Getah Virus in Aedes vexans nipponii and Culex tritaeniorhynchus:
Vector Susceptibility and Ability to Transmit

By
I. Takashima¹, N. Hashimoto¹, J. Arikawa¹, and K. Matsumoto²

¹ Department of Veterinary Public Health, Faculty of Veterinary Medicine, and
² Institute for Animal Experiment, School of Medicine Hokkaido University, Sapporo, Japan
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Summary
Vector competences of Aedes (Ae.) vexans nipponii (nip.) and Culex (Cx.) tritaeniorhynchus to Getah virus were assessed by using a membrane feeding technique. The Getah virus was present at high titer in both species of mosquitoes after 21 days of extrinsic incubation at 28°C. Infection rates on 21 post-feeding were 100 per cent (4/4) for Ae. vexans nip. at a virus dosage of 10⁵.³ PFU/ml and 60 per cent (3/5) for Cx. tritaeniorhynchus at similar virus dosage. More than 10³.⁵ PFU of virus was detected in salivary glands of both species of mosquitoes on day 21 of extrinsic incubation. Forty percent (2/5) of Ae. vexans nip. transmitted the virus into serum-agar after ingesting 10⁴.³ PFU/ml of virus blood mixture. In experiments with Cx. tritaeniorhynchus ingesting 10⁷.⁵ PFU/ml of virus blood mixture, 57 per cent (4/7) were able to transmit the virus to suckling mice and 59 per cent (10/17) transmitted the virus into serum-agar.

Introduction
Getah virus, a Alphavirus of the family Togaviridae, was isolated from mosquitoes in Japan (8, 10, 13, 18), Australia (1, 2), and Malaysia (14). The virus has been isolated from pigs in Japan (7) and antibody has been detected in human, cattle, horse, pig and several other animals (3, 9, 10, 13, 17). However, the pathogenesis of the virus for mammalian hosts other than small laboratory animals (11) has been unclear until recently, when an enzootic of Getah virus occurred among racehorses in Japan (6, 12). The affected horses exhibited pyrexia, urticarial rash and edema of the hind legs. The viruses were isolated from blood plasma of the racehorses involved in the enzootic. Although Getah virus had been isolated from Ae. vexans nip. and Cx. tritaeniorhynchus in Japan (8, 10, 13, 18), vector competences of these mosquitoes were unstetned.
The present study was undertaken to determine whether *Ae. vexans nip.* and *Cx. tritaeniorhynchus* were susceptible to Getah virus infection and were able to transmit the virus after oral ingestion of the virus.

**Materials and Methods**

**Virus**

The virus used was Getah virus, strain AMM 2021, which was kindly supplied by Dr. A. Oya, National Institute of Health of Japan, Tokyo. The virus strain had been passed 19 times intracerebrally in suckling mice prior to use in these experiments. Ten per cent suspensions of infected suckling mouse brain were prepared in phosphate buffered saline (PBS) containing 5 per cent fetal calf serum (FCS). The suspension was centrifuged at 10,000 rpm at 4°C for 20 minutes and the supernatant was stored at -80°C as virus working stock.

**Mosquitoes**

*Ae. vexans nip.* (Theobald) was reared from the larvae collected in the field at the Hokkaido National Agricultural Experiment Station, Sapporo, Japan. *Cx. tritaeniorhynchus* (Giles), strain Kyoto, was kindly supplied by Dr. M. Takahashi, National Institute of Health of Japan, Tokyo. This strain had been maintained in the laboratory for 149 generations prior to its use in these experiments. All mosquitoes were reared in an insectary at 28°C, with relative humidity of 75 to 85 per cent, and a 16 hour photoperiod. The larval diet consisted of finely ground insect food. A 2 per cent sucrose solution was provided continuously to adult mosquitoes. The age of mosquitoes at onset of their use in the experiments ranged from 3 to 10 days.

**Infection of Mosquitoes**

Serial ten-fold virus dilutions were made in defibrinated rabbit blood. A porcine skin membrane was fitted to the glass flask by a rubber band. The glass flask was kept at 35°C in a water bath for 5 minutes prior to feeding. A cage containing the mosquitoes was placed against the membrane at the bottom of the glass flask. The virus in the blood meal was titrated by a plaque formation on Vero cells.

Five mosquitoes were removed immediately after ingestion of blood. The remainder of the infected mosquitoes were held at 28°C with a relative humidity of 75 to 85 per cent. Five mosquitoes were removed at intervals and were stored at -80°C prior to dissection and titration of the virus.

**Dissection of Mosquitoes**

Each mosquito was separated into an abdomen part and a head-thorax part. The salivary gland was also removed from mosquitoes with minute pins. Dissected mosquito body parts were triturated in PBS containing 5 per cent FCS and 400 µg/ml of kanamycin. The triturated suspension was centrifuged at 6000 rpm for 20 minutes. The supernatant was stored at -80°C prior to virus titration.

**Virus Titration**

Vero cells were maintained in Eagle's minimum essential medium (MEM) containing 5 per cent calf serum, 100 IU/ml of penicillin, 100 µg/ml of streptomycin, and 2 mM of L-glutamine. The Vero cell monolayer formed in the 24 well plastic plates (Nunc, Denmark) was inoculated with 0.1 ml of each decimal dilution of mosquito specimens or blood meals. The plate was incubated at 37°C in 5 per cent CO₂ for 60 minutes and washed three times with PBS. Each well was overlaid with 1 ml of Eagle's MEM medium containing 1 per cent calf serum, 1.5 per cent carboxy-methylcellulose, 0.3 per cent tryptose phosphate broth, 2 mM of L-glutamine and antibiotics. The plates were incubated at 37°C in 5 per cent CO₂ for 4 days. After incubation, the medium in the wells was removed and the plate was fixed with the solution containing