RECOVERY OF JAPANESE ENCEPHALITIS VIRUS FROM WILD CAUGHT MOSQUITOES IN THAILAND

PHINIT SIMASATHIEN*, SKON ROHITAYODHIN**, ANANDA NISALAK***,
PRICHA SINGHARAJ*, SCOTT B. HALSTEAD*** and PHILIP K. RUSSELL***

*Department of Microbiology, Faculty of Public Health, Mahidol University, Bangkok,
**The Thai Red Cross Society, Queen Saovabha Memorial Institute and
***Department of Virology, the U.S. Army Medical Component,
SEATO Medical Research Laboratory, Bangkok, Thailand.

INTRODUCTION

Japanese encephalitis (JE) virus, a member of the B group of arboviruses, was first isolated from the brain of a fatal human case of encephalitis in Tokyo, Japan in 1935 (Taylor, 1967). Subsequent isolations of this virus have come from many species of vertebrates in addition to man, including horses, pigs and wild birds, and from several species of mosquitoes. Natural vectors of JE virus include *Culex tritaeniorhynchus* in Northeastern Asia and *Culex gelidus* in Southeast Asia. Human encephalitis due to infection with JE virus occurs in Siberia, Japan, Korea, Okinawa, Taiwan, China, Malaysia, Singapore and India (Halstead and Grosz, 1962; Taylor, 1967). Prior to 1963, JE virus was suspected by clinicians as the cause of some of the encephalitis and aseptic meningitis seen in Thailand, but isolation of the virus had not yet been reported from this country. In 1962 a study of the ecology of arbovirus infections in horses was undertaken at the Queen Saovabha Memorial Institute at Bangpra, Cholburi, in Southeastern Thailand in cooperation with the U.S. Army Medical Component, SEATO Medical Research Laboratory. This paper reports the results of attempts to isolate JE virus from pools of mosquitoes collected during the study between 1962 and 1968.

MATERIALS AND METHODS

Mosquito Collections : Most of the mosquitoes were collected in New Jersey type and CDC light traps, and from a horse-baited Magoon trap. Female mosquitoes were identified, and combined by species into pools of up to 200 mosquitoes. Pooled mosquitoes were kept frozen in dry ice until virus isolation attempts were performed.

Virus Isolation : Mosquito pools of from 100 to 200 mosquitoes were triturated in 4 ml of 0.75% bovine serum albumin in phosphate buffered saline pH 7.4. In pools containing less than 100 mosquitoes, the buffered saline was used at the rate of 1 ml per 25 mosquitoes. Mosquito suspensions were subjected to centrifugation at 10,000 rpm in a refrigerated Sorvall centrifuge for 30 minutes. The supernatant fluids were inoculated both intracerebrally (0.01 ml) and intraperitoneally (0.02 ml) into 1- to 2-day old suckling mice. Inoculated mice were kept under observation for sign of illness for 21 days. Details of the procedure have been previously described (Singharaj *et al.*, 1966).

Virus Identification : Virus isolates were identified by neutralization test in primary hamster kidney cells and by plaque reduction neutralization test (PRNT) in LLC-MK_2 cells (Russell and Nisalak, 1967). Antiserum used for identification was prepared in rabbits by four intravenous inoculations of JE virus (Nakayama strain) containing 10^5 PFU given at one week intervals.

Table 1

Japanese encephalitis virus isolated from mosquitoes during October 1962 to October 1968.

Virus Strains	Mosq. Species	Number in pool	Date collected	Date of isolation
Ct-Bt-1	*C. tritaeniorhynchus*	100	10/14/62	10/15/62
Cg-Bt-10	*C. gelidus*	110	10/14/62	10/15/62
Cg-Lt-75	*C. gelidus*	180	11/7/62	11/8/62
Cg-Lt-77	*C. gelidus*	155	11/11/62	11/12/62
Cg-Lt-251	*C. gelidus*	200	11/10/63	11/10/63
BKM-210-64	*C. tritaeniorhynchus*	80	5/24/64	5/28/64
BKM-218-64	*C. tritaeniorhynchus*	200	5/28/64	5/29/64
BKM-229-64	*C. tritaeniorhynchus*	200	6/3/64	8/29/64
Ct-Lt-273	*C. tritaeniorhynchus*	180	6/21/64	6/22/64
Cg-Lt-520	*C. gelidus*	200	3/22/65	3/23/65
Cg-Lt-541	*C. gelidus*	200	4/21/65	4/22/65
Cg-Lt-549	*C. gelidus*	200	5/18/65	5/19/65
Ct-Lt-474	*C. tritaeniorhynchus*	200	5/19/65	5/20/65
Ct-Lt-475	*C. tritaeniorhynchus*	200	5/20/65	5/21/65
Cg-Lt-570	*C. gelidus*	200	6/8/65	6/9/65
Ct-Lt-499	*C. tritaeniorhynchus*	200	6/22/65	6/23/65
BKM-269-65	*C. gelidus*	13	9/12/64	11/18/65
BKM-426-65	*C. gelidus*	45	4/17 + 21/64	12/27/65
Cg-Lt-778	*C. gelidus*	160	3/2/66	3/3/66
BKM-64-66	*C. gelidus*	264	3/3/66	4/2/66
Cg-Lt-787	*C. gelidus*	170	3/13/66	3/14/66
Ct-Lt-627	*C. tritaeniorhynchus*	190	5/5/66	5/6/66
Cg-Lt-908	*C. gelidus*	200	9/12/66	9/13/66
Cg-Lt-1087	*C. gelidus*	200	5/1/67	5/2/67
BKM-562-67	*C. tritaeniorhynchus*	60	5/28/67	6/21/67
Cg-Lt-1240	*C. gelidus*	200	11/27/67	11/28/67
Ct-Lt-999	*C. tritaeniorhynchus*	200	11/28/67	11/29/67
Cg-Lt-1270	*C. gelidus*	200	12/25/67	12/26/67
Ct-Lt-1076	*C. tritaeniorhynchus*	170	4/30/68	5/1/68
Ct-Lt-1079	*C. tritaeniorhynchus*	200	5/7/68	5/8/68
Cg-Lt-1545	*C. gelidus*	200	10/17/68	10/17/68

RESULTS

From October 1962 to October 1968, a total of 31 strains of JE virus were isolated and identified. Virus strains, mosquito species, number of mosquitoes per pool, date of collection, etc. are presented in Table 1.

DISCUSSION

Culex tritaeniorhynchus has been proved a major vector of JE virus in Japan, Okinawa, Korea, Taiwan, Malaysia, China, India, and the USSR (Barnett and Gould, 1964; Buescher and Scherer, 1959). In Southeast Asia, particularly in Malaysia, both *Culex gelidus* and *Culex tritaeniorhynchus* serve as vectors of this virus. Results from this study indicate that both species are also natural vectors of JE virus in Thailand. *C. gelidus* may be the more important species of the two species in Southeastern Thailand because of the fact that more virus strains were recovered from this species although the total number tested was less than that of *C. tritaeniorhynchus*. Recovery of JE virus from mosquitoes in Thailand clearly indicates that this virus does exist in the country as formerly suspected (Taylor, 1967). The natural vertebrate hosts for the virus remain to be determined before a complete understanding of infectious cycle of JE virus in Thailand can be achieved.

ACKNOWLEDGEMENTS

The authors are deeply indebted to Drs. D.J. Gould and J.E. Scanlon, Chief and Former Chief, respectively, of the Medical Entomology Department, SEATO Medical Research Laboratory who had done some part of the mosquito collection in this study. Special thanks is due Dr. D.J. Gould for his suggestions and help in preparing this manuscript. The assistance of Mr. Anan Boonkanok and Mr. Ming Choohong is gratefully acknowledged.

REFERENCES

BARNETT, H.C. and GOULD, D.J., (1964). Vector-reservoir relationships in the ecology of Japanese B. Encephalitis. *Proc. Seventh Intern. Congr. Trop. Med. & Malaria, 3* : 156.

BUESCHER, E.L. and SCHERER, W.F., (1959). Ecologic Studies of Japanese Encephalitis virus in Japan. *Amer. J. Trop. Med. Hyg., 8* : 719.

HALSTEAD, S.B. and GROSZ, C.R., (1962). Subclinical Japanese Encephalitis. I. Infection of Americans with limited residence in Korea. *Amer. J. Hyg., 75*: 190.

RUSSELL, P.K. and NISALAK, ANANDA., (1967). Dengue virus identification by the plaque reduction neutralization test. *J. Immun., 99* : 291.

SINGHARAJ, P., SIMASATHIEN, P., SUKHAVACHANA, P., HALSTEAD, S.B. and SCANLON, J.E., (1966). Recovery of Dengue and other viruses in mice and tissue culture from Thai mosquitoes. *Bull. WHO, 35* : 67.

TAYLOR, R.M., (1967). Catalogue of Arthropod borne Viruses of the World. J (B) E. First Edition. US Government Printing Office, p. 549-552.