Isolation of Japanese encephalitis virus from *Anopheles annularis* and *Anopheles vagus* in Lombok, Indonesia

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Abstract

Three strains of Japanese encephalitis (JE) virus were recovered from mosquitoes collected in Lombok, Indonesia, during March 1979, from pools of *Anopheles vagus, An. annularis* and *Culex tritaeniorhynchus* respectively. This is believed to be the first report of isolation of JE virus from *An. vagus*. The frequencies of JE viral infection in zoophilic *Anopheles* species were higher than in *Cx tritaeniorhynchus*, the principle vector of JE virus in Asia. The low frequency of infection in *Cx tritaeniorhynchus* and the relatively infrequent raising of pigs may account for the low prevalence of JE neutralizing antibodies in the human populations of Lombok.

Introduction

Serological studies by KANAMITSU *et al.* (1979) identified neutralizing antibodies to Japanese encephalitis (JE) virus in 16% of sera obtained from human residents of Lombok Island, Indonesia. OLSON *et al.* (1983) showed by serological testing that domestic animals had been infected with JE virus, but that infections in man were rare.

Attempts to recover virus from arthropods collected in Lombok have been limited. In May 1978 a total of 15,607 mosquitoes were collected, pooled by species into 258 pools and virus isolation attempted. A single strain of JE was recovered from a pool of *Culex tritaeniorhynchus* collected from a cattle shed in West Lombok.

In 1979 we returned to West Lombok where antibody prevalences by a previous survey were highest and where the only pool of mosquitoes which yielded virus was collected. Collections were concentrated around animal sheds and scheduled for March after the annual rainy season was well under way when we expected large populations of *Cx tritaeniorhynchus*. Our objective was to estimate the prevalence of JE viral infection in mosquitoes in an attempt to explain the low prevalence of neutralizing antibodies in man (OLSON *et al.*, 1983).

Materials and Methods

Study Area

Lombok is one of a chain of islands which makes up the Lesser Sundas. It is located immediately to the east of Wallace's Line. Collections were made in three villages near Gerung, West Lombok. The human populace was engaged in subsistence agriculture supplemented by animal husbandry. Principal food crops included rice, corn and cassava. Domestic animals were housed adjacent to family dwellings and screening or other means to deny arthropods access to living areas was not evident. Mean annual rainfall exceeds 1500 mm with the bulk of precipitation between October and May. The rainy season was well under way when we arrived.

Arthropod collections

Mosquitoes were very abundant in the study area. Collections were made by means of CDC miniature light traps and resting/sweeping captures near animal stables in villages. All mosquitoes which had fed were held for two days to allow for digestion of blood meals. Mosquitoes were pooled by species in groups of about 100 and stored at or below −60°C. Each pool was thawed and triturated as previously described (TAN *et al.*, 1981).

Virus isolation

Each triturated pool was inoculated in stationary roller tubes which contained washed monolayers of vervet monkey kidney (Vero) and baby hamster kidney (BHK-21) cells. Cells were observed at two to three day intervals for evidence of cytopathic effect (CPE) and blind passaged after 10 days. 14 days after subpassage, specimens which showed no CPE were considered negative.

Virus identification

Viruses were identified in microneutralization (MNT) tests (KSIAZEK & LIU, 1980) after passage of virus by intracranial inoculation of suckling mice to increase the virus titre. Representative viruses known to be active in Southeast Asia and the Western Pacific were obtained from the Yale Arbovirus Research Unit (YARU) Arbovirus Reference Collection and the National Institute of Allergy and Infectious Diseases (NIAID). Hyperimmune mouse ascitic fluids (HMAFs) were prepared to each reference virus and field isolate using the modified method of BRANDT *et al.* (1967) at the NAMRU-2 laboratory in Taipei, Taiwan. Two isolates were tested by complement fixation (CF) as described by SHOPE & SATHER (1979).

Results

Table I lists the mosquitoes collected in west Lombok by species and the results of attempts to recover virus from each. A total of 144,545 mosquitoes belonging to three genera and 14 species were pooled by species. *Cx tritaeniorhynchus* was most frequently collected followed by *Cx vishnui* and *Cx whitmorei*.

Three strains of JE virus were recovered from three different mosquito species collected near Bilekedit.

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Table I—Mosquitoes collected in West Lombok, Indonesia during March 1979

Species	No. of mosquitoes tested (% of total)	No. pools tested (% of total)	No. pools positive %	Minimum frequency of infection* (expressed/1000) mosquitoes tested
Culex tritaeniorhynchus	112,398 (78)	596 (52)	1 (0·2)	0·01
Cx vishnui	15,820 (11)	104 (9)	0	
Cx whitmorei	7,050 (5)	62 (5)	0	
Cx fuscocephala	4,321 (3)	49 (4)	0	
Cx bitaeniorhynchus	360 (0·2)	13 (1)	0	
Cx pseudovishnui	138 (0·1)	7 (1)	0	
Anopheles vagus	2,700 (2)	42 (4)	1 (2)	0·37
An. tessellatus	581 (0·4)	33 (3)	0	
An. annularis	250 (0·2)	28 (2)	1 (4)	4·00
An. barbirostris	208 (0·1)	27 (2)	0	
An. kocki	104 (0·1)	15 (1)	0	
An. lineatopennis	383 (0·1)	24 (2)	0	
Aedes vexans	194 (0·1)	16 (1)	0	
Ae. poilcilius	38 (0·0)	8 (1)	0	
TOTAL	144,545	1,141	3	

*Minimum frequency of infection was calculated by dividing the number of pools positive for virus by the number of mosquitoes tested and then multiplying by 1000.

Table II—Results of cross-neutralization test employing a micro neutralization procedure*

Hyperimmune mouse ascitic fluid (HMAF)	Virus 1724	2254	2267	JE	MVE	TMU	SEP
1724	**≥640***	160	20	160	10	5	5
2254	≥640	**320**	40	320	20	< 5	< 5
2267	40	20	**40**	40	5	5	5
JE	≥640	≥640	**≥640**	**≥640**	160	< 5	< 5
MVE	10	10	5	5	**160**	5	< 5
TMU	10	20	10	5	80	**320**	< 5
SEP	< 5	< 5	< 5	< 5	< 5	< 5	**320**

*Reciprocal of highest dilution neutralizing test dose of virus containing 1·5-2·5 $\log_{10}$ $TCID_{50}$.

Isolate JKT Arbo No. 1724 was obtained from a pool of 200 female *Cx tritaeniorhynchus* feeding on domestic buffalo (*Bubalus bubalis*), or resting in buffalo stables, on March 22, 1979. Isolate JKT Arbo No. 2254 was recorded from a pool of 21 female *An. annularis* collected from buffalo stables on March 24, 1979. Isolate JKT Arbo No. 2267 was obtained from a pool of 70 *An. vagus* females which were collected by CDC light traps on March 24 and 25, 1979. Only three strains of JE virus were recovered from mosquitoes captured on Lombok in 1979. The MIF of JE virus was higher in *An. annularis* (4·0) and *An. vagus* (0·37) than in *Cx tritaeniorhynchus* (0·1), the principle vector of JE in most of Asia (BUESCHER *et al.*, 1959).

Table II shows the results of microneutralization testing with HMAFs prepared against the three strains of virus recovered in Lombok and several flavivirus which are present in Southeast Asia. Strains 2254 and 2267 were tested by CF and found to be closely related to or indistinguishable from JE virus.

Discussion

The frequency of JF virus infection in *Cx tritaeniorhynchus* mosquitoes in Lombok is low relative to hyperendemic foci in Java, Indonesia (VAN PEENEN *et al.*, 1975a, b), Sarawak, Malaysia (SIMPSON *et al.*, 1970, 1974) and other Asian countries (MAEDA *et al.*, 1978; FUKUMI *et al.*, 1975; BUESCHER *et al.*, 1959). This low frequency of infection may account for the paucity of serological evidence of human infection in Lombok (OLSON *et al.*, 1983). We have no information on whether stain differences occur among the JE isolates from Lombok and elsewhere.

The epidemiology of JE virus in Lombok is unique in that pigs are not raised in large numbers and are conspicuously absent from the area surrounding the study area. Large domestic animals including buffalo, cattle and horses residing in the area had serological evidence of infection with JE virus (OLSON *et al.*, 1983). Previous studies have shown that the domestic horse may develop sufficient viraemia (GOULD, 1964) to infect mosquitoes, but it is unclear whether horses act as amplifying host for JE virus in Lombok. Domestic ducks are an important food source and are raised in large numbers throughout the Island. Serological testing of a smaller number of sera from ducks and Java Pond Herons revealed no evidence of JE antibody.

The infection frequency of JE virus was low in *Cx tritaeniorhynchus* but was higher in *An. annularis* and *An. vagus* (Table I). The only previous evidence implicating either of the two last-named mosquito species as vectors of JE virus is one isolation from *An.*

annularis (see KSIAZEK *et al.*, 1980). Experimental transmission experiments have only been attempted with *An. tessellatus* (see BANERJEE *et al.*, 1977) and with *An. sinensis* (see PAVRI, 1979) which were both successful. The virus has also been recovered from *An. barbirostris* (see BANERJEE *et al.*, 1979), *An. hyrcanus, An. subpictus* and *An. sinensis* (see PAVRI, 1979).

Further studies are needed on the zoophilic *An. annularis* and *An. vagus* to determine whether they can transmit JE virus. It is possible that one or both these mosquito species could be a participant in an amplification cycle with an as yet unknown vertebrate or, less likely, that they are maintenance vectors in transmission cycles with birds.

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