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MOSQUITOES AS VECTORS OF THE VIRUS OF EQUINE ENCEPHALOMYELITIS*

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At a seminar at the Army Medical School on March 17, 1933, and subsequently at a meeting of the Washington Branch of the Society of American Bacteriologists held March 21, 1933, the author presented a preliminary report on the ability of mosquitoes to transmit the virus of equine encephalomyelitis to guinea pigs. At the time the report was made to the two organizations mentioned, a mosquito-feeding experiment was under way with a horse, but up until that time the animal had shown no ill effects from the mosquito bites. Subsequently, however, this animal developed encephalomyelitis and died as a result thereof. Thus, we are now in a position to state definitely that mosquitoes are capable of transmitting the virus of equine encephalomyelitis, not only to guinea pigs but also to horses.

There is presented in the following paragraphs a brief summary of this work. A full, detailed treatise will be published at a later date.

The virus employed in these experiments was originally obtained from a natural case of the disease occurring in a horse in August, 1932, in South Dakota. It was furnished the Army Medical School in September, 1932, by Dr. H. W. Schoening, Chief of the Division of Pathology, U. S. Bureau of Animal Industry.

The mosquitoes used were *Aedes aegypti,* from stock which has been maintained by the Army Medical School, under laboratory conditions, since 1925, the original stock having been brought from the Philippine Islands.

The guinea pigs used weighed between 250 and 300 grams each. They were from carefully selected stock and had undergone our usual period of quarantine and observation before being placed in the experiments.

Following numerous passages of the virus through guinea pigs, and preliminary studies to determine the periods the blood of infected guinea pigs contained virus, three guinea pigs were inoculated for use in the mosquito-transmission experiments. One of these three pigs was inoculated both intracutaneously

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and intracerebrally. The other two were inoculated only by the intracutaneous route.

Commencing 48 hours after the infection of the guinea pigs, a lot of 50 *Aedes aegypti* was permitted to feed on these three guinea pigs. The following day—72 hours after the inoculation of the guinea pigs—a second lot of 50 mosquitoes was fed on the pigs. This procedure was repeated the following day with another lot of mosquitoes. On the morning of the fifth day following the inoculation of the guinea pigs, the animal that had received both the intracutaneous and intracerebral injections was dead. Hence, our next lot of mosquitoes fed only on the two pigs which had received the intracutaneous inoculations. The following day—144 hours after the infection of the guinea pigs—another lot of 50 mosquitoes was permitted to feed on the pigs, which were then in the late stages of the disease. By the following morning, both of these guinea pigs were dead.

Thus, we had five lots, each consisting of fifty mosquitoes, which had fed on guinea pigs infected with equine encephalomyelitis virus, 48, 72, 96, 120 and 144 hours respectively, subsequent to the inoculation of the pigs.

Experience has shown that this species of mosquito, under laboratory conditions, will draw blood approximately every six or seven days. Hence, we permitted the mosquitoes of each of our five lots to feed on a normal guinea pig on the sixth day following their initial feeding on the infected guinea pigs. The picture can be presented best by giving consecutively the results obtained with one lot of mosquitoes before taking up the next lot.

Starting with the 48-hour lot of mosquitoes, it should be stated that we killed two of the mosquitoes of this lot immediately after they had fed on the infected guinea pigs. These two mosquitoes were then ground in a little physiological saline solution and injected intracutaneously into a guinea pig. This guinea pig died of encephalomyelitis eight days later. Six days subsequent to the initial feeding of this lot of mosquitoes on the infected pigs, they were fed on a normal guinea pig. This pig died of encephalomyelitis eight days later. After an additional six days (twelve days after the original infective meal), this lot of mosquitoes was fed on another normal guinea pig. This animal died of the disease on the ninth day following, but showed symptoms of paralysis as early as the sixth day. Then, eighteen days after this lot of mosquitoes had partaken of their original infective meal, they were permitted to feed on two normal guinea pigs. Both of these pigs died of encephalomyelitis on the sixth day following the exposure to the mosquitoes.

The mosquitoes of the 72-hour lot were fed on a normal guinea pig on the sixth day subsequent to their meal on the infected guinea pigs. This pig died of encephalomyelitis six days later. The intracutaneous inoculation of a small amount of an emulsion of the brain of this animal into two normal guinea pigs produced encephalomyelitis, and death in both cases on the seventh day. The brains of these two animals were then emulsified and a small amount inoculated intracutaneously into each of three normal guinea pigs. Two of these three animals died of the disease, one on the sixth day and the other on the ninth day. Twelve days after their infective meal, this lot of mosquitoes was fed on three normal guinea pigs, and at the same time two of the mosquitoes were killed and inoculated intracutaneously into a fourth normal guinea pig. This latter pig died of encephalomyelitis eight days later. During the feeding of the mosquitoes on the other three pigs, it was noted that one of these pigs (a white one) was bitten by only 3 or 4 mosquitoes, the insects preferring to feed on the other two animals which were dark in color. These two dark-colored pigs died of encephalomyelitis on the fourth and sixth days respectively, following the mosquito bites. The white pig survived. When the time for the third test-feeding of this group of mosquitoes arrived, we had obtained a horse, so, instead of feeding the mosquitoes on one or more guinea pigs, we placed them on the horse. The results with the horse will be discussed a little farther along.

On the sixth day following their feeding on the original three infected guinea pigs, the mosquitoes of the 96-hour lot were fed on a normal guinea pig. This animal died of encephalomyelitis six days subsequently. Twelve days subsequent to the feeding on the infected guinea pigs, this lot of mosquitoes was permitted to feed on three guinea pigs. All three of these latter animals have remained healthy. When the time for the third test-feeding of this group of mosquitoes arrived (18th day), this lot, like the 72-hour lot, was fed on the horse.

The mosquitoes of the 120-hour lot were fed on one normal guinea pig on the sixth day following their feeding on the original two intracutaneously-injected guinea pigs. This pig died of encephalomyelitis on the tenth day following. Then, after another period of six days, this lot of mosquitoes was fed on two normal guinea pigs. One of these died of encephalomyelitis six days later; the other has remained healthy. As with the two former lots of mosquitoes, the 120-hour lot was fed on the horse rather than on guinea pigs, when the time for the third test-feeding arrived.

The mosquitoes of the 144-hour lot were fed on normal guinea pigs 6, 12, 18 and 24 days respectively, after their initial feeding on the originally infected guinea pigs. The guinea pigs bitten by this lot of mosquitoes have all remained healthy.

As indicated above, the horse used in this experiment was bitten by mosquitoes of the 72-hour lot, 96-hour group, and 120-hour lot. In the meantime, by the procedure we had previously employed, we fed four new lots of mosquitoes (50 in each lot) on infected guinea pigs. Thus, these four lots consisted of mosquitoes which had fed 48, 72, 96 and 120 hours respectively, following the inoculation of the guinea pigs used to infect the insects. Then, mosquitoes of each of these four lots were fed on the horse on the sixth day following their initial feeding on the infected guinea pigs. The horse we used was thus exposed to a total of seven groups of mosquitoes within 16 days. The number of mosquitoes in these seven groups which engorged on the horse totaled approximately 110.

The horse showed a slight temperature rise (2 degrees) on the eleventh day following the date on which the first lot of mosquitoes fed on it, but manifested no other evidence of abnormality. However, on the 22nd day subsequent to the date we fed the first group of mosquitoes on this animal, it developed a temperature rise which, within 24 hours, reached 106° F. Shortly following the onset of fever, the horse developed the classical symptoms of encephalomyelitis, and died as a result of the disease on the fifth day.

Obviously, from this experiment, we do not know which of the seven lots of mosquitoes was responsible for the disease in the horse. Our object in this particular test was to determine whether or not mosquitoes could transmit the disease to the equine genus. Our results make it possible to answer this in the affirmative. Supplementary experiments, now in progress, should clear up questions of various details.

In conclusion, it may be stated that we have produced encephalomyelitis, due to the filtrable virus of the equine disease, in numerous guinea pigs, and in a horse, through the agency of mosquitoes of the species *Aedes aegypti*. The mosquitoes proved infectious as early as the sixth day following feeding on an infected animal, and remained infectious for at least 18 days subsequent to the infective meal. It is possible that the mosquitoes may be capable of transmitting the disease earlier than the sixth day after an infective feeding, and it is highly probable that they remain infectious for a much longer period than 18 days. Tests to clear up these points are in progress.

In our guinea-pig tests, mosquitoes were infected by feeding on artifically inoculated guinea pigs 48 and 72 hours subsequent to the inoculation of the pigs, and to a lesser extent, 96 and 120 hours after the infection of the guinea pigs. Mosquitoes, fed on the inoculated guinea pigs 144 hours subsequent to the inoculation, failed to become infective. These periods of infectivity of the inoculated guinea pigs for the mosquitoes followed in general the period of fever in the pigs.

Since the classical work of Meyer, Haring and Howitt, proving that the American type of equine encephalomyelitis is due to a filtrable virus, speculation has existed as to the natural mode of infection. We believe that the results we have reported, coupled with epizoölogical observations, indicate that the disease is naturally transmitted by insect vectors—very probably mosquitoes. While it is understood that the particular species of mosquito (*Aedes aegypti*), which figured in our experiments, is not likely to be found in a number of the areas where encephalomyelitis has been enzoötic, other species of this same genus (Aedes) do occur in such areas and in all probability are as capable of transmitting the disease as the *aegypti* species.

California Has New State Veterinarian

Dr. C. U. Duckworth (Ind. '21), of Los Angeles, Calif., has succeeded Dr. Joseph J. King (San Fran. '13) as Chief of the Division of Animal Industry, California Department of Agriculture, Sacramento. Dr. Duckworth reënters state control work in California at a critical time in the progress of its tuberculosis eradication program, and it is believed that his experience in matters relating to the dairy industry, both in private and public connections, will be of great value to all interests concerned.

Doctor Youngberg in New Role

Dr. Stanton Youngberg (O. S. U. '07) completed his term of office as Director of the Bureau of Animal Industry, Philippine Department of Agriculture, on December 31, 1932, and is now employed as a technical adviser on the staff of the Governor-General, detailed to the Department of Agriculture and Commerce. On March 29, Dr. Youngberg left Manila for Hong Kong, Canton and Shanghai, on official business for the Government of the Philippine Islands.

Dr. Victor Buencamino (Corn. '11) has succeeded Dr. Youngberg as Director of the Bureau of Animal Industry.