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Studies on Dengue Virus Infection in the Mosquitoes of Taiwan Rock Monkey Habitats at Gushan, Kaohsiung

Abstract

From Apr. 1992 to Jun. 1993, forest mosquitoes, larvae and eggs were collected by sweep-net and ovitraps once a month in the area of Taiwan rock monkey habitats at Gushan, Kaohsiung, and were processed for isolation of dengue viruses. Collected adults were kept alive at least 7 days and then triturated for virus isolation through use of *Aedes albopictus* cells C6/36 and also through inoculation of *Toxorhynchites amboinensis* mosquitoes. The inoculated cell cultures were kept at 32°C for 7 days, and the inoculated mosquitoes were kept at 28±1°C for 14 days. The cultures or mosquito head squash were examined for viruses with a fluorescent antibody technique. A total of 77 pools consisting of 2,670 female and 915 male mosquitoes were processed for virus isolation. The processed specimens were composed of 10 species of mosquitoes in 4 genera. No viruses were detected. It was suggested that the viruses didn't spread 1 kilometer away from downtown to forest area, although the Kaohsiung City had been outbreaked dengue fever many times.

1. Introduction

Dengue fever had become an important health issue in Taiwan after several outbreaks in the recent years. The mode of transmission of the indigenous outbreaks was through the bite of dengue fever patients by either *Aedes aegypti* or *Aedes albopictus*, and to the healthy individuals. The natural hosts of dengue virus were, in addition to man and the *Aedes* mosquitoes, some other mammals such as chimpanzees, gibbons and macaques. Simmons et al. first demonstrated that dengue virus could be transmitted from monkeys to monkeys or from monkeys to men⁽¹⁾. In 1965, monkeys in Malaysian forests were found to carry dengue virus antibody⁽²⁾. Later, dengue type 4 virus was isolated from the *Aedes* (*Finlaya*) *niveus* complex collected from the forest areas⁽³⁾. Dengue type 2 virus was also isolated from the *Aedes* (*Diceromyia*) *taylori* male mosquitoes collected from the forest areas of Africa, and Yuwono et al. identified dengue type 4 virus antibody in monkeys in the forest areas of Malaysia, Vietnam, Cambodia,

Indonesia and the Philippines⁽⁴⁾. These findings made one suspect if Taiwan monkeys were also infected with dengue virus. There were three major outbreaks in Taiwan in 1915, 1931 and 1942. In the 40 years after the World War II, no outbreak was noted until 1981 when an outbreak of type 2 dengue fever occurred in an off-shore island, Liouchyou, of Pingtung County^(5,6). In 1987, a minor outbreak occurred in Tungkang of Pingtung County and Kaohsiung City with 1,387 reported cases, primarily of type 1 virus and 5 cases of type 2 virus. In 1988, a major outbreak^(7,8) occurred in the southern part of Taiwan with 10,420 reported cases, primarily of type 1 virus^(9,10). In 1991, a minor outbreak occurred in the Sanmin District of Kaohsiung City with 431 reported cases, again primarily of type 1 virus. Because monkeys were also natural hosts of dengue virus, whether these outbreaks had brought virus to monkeys in the mountains and whether some infections began with monkeys to mosquitoes and to men require further investigations.

2. Materials and Methods

Collection of Mosquitoes:

Mosquitoes were collected once every month at the Peishou Mountain of Gushan District, Kaohsiung City, with sweep-net or human baits. They were kept in paper cups for at least 7 days under $28 \pm 1^\circ\text{C}$ for them to digest the blood they intook. 10 ovitraps were also placed at different places. These ovitraps are made of black plastic materials of 17 cm high and 12.5 cm in diameter with lids. On each an opening of 4 cm in diameter was made to allow mosquitoes to fly in. Along the inner wall of the container was a piece of 11.5×40 cm non-woven cloth dipped in water half way of the container to allow mosquitoes to lay eggs. Eggs, larvae and pupae were collected once every month, kept till adult mosquitoes in the laboratory and then grounded for virus isolation.

Isolation and Tests for Virus:

1) Grouping: Mosquitoes in paper cups were placed in a freezer for 2 to 3 minutes. Faint mosquitoes were then placed on filter paper for grouping under microscope. Mosquitoes of same sex and same species were collected in one tube of no more than 100 mosquitoes at one pool. Name of species, sex, amount and serial number were labelled on the tube. The tubes were then placed in the freezer under -70°C for virus isolation.

2) Grinding: Each batch of adult mosquitoes was placed in a tissue grinder, added 1 ml of HANK's MEM media, supplemented with 0.8% bovine serum albumin (BSA), ground and centrifugated at 10,000 rpm for 1 minute. The upper suspension was collected, freezeed and kept in -70°C .

3) Inoculation: Rosen and Gubler's method was used to inoculating viruses. The upper suspension was injected into the membranous area anterior to the mesepisternum

and below the spiracle of the *Toxorhynchites amboinensis*⁽¹¹⁾ at 0.51 μl per mosquito and 3-5 mosquitoes per batch. They were kept in paper cup for 14 days under 28-32°C.

4) Culture: The upper suspensions were filtered with 0.45 μm minipore, then the filtrates were diluted in HANK'S MEM media, supplemented with 0.8% BSA, and 50 μl aliquots were dispensed into 96-well microtitre plates. Stock C6/36 cells were then suspended in HANK'S MEM media with 5% fetal calf serum and 100 μl aliquots were added to each well which provided approximately 1×10^5 cells per well. The plates were then sealed with a sterile plate sealer and incubated at 32°C for 7 days.

5) Examination: The head squash immunofluorescence technique (IFT) was used to assay for infection of brain tissue⁽¹²⁾. Additionally, a small quantity of the cells in the microtitre plate removed with 20 μl phosphate buffer saline, and smeared on a piece of slide, then were examined by IFT. The antibodies used were group antibody to dengue: ATCC HB-114, group antibody to flavivirus: ATCC HB-112, FITC goat antimouse IgG(H+L) Zymed 62-6511.

3. Findings

Between April 1992 and June 1993, from Peishou Mountain of the Gushan District, Kaohsiung City, 4,018 mosquito larvae of 4 genera and 13 species (see Table 1) had been collected. Ninety four percent of them were *Aedes albopictus*. Peaks of collection were May to June and October to November. Other mosquitoes collected were: *Aedes pseudalbopictus*, *Aedes albolineatus*, *Culex pallidothorax*, *Aedes albopictus*, *Tripteroides arandoides*, *Tripteroides bambusa*, *Culex halifaxi*, *Culex bicornutus*, *Culex brevipalpis*, *Aedes annandalei*, *Heizmannia taiwanensis*, *Aedes malikuli*. In addition, by human baits and sweep-net, 88 female and 42 male mosquitoes of 3 genera and 4 species had been collected. 98% of them were *Aedes albopictus*. They were collected every month, and the collections were not related to seasons. In addition, one adult of each *Aedes desmotes*, *Culex pallidothorax* and *Armigera subalbatus* had also been collected. These mosquitoes were isolated for virus. A total of 77 pools in 2,670 female and 915 male mosquitoes of 4 genera and 10 species had been treated. The results were all negative.

4. Discussion

In Taiwan, of all *Aedes* mosquitoes, both *Aedes aegypti* and *Aedes albopictus* were in frequent contact with men. In the 1987-1990 outbreak in the southern part of Taiwan, dengue virus had already been isolated from the adult *Aedes aegypti*, *Aedes aegypti* was hence considered to be the vector of dengue fever in Taiwan. No dengue virus, however, had ever been isolated from *Aedes albopictus*. Many, Rosen (1985) and Foliart (1986) for instance, thought that *Aedes albopictus* was an important vector⁽¹³⁾. Rosen in 1983 demonstrated that four types of dengue virus could be vertically transmitted to the next generation⁽¹⁴⁾. Chen in 1993 also showed that *Aedes albopictus* in

Kaohsiung area was capable of transmitting type 1 virus at a rate of 3%⁽¹⁵⁾. *Aedes albopictus* was more widely distributed in Taiwan than *Aedes aegypti* with higher density in most areas. Though no virus had ever been isolated, this mosquito should be considered as one of the vectors of dengue fever.

Of the mosquitoes collected by the present study, 94% were *Aedes albopictus*. *Aedes aegypti* was a household mosquito and was not found in the mountain area. Two methods, injection into were used *Toxorhynchites amboinensis* and C6/36 for the propagation of virus. Of them, 130 adults and 3,455 larvae were tested, ending in all negative. As the sample size was relatively small, no conclusion should be made as to the non-existence of virus in mosquitoes and monkeys in these areas. To demonstrate the existence of dengue fever virus in these areas, future studies should try to increase the sample size and to improve the laboratory techniques.

References

1. Simmons JS, John JH, Reynolds FHK et al. Experimental studies of dengue, Philipp J Sci 1931;44:1-247.
2. Rudnick A. Studies of the ecology of dengue in Malaysia: A preliminary report. J Med Entomol 1965;2(2):203-208.
3. Rudnick A. Ecology of dengue virus. Asian J Infec Dis 1978;2:156-160.
4. Yuwono J, Suharhono W, Koiman I et al. Seroepidemiological survey on dengue and Japanese encephalitis virus infection in Asian monkeys. Southeast Asian J Trop Med Pub Hlth 1984;15:194-200.
5. Hsieh WC, Chen MF, Lin KT et al. Study of outbreak of dengue fever on Liouchyou Shiang, Pingtung County in 1981. J Formosan Med Assoc 1982; 81:1388-1395.
6. Wu YC. Epidemic of dengue 2 on Liouchyou Shiang, Pingtung, Taiwan, in 1981. Chin J Microbiol Immunol 1986;19:203-211.
7. Ko YC. Epidemiology of dengue fever — Epidemic of dengue fever in Taiwan. Kaohsiung J Med Sci 1989;5:1-11.
8. Harn ML. A clinical study of dengue fever in Kaohsiung from 1987 to 1988. Kaohsiung J Med Sci 1989;5:58-65.
9. Wu YC, Yueh IY, Lin TL et al. Laboratory diagnosis of dengue fever in Taiwan. Symposium on dengue fever, February 25-26, 1989, Taipei, Taiwan: Department of Health, Executive Yuan 1989;19-20.
10. Chuang CH. Dengue epidemics in Taiwan, 1981-1990. World Health Organization Dengue Newsletter 1991;16:31.
11. Rosen L, Gubler DJ. The use of mosquitoes to detect and propagate dengue viruses. Am J Trop Med Hyg 1974;23:1153-1160.
12. Kuberski TT, Rosen L. A simple technique for the detection of dengue antigen in mosquitoes by immunofluorescence. Am J Trop Med Hyg 1977;26:533-537.
13. Rosen L, Roseboom E, Gulber DJ et al. Comparative susceptibility of mosquito species and strains to oral and parenteral infection with dengue viruses and Japanese encephalitis B virus. Am J Trop Med Hyg 1989;34:603-614.
14. Rosen L, Shroyer DA, Tesh RB et al. Transovarial transmission of dengue viruses

