TRANSMISSION OF THE VIRUS OF EQUINE ENCEPHALOMYELITIS BY AEDES TAENIORHYNCHUS*

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In 1933, the author[1] found that the virus of equine encephalomyelitis could be transmitted from animal to animal by the mosquito *Aedes aegypti*. This constituted the first proof of the ability of mosquitoes to transmit the disease. The results were further interesting in that it was the first time that a filtrable virus, preëminently neurotropic in character, was found to be transmissible by mosquitoes. Since the publication of these findings, the results have been confirmed by a number of investigators and, in addition, several other species of mosquitoes have been proved capable of transmitting the virus.

In June, 1934, Simmons, Reynolds and Cornell[2] announced that they had transmitted the western type virus of equine encephalomyelitis from guinea pig to guinea pig through the agency of *Aedes albopictus*. The details of their experiments were published in 1936.[3]

Merrill, Lacaillade and Ten Broeck,[4] in September, 1934, reported transmission of both the eastern and western virus by *Aedes sollicitans* and the eastern type by *Aedes cantator*.

In May, 1935, Madsen and Knowlton[5] published the results of experiments in which they indicated transmission of the western type virus by *Aedes nigromaculis* and *Aedes dorsalis*. In a footnote in connection with this report it was stated that there was some question regarding the purity of the *A. nigromaculis* employed, it being possible that some *A. sollicitans* were present. However, in a later publication by Madsen, Knowlton and Rowe[6] additional experiments were reported in which apparently definitely identified *A. nigromaculis* and *A. dorsalis* transmitted the disease.

Early in 1935, the author conducted several transmission tests with the western type virus and *Aedes vexans* raised from eggs contained in dried earth collected from areas where this species of mosquito had been breeding. Considerable difficulty was experienced in keeping these mosquitoes alive over the time period ordinarily required in transmission experiments. However, out

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of one relatively large lot of mosquitoes which had fed on three infected guinea pigs, some 35 or 40 survived for eight or nine days subsequent to their infective meals. During this period of survival they were given an opportunity to feed on a total of three normal guinea pigs. One of these three guinea pigs developed encephalomyelitis as a result of the mosquito bites. It would appear, therefore, that *A. vexans* is capable of transmitting the virus. It was highly desirable to conduct additional transmission tests with this species of mosquito to supplement the one positive instance. However, because the supply of mosquito eggs had been exhausted, it was possible to go on with such tests at that time and before more eggs or larvae could be obtained the writer left the United States for work in Panama. In Panama, *A. vexans* does not occur. Consequently work with this species could not be readily undertaken here.

In July, 1936, the author[7] identified what was apparently the first definitely proved case of equine encephalomyelitis in Panama. The virus in this case was found to be of the so-called eastern type.

With the discovery of the presence of the virus of equine encephalomyelitis in Panama, transmission tests were undertaken with *Aedes taeniorhynchus*. There are 15 known species of *Aedes* in Panama and the Canal Zone, the two most common being *A. taeniorhynchus* and *A. aegypti*. As *A. aegypti* was the species originally used in investigations concerning the mosquito transmission of equine encephalomyelitis, and ample evidence having been accumulated to establish its ability to transmit the virus of the disease, it was decided to conduct experiments with the other common species—*A. taeniorhynchus*.

Through the coöperation of the Army sanitary squad under Mr. J. B. Shropshire, adult *A. taeniorhynchus* and larvae of this species were obtained for the experiments.

It was found that, for some reason, mosquitoes raised from larvae kept under laboratory conditions did not survive nearly so long as adults caught in the field. Because of this, and in view of the fact that adult mosquitoes could be caught in fair numbers in certain localities, most of the experiments covered by this report were carried out with *A. taeniorhynchus* thus obtained.

The transmission experiments were commenced with the eastern type of virus recovered from the case of encephalomyelitis discovered in Panama. The plan of the experiment was, in general, similar to that initially used in the writer's original work[1] with *A. aegypti*.

Adult mosquitoes delivered to the laboratory were placed in feeding cages of the type employed in previous investigations. These cages measure 22x12x12 inches and are constructed of a light wood frame covered with a fine mesh netting (bobinette). The floor of the cage is made of a light-weight insulating board which, together with the wooden framework, is painted white. At one end of the cage the netting is extended about one foot, bunched, and tied by a piece of binding tape. This serves as the door of the cage.

Guinea pigs weighing approximately 250 gm each were used throughout these experiments. Experience has shown that this size pig is best adapted for work with the virus of equine encephalomyelitis.

In attempting to infect the mosquitoes, two guinea pigs were inoculated both intracerebrally and subcutaneously with an emulsion of guinea pig brain tissue containing the encephalomyelitis virus. The subcutaneous inoculation, augmenting the intracerebral injections, was made with the idea that such treatment might make a difference in the time of appearance and persistence of the virus in the blood.

The method utilized for the feeding of the mosquitoes on the infected guinea pigs may be briefly described as follows: Within an hour after inoculation, the two guinea pigs, with their abdomens shaved, are tied out on their backs on an animal-board, a single board being used for the two pigs. The board is then placed in the cage and the mosquitoes given from 20 to 30 minutes to feed. Following this period, the pigs are carefully removed from the cage, precautions being taken to prevent the escape of mosquitoes. It has been our practice to do all of our feeding experiments in a small, well-screened room. Following the feedings, the individual cages containing the mosquitoes are moved out and the room sprayed with an insecticide and closed for about one hour. Except during feeding, the mosquitoes are kept in an insectary, the individual cages, containing supposedly infected mosquitoes, being placed in slightly larger copper-screened cages as an added precaution. A Petri dish containing a fresh 10 per cent glucose solution is routinely placed in each mosquito cage daily.

Feeding of the same lots of mosquitoes on the same guinea pigs is repeated 24 hours after inoculation of the pigs, and thereafter at 24-hour intervals until the pigs are *in extremis* or dead. This will ordinarily vary from three to six days, depending upon the virulence of the virus with which the guinea pigs are inoculated.

In a number of the experiments entering into this investigation, individual lots of mosquitoes, after having fed daily during the course of the disease on one group of two pigs, were subsequently permitted to feed on one or more additional groups of infected guinea pigs before being placed on normal animals. This was done to increase the chance of the maximum number of mosquitoes actually ingesting blood containing virus. As but a portion of each lot of *A. taeniorhynchus* would feed on the same day, it has been evident that more mosquitoes will receive infected blood if the procedure indicated above is followed. However, this cannot always be done with small lots of mosquitoes, as the mortality rate of *A. taeniorhynchus* in captivity is high and if repeated feedings on successive groups of infected guinea pigs are carried too far, there may be few, if any, mosquitoes surviving when it comes time to fed on normal pigs.

The first transmission experiment was commenced on August 25, 1936, starting with a lot of approximately 40 *A. taeniorhynchus* caught on the Fort Amador and Fort Randolph reservations. From August 25 to August 28, both dates inclusive, these mosquitoes were given daily opportunities to feed on two guinea pigs infected with the Panama eastern virus. The pigs were inoculated on August 25 and were dead on August 29.

On August 29, the mosquitoes of this lot were fed on two normal guinea pigs and such feeding continued daily until September 15, 1936, when the last of the mosquitoes died. The result of this experiment was negative, the mosquitoes failing to transmit the disease to the normal guinea pigs.

Between August 28 and September 5, 1936, four additional lots of 35, 46, 52, and 27 mosquitoes, respectively, were used in transmission experiments with Panama eastern virus, with negative results.

On September 7, 1936, a sixth lot of 75 mosquitoes was started in another transmission experiment with the Panama eastern virus. Between September 7 and September 18, 1936, these mosquitoes were given an opportunity to feed on four infected guinea pigs. Commencing September 19, 1936, this lot of mosquitoes was given daily opportunities to feed on a normal guinea pig. On October 1, 1936, this guinea pig showed symptoms of encephalomyelitis and died the following day. Two additional normal guinea pigs were exposed to the bites of this lot of mosquitoes, starting on October 1, 1936. One of these two pigs was dead of encephalomyelitis on October 6 and the second died of the disease on October 8.

Up to this time, the identification of the mosquitoes, as made by the collectors, was accepted by the laboratory and accurate check of individual mosquitoes in a given lot was made only as the mosquitoes died. This was done to avoid increasing the mortality rate which would have resulted from an additional handling of each mosquito when first received. However, a finding in connection with the positive transmission experiment just referred to indicated the necessity of identifying the mosquitoes individually as received, in spite of increasing the mortality rate. A careful check of each mosquito in lot 6, which had proved positive in the transmission experiments, revealed two *A. aegypti*, the others all being *A. taeniorhynchus*. This nullified the particular experiment in so far as transmission by *A. taeniorhynchus* was concerned, because subsequent transmission tests were negative with the *A. taeniorhynchus* in the lot and positive with the two *A. aegypti*. Following this experience, all subsequent tests were carried out with mosquitoes which were individually identified before being used in experiments. This definitely insured that only *A. taeniorhynchus* were used.

As it has been found that certain species of mosquitoes will readily transmit one type of encephalomyelitis and not the other, transmission experiments with the western type of virus and *A. taeniorhynchus* were included in our studies. Within a short time, positive results were obtained with *A. taeniorhynchus* and the western type of virus. A preliminary announcement of this finding was made in February, 1937,[7] and a more detailed account presented before the Medical Society of the Isthmian Canal Zone in April of the same year.

When it became evident that *A. taeniorhynchus* was transmitting the western type of virus, work with the eastern type was temporarily suspended in order that all mosquitoes of the *taeniorhynchus* species received at the laboratory could be utilized in tests to establish clearly the ability of this mosquito to transmit the western type of disease. Thus, between October 5 and December 17, 1936, 14 transmission tests were conducted with *A. taeniorhynchus* and the western type of virus. The number of mosquitoes figuring in each of these tests varied with the supply from daily catches. In a number of instances—as the daily feedings on infected guinea pigs progressed—additional mosquitoes were added to those originally started in a particular lot. This was done to offset the relatively high mortality rate, so that when it eventually came time to feed on a normal guinea pig, there would be in each lot a fair number of mosquitoes which had previously fed on infected guinea pigs.

TABLE I—*Transmission of western type encephalomyelitis virus by Aedes taeniorhynchus.*

Mosquito Lot	Mosquitoes Used	Period of Feedings on Infected Guinea Pigs	Engorgements	Survivals for Test on Normal Guinea Pigs	Period of Feedings on Normal Guinea Pigs	Engorgements	Results
1	30	10/7–14	21	5	10/15–27	14	Negative
2	68	10/12–21	97	11	10/27–11/5	27	G. P. dead 11-20-36*
3	61	10/13–21	37	3	10/22–26	6	Negative
4	65	10/14–23	59	6	10/24–11/1	31	G. P. dead 11-2-36*
5	115	10/16–23	94	15	10/24–11/3	46	G. P. dead 11-4-36†
6	130	10/17–26	122	17	10/27–11/15	35	G. P. dead 11-16-36†
7	112	10/21–29	106	23	10/30–11/22	89	G. P. dead 11-23-36†
8	153	10/26–11/9	152	15	11/10–12/4	32	G. P. dead 12-21-36*
9	197	10/28–11/9	118	16	11/10–29	50	G. P. dead 12-3-36†
10	130	11/1–9	88	9	11/10–12/2	56	G. P. dead 12-8-36†
11	107	11/5–14	55	11	11/15–23	7	G. P. dead 12-9-36†
12	89	11/6–14	46	7	11/15–12/8	18	Negative
13	129	11/11–17	59	20	11/18–30	45	G. P. dead 12-16-36†
14	127	11/15–17	33	44	11/18–27	34	G. P. dead 12-7-36†

*All mosquitoes of the lot dead when guinea pigs developed symptoms of encephalomyelitis.
†Surviving mosquitoes produced encephalomyelitis in additional guinea pigs.

The total number of mosquitoes entering into each of these 14 transmission tests were respectively as follows: 30, 68, 61, 65, 115, 130, 112, 153, 197, 130, 107, 89, 129 and 127. Table I indicates the results.

As indicated in table I, transmission of the western type of encephalomyelitis from guinea pig to guinea pig by *A. taeniorhynchus* occurred in eleven out of the 14 tests conducted. In the three negative tests there were surviving but 5, 3 and 7 mosquitoes, respectively, when it came time to feed on normal guinea pigs. Apparently it so happened that these particular mosquitoes did not feed on the infected guinea pigs at a time when virus was present in the blood of such pigs. On the other hand, in two of the other tests in which only a few mosquitoes (6 and 9, respectively) survived sufficiently long to test them on normal guinea pigs, the disease was produced.

In three out of the eleven lots of mosquitoes which transmitted the disease (lots 2, 4 and 8), all of the mosquitoes had died before the normal guinea pigs upon which they were feeding developed symptoms of encephalomyelitis. Thus, the mosquitoes of each of these three lots had opportunities to infect respectively but one guinea pig per lot. With mosquito lots 5, 6, 7, 9, 10, 11, 13 and 14, however, some of the mosquitoes in each lot survived sufficiently long to test their ability to transmit the disease to additional guinea pigs. In every instance mosquitoes which had transmitted encephalomyelitis to one guinea pig transmitted it to others upon which they subsequently fed.

The experiments do not indicate the time period elapsing between the ingestion of blood containing virus and ability of the mosquito to transmit the disease. Further tests will be necessary to establish this factor.

It will be noted (table I) that the earliest death occurring in the eleven positive tests was on the ninth day following the exposure of a normal guinea pig to the mosquitoes of lot 4. The mosquitoes of this lot had had daily opportunities to feed on infected guinea pigs over a period of nine days before feedings were started on the normal guinea pigs. At the other extreme it will be seen that the guinea pig exposed to the bites of the mosquitoes of lot 8 did not die of encephalomyelitis until 41 days following the start of exposure to these mosquitoes.

While, in general, a relatively long incubation period occurred with the initial normal guinea pig exposed to the respective lots of mosquitoes, in those positive lots in which there were survivals to test on additional guinea pigs the periods of incubation

were invariably much shorter. It thus appears that multiplication or maturation of the virus in *A. taeniorhynchus* takes place at a relatively slow rate—at least under the conditions obtaining in our experiments. By comparison with the results obtained by the writer[1] in previous experiments with *A. aegypti*, the rate of development of the virus in this species is considerably greater than in *A. taeniorhynchus*. On the other hand, once the multiplication or maturation of the virus in *A. taeniorhynchus* has reached the proper point, the disease is promptly transmitted with fatal results within a few days.

It so happened that in lot 5 a single mosquito survived for ten days after all the others of the lot had succumbed. This lone mosquito was fed once on a normal guinea pig. The pig developed encephalomyelitis and was dead on the fifth day following the bite. This mosquito was then permitted a single engorgement on a second normal guinea pig, resulting in the production of the disease and death of this pig on the sixth day following the bite. With other positive lots it was not uncommon to have several mosquitoes, after finally becoming infectious, bite a normal guinea pig but once and produce encephalomyelitis and death within five to seven days.

All positive transmission results were confirmed by serial passage of the virus through additional guinea pigs, and by histopathologic examination of brain preparations.

Summary and Conclusions

The mosquito *Aedes taeniorhynchus* is capable of transmitting the western type of encephalomyelitis virus from guinea pig to guinea pig.

Transmission is not mechanical but occurs after multiplication, maturation or, less probably, cyclic change of the virus within the mosquito.

While, under the conditions of these experiments, the precise period of time necessary between the ingestion of virus-containing blood and ability of the mosquito to transmit the disease was not established, it appears that such period is longer in *Aedes taeniorhynchus* than in some other species of *Aedes*, especially *Aedes aegypti*. The evidence, however, indicates that once *A. taeniorhynchus* becomes fully infectious it will readily transmit the disease to guinea pigs with fatal results in a few days. A single mosquito biting a normal guinea pig but once produced encephalomyelitis and death of the pig in five days.

Difficulty in maintaining *A. taeniorhynchus* in captivity over extended periods of time prevented determination of the dura-

tion of infectivity of this species of mosquito. In our laboratory the longest period of time it has been possible to keep *A. taeniorhynchus* alive, subsequent to proving them infectious, is 32 days. Such mosquitoes proved infectious during this period.

Incrimination of *A. taeniorhynchus* brings to eight the total number of species of *Aedes* found capable of transmitting the virus of equine encephalomyelitis.

Several transmission tests conducted in the beginning of this investigation with *A. taeniorhynchus* and the eastern type of encephalomyelitis virus proved negative. However, further tests on this phase of the subject are necessary, and will be carried out, before a definite conclusion is drawn as to the ability or inability of *A. taeniorhynchus* to transmit the eastern type of virus.

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REFERENCES

[1]Kelser, R. A.: Mosquitoes as vectors of the virus of equine encephalomyelitis. Jour. A. V. M. A., lxxxii (1933), n. s. 35 (5), pp. 767-771.
[2]Simmons, J. S., Reynolds, F. H., and Cornell, V. H.: An. Rpt., Surgeon General, U. S. Army (1934), p. 185.
[3]Simmons, J. S., Reynolds, F. H., and Cornell, V. H.: Amer. Jour. Trop. Med., xvi (1936), 3.
[4]Merrill, M. H., Lacaillade, C. W., Jr., and Ten Broeck, C.: Mosquito transmission of equine encephalomyelitis. Sci., lxxx (1934), 2072, pp. 251-252.
[5]Madsen, D. E., and Knowlton, G. F.: Mosquito transmission of equine encephalomyelitis. Jour. A. V. M. A., lxxxvi (1935), n. s. 39 (5), pp. 662-666.
[6]Madsen, D. E., Knowlton, G. F., and Rowe, J. A.: Further studies on transmission of equine encephalomyelitis by mosquitoes. Jour. A. V. M. A., lxxxix (1936), n. s. 42 (2), pp. 187-196.
[7]Kelser, R. A.: Equine encephalomyelitis in Panama. U. S. Army Vet. Bul., xxxi (1937), 1, pp. 19-21.

Silly

"What's wrong, Henry?" asked his wife.

"My razor," boomed the voice within the bathroom. "It doesn't cut at all."

"Don't be silly. Your beard can't be tougher than the linoleum."

Pennsylvania Farmer.