

Experimental Transmission of Japanese Encephalitis Virus by Culex fuscocephala.

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OBJECTIVE: To determine if Culex fuscocephala mosquitoes can be infected by and transmit JE virus to a susceptible animal host.

BACKGROUND: JEV mosquito vectors in Thailand have been previously identified as Culex tritaeniorhynchus Giles and Culex gelidus Theobald. In 1970, during a JE epidemic, JE virus was isolated from 2 pools of female Culex fuscocephala Theobald collected in Chiangmai, a northern province of Thailand. In this epidemic area, C. fuscocephala were found to be as abundant as C. tritaeniorhynchus, a known JE vector. Furthermore, their feeding and breeding habits were very similar. They also feed on a wide variety of wild and domestic animals including man. Except for 2 previous reports from Taiwan in 1958 of JEV isolations from this mosquito species, C. fuscocephala has not been implicated as a potentially important JEV vector. To our knowledge, no published study has evaluated its efficiency as a vector of JEV.

MATERIAL & METHODS: JE virus strain, BKM-984-70, was isolated from a pool of 100 Culex fuscocephala females collected in Chiangmai in 1970. The stock virus had undergone 3 passages in suckling mice and was prepared as a 10% mouse brain suspension in bovine albumin phosphate saline (BAPS). First generations of wild-caught Culex tritaeniorhynchus and Culex fuscocephala females were allowed to feed on viremic white leghorn chicks which had been inoculated 55 hours earlier with 1,700 PFU of JEV subcutaneously. Engorged mosquitoes were then collected and maintained for transmission experiments. At selected days after the infectious blood meal, each of 8-10 presumably infected mosquitoes from each species was induced to feed on 1 day old chicks at night. After overnight exposure, each mosquito was inspected for presence of blood in its midgut under a dissecting microscope and tested for the presence of virus in LLC-MK2 cell culture and suckling mice. Chicks which were exposed to presumably infected mosquitoes were kept for 2-3 days before their blood was drawn and tested for viremia. Thus, one group of presumably infected mosquitoes was individually triturated and tested for virus after each transmission attempt while another group from the same cohort was similarly triturated without being allowed to feed on chicks. In addition, plaque reduction neutralization tests were performed on all virus isolates from the chick sera in order to confirm JE viremia.

RESULTS: 1) JE virus multiplication in C. tritaeniorhynchus and C. fuscocephala: Chart on page 38 shows that JE virus multiplies equally well in both mosquito species.

2) Infection Rates: In Table 1, the data demonstrates that more than 90% of both mosquito species are infected with JEV after feeding on viremic chicks and the infection remains at high level for at least 27 days.

3) Transmission Rates: In Table 2, both mosquito species show similar ability in transmitting JE virus to baby chicks. All virus isolates from chick sera were proven to be JEV by neutralization test.

4) Relationship between Mosquito Engorgement and Virus Transmission: Data in Table 3 shows that JE virus transmission could occur either by feeding until blood engorgement occurs or by feeding without engorgement (probing only).

DISCUSSION & CONCLUSIONS: It was demonstrated that more than 90% of C. tritaeniorhynchus and C. fuscocephala became infected after ingesting very small amounts of JEV (average infecting dose = 8 PFU/mosquito). JE virus multiplied in both mosquito species to comparable, high titers (above 10^5 PFU/mosquito) within 10 days after the infectious blood meal. Once infected, both species maintained high virus titers through at least four weeks. The average transmission rates in both mosquito species were found to be similar (16% and 17%). These low transmission rates might be due to several factors, such as low concentration of JE virus initially ingested (8 PFU/mosquito), somewhat low insectary temperature (25°–27°C), or the combination of first generation of wild-caught mosquitoes and JE virus of low passage. Evidence suggests that virus transmission occurred after mosquito probing as well as after feeding to repletion.

Thus, we have demonstrated a high vector efficiency of C. fuscocephala in the laboratory when compared with C. tritaeniorhynchus, a confirmed JEV vector. Together with field observations, these data provide strong evidence to incriminate Culex fuscocephala as a potentially important vector of JE virus in the northern part of Thailand.

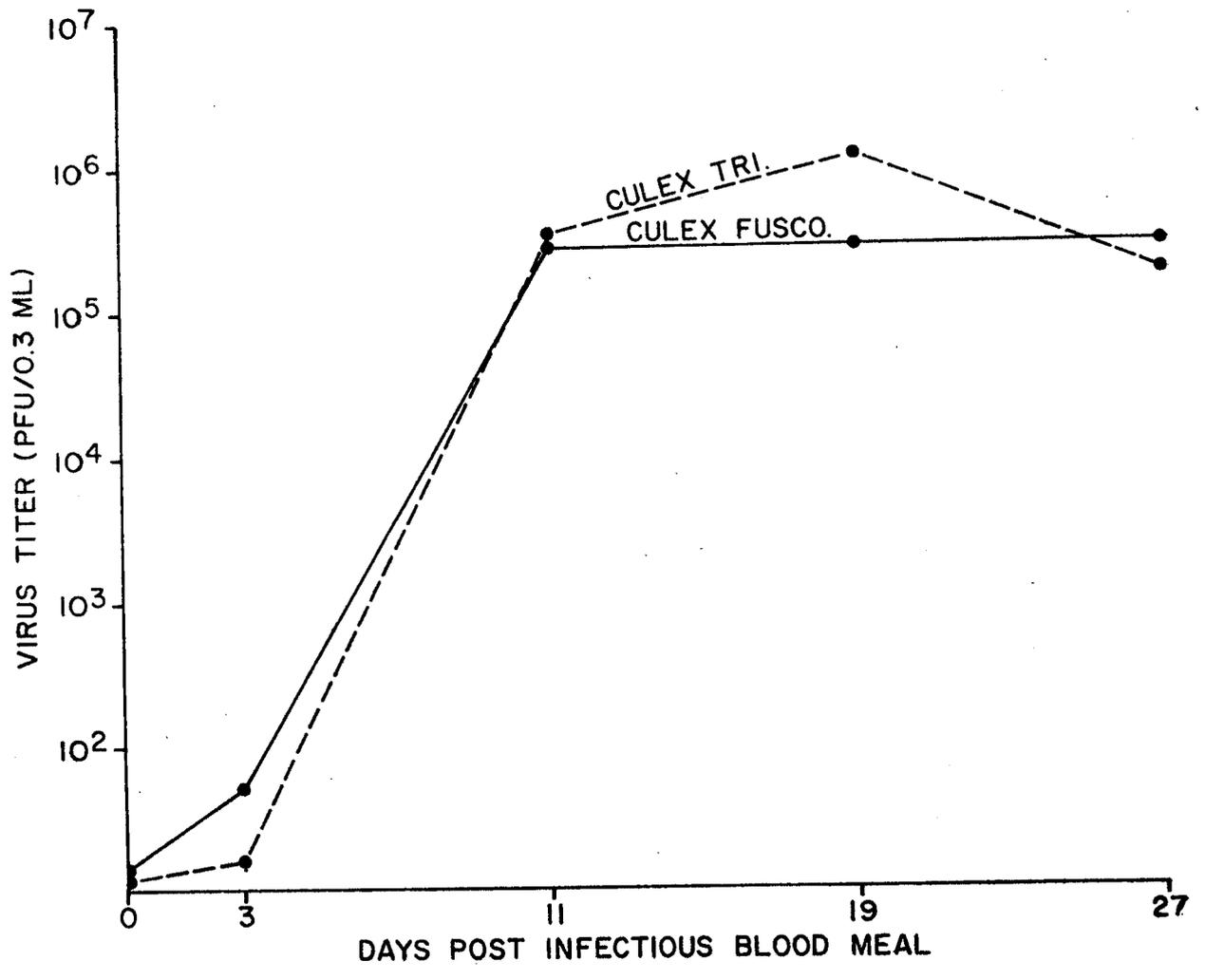


Table 1.
 Number of Culex tritaeniorhynchus and Culex fuscocephala infected
 with JE virus at various days after the infectious blood meal

Days after the infectious blood meal	<u>C. fuscocephala</u>	<u>C. tritaeniorhynchus</u>
10	19/20*	20/20*
11	20/20	18/20
19	20/20	18/20
27	14/15	18/20
TOTAL	73/95(97%)	74/80(92%)

* $\frac{\text{No. of mosquitoes infected}}{\text{Total mosquitoes tested}}$

Table 2.
 Comparison of the transmission rates of JEV-infected Culex fuscocephala
 and Culex tritaeniorhynchus mosquitoes*

Days after the infectious blood meal	% Transmission rate			
	<u>C. fusco</u>	<u>C. tri</u>	<u>C. fusco</u>	<u>C. tri</u>
10	1/10**	0/10**	10%	0%
11	2/10	1/10	20%	10%
19	2/10	4/10	20%	40%
27	1/8	2/10	12%	20%
TOTAL	6/38	7/40	16%***	17%***

* Each mosquito was tested for the presence of virus immediately after the transmission attempt and all were found to be infected.

** $\frac{\text{No. of viremic chicks}}{\text{total no. chicks exposed}}$

*** Average transmission rate

Table 3.
Relationship between mosquito engorgement and transmission of JE virus to 1–2 day old chicks

Virus transmission	<u>Engorged Mosquitoes*</u>		<u>Unengorged Mosquitoes</u>	
	<u>C. fusco.</u>	<u>C. tri.</u>	<u>C. fusco.</u>	<u>C. tri.</u>
positive	3	5	3	2
negative	3	14	29	19
Total	6	19	32	21

* Mosquitoes with presence of blood in the abdomen after an overnight exposure to 1–2 day old chicks