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Citation	Japanese Journal of Veterinary Research, 36(2), 149-149
Issue Date	1988-05-20
Doc URL	<a href="https://hdl.handle.net/2115/3093">https://hdl.handle.net/2115/3093</a>
Type	departmental bulletin paper
File Information	KJ00002377077.pdf



EXPERIMENTAL STUDY ON THE TRANSTADIAL TRANSMISSION  
OF JAPANESE ENCEPHALITIS VIRUS IN MOSQUITO LARVAE

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Transstadial transmission is a vital process involved in the "transovarial transmission" of arthropod-borne viruses in vectors. In this study, basic conditions required for the transstadial transmission of Japanese encephalitis (JE) virus were examined by adopting two experimental infection methods to mosquito larvae.

First, the 3rd and 4th instar larvae of *Culex tritaeniorhynchus* and the 4th instar larvae of *Aedes japonicus* were injected with JE virus ( $10^{1.5}$  to  $10^{1.8}$  PFU/insect) intracranially. Rearing larvae at 28°C, viral antigen was detected 4 to 7 days after inoculation in headsmears from all adults and larvae using the indirect immunofluorescent antibody (IFA) technique. On the other hand, by immersion of larvae in culture fluid containing JE virus, viral multiplication was observed in each 4 instar of *Culex tritaeniorhynchus*.

These results confirmed the complete transstadial transmission of JE virus during the metamorphic process from larvae to adults in mosquitoes. The IFA method was revealed to be convenient for the screening of insects infected with the virus.

Furthermore, the susceptibility of 3 mosquito species in Japan to JE virus was examined by means of immersion in virus suspension at the 4th instar. Colonized strains *Culex tritaeniorhynchus* (Amami) and *Culex pipiens pallens* (Nagasaki) and field strains of *Aedes japonicus* (collected near Sapporo) had the same level of susceptibility to the virus except for a field strain of *Culex pipiens pallens* (collected near Sapporo) which showed relatively low susceptibility. It was also discovered that the infection rates and the virus titers in *Aedes japonicus* reared at 20°C after immersion in the virus suspension at the 4th instar were similar to those at 28°C.

These results suggest that *Aedes japonicus* may act as the main vector of JE virus in Hokkaido where this species predominates and *Culex tritaeniorhynchus*, known as the common vector in Honshu, inhabits rarely.