CORRELATION OF POPULATION INDICES OF FEMALE *CULEX TRITAENIORHYNCHUS* WITH JAPANESE ENCEPHALITIS VIRAL ACTIVITY IN KAPUK, INDONESIA

JAMES G. OLSON,* THOMAS G. KSIAZEK**, RATNA TAN, SOEROTO ATMOSOEDJONO, VERNON H. LEE and JAMES D. CONVERSE

U.S. Naval Medical Research Unit No. 2 Jakarta Detachment, APO San Francisco 96356

INTRODUCTION

Japanese encephalitis (JE) virus was first isolated from Java, Indonesia from pooled female *Culex tritaeniorhynchus* mosquitoes collected in Kapuk, an area near Jakarta where pigs are raised (Van Peenen *et al.*, 1974a). Further studies (Koesharyono *et al.*, 1973; Van Peenen *et al.*, 1974b; 1975) of the ecology of JE in this focus incriminated swine as amplifying hosts and further implicated *Cx. tritaeniorhynchus* as the principal vector species. The present study was designed to determine if increased relative abundance of *Cx. tritaeniorhynchus* was associated with increased JE activity.

MATERIALS AND METHODS

Mosquitoes were collected in 3 CDC miniature light traps on each of 2 nights per month at 2-week intervals from October 1978 through April 1980. Location of the light traps remained constant throughout the study. All blood fed females were held for two days to permit digestion of blood meals. Female mosquitoes were chilled on a cold table, identified, separated into pools of 50 each, and stored at −60°C. Each pool was thawed and triturated as previously described (Tan *et al.*, 1981).

Each triturated pool was inoculated into tubes which contained a washed monolayer of vervet monkey kidney (VERO) and baby hamster kidney (BHK–21) cells. Cells were observed at 2-3 day intervals for evidence of cytopathic effect (CPE) and blind passaged after 10 days. Fourteen days subsequent to subpassage, specimens which showed no CPE were considered negative.

Viruses were identified in micro-neutralization (Nt) tests after intracranial inoculation of suckling mice to increase the virus titer (Trosper *et al.*, 1980). Hyperimmune mouse ascitic fluids (HMAFs) to viruses known to be active in Southeast Asia sere prepared by the method of Brandt *et al.*, (1967) using stock virus supplied by the Yale Arbovirus Rseearch Unit, the National Institutes of Health or Dr. Leon Rosen. The strains of virus used to prepare these HMAFs have been given in a previous publication (Ksiazek and Liu, 1980).

Histograms were constructed of monthly trap indices (LTI = Log_{10} number of female *Cx. tritaeniorhynchus* per light trap per night for each month). Arcsine square root transformed percentages of pools from which JE was recovered and the minimum infection frequency (MIF), defined as the number of

This study was supported by funds provided by the U.S. Naval Medical Research and Development Command, Navy Department for Work Unit MR041. 09-001-0166 and MR041. 09-001-0162.

The opinions and assertions contained herein are those of the authors and are not to be construed as official or as reflecting the views of the U.S. Navy Department.

Reprint requests to Publications Office, U.S.Naval Medical Research Unit No. 2, APO San Francisco 96528.

* Present address: U.S. Naval Medical Research Institute Bethesda, Maryland 20814-5055

**U.S. Naval Medical Research Unit No. 3 FPO New York 09527.

pools positive divided by the number of mosquitoes per 1,000 in the pool, were calculated. Transformed data were tested for closeness of fit to several curves using a Radio Shack TRS-80 Color Computer and regression programs. The variation in frequency of recovering JE (Y) which was due to the variation of *Cx. tritaeniorhynchus* population indices (X) is measured by r. Those models (Table 3) which had highest r values fit the data best. Correlation coefficients (r) were tested for significant departure from 0 using Student's t-test (Swinscow, 1976).

Three strains of virus recovered from *Cx. tritaeniorhynchus* were not reisolated from the lyophilized material received at Yale.

Table 1

Virus strains recovered from mosqitoes collected in light traps, Kapuk, Indonesia, 1978-1980.

Jakarta Arbo Log #	Host species	Month collected	Virus
654	*Cx. tritaeniorhynchus*	Oct. 78	JE
657	*Cx. tritaeniorhynchus*	Oct. 78	JE
475	*Cx. tritaeniorhynchus*	Dec. 78	JE
788	*Cx. tritaeniorhynchus*	Jan. 79	JE
792	*Cx. tritaeniorhynchus*	Jan. 79	JE
811	*Cx. tritaeniorhynchus*	Jan. 79	JE
813	*Cx. fuscocephala*	Jan. 79	JE
1105	*Cx. gelidus*	Feb. 79	JE
1110	*Cx. tritaeniorhynchus*	Feb. 79	JE
1729	*Cx. tritaeniorhynchus*	Apr. 79	JE
1749	*Cx. tritaeniorhynchus*	Apr. 79	JE
1754	*Cx. tritaeniorhynchus*	Apr. 79	JE
1977	*Cx. tritaeniorhynchus*	May 79	Virus not yet identified
1989	*Cx. vishnui*	May 79	Non-viable*
2159	*Cx. tritaeniorhynchus*	Aug. 79	Not received
2204	*Cx. tritaeniorhynchus*	Oct. 79	Non-viable
2212	*Cx. tritaeniorhynchus*	Oct. 79	JE
2219	*Cx. tritaeniorhynchus*	Oct. 79	JE
2303	*Cx. tritaeniorhynchus*	Nov. 79	JE
2329	*Cx. tritaeniorhynchus*	Nov. 79	JE
2352	*Cx. tritaeniorhynchus*	Nov. 79	JE
2362	*Cx. tritaeniorhynchus*	Nov. 79	JE
2363	*Cx. tritaeniorhynchus*	Nov. 79	JE
2380	*Cx. vishnui*	Nov. 79	JE
4312	*Cx. gelidus*	Dec. 79	JE
4331	*Cx. tritaeniorhynchus*	Dec. 79	JE
4332	*Cx. tritaeniorhynchus*	Dec. 79	JE
4393	*Cx. tritaeniorhynchus*	Jan. 80	Non-viable*

* Virus was not recovered from lyophilized material.

RESULTS

We attempted to isolate virus from 18,486 female *Cx. tritaeniorhynchus* in 359 pools, 7,144 *Cx. gelidus* in 154 pools and 7,569 mosquitoes of other species in 192 pools. A total of 19 strains of JE were isolated from *Cx. tritaeniorhynchus* (MIF = 1.30, 5.2% positive pools). Two strains of JE (Table 1) were recovered from *Cx. gelidus* (MIF = 0.28, 1.3%, positive pools) and one strain each from *Cx. fuscocephala* (MIF = 1.00, 3.3% positive pools) and *Cx. vishnui* (MIF = 0.20, 1.0% positive pools).

Monthly frequencies of JE isolation and LTIs of female *Cx. tritaeniorhynchus* are shown in Table 2. Figures 1 and 2 show the correlation of monthly frequencies of JE isolation with *Cx. tritaeniorhynchus* LTIs. LTIs (X) were significantly correlated ($p < 0.05$) with the transformed percentage of pools positive for JE (Y) when fitted to several models tested (Table 3). Those models, which gave the closest fit (inverse linear power and exponential) result in accelerating increases of Y values, with increases of X. Similarly, *Cx. tritaeniorhynchus* LTIs were significantly correlated ($p<0.01$) with the MIF of JE (Y) when fitted to each of the

Table 2

Female *Culex tritaeniorhynchus* collected in light traps, Kapuk, Indonesia, 1978-1980.

Month of collection	Light trap index*	No. mosquitoes tested	No. pools tested	No. pools positive (%)	Minimum infection frequency**
Oct	167	772	16	2(12)	2.59
Nov	137	599	12	0	
Dec	387	1,473	31	1(3)	0.68
Jan 1979	341	1,790	36	3(8)	1.68
Feb	860	1,504	31	1(3)	0.66
Mar	186	433	9	0	
Apr	192	812	16	3(19)	3.69
May	190	319	5	0	
Jun	93	228	4	0	
Jul	155	275	5	0	
Aug	452	1,501	30	1(3)	0.67
Sept	144	245	1	0	
Oct	593	1,449	20	3(15)	2.07
Nov	1,036	2,859	57	5(9)	1.75
Dec	486	1,639	33	2(6)	1.22
Jan 1980	759	1,418	28	1(4)	0.71
Feb	258	810	16	0	
Mar	48	249	5	0	
Apr	32	111	4	0	
Total		18,486	359	22(6)	1.03

* Light trap index is the number of female *Culex tritaeniorhynchus* collected/trap/night during one month.
** Minimum infection frequency is the number of strains of JE recovered/1,000 mosquitoes tested.

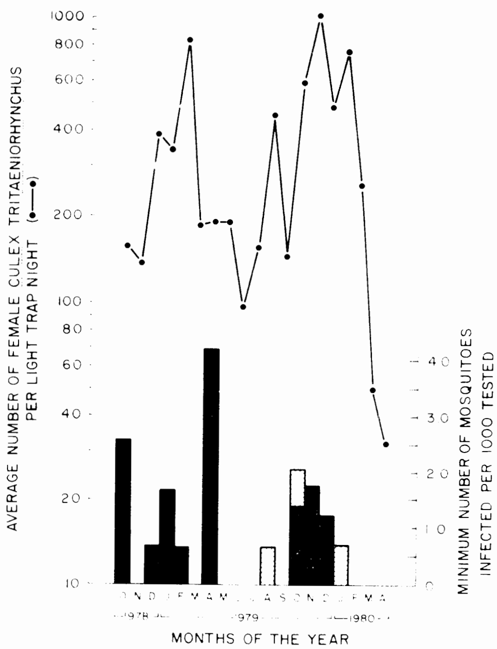

Fig. 1—Minimum frequency of Japanese encephalitis virus infection and abundance in female *Culex tritaeniorhynchus*, Kapuk, Indonesia.

■ confirmed positive by reisolation of virus

positive but virus not reisolated from lyophilized material presumed

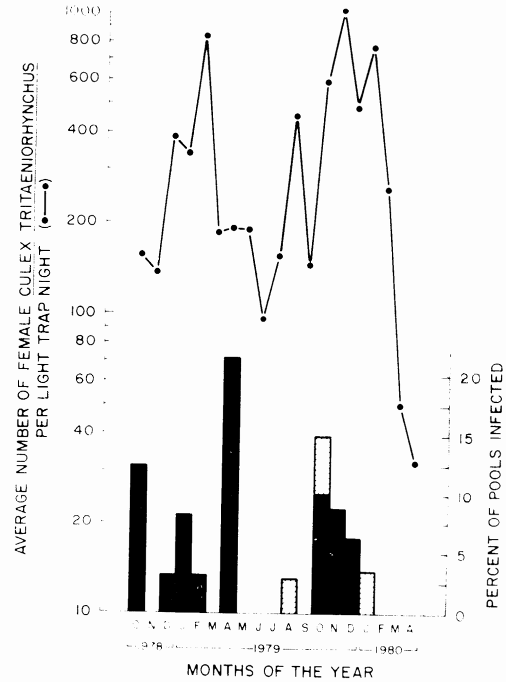

Fig. 2—Prevalence of Japanese encephalitis virus and abundance in female *Culex tritaeniorhynchus*, Kapuk, Indonesia.

■ confirmed positive by reisolation of virus

presumed positive but virus not reisolated from lyophilized material

Table 3

Correlation of *Culex tritaeniorhynchus* light trap indices* with virus activity.**

Type of model	Formula	%/MIF Measure of fit(r)	%/MIF level of significance (p)
inverse linear	1/Y = a + bX	0.736/0.740	< 0.001/ < 0.001
power	Y = aX^b	0.677/0.597	< 0.001/ < 0.01
exponential	lnY = ln a + bX	0.574/0.614	< 0.02/ < 0.01
linear	Y = a + bX	0.550/0.388	< 0.02/NS***
parabolic	Y = a + bX^2	0.548/0.379	< 0.02/NS
logarithmic	Y = a + b lnX	0.543/0.391	< 0.02/NS
hyperbolic	Y = a + b/X	0.526/0.389	< 0.05/NS

* data were transformed ($\log_{10}$ LTI)

** data were transformed (arcsine square root percent pools positive)

*** NS = p > 0.5

models tried. The best fit was observed with the same three models using the percentage of pools positive for JE.

DISCUSSION

The close fit of the monthly Log_{10} transformed mosquito population data (X) and monthly frequencies of JE virus infection in the mosquitoes (Y) to models suggests that as *Cx. tritaeniorhynchus* populations increase, the frequency of JE viral infection in the mosquitoes also increases. This relationship further suggests that JE viral infection frequencies increase proportionately with increased abundance of *Cx. tritaeniorhynchus*.

These observations have practical significance, since from this model targets for vector control can be derived. Calculation of the LTI of *Cx. tritaeniorhynchus* (X) which is associated with a very low frequency of infected mosquitoes (Y), provides a LTI below which infection of vector mosquitoes is rare and thereby the risk of transmission of virus to vertebrate hosts is extremely unlikely. When the population of *Cx. tritaeniorhynchus* reaches 66 females per light trap night only one per million females is infected with JE virus. Clearly, the risk of a vertebrate becoming infected depends upon many factors (Reeves, 1967), but the frequency of viral infection in the vector population has an important effect on the probability of viral transmission (Buescher and Scherer, 1959).

For the 19 month period of study, monthly LTIs of female *Cx. tritaeniorhynchus* varied from a low of 32 per light trap night to a high of 1,036. Of the 9 months during which population indices were lowest (LTI < 190), only 2 strains of JE virus were recovered from 3,328 *Cx. tritaeniorhynchus* comprising 64 pools (0.60 MIF, 3.1 % pools positive). From the 15,107 *Cx. tritaeniorhynchus* (295 pools) collected during the 10 months during which the LTI exceeded 190 females per trap night, 20 strains of JE virus were isolated (1.3 MIF, 6.8 % pools positive). At least one strain of JE was recovered during 9 of the 10 months when the LTI exceeded 190 females.

SUMMARY

Nineteen consecutive monthly light trap collections of mosquitoes were made between October 1978 and April 1980 in Kapuk, Indonesia. Kapuk is a small suburb of Jakarta where pigs are raised in close proximity to rice paddies which are breeding sites for *Culex tritaeniorhynchus*. Japanese encephalitis (JE) virus is believed to be endemic and has been recovered from mosquitoes and pigs in the area on several occasions. A total of 18,435 female *Cx. tritaeniorhynchus* were allocated to 359 pools of approximately 50 per pool. Virus isolations were attempted in both Vero and BHK-21 cells and agents producing cytopathic effect were identified in a micro-neutralization test. Nineteen strains of JE were recovered from the 359 pools of *Cx. tritaeniorhynchus* tested. The light trap index of female *Cx. tritaeniorhynchus* (X) and the relative frequency of pools positive for JE (Y) for each month of the study were plotted and correlation coefficients (r) calculated after transforming the mosquito population data logarithmically and the relative frequencies of isolation by arcsine square root. The close fit of the data ($p < 0.001$) to an inverse linear model ($1/y = a + b \log_{10}X$) suggests a close dependence of JE viral activity on the population dynamics of *Cx. tritaeniorhynchus*.

Three additional strains of JE were recovered from other *Culex* spp. at the same study site. One strain each was isolated from individual pools of *Cx. gelidus*, *Cx. vishnui* and *Cx. fuscocephala*. *Cx. tritaeniorhynchus* was more frequently infected with JE than the other species tested.

ACKNOWLEDGEMENTS

The authors wish to thank Chairin Ma'ruf, Woro Djuarti, Hardianto Saipan, Masran Masri, Iswardi Rachman and Jam Winata of the Virology Department, Aep Suhaepi, Nasir Hamzah and Umi Tri Indiah of the Entomology Department and Sukeri Sarbini and Suryatman Surdja of the Zoonoses Department for their assistance in collection, identification and preparation of mosquitoes and for technical aid provided in isolation, preparation and lyophilization of the virus strains. Special thanks to Twanda Thirkill who identified selected virus strains at Yale Arbovirus Research Unit. Sincere thanks to Dr. Gindo Simanjuntak and Dr Koesharyono of the Zoonoses Division of the Directorate General for Communicable Disease Control of the Ministry of Health for their support throughout the study.

REFERENCES

BRANDT, W.E., BUESCHER, E.L. and HETRIZK, F.M., (1967). Production and characterization of arbovirus antibody in mouse ascitic fluid. *Amer. J. Trop. Med. Hyg., 16*: 339.

BUESCHER, E.L. and SCHERER, W.F., (1959). Ecologic studies of Japanese encephalitis virus in Japan. IX. Epidemiologic correlations and conclusions. *Amer. J. Trop. Med. Hyg., 8*: 719.

KOESHARYONO, C., VAN PEENEN, P.F.D., JOSEPH, S.W., SAROSO, J.S., IRVING, G.S. and DURFEE, P.T., (1973). Serologic survey of pigs from a slaughter house in Jakarta, Indonesia. *Bull. Hlth. Stud. Indonesia, 1*: 8.

KSIAZEK, T.G. and LIU, J.Y.,(1980). A microneutralization test for flavivirus antibodies. *Southeast Asian J. Trop. Med. Pub. Hlth., 11*: 189.

REEVES, W.C., (1967). Factors that influence the probability of epidemics of Western equine, St. Louis and California encephalitis in California. *Vector Views, 14* : 13.

SWINSCOW, T.D.V., (1976). Statistics at square one. British Medical Association, London, pp. 86.

TAN, R., KSIAZEK, T.G. and OLSON, J.G., (1981). Comparative sensitivity of mosquito inoculation and mammalian cell culture for isolation of some arboviruses in Indonesia. *Southeast Asian J. Trop. Med. Pub. Hlth., 12* : 544.

TROSPER, J.H., KSIAZEK, T.G., CROSS, J.H. and BASACA-SEVILLA, V., (1980). Isolation of Japanese encephalitis virus from the Republic of the Phillipines. *Trans. Roy. Soc. Trop. Med. Hyg., 74* : 292.

VAN PEENEN, P.F.D., IRSIANA, R., SAROSO, J.S., JOSEPH, S.W., SHOPE, R.E. and JOSEPH, P.L., (1974 a). First isolation of Japanese encephalitis virus from Java. *Milit. Med., 139* : 821.

VAN PEENEN, P.F.D., JOSEPH, P.L., ATMOSOEDJONO, S., IRSIANA, R. and SAROSO, J.S., (1975). Japanese encephalitis virus from pigs and mosquitoes in Jakarta, Indonesia. *Trans. Roy. Soc. Trop. Med. Hyg., 69*: 477.

VAN PEENEN, P.F.D., JOSEPH, S.W., ATMOSOEDJONO, S., IRSIANA, R., SAROSO, J.S. and SAARONI, O., (1974 b). Group B arbovirus antibodies in sentinel pigs near Jakarta, Indonesia. *Southeast Asian J. Trop. Med. Pub. Hlth., 5* : 1.