ADDITIONAL ISOLATIONS OF JAPANESE ENCEPHALITIS VIRUS FROM THE PHILIPPINES

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INTRODUCTION

Japanese encephalitis virus (JEV) has been isolated in most areas of Asia (Berge., 1975) and has recently been isolated for the first time from mosquitoes collected in the Philippines (Trosper *et al.*, 1980). This paper reports additional isolations of JEV from Nueva Ecija Province, South Central Luzon, Republic of the Philippines at a location geographically separated from the site of the first reported isolation.

MATERIALS AND METHODS

Mosquitoes were collected in a previously described area near San Jose (Ksiazek *et al.*, 1980). Collections were made primarily by operated CDC light traps supplemented with dry ice although some were also made by sweep net collections from tethered carabao. Mosquitoes were processed for virus isolations using techniques previously described (Trosper *et al.*, 1980). Virus identification was performed using a microneutralization test employing a line of PS cells (porcine kidney)

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and reference hyperimmune mouse ascitic fluids prepared according to the method of Brandt *et al*, (1967).

RESULTS

A total of 79,177 mosquitoes representing 16 species was collected over a one week period during August 1977 and divided into 913 pools (Table 1). Three mosquito pools yielded viral agents: one from a pool of 50 *Culex tritaeniorhynchus*, one from a pool of 28 *Cx. bitaeniorhynchus*, and one from a pool of 92 *Anopheles annularis*. All three isolates were identified as JEV in a microneutralization test using both Nakayama strain hyperimmune mouse ascitic fluid and Nakayama reference virus for comparative purposes.

DISCUSSION

These findings offer further evidence that JEV is present and widely distributed in the Philippines. Isolation of JEV from Cx. tritaeniorhynchus is very common elsewhere in Asia (Berge, 1975) and isolation of JEV from this species in the Philippines has been previously reported (Trosper et al., 1980). The significance of isolation of JEV from Cx. bitaeniorhynchus and An. annularis is unknown although there are previous reports of isolations or experimental transmission in Cx. bitaeniorhynchus and several Anopheles spp. An isolation of JEV from field collected Cx. bitaeniorhynchus has been reported from India (Banerjee et al., 1979) and experimental infection and transmission of Cx. bitaeniorhynchus with JEV has also been reported (Dhanda

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Table 1

Species	No. of mosq. (%)	No. of pools	No. posit. pools
Culex vishnui	50,652(63.8)	525	0
Cx. tritaeniorhynchus	11,396(14.4)	139	1*
Cx. fuscocephala	4,183(5.3)	57	0
Cx. annulirostris	2,801(3.5)	33	0
Cx. bitaeniorhynchus	1,744(2.2)	29	1*
Cx. whitmorei	1,734(2.2)	28	0
Cx. gelidus	820(1.0)	14	0
Cx. fuscanus	117(0.2)	4	0
Cx. quinquefasciatus	36(0.0)	1	0
Anopheles annularis	2,491(3.2)	32	1*
An. peditaeniatus	1,936(2.5)	27	0
An. indefinitis	300(0.4)	3	0
An. tessellatus	220(0.3)	4	0
Aedes vexans	276(0.4)	7	0
Ae. lineatopennis	164(0.2)	3	0
Mansonia uniformis	307(0.4)	7	0
Total	79,177(100.0)	913	3

Mosquitoes collected near San Jose during August 1977 from which virus isolations were attempted.

*JEV isolates, Cx. tritaeniorhynchus, pool of 50; Cx. bitaeniorhynchus pool of 28; An. annularis pool of 92.

et al., 1977). JEV has been isolated from field collected An. hyrcanus and An. barbirostris (Banerjee, 1975) and experimental transmission of JEV by An. tessellatus has also been reported (Banerjee et al., 1977). Cx. bitaeniorhynchus and An. annularis have not been previously demonstrated to be important vectors in the endemic or epidemic cycles of JEV and, for the moment, isolation from these species would probably best be considered incidental.

The apparent absence of clinical cases of Japanese encephalitis in the Philippines may be due to a variety of reasons (Trosper *et al.*, 1980). In view of these additional isolations of JEV and previous serologic evidence of

its wide distribution in the Philippines, perhaps clinical diagnostic techniques should be employed more aggressively to determine the role of JEV as a cause of encephalitis.

SUMMARY

Japanese encephalitis virus was isolated from *Culex tritaeniorhynchus*, *Culex bitaenorhynchus* and *Anopheles annularis* mosquitoes collected from San Jose, Nueva Ecija, South Central Luzon in the Philippines. This is the second report of the isolation of the virus from mosquitoes in the Philippine Islands.

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