ST. LOUIS ENCEPHALITIS

TRANSMISSION OF VIRUS TO CHICKENS BY INFECTED MITES DERMANYSSUS GALLINAE AND RESULTING VIREMIA AS SOURCE OF VIRUS FOR INFECTION OF MITES*

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Chicken mites (*Dermanyssus gallinae*) collected in several localities in St. Louis County have been found to be infected with the virus of St. Louis encephalitis (1, 2). The infection is probably permanent since congenital transfer of the virus in these mites has been demonstrated. Persistence of the virus in nature is indicated likewise by isolation of the virus from mites collected at a single site at intervals of 6, 8, and 16 weeks and by persistence of the virus for many months in laboratory colonies, one established with mites found infected in nature (31 months) and one established with experimentally infected mites (21 months). Experimental infection of mites from a homogeneous colony of uninfected mites derived from a single female, was accomplished by allowing them to feed on chickens having viremia following subcutaneous inoculation of the virus. Transovarian passage in these experimentally infected mites has been demonstrated (3).

However, before these observations can be considered significant in the epidemiology of St. Louis encephalitis, it is necessary to show that infected mites are capable of transferring the virus of St. Louis encephalitis to chickens and that such chickens can serve as the source of virus for a blood-sucking vector. The present paper reports findings which show that infected mites, both those found infected in nature and those infected experimentally in the laboratory, are capable of producing viremia in chickens by bite and that these chickens in turn can serve as a source of the St. Louis virus for the infection of mites.

Materials

Mite Colonies Free of the St. Louis Encephalitis Virus.—The colonies of uninfected mites (*Dermanyssus gallinae*) used in this investigation were subcolonies from a homogeneous parent colony, shown by repeated tests to be free from the virus of St. Louis encephalitis. The establishment of this original homogeneous colony from a single adult female and her nymph offspring has been described in a previous communication (3).

Mite Colonies Infected with the St. Louis Encephalitis Virus.—Four colonies of chicken

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mites have been used: one colony derived from mites found infected in nature with the St. Louis virus (Rippy strain), and three colonies of mites infected experimentally in the laboratory with three strains of St. Louis encephalitis virus. The colony derived from mites found infected in nature has been maintained in the laboratory since October 5, 1944, by feeding on normal chickens. The colonies of experimentally infected mites were established with mites from the homogeneous uninfected colonies after they had been allowed to feed upon chickens inoculated subcutaneously with strains of the St. Louis virus. The method of infecting mites and the testing of these mites for the presence of virus have been described previously (3). Mites were infected experimentally with three different strains of the St. Louis virus: (*a*) the Rippy strain (RN_6), a mouse brain virus isolated from mites found infected in nature; (*b*) the Hubbard egg membrane strain, an egg membrane strain of St. Louis virus (Hubbard) isolated in mice from human brain tissue in 1937 and maintained since 1938 on the chorioallantoic membrane of the developing hen's egg (4); (*c*) the Mullen strain, isolated in mice in 1945 from the blood of a patient who recovered (5).

Chickens.—The chickens used in these experiments were New Hampshire Reds, 8 to 20 days of age. All chickens were hatched in the laboratory and carefully protected at all times from possible exposure to arthropods.

Transmission of the Virus of St. Louis Encephalitis to Chickens by Infected Mites

Many preliminary attempts to demonstrate viremia in chickens following the bite of infected mites were made by direct inoculation (0.03 to 0.04 ml.) of serum or whole blood intracerebrally into young Swiss mice. In no instance did either serum or whole blood produce any signs suggestive of encephalitis. Previous experience (6) had shown, however, that small amounts of the St. Louis encephalitis virus, insufficient in quantity to produce signs of encephalitis in mice even by the intracerebral route, could increase sufficiently by chorioallantoic passage in the developing hen's egg to produce signs of encephalitis when the egg membrane material was transferred intracerebrally to mice. In the light of this experience it seemed possible that virus might be present in the blood of chickens on which infected mites had fed but not in sufficient quantities to be detected by the direct inoculation of mice.

Accordingly, in a further series of experiments heparinized blood drawn from the heart of chickens at intervals after the beginning of the period during which the infected mites had fed, was injected intracerebrally into Swiss mice (0.03 ml.), and simultaneously 0.08 to 0.1 ml. of the same blood sample was inoculated on the chorioallantoic membrane of the developing hen's egg. Four days after inoculation these membranes were harvested and ground with a small amount of tryptose phosphate broth. The resulting suspension was centrifuged at low speed for 2 minutes, and the supernatant fluid was passed to a second series of embryonated eggs and at the same time was inoculated intracerebrally in 0.03 ml. amounts into white Swiss mice. As before, the passage membranes were harvested after a 4 day incubation period and were ground with broth. After centrifugation, the supernatant fluid was injected intracerebrally in 0.03 ml. amounts into white Swiss mice.

The colony of infected mites used in this series of experiments was derived from mites found infected in nature, the so called Rippy colony. The first three chickens tested for viremia by the above method were bled as follows: one at 18, one at 21, and one at 26 hours following

contact with the Rippy colony of infected mites. Virus was isolated from the blood of each of these three chickens by egg membrane passage and subsequent intracerebral inoculation of mice, but in no instance did any of the mice injected directly with fresh heparinized blood show signs of illness.

A fourth chicken was exposed to the Rippy colony of infected mites in the same way as before. This chicken was bled on three occasions—at 48, 72, and 93 hours counting from the time that the mites first had opportunity to feed. Virus was isolated from the blood obtained at each of these intervals by egg passage technique. Results obtained by direct inoculation of mice were negative.

In order to obtain some idea concerning the duration of viremia, a fifth chicken was bled at 20, 48, 67, 97, and 115 hours after the infected mites were given opportunity to feed. Virus was isolated from the blood of this chicken at 20, 48, 67, and at 97 hours, but not at 115 hours. Here again in no instance were signs of encephalitis observed in mice injected with the fresh heparinized blood. However, after two egg passages the presence of the virus was demonstrated readily by the intracerebral inoculation of mice with egg membrane material. Details of this experiment are given in Fig. 1. By means of the same method combining egg and mouse inoculation, the blood of this chicken (No. 5) was tested again for virus at 18 days and at 32 days following the first exposure to infected mites. Both tests gave negative results. After the second of these negative bleedings (at 32 days) this chicken was placed in contact with the infected mites for a second exposure in the same manner as that described for the first exposure. On this occasion the chicken was bled at 24, 48, 67, 100, and 123 hours after the beginning of the feeding period. By means of chorioallantoic passage, virus was detected in blood samples drawn at 48 and 67 hours, but not in those drawn at 24, 100, and 123 hours. Thus viremia occurred a second time in this chicken, resulting apparently from reexposure to infected mites 32 days after the first exposure.

The virus isolated from blood drawn at the 48 hour period in each of the above series of bleedings was identified by mouse protection tests, using known St. Louis immune rabbit sera, as the virus of St. Louis encephalitis.

Subsequently, virus was isolated by the egg passage technique from fifteen other chickens after exposure to the naturally infected mites (Rippy colony). Results obtained in these experiments are shown in Table I.

Viremia in Chickens Owing to the Bite of Experimentally Infected Mites

The first attempt to demonstrate virus in the blood of a chicken exposed to the experimentally infected mites (RN_6 strain) was not successful even by chorioallantoic passage. However, in later experiments virus was isolated with regularity from the blood of eighteen chickens fed upon by experimentally infected mites. Such viremia was produced by mites infected with the Rippy strain (RN_6) of virus and also with two other strains of the St. Louis virus, the Hubbard egg membrane strain and the Mullen strain, one isolated from the brain of a fatal case of encephalitis and the other isolated from the blood of a patient (4, 5). Three control experiments, in which the same procedures were followed but in which the chickens were exposed to mites free of the St. Louis virus, gave negative results. A summary of these results is included in Table I. During this phase of the experimental work many of the chickens tested

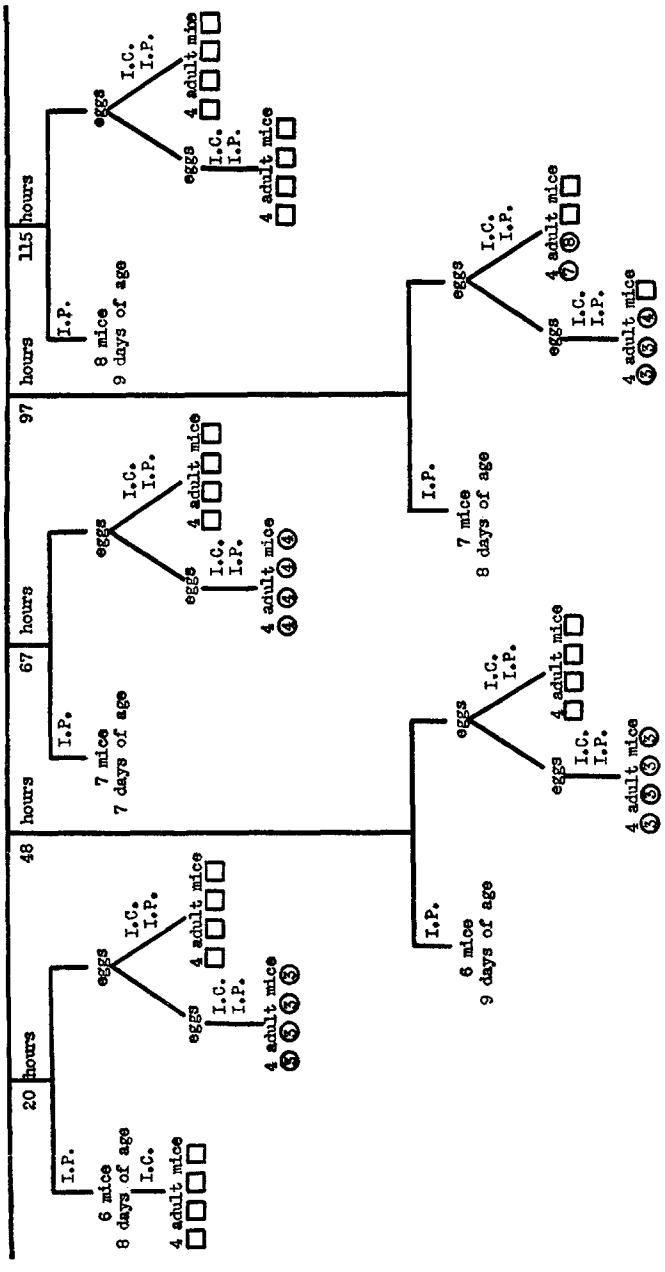

FIG. 1. Transmission of virus of St. Louis encephalitis to normal chickens by bite of infected chicken mites (*Dermanyssus gallinae*). Chicken, 20 days of age, fed upon by infected mites (Rippy colony) for period of 4 hours; heparinized blood drawn from heart at intervals following beginning of exposure period. Inoculations: 0.06 to 0.07 cc. heparinized blood on chorioallantoic membrane; 0.05 cc. heparinized blood injected intraperitoneally into young mice, 6 to 9 days of age.

for viremia were being used in connection with an investigation dealing with the mosquito transmission of the virus of St. Louis encephalitis.[1]

It is well to reemphasize that viremia in chickens bitten by infected mites is demonstrated with comparative ease by means of the chorioallantoic passage technique but that viremia has not been shown by the direct inoculation of serum or of fresh heparinized blood intracerebrally into mice. Multiplication

TABLE I

Transmission of the Virus of St. Louis Encephalitis to Chickens by Bite of Infected Mites (Dermanyssus gallinae). Viremia Demonstrated by Chorioallantoic Passage

Strain of virus infecting mites	No. of chickens tested	Viremia in chickens at intervals following the beginning of feeding by mites			
		24 to 30 hrs.	44 to 54 hrs.	72 to 77 hrs.	90 to 97 hrs.
Rippy strain (infected in nature)	3	0	+	+	+
	1	0	−	+	+
	6	0	+	0	0
	5	0	+	0	+
RN_6 strain	1	−	−	−	−
	1	+	+	+	0
	1	0	−	+	+
	2	0	+	0	+
	5	0	+	0	0
Hubbard egg membrane strain	1	0	+	+	+
	1	+	0	+	0
	2	0	+	+	0
Mullen strain	3	0	+	0	+
	1	0	+	0	0
	1	0	+	+	0
Control (normal mites)	3	0	−	0	−

+ = virus present in blood.
− = virus not present in blood.
0 = blood not tested.

of the virus by egg membrane passage seems to be required before its presence can be demonstrated by the intracerebral inoculation of mice.

Chickens Bitten by Infected Mites, as a Source of Virus for the Infection of Other Mites

Since it was evident that the amount of virus present in the blood of chickens following the bite of infected mites is small, it was of interest to ascertain

[1] Data to be published.

whether this small amount of virus circulating in the blood could serve as a source of virus for other (uninfected) mites.

When such a chicken infected by the bite of mites is used as a possible source of virus for uninfected mites, there is always the probability that mites from the infected colony remaining on the chicken will be carried over to the uninfected colony, defeating the purpose of the experiment at the outset. If, however, mites of the adult stage only are used to infect the chicken, and this chicken is exposed within the next 48 hours to mites of an uninfected colony, one can assume that all engorged first stage nymphs which are collected from the chicken must have come from the uninfected colony of mites constituting the second exposure. Nymph offspring of the adult infected mites which were used to infect the chicken cannot be present owing to the fact that insufficient time has elapsed for the development of nymphs from eggs laid by these infected adults, whereas nymphs found on the chicken are from the general uninfected colony composed of mites of all stages of development. Accordingly, the following experiments were carried out.

In order to obtain adult infected mites only, a normal chicken, 10 days of age, was placed in a container housing a colony of mites experimentally infected with the RN_6 strain of St. Louis virus. After 2 hours the chicken was removed and placed in a covered crock. During the next several hours, large engorged adult mites which dropped from the chicken were collected from the crock and placed in glass tubes. After egg laying the adults were separated from their eggs. Four or 5 days later the adult mites were placed on black paper, examined, and collected again in tubes. This procedure was followed in order to make sure that unfed nymphs which might have hatched from undetected eggs were eliminated. For each experiment 800 to 1000 adult infected mites were collected in the above manner, being placed each time in an autoclaved container.

The experimental procedure consisted in placing a normal chicken approximately 2 weeks of age in contact with these infected adult mites in the late afternoon and allowing exposure to continue during the night. Twenty-four hours after the beginning of this feeding period the chicken was used as a possible source of infection for uninfected mites. It was placed in a container housing a colony of uninfected mites, allowed to remain in contact with the mites for 2 hours in the late afternoon, and then removed to a covered crock. Mites remaining on the chicken continue to feed during the night. Approximately 16 hours later the chicken was removed, and the engorged mites of the smallest size, all believed to be first stage nymphs, were collected from the crock. These first stage nymphs were placed in glass tubes and allowed to moult to the second nymph stage. All of the engorged nymphs must have come from the uninfected colony since the mites used to infect the chicken were adults, and sufficient time had not elapsed for nymphs to have developed from eggs laid by these adults. Five to 7 days after these mites had been collected as engorged first stage nymphs, they were tested for virus.

In the course of four experiments of this type, details of which are given in the next paragraph, samples of approximately 150 to 920 mites were triturated in small amounts of tryptose phosphate broth and the resulting suspensions were inoculated intraperitoneally into young Swiss mice under 10 days of age.

These young mice were observed closely. While none developed definite convulsions, signs of illness such as roughening of the fur, unusual excitability or lethargy with failure to nurse appeared within 12 to 13 days after intraperitoneal inoculation. The brain of each mouse showing signs of illness was removed and passed by intracerebral inoculation to four or five adult mice. All the brains proved bacteriologically sterile on culture. In the experiments giving positive results the adult passage mice developed convulsions in 3 to 5 days following intracerebral inoculation. The infectious agent thus recovered from mites was identified in each instance as the virus of St. Louis encephalitis by means of the mouse protection test using the serum of a rabbit immunized with the virus of St. Louis encephalitis (Hubbard strain).

Results obtained in the four experiments are summarized briefly as follows:—

In the first experiment a suspension of approximately 850 nymphs was inoculated intraperitoneally into eight mice, 8 days of age. Two of the eight mice appeared ill on the 13th day following inoculation and virus was recovered from the brain of each. In a second experiment two samples of nymphs consisting of approximately 700 and 150 respectively were tested for virus. The suspension of 700 nymphs was inoculated intraperitoneally into six mice, 7 days of age. Two of the six began on the 12th day to show alternate periods of excitability and lethargy. Virus was recovered from the brain of each of these two by intracerebral passage to adult mice. The suspension of 150 nymphs was inoculated intraperitoneally into six mice, 12 days of age. One of the six showed definite signs of illness on the 12th day. It was killed on the 13th day and virus was recovered from the brain. A third experiment gave unsatisfactory results: Two of eight mice, 9 days of age, which had been inoculated intraperitoneally with a suspension of approximately 920 nymphs, were found dead and partially eaten on the 13th day. The brain of each of the other six young mice was tested for virus by intracerebral passage to adult mice with negative results. In a fourth experiment two samples of nymphs, 720 and 325, were tested for virus. The suspension of 720 nymphs was tested by intraperitoneal inoculation of seven mice, 8 and 9 days of age. Four of the seven mice appeared ill on the 12th and 13th days. Virus was recovered from the brain in the case of two of the four. The suspension of 325 nymphs in this experiment was tested by intraperitoneal inoculation of six mice, 8 to 9 days of age. Virus was recovered from the brain of each of two, which appeared ill on the 10th day.

Thus in three of four experiments the virus of St. Louis encephalitis, identified by neutralization with specific immune serum, was recovered from mites which had acquired the virus by feeding on a chicken previously bitten by infected mites.

As a control for these experiments and as verification of earlier results which had indicated that the colonies believed to be uninfected were still free of virus, two samples of mites from uninfected colonies were tested for virus. These two control samples consisted of 700 and 200 mites. The suspension of each sample was inoculated intracerebrally into six mice, 7 days of age. The brain of each of the twelve mice thus inoculated was passed intracerebrally to adult mice on the 12th or 13th day. None of these adult passage mice developed signs of illness.

DISCUSSION

Hammon and his associates (7) have presented data from field and laboratory investigations which support the hypothesis that both St. Louis encephalitis and Western equine encephalomyelitis are mosquito-borne, and that the source of the infection for the mosquito is an inapparent reservoir among vertebrates, particularly the domestic fowl. However neither the virus of St. Louis encephalitis nor that of Western equine encephalomyelitis has been isolated from hibernating mosquitoes, nor is there evidence that these viruses persist in the blood of experimentally infected fowls for longer than a few days. Thus the means by which these viruses persist from year to year in an endemic area is unexplained.

Isolation of the virus of St. Louis encephalitis from chicken mites (*Dermanyssus gallinae*) in several localities in St. Louis County during a non-epidemic year (1, 2), and demonstration that the virus persists in these mites for many months by transovarian passage (3) have pointed to the possible rôle of this arachnid vector in maintaining an endemic focus in nature.

However, as previously pointed out, these observations cannot be considered significant in the epidemiology of St. Louis encephalitis unless infected mites transfer the virus to chickens by bite and unless the blood of such chickens can serve as a source of virus for blood-sucking vectors. Results described in the present report demonstrate that both naturally infected and experimentally infected chicken mites are capable of transmitting the virus of St. Louis encephalitis to normal chickens by bite. The amount of virus in the blood of chickens thus infected appears to be small since viremia was demonstrated by means of the chorioallantoic passage technique but not by direct inoculation of serum or fresh heparinized blood intracerebrally into mice. However, the amount of virus in the blood of chickens bitten by infected mites was sufficient to be acquired by uninfected chicken mites which fed upon the chicken during the period of viremia. Since one blood-sucking vector, the mite, can acquire virus from chickens fed upon by infected mites, it seems possible that other blood-sucking vectors such as the mosquito may acquire sufficient virus from such chickens to infect other animals, mammals or birds, by bite.

A complex cycle of this type may be applicable also in the epidemiology of Western equine encephalomyelitis since Sulkin (8) has succeeded in isolating the virus of Western equine encephalomyelitis from *Dermanyssus gallinae* collected in nature, and more recently Reeves *et al.* (9) have isolated the virus of Western equine encephalomyelitis from another mite, *Liponyssus sylviarum.*

SUMMARY

Transmission of the virus of St. Louis encephalitis to normal chickens by the bite of infected mites (*Dermanyssus gallinae*) has been demonstrated. Both experimentally infected and naturally infected mites were shown to be

capable of transferring the virus of St. Louis encephalitis to chickens by bite. Virus is present in the blood of such chickens in small amounts, so that demonstration of viremia was possible only by utilizing chorioallantoic passage in hens' eggs. However, there is sufficient virus present in the blood for uninfected chicken mites to acquire the virus by feeding on chickens in which viremia has resulted from previous bite of infected mites. Thus it has been shown that the arachnid vector *Dermanyssus gallinae* is capable of transmitting the virus of St. Louis encephalitis to normal chickens by bite and that such chickens can serve as a source of virus for uninfected mites.

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