# EXPERIMENTAL STUDIES ON THE MECHANICAL TRANSMISSION OF CHIKUNGUNYA VIRUS BY *AEDES AEGYPTI*

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Although biological transmission is the well understood mechanism of transmission of arboviruses under natural conditions, there is a theoretical possibility that mechanical transmission could also take place in certain circumstances, particularly where the vector bites more than once at short intervals to complete a blood meal.

Mechanical transmission of some mosquito-borne viruses has previously been demonstrated in the laboratory (Barnett, 1956; Woodall and Bertram, 1959; Chamberlain and Sudia, 1961; Scherer and Hurlbut, 1967), but its significance in the epidemiology of arbovirus infections has not been assessed. The role of mechanical transmission in the spread of myxomatosis, not an arbovirus, is however well documented (Fenner and Ratcliffe, 1965).

Laboratory studies reported here were undertaken to test the possibilities of mechanical transmission of chikungunya virus by *A. aegypti* as a factor contributing to the rapid build-up of human infections noted in the recent epidemics of chikungunya infection in Madras and Vellore cities, South India (Reuben, 1967).

MATERIALS AND METHOD. A strain of chikungunya virus (VRC #634029) isolated from a patient in Calcutta in 1963, was used in the present study.

Females of *A. aegypti* from a laboratory colony (original source, Vellore, S. India) were allowed to probe or partly feed on viraemic infant mice which had been inoculated 48 hours previously, intracerebrally with $10^3$ to $10^4 LD_{50}$ of chikungunya virus. The virus titre in the blood of these mice at the time of this experiment was found to be $10^{7.6} LD_{50}/0.02$ ml. The mosquitoes were exposed individually to the mice. Those which successfully probed, or fed partly, were disturbed to interrupt the feeding and later allowed to complete their blood meal, after various intervals on other susceptible infant mice (one mosquito per mouse). During this process the individual mosquitoes were held separately in 2½" x 1" glass tubes, which had one end covered by a fine mesh mosquito netting and the other end by an elastic rubber sheet with a small slit for introducing and removing the mosquitoes when necessary. The mosquitoes were allowed to probe or feed on viraemic or susceptible mice through the mosquito netting. By this technique it was possible to keep a complete check on the history of any particular mosquito.

The mosquitoes were held at room temperature (23° C.–25° C.) and 70–80 percent relative humidity. They were not given any kind of food during the interval between the initial probing or feeding and the second feeding. The fed mosquitoes were individually tested for the presence of chikungunya virus by intracerebral inoculation in 2-day-old mice.

The susceptible infant mice on which these mosquitoes had completed the second feeding were observed very closely for any sign of sickness and a further passage was made from the brain suspensions of sick mice, to confirm the presence of chikungunya virus. That the sickness in inoculated mice was due to chikungunya virus was confirmed by observing the pattern of sickness and by testing a few selected mouse brain suspensions in complement fixation tests.

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RESULTS. The results of this study are summarized in Table 1. Seventeen out of the 29 mosquitoes which had picked up detectable amounts of chikungunya virus were able to transmit the virus immediately. After half, one, two, four and eight hour-intervals, two out of eight, three out of nine, four out of eight and one out of three mosquitoes, respectively, transmitted the virus. There was no transmission of the virus by mosquitoes after lapse of 12, 24 and 30 hours after probing on the viraemic mice, although four out of eight at 12 hours'-interval, three out of 14 at 24-hours' interval and one out of eight at 30 hours' interval were positive for the virus.

TABLE 1.—Mechanical transmission of chikungunya virus by *A. aegypti*.

| Interval in hours between infecting probe on viraemic mice and feeding on susceptible mice | No. of mosquitoes completing the blood meal on susceptible mice and tested for the virus | No. of fed mosquitoes positive for the virus | No. of successful transmissions |
|---|---|---|---|
| 0 | 32 | 29 | 17 |
| ½ | 12 | 8 | 2 |
| 2 | 12 | 9 | 3 |
| 4 | 12 | 8 | 4 |
| 8 | 12 | 3 | 1 |
| 12 | 12 | 4 | 0 |
| 24 | 14 | 3 | 0 |
| 30 | 8 | 1 | 0 |
| Total | 114 | 65 | 27 |

DISCUSSION. The results of the present study indicate that chikungunya virus can be mechanically transmitted by *A. aegypti* within the first 8 hours after an infective bite. It has been a frequent observation of one of the authors (TRR) that this day-biting mosquito can bite more than once (sometimes three or more times) to feed to repletion. During the process of blood feeding the same mosquito might bite repeatedly the same person or others in the proximity. If the mosquito happens first to bite a viraemic person and its course of feeding is interrupted it may mechanically transmit the virus to another susceptible person on whom it tries to complete its blood meal. With this mechanism, *A. aegypti* is capable of transmitting chikungunya virus long before the mosquito becomes biologically infective on the fourth day after an infective feeding (Singh and Pavri, 1967).

A feature of the 1964 epidemics of chikungunya virus in Madras and Vellore was a very rapid build-up of the number of human cases. Within 12 weeks nearly 40 percent of the population had become infected. This phenomenon can be explained either because of a very high density of the vector, or because of an increase in the number of infective bites, or both. Mechanical transmission as a result of multiple bites by a mosquito before completing a blood meal can be one of the factors. It is particularly so with mosquitoes like *A. aegypti*. Because it bites during the day time, it is more likely to be disturbed by the hosts, who are also awake and active at the time, more so than in the case of a night-biting mosquito feeding on sleeping people. One of the authors (TRR) has actually observed mosquitoes landing on the face of a sick child being whisked away by the mother watching over it.

*A. aegypti* density can be considered to have been only moderately high during the epidemics (Reuben, 1967). Both density and mechanical transmission together could have contributed to the rapid build-up of the epidemics. However, an

assessment of the importance of the phenomenon of mechanical transmission in nature needs to be studied with factual quantitative observations made during an epidemic. The present studies have indicated such a possibility.

SUMMARY. Experiments were carried out to find out if chikungunya virus can be mechanically transmitted by *Aedes aegypti*. The mosquitoes were allowed to probe or partly feed on viraemic mice and then allowed to complete their blood feeding on other individual susceptible infant mice at various intervals.

The results showed that chikungunya virus can be transmitted by *A. aegypti* within the first eight hours after an infective bite. The epidemiological significance of the phenomenon of mechanical transmission of chikungunya virus by *A. aegypti* is briefly discussed.

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