LABORATORY TRANSMISSION OF WESTERN EQUINE ENCEPHALOMYELITIS VIRUS BY MOSQUITOES OF THE GENERA CULEX AND CULISETA*‡

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In previous communications (1–3) the epidemiological evidence pointing to mosquito transmission of Western equine encephalomyelitis virus has been outlined. Many workers have demonstrated laboratory transmission of this virus by *Aedes* mosquitoes (see review, 2), others by a tick *Dermacentor andersoni* Stiles (4, 5) and by a cone-nosed bug *Triatoma sanguisuga* (Le Conte) (6). With the isolation, during 1941, of 5 strains of this virus from naturally infected mosquitoes, *Culex tarsalis* Coquillett (7) (Fig. 1), attention was drawn to a species of mosquito previously not generally suspected or proven to be a vector of any virus. Again during 1942, repeated isolations of Western equine virus were made from *C. tarsalis*, and additional isolations were made from *Culiseta inornata* (Williston), *Culex pipiens* Linnaeus, and *Anopheles maculipennis freeborni* Aitken (8). Experiments are here reported which conclusively demonstrate the ability of *C. tarsalis* to act as a laboratory vector of the Western equine virus. Thus, this species is definitely incriminated as a natural vector. In addition the vector ability of 8 other species of mosquitoes from Western North America, species representing 4 genera, have been tested.

Methods

The techniques employed in these studies differ from those commonly employed by other experimental workers. The reason for these changes and the details of the methods are very similar to those described fully in the preceding paper on St. Louis

* Formerly *Theobaldia*. According to Freeborn and Brookman (10): "*Theobaldia* N-L. 1902 is invalidated by the prior use of *Theobaldia* Fischer 1887.... As *Culicella* and *Culiseta* were proposed...by Felt in 1904, *Culiseta* takes priority because it was used by the first reviewer (Dyar 1921)...."

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virus transmissions (9). These will be discussed again only in so far as differences occur. The virus employed was strain F-199, a Western equine virus within a few passages after isolation from mosquitoes (1). In many experiments, chickens and ducks were used as the experimental vertebrate host instead of the commonly employed guinea pig. These barnyard fowl on the basis of epidemiological evidence appear to be important natural vertebrate reservoirs (see review, 3).

It was found in preliminary experiments that following a subcutaneous injection of approximately 100 fifty per cent end point intracerebral mouse doses of virus in

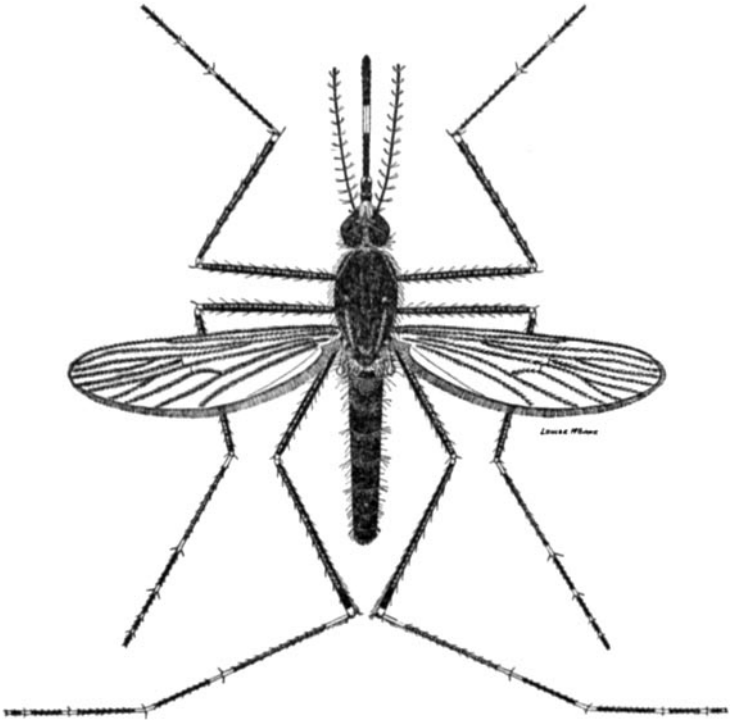

FIG. 1. *Culex tarsalis* Coquillett.

either chickens or ducks, 4 to 8 weeks of age, virus could usually be isolated from the blood serum from 18 to 64 hours later (11). These preliminary tests were made, unfortunately, with a "brain fixed" strain of Western equine virus, Howitt's California strain. Virus was most readily isolated at about 48 hours. 48 hours was therefore selected as the optimum time. However, just recently, since many lots of mosquitoes fed at 48 hours on chickens inoculated with F-199 strain virus failed to become infected, the earlier experiments were repeated with the new strain and it was found that the virus appeared in the blood much earlier and had usually disappeared or fallen to a very low titer 48 hours after the inoculation (12). This probably explains the high proportion of failures to infect mosquitoes from chickens.

Many of the mosquito transmission experiments here reported, were performed during the course of the summer of 1942 in field laboratories in the lower Rio Grande Valley, Texas, and in the Yakima Valley, Washington, in cooperative projects with the respective State Health Departments. Details of the first *Culiseta incidens* (Thomson) transmitted Western equine infection and a negative transmission with *Culex stigmatosoma* Dyar will also be reported. These were done in the spring of 1942 as a combined project of the Division of Entomology and Parasitology of the University of California, and the Hooper Foundation. Also reported are 8 experiments performed in San Francisco early in 1943.

FINDINGS

Experiments with Culiseta incidens (Thomson).—Two tests have been made of the ability of *Culiseta incidens* to act as a vector (Table I). The first experiment was completed before the techniques for utilizing fowl had been developed, and guinea pigs were the experimental animals. In the second experiment both guinea pigs and a chicken were used. In Experiment 1, transmission to a guinea pig occurred at 8 days' incubation and in Experiment 2, the mosquitoes infected a chicken at 12 days' incubation.

TABLE I

Experiments with Culiseta incidens

Holding temperature 24–26°C. Place, Berkeley, California. February, 1942.

Experiment No.	Source of virus	Days after infective meal when transmission or isolation was attempted	Mosquitoes feeding or tested	Material tested for virus	Result of virus test
1	Suspension	4, 6, 10	35, 31, 20	Guinea pig	0
		8	26	Guinea pig	+
		8	10	Mosquitoes	+
		10	20	Mosquitoes	0
2	Suspension	2, 6, 8, 10	9, 13, 15, 20	Guinea pig	0
		12	3	Chicken serum	+
		12	19	Mosquitoes	Contaminated

Experiments with Culiseta inornata (Williston).—Subsequent to the finding of *Culiseta inornata* infected in nature (8), 4 tests were made of the vector ability of this species (Table II). Individuals infected on a virus-blood suspension became infective as early as the 8th day and were demonstrated to be infective through 22 days (Experiment 4), by which time the longest experiment was terminated. A total of 7 transmissions occurred (Experiments 3, 4, and 5).

Experiment with Culex stigmatosoma Dyar.—In the fall of 1941, one experiment was performed with *Culex stigmatosoma* (Table III). It was able to

TABLE II

Experiments with Culiseta inornata

Holding temperature 24–29°C. Experiments 3, 4, 5, and 6. San Francisco, California. March, 1943.

Holding temperature 22–32°C. Experiment 7. Yakima, Washington. August, 1942.

Experiment No.	Source of virus	Days after infective meal when transmission or isolation was attempted	Mosquitoes feeding or tested	Material tested for virus	Result of virus test	Late neutralization test
3	Suspension	6, 8, 10, 14, 16, 18, 20, 22	27, 26, 27, 13, 5, 6, 43, 25	Chicken serum	0	
		12	23	Chicken serum	+	
		20	64	Mosquitoes	+	
4	Suspension	6, 10, 16, 18	10, 8, 11, 4	Chicken serum	0	
		8, 12, 14, 20, 22	11, 13, 9, 17, 4	Chicken serum	+	
		22	23	Mosquitoes	+	
5	Suspension	10, 14, 16	19, 9, 5	Chicken serum	0	
		12	13	Chicken serum	+	
		18	9	Mosquitoes	+	
6	Chicken	6, 8, 10, 12, 14	14, 9, 6, 7, 4	Chicken serum	0	
		14	8	Mosquitoes	0	
7	Suspension	6, 14	51, 4	Guinea pig	0	
		8, 12, 17	33, 6, 1	Chicken serum	0	0
		12	10	Mosquitoes	Contaminated	

TABLE III

Experiment with Culex stigmatosoma

Holding temperature 24–26°C. Place, Berkeley, California. November, 1941.

Experiment No.	Source of virus	Days after infective meal when transmission or isolation was attempted	Mosquitoes feeding or tested	Material tested for virus	Result of virus test	Late neutralization test
8	Suspension	5, 7, 8, 10, 11, 12	5, 5, 2, 1, 2, 2	Guinea pig	0*	+*
		5, 7	5, 5	Mosquitoes	0	
		12	18	Mosquitoes	+	

* A single guinea pig was used for all these feedings. 15 days after the last mosquito feeding its blood contained Western equine antibodies and the guinea pig resisted a challenge inoculation of this virus.

TABLE IV

Experiments with Culex tarsalis

Holding temperature 26–40°C. Experiment 9. San Benito, Texas. May, 1942.

Holding temperature 22–32°C. Experiments 10, 11, 12, 13, 14, 15. Yakima, Washington. July, August, 1942.

Holding temperature 24–29°C. Experiment 16. San Francisco, California. March, 1943.

Experiment No.	Source of virus	Days after infective meal when transmission or isolation was attempted	Mosquitoes feeding or tested	Material tested for virus	Result of virus test	Late neutralization test
9	Guinea pig	6, 10, 12, 15	8, 6, 6, 2	Guinea pig	0	
		15	10	Mosquitoes	0	
10	Duck	10, 16, 18	44, 12, 11	Chicken serum	+	+
		14	19	Chicken serum	0	0
		20	9	Guinea pig	0	
		21	5	Mosquitoes	+	
11	Suspension	2, 4, 7, 9	23, 10, 2, 1	Guinea pig	0	
		4, 7, 8	18, 4, 2	Chicken serum	0	0
12	Suspension	6, 8, 10, 12, 26	53, 25, 58, 28, 4	Guinea pig	0	
		14	21	Guinea pig	+	
		16, 18, 22, 30	29, 12, 17, 6	Chicken serum	+	+
		20	5	Chicken serum	+	
		28	5	Chicken serum	0	+
		16	10	Mosquitoes	+	
		22	10	Mosquitoes	0	
13	Suspension	5, 6, 7, 9, 13	43, 17, 39, 24, 13	Guinea pig	0	
		15	15	Chicken serum	+	+
		15	37	Mosquitoes	Contaminated	
14	Guinea pig	10, 15	45, 21	Chicken serum	0	0
		17	10	Chicken serum	+	+
		19	9	Chicken serum	+	
		21	10	Guinea pig	0	
		14, 22	10, 21	Mosquitoes	+	
15	Chicken	6, 10, 12	32, 12, 10	Chicken serum	0	0
		14, 16	16, 6	Chicken serum	0	
		8	34	Guinea pig	0	
		16	30	Mosquitoes	0	
16	Chicken	11	12	Chicken serum	0	
		16	11	Chicken serum	....	0
		13	8	Guinea pig	0	
		16	18	Mosquitoes	0	

harbor the virus for 12 days, and there was some evidence that transmission had occurred since the guinea pig on which all the mosquitoes had fed developed antibodies to Western equine virus and resisted an intracerebral challenge inoculation of the same virus. No experiment employing chickens was made.

Experiments with Culex tarsalis Coquillett.—As announced in an earlier report (3), *Culex tarsalis* fed on a virus-blood suspension, an infected guinea pig, and an infected duck transmitted the virus to chickens after incubation periods as short as 10 days and up till the end of the longest experiment at 30 days. One transmission to a guinea pig occurred.

TABLE V

Experiments with Culex pipiens

Holding temperature 22–32°C. Experiment 18. Yakima, Washington. September, 1942.

Holding temperature 24–29°C. Experiment 19. San Francisco, California. March, 1943.

Experiment No.	Source of virus	Days after infective meal when transmission or isolation was attempted	Mosquitoes feeding or tested	Material tested for virus	Result of virus test	Late neutralization test
18	Suspension	8	13	Guinea pig	0	
		10	4	Chicken serum	0	0
		12	4	Chicken serum	0	
		12	61	Mosquitoes	0	
19	Suspension	7, 14, 17, 20, 21	3, 2, 4, 1, 3	Chicken serum	0	
		21	22	Mosquitoes	0	

The details of these experiments and others are presented for the first time in Table IV (Experiments 9 to 16). Mosquitoes infected on a virus-blood suspension became infective by their bite to chickens and guinea pigs at a minimum of 14 days' incubation (Experiment 12) and remained infective until the end of the experiments, a period up to 30 days (Experiment 12).

It was demonstrated that specimens infected on an inoculated duck became infective to chickens at 10 days' incubation (Experiment 10) and remained infected up to the end of the experiment, 21 days' incubation. This species also became infected by feeding on an inoculated guinea pig and in subsequent feedings at 17 and 19 days' incubation, infected 2 chickens (Experiment 14).

The attempts to transmit the Western equine virus from chicken to chicken were unsuccessful. We now realize that with the strain of virus employed, virus was seldom present in adequate amount at the time the mosquitoes were permitted to feed on the inoculated chickens.

Experiments with Culex pipiens Linnaeus.—Two, limited tests (Table V)

were made of the vector ability of *Culex pipiens*. From these experiments, this species appeared to lose any virus content within a few days' incubation. Merrill and associates (15) also reported negative results. Additional tests,

TABLE VI

Experiments 20 and 21 with Psorophora confinnis and Experiment 22 with Psorophora ciliata

Holding temperature 26–40°C. Place, San Benito, Texas. May, June, 1942.

Experiment No.	Source of virus	Days after infective meal when transmission or isolation was attempted	Mosquitoes feeding or tested	Material tested for virus	Result of virus test
20	Suspension	3, 5, 7	20, 16, 6	Guinea pig	0
		7	9	Mosquitoes	+
21	Guinea pig	4, 6, 8, 10, 12, 15	20, 25, 19, 17, 18, 15	Guinea pig	0
		15	15	Mosquitoes	0
22	Guinea pig	2, 4, 6, 8, 10, 12, 15	32, 35, 16, 15, 32, 13, 26	Guinea pig	0
		12, 15	14, 35	Mosquitoes	0

TABLE VII

Experiment 23 with Mixed Anopheles maculipennis freeborni and Anopheles punctipennis and Experiment 24 with Anopheles maculipennis freeborni

Holding temperature 22–32°C. Experiment 23. Place, Yakima, Washington. August, 1942.

Holding temperature 24–29°C. Experiment 24. Place, San Francisco, California. March, 1943.

Experiment No.	Source of virus	Days after infective meal when transmission or isolation was attempted	Mosquitoes feeding or tested	Material tested for virus	Result of virus test	Late neutralization test
23	Suspension	6, 10	46, 28	Guinea pig	0	
		8, 12, 14	27, 17, 6	Chicken serum	0	0
		14	17	*A. maculipennis*	0	
		14	3	*A. punctipennis*	0	
24	Suspension	6, 8, 10, 12, 14, 16	21, 19, 19, 16, 11, 13	Chicken serum	0	
		16	18	Mosquitoes	0	

however, are indicated, since during 1942 this species was found naturally infected with Western equine virus in the Yakima Valley (8).

Experiments with Psorophora confinnis (Lynch Arribalzaga).—Psorophora confinnis has been shown to harbor Western equine virus for 7 days (Experiment 20), but transmission has not been demonstrated (Table VI, Experiments 20 and 21).

Experiment with Psorophora ciliata (Fabricius).—Psorophora ciliata was tested once, but transmission was not effected and virus was not recovered from the mosquitoes (Table VI, Experiment 22).

Experiments with Anopheles spp.—An early test employing a mixed group of *Anopheles maculipennis freeborni* Aitken and *Anopheles punctipennis* (Say) gave negative results (Table VII, Experiment 23). Subsequent to the finding of *A. maculipennis freeborni* infected in nature with Western equine virus (8), another test was made of its vector ability with completely negative findings (Table VII, Experiment 24). These tests cannot yet be accepted as conclusive.

DISCUSSION

By using a strain of Western equine virus originally isolated from mosquitoes, prior to its complete "adaptation" to direct mouse brain passage, and by using the presence of virus in the blood of chickens or development of clinical infection in guinea pigs as indications of transmission, 3 species of mosquitoes representing 2 genera (*Culex* and *Culiseta*) transmitted the virus by bite after being held for from 8 to 30 days' incubation at temperatures averaging above 25°C. (see summary Table VIII). Neither of these 2 genera has been previously reported capable of transmitting this virus. All earlier reports of mosquito transmissions were by the genus *Aedes*.

Since the Western equine virus has been isolated from *Culex tarsalis* repeatedly in the Yakima Valley, Washington, the proof of this mosquito's ability to transmit the virus assumes considerable importance. It must now be considered the first proven natural mosquito vector. As was previously reported in the case of St. Louis encephalitis virus (3, 10) the ability of this species to act as a vector to experimental birds and to acquire infection from them, greatly strengthens the other evidence that an infection cycle involving domestic fowl is the important one which occurs in nature. The feeding habits of *Culex tarsalis* (13, 14) in combination with its vector ability seems to explain satisfactorily much of the observed epidemiology of Western equine encephalomyelitis in the Yakima area.

The other species found naturally infected with Western equine virus in the Yakima Valley have been tested for their vector ability. *Culiseta inornata* has proven to be a capable vector. It therefore, can also be classified as a natural vector, though probably unimportant in this one area. Another species of this genus, *Culiseta incidens*, not yet found naturally infected can also transmit the infection in the laboratory. *Culex pipiens* and *Anopheles masculipennis freeborni* were found infected in nature but have not been incriminated as laboratory vectors. It is possible that those specimens of these 2 species infected in nature would never have become infective. They probably became temporarily infected by feeding on an infected animal and would either have lost their infection or never have developed to an infective stage.

It is interesting to note that *Psorophora confinnis* remained infected after 7 days' incubation. This genus has long been regarded with suspicion by certain workers and on further testing some species may prove capable of serving as vectors.

As has been previously demonstrated in studies with the yellow fever virus, St. Louis virus, and Japanese B virus, the mosquito vectors of virus diseases are not limited to the genus *Aedes* (10). For Western equine virus it has now been demonstrated that many species of *Aedes*, one species of *Culex*, 2 species

TABLE VIII

Summary of Mosquito Transmission Experiments

Mosquito	Source of infection	Minimum and maximum extrinsic incubation period in days	No. of times transmission effected	Days virus persisted in mosquito*
Culiseta incidens	Virus-blood suspension	8 to 12	2	12
Culiseta inornata	Virus-blood suspension	8 to 22	7	22
Culex tarsalis	Virus-blood suspension, duck, and guinea pig	10 to 30	12	30
Culex stigmatosoma	Virus-blood suspension	0	0 ?	12
Culex pipiens	Virus-blood suspension	0	0	0
Psorophora confinnis	Virus-blood suspension and guinea pig	0	0	7
Psorophora ciliata	Guinea pig	0	0	0
Anopheles maculipennis freeborni	Virus-blood suspension	0	0	0
Anopheles punctipennis	Virus-blood suspension	0	0	0

* In most instances in which there were positive results the figure represents the last day on which a test was made.

of *Culiseta*, one species of tick (*Dermacentor andersoni*), and one species of Reduviid (*Triatoma sanguisuga*) may act as experimental vectors.

CONCLUSIONS

1. Western equine virus has been successfully transmitted in the laboratory by 3 species of mosquitoes from 2 genera not previously reported as laboratory vectors: *Culex tarsalis*, *Culiseta inornata*, and *Culiseta incidens*.

2. Though transmission was not demonstrated, survival of the virus for more than a few days was shown to occur in *Culex stigmatosoma* and *Psorophora confinnis*. Possibly transmission occurred by the former.

3. In experiments with *Culex tarsalis*, infection of the mosquitoes occurred from feeding on an inoculated guinea pig, a duck, and virus-blood suspensions. After an incubation period of 10 to 30 days at a temperature above 25°C. these

mosquitoes infected chickens and a guinea pig by their bite and virus was in turn demonstrated in the blood of the chickens and in the brain of the guinea pig. A total of 12 transmissions occurred. The fact that mosquitoes can be infected from fowl and in turn transmit to fowl, together with much other supporting data from field and laboratory, is interpreted as strengthening evidence that fowl serve as reservoirs of virus in nature.

4. Since *Culex tarsalis* mosquitoes have been repeatedly found infected with Western equine virus and epidemiologic evidence supports their incrimination, the vector rôle of this species is now established, and it may be regarded as fully incriminated.

5. *Culiseta inornata* has also been found infected in nature and now proven a laboratory vector. This species does not fit the epidemiological picture in the Yakima Valley as well as *C. tarsalis*, but may play an important rôle elsewhere.

6. *Anopheles maculipennis freeborni* and *Culex pipiens* found naturally infected have not transmitted the virus under laboratory conditions.

BIBLIOGRAPHY

1. Hammon, W. McD., Reeves, W. C., Brookman, B., Izumi, E. M., and Gjullin, C. M., *Science*, 1941, **94**, 328.
2. Hammon, W. McD., Reeves, W. C., Brookman, B., and Gjullin, C. M., *J. Infect. Dis.*, 1942, **70**, 278.
3. Hammon, W. McD., Reeves, W. C., and Gray, M., *Am. J. Pub. Health*, 1943, **33**, 201.
4. Syverton, J. T., and Berry, G. P., *Science*, 1936, **84**, 186.
5. Syverton, J. T., *J. Exp. Med.*, 1941, **73**, 507.
6. Kitselman, C. H., and Grundmann, A. W., *Kansas Agric. Exp. Station Techn. Bull., No. 50*, 1940, 1.
7. Hammon, W. McD., Reeves, W. C., Brookman, B., and Izumi, E. M., *J. Infect. Dis.*, 1942, **70**, 263.
8. Hammon, W. McD., Reeves, W. C., and Brookman, B., data to be published.
9. Hammon, W. McD., and Reeves, W. C., *J. Exp. Med.*, 1943, **78**, 241.
10. Freeborn, S. B., and Brookman, B., in Identification guide to the mosquitoes of the Pacific Coast States, U. S. Public Health Service, Federal Security Agency, Malaria control in war areas, Atlanta, May, 1943.
11. Hammon, W. McD., and Izumi, E. M., unpublished data.
12. Hammon, W. McD. and Thorpe, A., data to be published.
13. Bang, F. B., and Reeves, W. C., *J. Infect. Dis.*, 1942, **70**, 273.
14. Reeves, W. C., and Hammon, W. McD., data to be published.
15. Merrill, M. H., Lacaillaide, G. W., and TenBroeck, C., *Science*, 1934, **80**, 251.