

Further Isolation of St. Louis Encephalitis Virus; Congenital Transfer of Virus in Chicken Mites (*Dermanyssus gallinae*).*

MARGARET G. SMITH, RUSSELL J. BLATTNER, AND FLORENCE M. HEYS.

From the Department of Pathology and the Department of Pediatrics, Washington University School of Medicine, St. Louis, Mo.

In a previous publication¹ the isolation of the St. Louis encephalitis virus from chicken mites (*Dermanyssus gallinae*) was reported. At that time the virus had been demonstrated on two occasions from separate collections of mites from a single chicken house in St. Louis County. Since our earlier report 3 further isolations of the virus have been accomplished from mites obtained from the same chicken house. Collections of chicken mites have also been made from 4 other localities in St. Louis County, and 2 of these have yielded the St. Louis virus. In addition, evidence of the congenital transfer of the virus in chicken mites has been secured.

On 8-20-44, 8-21-44, and 8-22-44 mites, some of which were freshly engorged with blood, were collected from the chicken house which had been the previous source of mites containing the St. Louis virus. The mites were identified and stored in glass tubes at the time of collection and on 8-22-44, 56 of these mites were triturated in an agate mortar with a small amount of tryptose phosphate broth. One-tenth of a ml of this inoculum was given intraperitoneally to each of 7 10-day-old mice. Two of these mice were killed when they showed signs of illness on the seventh day after inoculation. Three other mice developed convulsions on the eighth and ninth days. The brains of the latter 3 mice were stored for future passage and identification of the infective agent. The brain of each of the 2 mice killed on the seventh day after inoculation was emulsified and diluted 1:10 in broth. After centrifugation each supernatant fluid was inoculated intracerebrally into 4 adult Swiss mice. All the mice injected intra-

cerebrally with these inocula developed convulsions in 3 to 5 days.

On 8-29-44 and 9-7-44 mites were collected again from the same chicken house and stored in tubes. On 9-8-44, 78 of these mites were triturated in the manner described above and 8 ten-day-old mice were inoculated intraperitoneally with the mite suspension. Six of the 8 mice developed convulsions in 7 to 10 days. Intracerebral passage of brain tissue of each of the 6 to adult Swiss mice produced convulsions in 3 to 5 days.

Another collection from the above chicken house was made on 10-25-44. In this instance each engorged adult mite was placed in a separate glass tube to be observed for egg laying. Eggs were noted in most of the tubes. These eggs hatched into larvæ which metamorphosed into first stage nymphs within 24 to 30 hours. On 11-3-44 mothers from this collection were separated from their nymphs and were triturated in tryptose phosphate broth in the usual manner. Four nine-day-old mice and 2 eleven-day-old mice were inoculated intraperitoneally with .1 ml of the resultant suspension. Two of the 9-day-old mice developed convulsions on the fifteenth and sixteenth days following the intraperitoneal inoculation, and 1 of the eleven-day-old mice on the fifteenth day. From the brain of each an infective agent was recovered readily and was established in adult mice by intracerebral passage.

Similarly, 147 of the unfed first stage nymphs hatched in tubes from eggs laid by these mothers were triturated in broth and the resultant suspension inoculated intraperitoneally into 5 eight-day-old mice. Two of the 5 showed signs of illness with twitching on the thirteenth and fifteenth days. As before, an infective agent was recovered by secondary intracerebral passage and was

* Aided by a grant from The National Foundation for Infantile Paralysis, Inc.

¹ Smith, M. G., Blattner, R. J., and Heys, F. M., *Science*, 1944, **100**, 362.

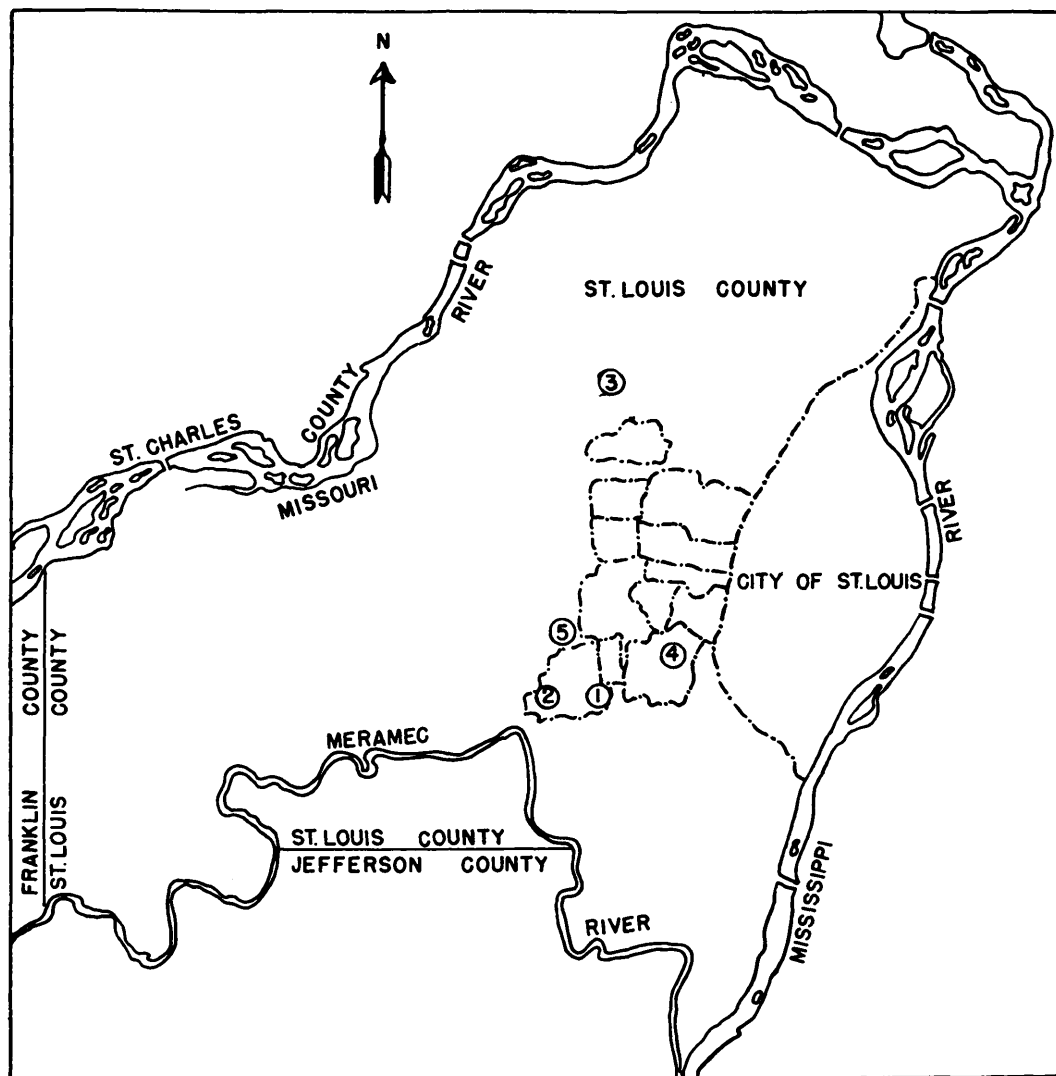


FIG. 1.

1, 2, 3—Sites from which St. Louis virus was isolated.
 4, 5 —Sites from which no virus was isolated.

established in adult mice. These results indicate that the infective agent was transmitted congenitally through the egg to the larval stage and through metamorphosis to the first stage nymphs. It should be emphasized that these nymphs were kept in glass tubes from the time that the eggs were laid and were unfed.[†]

The second chicken house (Fig. 1) from

[†] In *Dermanyssus gallinae* development from larva to the first stage nymph takes place without feeding.

which the St. Louis virus was isolated is approximately 2 miles distant from the site of the first isolation. On 8-18-44 mites, some freshly engorged with blood, were collected and stored. On 8-23-44, 60 of these mites were triturated in tryptose phosphate broth and 4 eight-day-old mice were inoculated intraperitoneally with the mite suspension. Three of the 4 mice developed convulsions on the sixth day following intraperitoneal inoculation. Secondary passage of the infective agent to adult Swiss mice by the intracerebral

route produced convulsions in mice in 3 to 4 days.

The third site from which mites containing the St. Louis encephalitis virus were collected is approximately 11 miles distant from the second chicken house and 10 miles distant from the original site of isolation. On 9-8-44, 72 mites which had been collected from this chicken house on 9-7-44 were triturated in broth. The suspension was injected intraperitoneally into 4 nine-day-old mice and into 3 eleven-day-old mice. In the case of the nine-day-old mice 3 developed convulsions on the seventh, ninth, and eleventh days following the intraperitoneal inoculation. The infective agent recovered from the brain of each of these mice was carried through 2 intracerebral passages in adult mice. One of the eleven-day-old mice receiving the mite suspension developed convulsions on the tenth day. The infective agent was passed intracerebrally to adult mice as before, producing convulsions in 3 to 6 days.

Routine cultures of the mouse brains in all instances were shown to be bacteriologically sterile. The infective agent isolated in each of the 5 instances was identified as the St. Louis encephalitis virus by means of mouse protection tests using immune St. Louis encephalitis serum. Immune sera were produced by the repeated inoculation of rabbits with 2 of the newly isolated strains. One of these 2 strains was isolated from site No. 1 and the other from site No. 2 (Fig. 1). The stock St. Louis virus (Hibbard strains) was neutralized by each of these 2 immune sera. Thus, the virus of St. Louis encephalitis has been isolated from chicken mites collected from one chicken house on 5 separate occasions, including the 2 previously reported¹ and from each of 2 additional chicken houses. The relative location of these sites is indicated on the map.

The 3 additional isolations (8-22-44, 9-8-44,

and 10-25-44) of the St. Louis encephalitis virus from one chicken house 6, 8, and 16 weeks after the initial isolation indicate the persistence of the virus in this population of chicken mites.

A colony established with mites collected from the above chicken house, site No. 1, has been maintained in the laboratory by frequent feeding on normal chickens from 10-5-44 to the present time, a period of 7 months. Two samples of this mite population were tested for the presence of virus on 3-5-45, 5 months after the colony was established, and from both samples the virus was recovered readily by the intraperitoneal inoculation of young mice.

The isolation of the St. Louis encephalitis virus from adult females and also from their unfed first stage nymphs indicates the congenital transmission of the virus in the chicken mite, *Dermanyssus gallinae*, and offers a plausible explanation for the persistence of the virus in mite populations, both in nature and under experimental conditions.

Summary. The virus of St. Louis encephalitis has been isolated from chicken mites (*Dermanyssus gallinae*) on 7 separate occasions from 3 of 5 sources in St. Louis County from which mites were collected. Two of these isolations from a single chicken house were reported in a previous publication. Since that time 3 further isolations have been accomplished from mites collected at this original site, and in addition one isolation has been made from each of 2 other chicken houses. All 3 sites are located in St. Louis County within a radius of 11 miles. Mites infected in nature have been shown to retain the virus after 5 months of propagation in the laboratory. Congenital transfer of the virus from the adult female through the egg into the first stage nymph has been demonstrated.