LABORATORY TRANSMISSION OF ST. LOUIS ENCEPHALITIS VIRUS BY THREE GENERA OF MOSQUITOES*

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In previous communications the epidemiological evidence pointing to mosquito transmission of St. Louis encephalitis virus has been outlined and the previous unsuccessful attempts by American workers to demonstrate transmission by mosquitoes in the laboratory have been reviewed (1, 2). The isolation of 3 strains of this virus from naturally infected mosquitoes, *Culex tarsalis* Coquillett (3, 4), encouraged further attempts to demonstrate transmission with this species and with others that occurred in large numbers in areas where this type of encephalitic infection was encountered. In the meantime, before *C. tarsalis* became available, transmission was effected with 2, more readily available mosquitoes, *Aedes lateralis* (Meigen) and *Culex pipiens* Linn. (1), the latter suspected in the St. Louis epidemic of 1933. In the following spring, in a temporary laboratory in Texas, successful preliminary transmission experiments were made with *C. tarsalis* and *Culex coronator* Dyar and Knab (2).

The success of these experiments in view of other failures, is attributed to two main factors: the use of a virus freshly isolated from mosquitoes, not brain passage "fixed," and the use of a bird as the experimental vertebrate host. Mice and monkeys had been unsuccessfully used as experimental animals by others whose criterion of transmission was based on development of visible signs of infection. It is now well known, however, that these animals are unlikely to manifest such signs when infected by a peripheral route unless the dose of the inoculum is inordinately large. In our work, on the basis of results of preliminary experiments, blood serum from the experimental bird host was injected into mice by the intracerebral route. The serum of the bird was taken 24 to 48 hours after infected mosquitoes had fed. By means of this test, infection of the bird was readily demonstrated by observing the development of en-

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cephalitis in the inoculated mice. Thus, viremia,[1] rather than observed signs of illness, was used as a criterion of infection in the experimental host. In no instance did the birds show any visible manifestation of disease. Most 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. In addition to these experiments the details of our first *Aedes* transmitted St. Louis infection will be reported. This was done in the fall of 1941 as a combined project of the Division of Entomology and Parasitology of the University of California and the Hooper Foundation. It involved transmission of the St. Louis virus to infant mice by *Aedes lateralis*. Two experiments with *Aedes nigromaculis* (Ludlow) and *Theobaldia*[2] *inornata* (Williston) were performed in San Francisco in 1943.

A total of 18 species of mosquitoes representing 5 genera have been tested. At least 2 species from each of 3 genera have transmitted the St. Louis virus.

Since, during a previous survey in the Yakima Valley, it had been found that 48 per cent of 113 domestic fowl tested had antibodies to the St. Louis virus (5), it seemed quite likely that these fowl were among the favored hosts of the vector. This percentage was higher than that found in any other group of domestic or wild vertebrates. It was important to know therefore whether or not birds could serve as efficient reservoirs of virus. *Culex tarsalis*, found naturally infected in the same area, included these among its hosts (6, 7). It was determined therefore to make some observations on the effect of inoculating a few of the more common domestic fowl by the subcutaneous route with such an amount of virus as a mosquito might be expected to transmit. If virus could be detected in the blood at some time thereafter, it would strengthen the evidence against them as reservoirs and at the same time would indicate that these fowl could serve as useful laboratory hosts for the demonstration of infection by mosquito bite. To determine these possibilities, two preliminary experiments were performed.

It was found that following a subcutaneous injection of approximately 100 fifty per cent end point intracerebral mouse doses of virus in either chickens or ducks 4 to 8 weeks of age, virus could frequently be isolated from the blood serum from 16 to 64 hours later. Virus could usually be isolated at about 48 hours from the undiluted serum and occasionally at a serum dilution of 10^{-2}. From an additional 5 chickens infected by mosquito bite, bleedings were made

[1] Virus present in the circulating blood.

[2] Since writing this paper Freeborn and Brookman have pointed out that *Theobaldia* has been invalidated due to prior use and that this genus must now be named *Culiseta*. Freeborn, S. B., and Brookman, B., Identification guide to the mosquitoes of the Pacific Coast States, U. S. Public Health Service, Federal Security Agency, Atlanta, May, 1943.

at 24 hours and 48 hours. In each instance virus was isolated at 48 hours, but twice it was not found at 24 hours. Some of these experiments were still in progress when mosquitoes became available in our field laboratories, so the methods employed in the earlier transmission experiments were somewhat different and less satisfactory.

Method

Strain F-103 of St. Louis virus, originally isolated from mosquitoes (3), was used in its third and fourth mouse brain passages. In addition to our identification of this virus, Dr. Casals and Dr. Webster have kindly checked the identification by means of the complement fixation reaction.

For preliminary transmission experiments the method of Merrill and TenBroeck (8), with certain modifications was followed. Cotton in a Petri dish cover was moistened with a mixture of a 10 per cent mouse brain virus suspension and defibrinated blood (rabbit or guinea pig) in the proportion of approximately 1:5. A little granulated sugar was then sprinkled on the blood soaked cotton. In most experiments carefully identified mosquitoes which had been reared from the larval stage were used. These were starved for at least 24 hours, then placed in lots of 50 or less in one-half pint glass fruit jars, the tops of which were covered with bobbinet. The jar was then inverted over the blood-virus soaked cotton to permit the mosquitoes to engorge. At intervals of half an hour, virus and blood which had been kept in the refrigerator were added to the cotton until a large proportion of or all of the mosquitoes had engorged. They were then released in a cage, and those which had fed were removed one at a time to another cage. Identification was again checked during this procedure. By this method of feeding it was usually possible to get almost all the mosquitoes of any species or genus to engorge on a blood-virus suspension.

Feeding on an experimentally infected animal (chicken, or duck, 48 hours after subcutaneous inoculation) though more similar to natural conditions was less frequently attempted, for in some instances, especially with the genus *Culex* only a very few mosquitoes could be induced to feed under laboratory conditions, and frequently none fed again at the desired intervals. Nevertheless this method was used a number of times. For feeding on an animal the mosquitoes were held in the same type of fruit jar and the bobbinet placed against a plucked area of the skin until all that would, had fed. By varying the amount of light, the time of day, and the temperature, at least a few could usually be induced to feed. These were then isolated and their identification again checked.

Following the infective meal all mosquitoes were held at a temperature averaging 80°F. or above in a room with a high relative humidity (average of 80 per cent or more). After varying periods, during which they were permitted to feed on cotton soaked with sugar water, the mosquitoes were replaced in a feeding jar and allowed to feed on a new experimental animal. These were usually 4 to 8 weeks old chickens, incubator-hatched, and previously protected from mosquitoes. The number of mosquitoes feeding was observed and recorded. 48 hours later, and occasionally also at 24 hours, about 2.0 cc. of blood were drawn from the fowl by cardiac puncture. This blood was permitted to clot in the refrigerator and at any time up to 24 hours, the serum was

separated, sealed in an ampoule, and frozen on dry ice. This serum was later tested for virus in our San Francisco laboratory by inoculating 0.03 cc. intracerebrally into each of 5 white Swiss mice. If one or more of these mice developed signs of encephalitis between the 5th and the 15th days, it was sacrificed and the brain passed to 3 other mice. If bacterial cultures were sterile and all 3 of the second passage mice developed typical encephalitis and died between the 5th and 10th days, transmission by mosquito bite was accepted as demonstrated. However, in most instances each fowl was again bled on the 15th day and the serum tested for the presence of antibodies to the Webster strain of St. Louis virus.

In two early experiments mosquitoes were permitted to feed on baby mice of less than 8 days of age. These were killed arbitrarily on the 5th or 6th day and brain passage made to other mice as in the case of chicken serum. By experiment we had previously found that mice of this age were relatively susceptible to encephalitis when inoculated by a peripheral route.

TABLE I

Experiment with Aedes lateralis

Holding temperature 77–81°F. Place, Berkeley, California. February, 1942.

Source of virus	Days after infective meal when transmission or isolation was attempted	Mosquitoes feeding or tested	Material tested for virus	Result
Suspension	6	40	Baby mice	+
	4 or 8	40,* 28*	" "	+
	10, 12	23, 16	" "	0
	12	22	Mosquitoes	0

* Only one of these was positive, but records were mixed.

At various intervals after an infective meal, or at the end of an experiment, lots of mosquitoes were sacrificed, ground in serum and broth, and tested by mouse inoculation for the presence of virus. Bacterial contamination frequently interfered with these tests and some of the results reported as negative might have been positive if heavy contamination had not been encountered. As a rule more difficulty from contamination was encountered in handling experimental mosquitoes than in those which were caught in the field.

RESULTS

Experiment with Aedes lateralis (Meigen).—A single test has been made of the ability of *Aedes lateralis* to act as a vector. The experiment was made before the techniques for utilizing fowl had been developed. Baby mice less than 10 days old were used. The brains from 9 mice fed on by 40, 40, and 28 mosquitoes at 4, 6, and 8 days' incubation respectively, were pooled and virus was isolated by mouse inoculation. Tests at 10 and 12 days' incubation were negative and no virus was isolated from 22 mosquitoes tested at 12 days' incubation (Table I).

Experiment with Culex stigmatosoma Dyar.—In the fall of 1941, one experiment was performed with *Culex stigmatosoma*, using a pigeon as the vertebrate host. Transmission was not demonstrated but virus persisted in the mosquitoes for at least 13 days.

Experiments with Culex quinquefasciatus Say.—In Experiment 1, of the summer of 1942, *Culex quinquefasciatus* mosquitoes were fed on a blood-virus suspension and in Experiments 4 and 6, on infected chickens. As shown in Table II, in no instance did transmission occur between the 2nd and the 10th days after the infective meal. However, infection persisted in the mosquitoes at least 11 days after feeding on an artificial mixture (Experiment 1), and at least

TABLE II

Experiments with Culex quinquefasciatus

Holding temperature 71–105°F. Place, San Benito, Texas. April and May, 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	Late neutralization test
1	Suspension	2, 4	20, 10	Baby mouse	0	
		2, 3, 4, 6, 10	2, 20, 12, 21, 2	Chicken serum	0	0
		4	155	Mosquitoes	0	
		3, 11	85, 10	"	+	
4	Chicken	3, 9	8, 6	Chicken serum	0	0
		9	26	Mosquitoes	+	
6	Chicken	4, 7, 8	3, 3, 2	Chicken serum	0	0
		9	23	Mosquitoes	+	

9 days after feeding on an infected chicken (Experiments 4 and 6). In each instance no test was made at a greater interval (Table II).

Experiments with Culex coronator Dyar and Knab.—In Experiments 2 and 13, *Culex coronator* mosquitoes were fed on a virus suspension and on a chicken, respectively. As seen in Table III, transmission to chickens in the former case occurred on the 8th and 10th days after the infective meal, and possibly on the 4th day as indicated by a positive neutralization test only. In Experiment 13, when the source of infection was a chicken, transmission did not occur and the 4 mosquitoes surviving till the 8th day did not contain virus. Too few mosquitoes participated in this latter experiment to make it significant (Table III).

Experiments with Culex tarsalis Coquillett.—As announced in a preliminary report (2), in Experiments 3 and 5 (Table IV), *Culex tarsalis* fed on a virus suspension transmitted the virus to chickens at 4, 6, 8, and 10 days. In one instance, transmission occurred through the bites of only 2 mosquitoes. As will

also be seen in Table IV, transmission was later effected by mosquitoes taking their infective meal on chicks (Experiments 17 and 18) and on a duck (Experiment 21). Transmission in these latter instances took place from the 8th to the 20th day after the infective meal, and possibly on the 6th day. The maximum temperature at which the mosquitoes were held was considerably lower in the latter experiments, probably accounting for the longer extrinsic incubation periods.

Experiments with Culex erraticus (Dyar and Knab).—Only one small experiment was performed with *Culex erraticus.* Five mosquitoes which had fed 8 days previously on an inoculated chicken failed to transmit infection when

TABLE III

Experiments with Culex coronator

Holding temperature 80–105°F. Place, San Benito, Texas. April and 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	Late neutralization Test
2	Suspension	4	3	Chicken serum	0	+
		6	3	" "	0	0
		8	5	" "	+	—
		10	4	" "	+	+
		11	25	Mosquitoes	+	
13	Chicken	3, 8	3, 1	Chicken serum	0	0
		8	4	Mosquitoes	0	

feeding on another. These 5 mosquitoes and the 3 other survivors were killed on the 8th day and tests for virus were negative.

Experiments with Aedes taeniorhynchus (Wiedemann).—In Experiment 7, no transmission or infection occurred from 84 *Aedes taeniorhynchus* which had engorged on an infected chicken, though large numbers fed at intervals up to the 8th day and 5 on the 10th. In Experiment 8, following engorgement on a virus-blood suspension, possible transmission occurred after 3 days and definitely occurred after 7 days when 99 fed on a chicken. Virus was isolated from both the 24 and 48 hour serum specimens on this chicken and neutralizing antibodies also were formed. The details of these two experiments are shown in Table V.

Experiments with Aedes aegypti (Linn.).—In Experiment 12, 35 *Aedes aegypti* fed on an inoculated chicken. After 3, 5, and 7 days at 80–105°F., 10, 14, and 8 respectively fed on chickens. No virus was isolated from these and no neutralizing antibodies were formed.

Experiment with Aedes dorsalis (Meigen).—In Experiment 15, 302 *Aedes*

dorsalis fed on a blood-virus suspension and after 2, 5, and 6 days at 82–92°F., no evidence of transmission was detected when 32, 2, and 1 fed, respectively. These mosquitoes did not survive for tests at a longer interval.

Experiment with Aedes vexans (Meigen).—In Experiment 16 (Table VI), *Aedes vexans* transmitted the virus to a chicken on the 5th day after engorging

TABLE IV

Experiments with Culex tarsalis

Holding temperature 80–105°F. Experiments 3 and 5. Place, San Benito, Texas. May, 1942.

Holding temperature 72–90°F. Experiments 17, 18, 21. Place, Yakima, Washington. August and September, 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	Late neutralization test
3	Suspension	8	2	Chicken serum	+	+
		15	2	Mosquitoes	+	
5	Suspension	4, 6, 10	8, 15, 6	Chicken serum	+	+
		9	5	" "	0	±
		10	18	Mosquitoes	+	
17	Chicken	6	10	Chicken serum	0	+
		8, 10, 12, 14	13, 14, 8, 13	" "	+	+
		16	9	" "	+	0
		16	9, 10, 15	Mosquitoes	+	
18	Chicken	11, 15, 21	51, 33, 6	Chicken serum	0	0
		17	3	" "	+	0
		19	9	" "	+	—
		22	17	Mosquitoes	Unsatisfactory	
21	Duck	10	55	Chicken serum	+	0
		16, 20	41, 15	" "	+	+
		14, 18	68, 51	" "	0	0
		21	53	Mosquitoes	+	

on a blood-virus suspension, as indicated by isolation of the virus from the chicken's serum and by the formation of neutralizing antibodies.

Experiment with Aedes nigromaculis (Ludlow).—In a single experiment, *Aedes nigromaculis* infected on a virus-blood suspension transmitted the virus to chickens at 10, 12, 14, 16, and 18 days' incubation (Table VII).

Experiment with Psorophora ciliata (Fabricius).—In Experiment 9, 41 *Psorophora ciliata* engorged on an inoculated chicken and were subsequently held at

TABLE V

Experiments with Aedes taeniorhynchus

Holding temperature 80–105°F. Place, San Benito, Texas. May, 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	Late neutralization test
7	Chicken	2, 4, 6, 8, 10	39, 52, 30, 23, 5	Chicken serum	0	0
8	Suspension	3	151	Chicken serum	0	+
		5, 9	102, 69	" "	0	0
		7	99	" "	+	+
		9, 11	30, 55	Mosquitoes	0	

TABLE VI

Experiment with Aedes vexans

Holding temperature 82–92°F. Place, Yakima, Washington. July, 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	Late neutralization test
16	Suspension	2, 6	22, 2	Chicken serum	0	0
		5	20	" "	+	+

TABLE VII

Experiment with Aedes nigromaculis

Holding temperature 78–95°F. Place, San Francisco, California. March and April, 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
5 B	Suspension	8	83	Chicken serum	0
		10, 12, 14, 16, 18	52, 37, 44, 28, 13	" "	+
		18	18	Mosquitoes	+

a temperature varying between 80°F. and 105°F. Feedings at 5, 7, and 9 days did not result in transmission, but virus was present in the remaining 17 mosquitoes on the 9th day (Table VII).

Experiment with Psorophora confinnis (Lynch Arribálzaga).—In Experiment 11, 20 *Psorophora confinnis* fed on an inoculated chicken and failed to transmit virus after 3, 5, and 7 days at 80–105°F. (Table VIII).

TABLE VIII

Experiments with Psorophora ciliata and Psorophora confinnis

Holding temperature 80–105°F. Place, San Benito, Texas. 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	Late neutralization test
9 (with *P. ciliata*)	Chicken	5, 7, 9	19, 20, 15	Chicken serum	0	0
		9	17	Mosquitoes	+	
11 (with *P. confinnis*)	Chicken	3, 5, 7	8, 5, 5	Chicken serum	0	0

TABLE IX

Experiment with Theobaldia incidens

Holding temperature 72–85°F. Place, 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	Late neutralization test
20	Suspension	6	10	Chicken serum	+	—
		8	6	" "	+	0
		10	2	" "	0	0
		10	4	Mosquitoes	0	—

TABLE X

Experiment with Theobaldia inornata

Holding temperature 78–93°F. 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
2 B	Suspension	6	10	Chicken serum	0
		8	11	" "	+
		10	8	" "	0
		12, 14	13, 9	" "	+
		16, 18	11, 4	" "	0
		20, 22	17, 4	" "	+
		22	23	Mosquitoes	+

Experiment with Anopheles maculipennis freeborni Aitken, and Anopheles punctipennis (Say), Mixed.—In Experiment 19, a mixed lot of 150 *Anopheles maculipennis freeborni* and *Anopheles punctipennis* engorged on a blood-virus suspension and after 6 and 8 days at 72–85°F. failed to transmit infection

when 6 and 5 fed, respectively. The 4 remaining *Anopheles punctipennis* were not demonstrated to contain virus on the 8th day, though the 7 *Anopheles maculipennis freeborni* were.

Experiment with Theobaldia incidens (Thomson).—In Experiment 20, transmission was effected by *Theobaldia incidens* 6 and 8 days after engorging on a blood-virus suspension. However, due probably to technical difficulties, no virus was recovered from 4 mosquitoes tested on the 10th day (Table IX).

Experiment with Theobaldia inornata (Williston).—In a single experiment with *Theobaldia inornata* transmission was effected at 8, 12, 14, 20, and 22 days after engorging on a blood-virus suspension (Table X).

DISCUSSION

By using a strain of St. Louis virus originally isolated from mosquitoes, prior to its complete "adaptation" to direct mouse brain passage, and by using the presence of viremia in a bird or fowl as an indication of transmission, 9 species of mosquitoes representing 3 genera (*Culex*, *Aedes*, and *Theobaldia*) transmitted the virus by bite after being held for from 3 to 22 days at temperatures averaging above 80°F. (see Summary, Table XI).

Since the St. Louis virus has been isolated from *Culex tarsalis* repeatedly in the Yakima Valley, Washington,[3] the proof of this mosquito's ability to transmit the virus assumes considerable importance. Various bits of evidence obtained from the Yakima Valley studies tended to incriminate birds, particularly those with domestic habits, as potential vertebrate reservoirs. Experimental evidence presented here indicates that *C. tarsalis* can readily acquire infection from a chicken or a duck and thereafter transfer it to another fowl following a short extrinsic incubation period at a high summer temperature. These findings greatly strengthen the evidence that this inapparent cycle is the important one which occurs in nature. Our observations indicate that *C. tarsalis* feeds readily on fowl, cattle, horses, and dogs and also feeds occasionally on man (6, 7), though it is not ordinarily a common house pest. These data seem to explain satisfactorily much of the observed epidemiology of the disease in the Yakima area and in a few other areas, specifically the Central Valley of California and Pinal County, Arizona.

The previous demonstration of the ability of *Culex pipiens*[4] to transmit this virus (1), offers a probable clue to the vector of the 1933 St. Louis epidemic (9) as this species was very common during this outbreak. Our experiments with a strain of *Culex quinquefasciatus*, from Texas, also a conspicuous species at the time of the St. Louis outbreak, although failing to demonstrate that it is able to transmit, do show that it can readily acquire the infection by feeding on

[3] Numerous isolations were again made in 1942 (to be published).

[4] Since writing this paper, *C. pipiens* has been found naturally infected with the St. Louis virus in the Yakima Valley (to be published).

a chicken and that the virus persists at least 11 days. There is a distinct possibility that another strain of this species may more readily transmit the virus.[5] There is considerable evidence that *C. quinquefasciatus* is very closely related to *C. pipiens*. Separation of adult females of *C. pipiens* and *C. quinquefasciatus* is oftentimes very difficult, especially in areas where both species occur.

Other mosquitoes employed in the above experiments have not been incriminated as vectors in any epidemiological studies, but it has seemed wise to in-

TABLE XI

Summary of Mosquito Transmission Experiments

Mosquito	Source of infection	Minimum and maximum extrinsic incubation period	No. of times transmission effected	Days virus persisted in mosquito*
		days		
Culex tarsalis	Virus-blood suspension, chicken and duck	4–20	14	21
" *coronator*	" "	8–10	2	11
Aedes lateralis	" "	4–8	2	8
" *taeniorhynchus*	" "	7	1	7
" *nigromaculis*	" "	10–18	5	18
" *vexans*	" "	5	1	5
Theobaldia incidens	" "	6–8	2	8
" *inornata*	" "	8–22	5	22
Culex stigmatosoma	" "	0	0	13
" *quinquefasciatus*	Virus-blood suspension and chicken	0	0	11
" *erraticus*	Chicken	0	0	0
Aedes aegypti	"	0	0	0
Psorophora ciliata	"	0	0	9
" *confinnis*	"	0	0	0
Anopheles maculipennis	Virus-blood suspension	0	0	8
" *punctipennis*	" "	0	0	0

* In most instances no satisfactory tests for virus were made at a longer interval of time.

vestigate their potentialities since they are found in large numbers in some epidemic areas. Since doing this work, viruses which on tentative identification appear to belong to the encephalitic group of infections have been isolated from a species of *Culex*[4] other than *C. tarsalis* and from a species of *Theobaldia*.

There is no evidence, from serological studies made on chickens in southeastern Texas (10), to suggest that for the past several years the fowl in this area have played any important rôle as hosts of the vector of St. Louis enceph-

[5] Since writing this, *C. quinquefasciatus* from Southern California have transmitted the St. Louis virus to chickens in our laboratory.

alitis, though antibodies were found frequently in cows and horses. Therefore, studies of other mosquitoes may be of considerable importance. Ticks and other arthropods, but principally those with biting habits limited to the hot season, should also be considered. Blattner and Heys have demonstrated laboratory transmission with *Dermacentor variabilis* (Say) (11).

Culex coronator was shown to be able to transmit the infection once it was acquired, but in a second small experiment failed to become infected or infective after feeding on an inoculated chicken. *Culex erraticus* was not adequately tested. The chicken on which the few mosquitoes fed may not have had a suitable viremia at the time of feeding. The same can be said for *Aedes aegypti* and *Psorophora confinnis*, which were fed on the same chicken for their infective meal.

Aedes lateralis, *Aedes taeniorhynchus*, *Aedes vexans*, and *Aedes nigromaculis* must all be considered as potential vectors since they transmitted after engorging on a virus suspension. In a single test *A. taeniorhynchus* failed to transmit after feeding on an infected chicken. The experiment with *Aedes dorsalis* unfortunately could not be carried beyond 5 days because of the untimely death of all mosquitoes.

It is interesting to observe, in connection with successful transmission of St. Louis virus by 4 species of *Aedes* mosquitoes, a genus which has previously been considered so potentially important in the transmission of the "equine" viruses, that this is another point in common between St. Louis encephalitis virus and the virus of Western equine encephalomyelitis. All 4 of these species have been reported as laboratory vectors of Western equine virus. Both infections occur simultaneously in the Yakima Valley, antibodies to both occur in an equal percentage of many species of animals, both were found in field-collected *C. tarsalis*, and both can be transmitted by *C. tarsalis*, and by certain *Aedes* and *Theobaldia*. However, up to this time no common antigenic factor between the viruses has been found to support a hypothesis of common parent source. Epidemiologically they appear to be as closely related or more so than Eastern and Western equine viruses.

Theobaldia incidens and *Theobaldia inornata*, represent the first 2 species of the third genus to be shown capable of transmitting the virus of St. Louis encephalitis. This is the first record of transmission of any virus by the genus.

Psorophora ciliata, though not a proven laboratory vector, does permit survival of the virus for at least 9 days. Neither this species nor *Psorophora confinnis* was satisfactorily tested, so negative results have little significance.

The experiment with mixed *Anopheles* was quite inadequate for negative conclusions, but it shows that *Anopheles maculipennis freeborni*, like *Anopheles quadrimaculatus* Say (12), can retain the virus for a short time at least.

As has been adequately demonstrated previously in studies with the yellow fever virus (13), it is obvious that mosquito vectors of virus diseases are not

limited to the genus *Aedes*, but that many genera have the ability to serve as vectors of these disease agents.

CONCLUSIONS

1. St. Louis virus has been successfully transmitted in the laboratory by the following 9 species of mosquitoes from 3 genera: *Culex tarsalis*, *Culex pipiens*, *Culex coronator*, *Aedes lateralis*, *Aedes taeniorhynchus*, *Aedes vexans*, *Aedes nigromaculis*, *Theobaldia incidens*, and *Theobaldia inornata*.

2. Though transmission has not been demonstrated, survival of the virus for more than a few days was shown to occur in *Culex quinquefasciatus*, *Culex stigmatosoma*, *Psorophora ciliata*, and *Anopheles maculipennis freeborni*.

3. In experiments with *Culex tarsalis*, infection occurred from feeding on chickens and ducks which had been previously inoculated by the subcutaneous route. After an incubation period these mosquitoes infected other chickens and virus was in turn demonstrated in the blood of these. This is interpreted as proof that fowl may serve as reservoirs of virus in nature.

Since mosquitoes have been repeatedly found naturally infected with St. Louis virus and epidemiologic evidence supports their incrimination, their rôle as vectors is now established. The fully incriminated species is *Culex tarsalis*.[6]

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[6] *Culex pipiens* can now be added as a fully incriminated species (to be published).