffering from DHF and dengue shock syndrome (DSS).[9, 23] It was reported that DEN-3 virus was isolated in comparatively high frequencies from the sera of DHF patients.[5, 26]

Primarily DHF was one of the urban diseases in Indonesia. Most cases were reported from large cities in Java island such as Surabaya, Jogyakarta and Jakarta. However, DHF epidemics in 1980 were reported from smaller cities and many rural areas in all provinces except Timor Timur (East Timor).[22] Up to the present time, DEN viruses have been isolated only from *Aedes aegypti* mosquitoes in Indonesia.

Chikungunya (CHIK) virus, an alphavirus widely distributed in

Tropical Africa and Southeast Asia, has been isolated from various species of mosquitoes including *Aedes aegypti, Aedes africanus, Culex fatigans* and *Culex tritaeniorhynchus.*[1] It has been reported that the multiplication of DEN and CHIK viruses in *Aedes aegypti* and *Aedes albopictus* were of similar pattern, and it is well known that there are wide variations in susceptibility to infection with these viruses among different species of mosquitoes and different strains of the same species.[3, 4, 14, 29]

In the transmission of arboviruses, the salivary gland plays an important role in producing and maintaining high virus titers throughout the life of the vector.[27] The mechanism of transmission of these viruses in *Aedes* mosquitoes was not clarified yet.

The present paper reports on the biological characteristics of Indonesia colonies of *Aedes aegypti* and *Aedes albopictus* mosquitoes, susceptibility to oral infection with DEN and CHIK viruses and electronmicroscopic observations of the salivary glands of these mosquitoes infected with CHIK virus in order to clarify the roles of *Aedes* mosquitoes in epidemiology of DEN and CHIK viruses.

MATERIALS AND METHODS

Mosquitoes

Colonies of *Aedes aegypti* (Surabaya strain), *Aedes albopictus* (Malang strain) were mainly used. For electron microscopy, *Aedes albopictus* (Oahu strain) was used as a standard of highly-susceptible strain.[14] To make biological studies, the insectary was maintained at 28C-29C in 50-60% relative humidity (RH) and 12 hr:12 hr (L:D) photoperiod and larvae were given extract yeast powder. For susceptibility experiments and electron-microscopic observations, mosquitoes were held at 27C in 70-80% RH and 16 hr:8 hr (L:D) photoperiod and larvae were given a diet of mouse pellets. Adult mosquitoes were provided with 0.7% sucrose solution.

Viruses

DEN-3 virus, H87 strain, was provided from the Department of Microbiology of Kobe University, at the 32th suckling mouse brain passage. The seed virus was obtained from 10% homogenate of

infected brain speciments in phosphate-buffered saline (PBS). An African strain of CHIK virus was also used for mosquito infection, in which the seed virus was the culture fluid obtained from infected BHK-21 monolayer cultures.[13)]

Biological studies

Eggs of the mosquitoes were submerged in distilled water. After hatching, larvae that reached at the pupal stage were observed daily. Five-day-old female adult mosquitoes were given once blood feeding on young chicken. The total number of eggs produced by each female mosquito was counted. The time needed for feeding on arms of a volunteer until mosquitoes became fully engorged was recorded to compare the speed of feeding.

Infection of mosquitoes

For infection of mosquitoes with DEN-3 and CHIK viruses, 4- to 10-day-old female mosquitoes were allowed to feed on virus-defibrinated sheep blood mixtures with an appropriate titer of infective virus. At 1- to 2-day intervals, 5 to 12 mosquitoes of each species were frozen at -80C until use for viral assay.

Virus assay

Each pool of mosquitoes was homogenized in 2 ml of culture medium (Eagle's minimum essential medium supplemented with 0.03% sodium bicarbonate, 5% heat-inactivated calf serum and 0.03% L-glutamine), centrifuged at 700 x g for 5 min and then filtered through a Minisart NP membrane with 0.45 μm porosity. The filtrate was titrated for viral infectivity by the plaque assay method[13)] or by the indirect fluorescent antibody technique essentially based on the original focus-counting method.[10)] The titer was expressed as plaque forming unit (PFU) or focus forming unit (FFU).

RESULTS

Biological studies

A total of 148 *Aedes aegypti* larvae and 112 *Aedes albopictus* larvae (Malang strain) were observed. Their mean times of larval development were 7.62 ± 0.64 days in *Aedes aegypti* and 9.20 ± 1.03

days in *Aedes albopictus* (Table 1). As shown in Fig. 1, pupation in *Aedes aegypti* began at the 4th day after egg hatching and lasted for 8 days; 52.7% of the larvae pupated within 3 days and 94.6% within 6 days. In *Aedes albopictus*, pupation began at the 4th day after hatching and lasted for 16 days; 55.4% of the larvae pupated within 5 days, and 92.9% within 7 days. Mean pupation times for *Aedes aegypti* and *Aedes albopictus* were 1.63 ± 0.07 days and 1.45 ± 0.08 days, respectively. Mean emergence time was 1.61 ± 0.10 days for female *Aedes aegypti* and 1.66 ± 0.10 days for male mosquitoes. In *Aedes albopictus*, the mean emergence times for female and male mosquitoes were 1.44 ± 0.12 days and 1.46 ± 0.11 days, respectively (Table 1).

Egg production

As shown in Table 2, the number of eggs deposited by individual female *Aedes aegypti* and *Aedes albopictus* were 66.2 ± 31.6 and 57.5 ± 17.3, respectively.

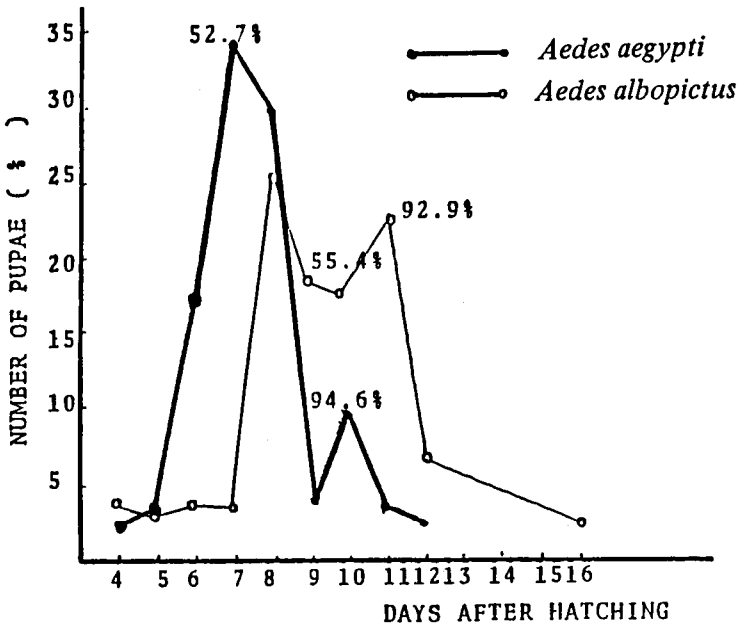

Fig. 1 Pupal development in *Aedes aegypti* and *Aedes albopictus* in a room condition (temperature: 28C-29C, RH: 56%) (Reproduced with written permission from Machfudz, Subagyo, Soewignjo, A., Yamanishi, H., Matsumura, T. and ICMR Annals).

Table 1 Development time (in day) of *Aedes aegypti* and *Aedes albopictus* at the room temperature 28C-29C and RH 56% (Reproduced with written permission from Machfudz, subagyo, Soewignjo, A., Yamanishi, H., Matsumura, T. and ICMR Annals).

Developmental stage	*Aedes aegypti* (in day)	*Aedes albopictus* (in day)
Larval development	7.62 ± 0.64	9.20 ± 1.03
Mean pupation	1.63 ± 0.07	1.45 ± 0.08
Mean emergence (female)	1.61 ± 0.10	1.44 ± 0.12
Mean emergence (male)	1.66 ± 0.10	1.46 ± 0.11

Table 2 Eggs production in *Aedes aegypti* and *Aedes albopictus* by a single blood meal (Reproduced with written permission from Machfudz, Subagyo, Soewignjo, A., Yamansihi, H., Matsumura, T. and ICMR Annals).

Species of mosquito	Number of mosquitoes observed	Total eggs production	Average number of eggs per female
Aedes aegypti	30	1986	66.2 ± 31.6
Aedes albopictus	30	1725	57.5 ± 17.3

Feeding speed

Out of 30 females of *Aedes aegypti* used in this experiments, 8 mosquitoes needed 2 min until they became fully engorged, 13 mosquitoes 3 min, 3 mosquitoes 4 min, 4 mosquitoes 5 min and 2 other mosquitoes 8 min. The mean time of the feeding speed in these female mosquitoes was 3.43 ± 0.93 min. Out of 30 females of *Aedes albopictus,* 3 mosquitoes needed 2 min until they became fully engorged, 12 mosquitoes 3 min, 9 mosquitoes 4 min, 5 mosquitoes 5 min and 1 mosquito 6 min. The mean time of the feeding speed in *Aedes albopictus* was 3.60 ± 0.6 min.

Susceptibility experiments

Comparative growth of CHIK virus in *Aedes aegypti* and *Aedes albopictus* is shown in Fig. 2. In this experiment, mosquitoes received an infective meal with a titer of $10^{7.2}$ PFU per ml. On day 0 the virus titers were $10^{3.6}$ PFU in *Aedes albopictus* and $10^{3.7}$ in *Aedes aegypti.* On day 2 the titers dropped to $10^{3.1}$ PFU

in *Aedes albopictus* and $10^{1.0}$ PFU in *Aedes aegypti*. After that, the titers started to increase in both species and reached the maximum titers of $10^{5.4}$ PFU on day 13 in *Aedes albopictus* and of $10^{4.6}$ PFU on day 10 in *Aedes aegypti*. The virus titer in *Aedes aegypti* then dropped quickly and became undetectable on day 20. On the other hand, the virus titer in *Aedes albopictus* declined slowly to $10^{3.9}$ PFU on day 20.

Growth of DEN-3 virus in three *Aedes* mosquitoes was studied after feeding on virus at $10^{7.7}$ FFU per ml. As shwon in Fig. 3, the virus titer on day 0 was $10^{4.6}$ FFU in both species. On day 2 the titers dropped to $10^{3.9}$ FFU in *Aedes aegypti* and $10^{2.6}$ FFU in *Aedes albopictus*. The titer of DEN-3 virus reached a maximum of $10^{8.2}$ FFU in *Aedes aegypti* and $10^{7.5}$ FFU in *Aedes albopictus* both on day 12. Growth of CHIK virus in *Aedes aegypti* infected with virus at $10^{8.2}$ FFU per ml was also obtained as a control of this experiment. It showed remarkable initial drop of the virus titer from $10^{5.4}$ FFU on day 0 to $10^{1.7}$ FFU on day 2. The peak titer of CHIK virus was reached at $10^{6.9}$ FFU on day 9.

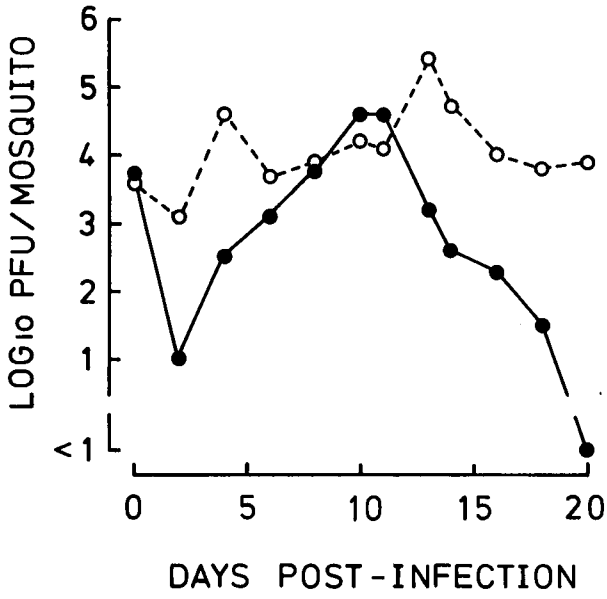

Fig. 2 Growth of CHIK virus in Indonesia colonies of *Aedes aegypti* (●) and *Aedes albopictus* (○) after feeding on virus at $10^{7.2}$ PFU per ml. Mean virus titer obtained from 5-12 mosquitoes per pool was represented as PFU per mosquitoes (Reproduced with written permission from Konishi, E., Matsumura, T. and Kone J. Med. Sci.).

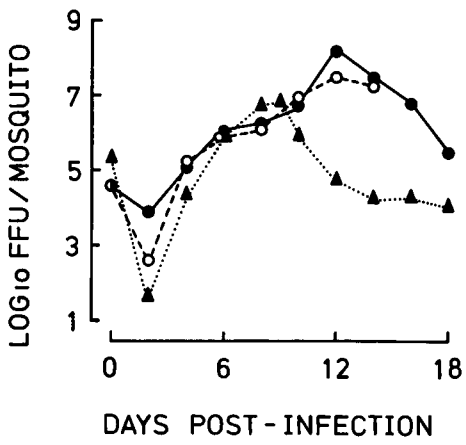

Fig. 3 Growth of DEN-3 virus in Indonesia colonies of *Aedes aegypti* (●) and *Aedes albopictus* (○) after feeding on virus at $10^{7.7}$ FFU per ml, compared with growth of CHIK virus in *Aedes aegypti* (▲) after feeding on virus at $10^{8.2}$ FFU per ml. Mean virus titer obtained from 5-12 mosquitoes per pool was represented as FFU per mosquito (Reproduced with written permission from Konishi, E., Matsumura, T. and Jpn. J. Trop. Med. Hyg.).

Electron microscopy

Observation at low magnification of CHIK virus-infected salivary glands of *Aedes aegypti* and *Aedes albopictus* obtained by feeding them on blood-virus-mixture at a titer about $10^{7.9}$ FFU/ml, showed the increasing number of vacuoles. The proximal portion in the lateral lobe of salivary glands of these *Aedes* mosquitoes showed a numerous number of enveloped CHIK virus particles in intracytoplasmic areas (Fig. 4). These virions were found in the perinuclear area and not in the nucleus of the cells (Fig. 5). The diameter of virion ranged from 55 nm to 69 nm with the electron-dense internal structure of 40 nm-48 nm. Especially closed to virion's accumulated area (crystalloid structure), a large number of mitochondria were seen (Fig. 6). Observation underneath the surface membrane of the lateral lobe cell in the salivary glands of *Aedes aegypti* and *Aedes albopictus* showed enveloped CHIK virions clearly remaining (Fig. 7). No virus budding from the cell surface in these regions were found (Fig. 8). Precursor particles of CHIK virus[6, 18] were not clearly observed in these salivary glands.

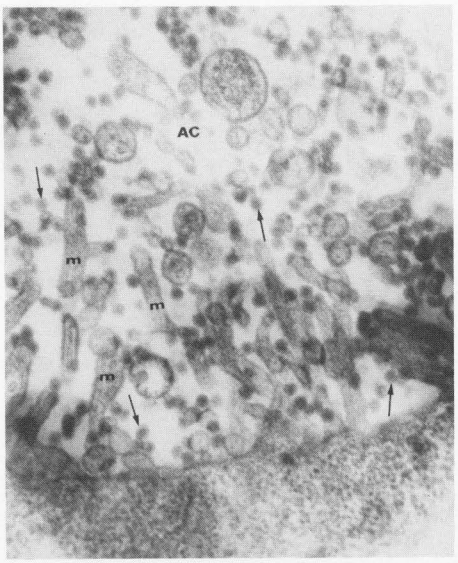

Fig. 4
Thin section of proximal portion in the lateral lobe of salivary glands of *Aedes albopictus* (Malang strain) infected with CHIK virus by oral feeding, showed a numerous number of virions in the apical cavity (Magnification: x 30,556: AC=apical cavity, m=microvilli. Arrows show virions).

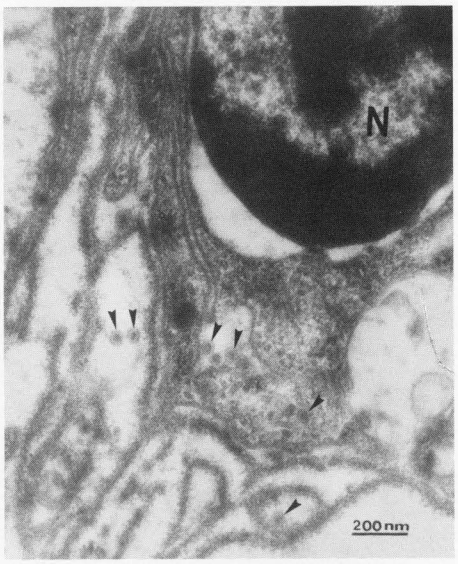

Fig. 5
Virions were found in the perinuclear area and not in the nucleus of infected cells. The diameter of enveloped virions was between 55 nm-69 nm with electron-dense internal structure of 41 nm-48 nm. (*Aedes albopictus*, Oahu strain, salivary glands infected with CHIK virus. Magnification: x 35,396; Arrows show virions).

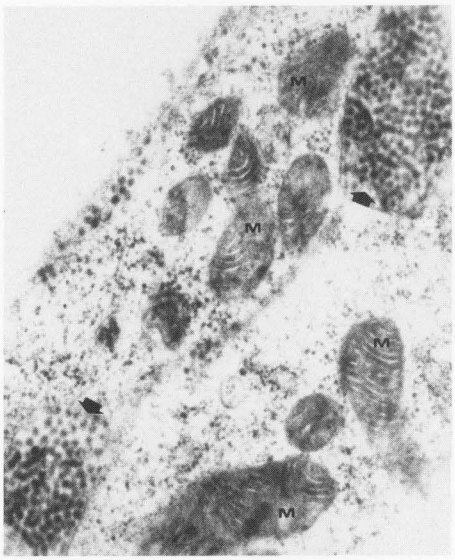

Fig. 6
Closed to the virion's crystalloid structure, a numerous number of mitochondria were clearly seen (*Aedes aegypti* salivary glands infected with CHIK virus. Magnification: x 18,375; M=mitochondria. Arrows show virion's crystalloid structure) (Reproduced with written permission from Matsumura, T. and Jpn. J. Trop. Med. Hyg.).

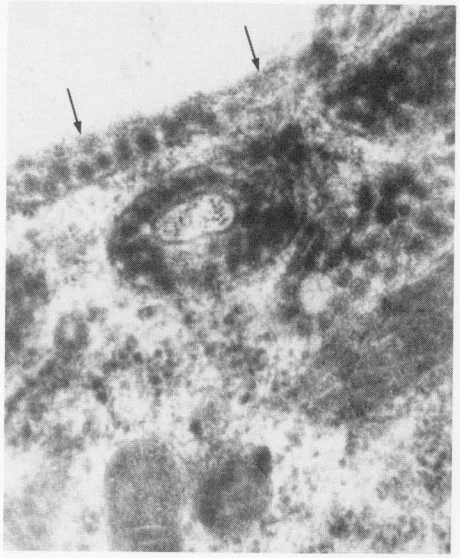

Fig. 7
Mature enveloped chikungunya viruses lined along underneath the cell surface membrane (arrows), withoug any budding process (*Aedes aegypti* salivary glands; Magnification: x 42,553) (Reproduced with written permission from Matsumura, T. and Jpn. J. Trop. Med. Hyg.).

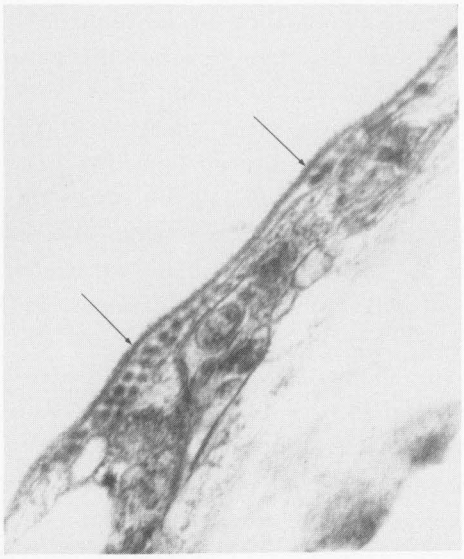

Fig. 8 Salivary glands of *Aedes albopictus* infected with chikungunya virus by oral feeding showed mature virions underneath the cell surface membrane (arrows). No findings of virus budding from this salivary glands were also seen. Magnification: x 31,935 (Reproduced with written permission from Matsumura, T. and Jpn. J. Trop. Med. Hyg.).

DISCUSSION

Pattern of DEN-3 virus multiplication in *Aedes aegypti* showed that the virus titer on day 2 was one fifth of the titer on day 0, while in *Aedes albopictus* the virus titer on day 2 was 100 times as low as that on day 0. On the other hand, the initial drop was remarkable in CHIK virus-infected *Aedes aegypti* in which in the titer on day 2 was 5,000 times lower than the virus titer on day 0. The initial drop observed in this study was consistent with previous reports.[4, 14,] Janzen et al[11] reported that on the 2nd day of oral infection, no CHIK virus was recovered from hemolymph of *Aedes aegypti*. Presence of virus antigen was restricted in the posterior midgut cells of *Aedes albopictus* infected with DEN-2 virus as reported by Kuberski.[16] These results suggests "gut barrier" was present in the midgut of mosquitoes infected with virus by oral feeding.

Titers of CHIK and DEN-3 viruses in infected mosquitoes

increased after the second day of infection. In *Aedes aegypti* infected with DEN-3 virus, the maximum titer was 4000 times as high as the titer on day 0, while in *Aedes albopictus* the maximum titer was 800 times higher than the titer on day 0. In *Aedes aegypti* and *Aedes albopictus* mosquitoes infected with CHIK virus, the maximum titers were 8 times and 60 times as high as the titer on day 0, respectively. After reaching the maximum, the titer began to decrease on the next day, indicating a limited period of efficient virus multiplication in the mosquito body. The maximum titers of CHIK virus in the Surabaya strain of *Aedes aegypti* and the Malang strain of *Aedes albopictus* were lower than that obtained with the Oahu strain of *Aedes albopictus*, but higher than or equal to those obtained with any other strains of *Aedes albopictus*. Although CHIK virus titers in *Aedes aegypti*, in general, tended to be lower than those in *Aedes albopictus*, the maximum titers were almost of the same magnitude in both *Aedes* species. Differences in some experimental conditions may affect the virus multiplication in these mosquitoes. High DEN-3 virus titers observed in the present study using *Aedes aegypti* and *Aedes albopictus* were similar to those reported by Gubler and Rosen[3] and Gubler et al.[5] with several other geographic strains.

Electron microscopic observations on salivary glands of *Aedes aegypti* and *Aedes albopictus* infected with CHIK virus by oral feeding showed that maturation process of the virus in this organs was almost similar in both species. A numerous number of enveloped CHIK viruses were seen in the intracytoplasmic areas of the salivary glands, especially in perinuclear area, and also were densely concentrated extracellularly in the apical cavity. The results so far obtained indicate that in *Aedes aegypti* as well as *Aedes albopictus* infected with CHIK virus, the maturation sites of salivary glands consisted in intracytoplasmic membranous structures such as cysternae of endoplasmic reticulum and vesicles as already shown in mammalian culture cells infected with DEN-1 and DEN-2 viruses[17, 19] and in *Culex tritaeniorhynchus* salivary glands infected with Japanese encephalitis virus.[27]

In the present study, also showed no picture of virus budding from the cell surface membrane of the salivary glands of CHIK-infected *Aedes aegypti* and *Aedes albopictus* as shown in DEN-infected ones; this finding was in contrast to those in the

mammalian cells such as Vero,[8] KB, IMR, J-111 and BHK-21 cells infected with CHIK or DEN viruses.[6, 18, 19] On the other hand, crystalloid structures of virions as seen in Vero and BHK-21 cells infected with DEN-1 and DEN-2 viruses[17, 19] were observed in the salivary glands. These results suggested that the salivary glands infected with CHIK virus play an important role in producing and maintaining a high titer of CHIK virus, similarly to as in the case of DEN virus.[25]

In connection with the role as a transmitter of viral diseases, relative abundance of a mosquito species, its relation to zoonoses and specificity of blood-feeding behaviors must be elucidated along with experimental results in the laboratory.[2] Differences in blood-feeding behaviors, for instance, may lead to different vectorial capacities of mosquito population.[20]

Field observations by Seawright et al. in North Florida, USA (temperature 21C-35C, constant weather condition) showed that female *Aedes aegypti* usually sucked blood on the 3rd day after mating and began ovipositing on the 6th day.[24] By using the Liverpool strain of *Aedes aegypti*, Judson reported that each female mosquito produced 70-90 egg per female by a single blood meal.[12] In our experiments of egg production, a female *Aedes aegypti* produced 66.2 ± 31.6 eggs in average by one blood feeding, while a female *Aedes albopictus* (Malang strain) produced 57.5 ± 17.3 eggs totally under the same conditions.

It is concluded from these results that these two *Aedes* species serve as potent vectors of DEN-3 and CHIK viruses. Especially, *Aedes aegypti* is important in urban areas and *Aedes albopictus* in rural areas. Epidemiological, ecological and experimental studies of *Aedes albopictus* and *Aedes aegypti* mosquitoes should be done more intensively and in comparison with each other. Such data will be useful to clarify the the recent pattern of DHF epidemics in Indonesia.

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REFERENCES

1. Berge, T.O.: The National Institute of Allergy and Infectious Diseases, National Institute of Health, Bethesda, Maryland. 1975. International catalogue of arboviruses.
2. Chamberlain, R.W., and Sudia, W.D.: Method in Virology. Vol. I, Academic Press, New York and London, 1967.
3. Gubler, D.J., and Rosen, L.: Am. J. Trop. Med. Hyg. 1976. 25. 318/325. Variation among geographic strains of *Aedes albopictus* in susceptibility to infection with dengue viruses.
4. Gubler, D.J., Nalim, S., Tan, R., Saipan, II., and Saroso, J.S.: Am. J. Trop. Med. Hyg. 1979a. 28. 1045/1052. Variation in susceptibility to oral infection with dengue viruses among geographic strains of *Aedes aegypti*.
5. Gubler, D.J., Suharjono, W., Lubis, I., Eram, S., and Saroso, J.S.: Am. J. Trop. Med. Hyg. 1979b. 28. 701/710. Epidemic dengue hemorrhagic fever in rural Indonesia. I. Virological and epidemiological studies.
6. Higashi, N., Matsumoto, A., Tabata, K., and Nagatomo, Y.: Virology. 1967. 33. 55/69. Electron microscope study of development of chikungunya virus in green monkey kidney Stable (Vero) cells.
7. Horsfall, W.R.: Mosquitoes. Their bionomics and relation to diseases. The Ronald Press Company, New York. 1955.
8. Hotta, S., and Matsumura, T.: Abstract of the Annual Meeting 1979, American Society for Microbiology. 1979. S(H)34. Studies on the release of togaviruses (Dengue and Chikungunya).
9. Igarashi, A., Fujita, N., Okuno, Y., Oda, T., Funahara, Y., Shirahata, A., Ikeuchi, H., Hotta, S. Wiharta, A.S., Sumarmo

and Sujudi: ICMR Annals. 1982. 2. 7/17. Isolation of dengue viruses from patients with dengue haemorrhagic fever (DHF) and those with fever of unknown origin (FUO) in Jakarta, Indonesia, in the years of 1981 and 1982.

10. Igarashi, A., and Mantani, M.: Biken J. 1974. 17. 87/93. Rapid titration of dengue virus type 4 infectivity by counting fluorescent foci.
11. Janzen, H.G., Rhodes, A.J., and Doane, F.W.: Can. J. Microbiol. 1970. 16. 581/586. Chikungunya virus in salivary glands of *Aedes aegypti* (L.): an electron microscope study.
12. Judson, C.L.: J. Med. Ent. 1968. 3. 21/23. Physiology of feeding and oviposition behavior in *Aedes aegypti* (L.).
13. Konishi, E., and Hotta, S.: Microbiol. Immunol. 1979. 23. 659/677. Effects of tannic acid and its related compounds upon chikungunya virus.
14. Konishi, E., and Yamanishi, H.: J. Med. Entomol. 1986. 23. 92/98. Titer distribution analysis of chikungunya virus in *Aedes albopictus* (Diptera: Culicidae).
15. Ko, K.K., Igarashi, A., and Fukai, K.: Archives of Virology. 1979. 62. 41/52. Electron microscopic observation on *Aedes albopictus* cells infected with dengue virus.
16. Kuberski, T.: J. Med. Entomol. 1979. 16. 343/349. Fluorescent antibody studies on the development of dengue-2 virus in *Aedes albopictus* (Diptera: Culicidae).
17. Matsumura, T., Stollar, V., and Schelesinger, R.W.: Virology. 1971. 46. 344/355. Studies of the nature of dengue viruses. V. Structure and development of dengue virus in Vero Cells.
18. Matsumura, T., Shiraki, K., Hotta, S., and Sashikata, T.: Proc. Soc. Exp. Biol. Med. 1972. 141. 599/605. Release of arboviruses from cells cultivated with low ionic strength media.
19. Matsumura, T., Shiraki, K. Sashikata, T., and Hotta, S.: Microbiol. Immunol. 1977. 21. 329/334. Morphogenesis of dengue virus in cultures of human leukemic leukocyte (J-111).
20. Mellink, J.J.: Mosquito News. 1981. 41. 119/125. Selections for blood feeding efficiency in colonized *Aedes aegypti*.
21. Mogi, M.: Mosquito News. 1982. 42. 196/201. Variation in oviposition, hatch rate and setae morphology in laboratory strain of *Aedes albopictus*.

22. Ministry of Health of Republic Indonesia: Hemorrhagic fever, diagnosis and patients care. 1981 (In Indonesian).
23. Oda, T., Igarashi, A., Hotta, S., Fujita, N. Funahara, Y., Djakaria, S., Hudojo, R., Isfarain, A., and Djohor, D.: ICMR Annals. 1984. 3. 31/35. Studies on bionomics of *Aedes aegypti* and *Aedes albopictus* and dengue virus isolation in Jakarta, Indonesia, 1983.
24. Seawright, J.A., Kaiser, P.E., Willies, J.L., and Dame, D.A.: J. Med. Ent. 1976. 13. 208/221. Field competitiveness of double translocation heterozygote males of *Aedes aegypti* (L.).
25. Sriurairatna, S., and Bhamarapravati, N.: J. Virol. 1977. 26. 275/283. Replication of dengue-2 virus in *Aedes albopictus* cell culture.
26. Sunoto, Van Peenen, PCFPD., Sumarno, Sarno Sinto, and Joseph, P.L.: Paed. Indonesiana. 1975. 15. 143/150. Studies on dengue in children at Dr. Cipto Mangunkusumo General Hospital, Jakarta (May 1973 - January 1974).
27. Takahashi, M., and Suzuki, K.: Am. J. Trop. Med. Hyg. 1979. 28. 122/135. Japanese encephalitis virus in mosquito salivary glands.
28. Tesh, R.B., Gubler, D.J., and Rosen, L.: am. J. Trop. Med. Hyg. 1976. 25. 326/334. Variation among geographic strains of *Aedes albopictus* in susceptibility to infection with chikungunya virus.
29. Yamanishi, H., Konishi, E., Sawayama, T., and Matsumura, T.: Jpn. J. Sanit. Zool. 1983. 34. 229/233. The susceptibility of some mosquitoes of chikungunya virus. (In Japanese with English Summary).