# A PRELIMINARY INVESTIGATION OF POSSIBLE ORAL AND TRANSOVARIAL TRANSMISSION OF ROSS RIVER VIRUS BY *AEDES NORMANENSIS* (TAYLOR) FROM THE NORTHERN TERRITORY

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## SUMMARY

Wild caught females of *Aedes normanensis* from Mataranka in the Northern Territory were fed on new born mice intracerebrally inoculated with Ross River (RR) virus isolated from the Northern Territory. Classic RR virus symptoms were produced in litters of new born mice from five to eight days after exposure to the infected mosquitoes. Tests on the mice showed cytopathic effects and the presence of alpha virus. Eggs laid by the caged mosquitoes reared to adults failed to show any evidence of transovarial transmission at least at the 0.46% level of individuals examined.

## INTRODUCTION

Relatively high isolation rates of RR virus have been made from wild caught *Ae. normanensis* at Mataranka, approximately 380 km south of Darwin in the Northern Territory (Whelan and Shorthose, unpublished), suggesting that this species is a potential vector of RR virus in the Northern Territory. Ross River virus has been previously isolated from *Ae. normanensis* in Queensland (Doherty et al 1979) but there have been no reports on the vector competence of *Ae. normanensis* and RR virus.

*Aedes normanensis* is a very widespread mosquito species in the Northern Territory, from the sub-coastal areas, inland to at least Tennant Creek, approximately 800 km south of Darwin, and can reach very high population levels soon after the start of the rainy season (N.T. Health Department records). If *Ae. normanensis* is confirmed as a vector of RR virus, it would have important significance to the transmission of human disease in the Northern Territory and neighbouring states. Part of this paper examines the possible oral transmission of RR virus by *Ae. normanensis* under laboratory conditions.

The high rate of RR virus isolations from the semi-arid, seasonal rainfall area around Mataranka were made in January 1984, soon after the beginning of the rainy season. This was at a time when numbers of *Ae. normanensis* were relatively high and the numbers of most other species including *Culex annulirostris* were relatively low. This suggests that RR virus could possibly be surviving the dry season in the drought resistant eggs of *Ae. normanensis* or another of the ground pool breeding *Aedes.*

Transovarial transmission of arboviruses have been well documented, with La Crosse virus by *Ae. atropalpus* (Freier and Beier 1984), St. Louis Encephalitis virus by *Ae. atropalpus* and *Ae. epactius* (Hardy et al 1984), Yellow fever virus by *Ae. aegypti* and *Ae. mascarensis* (Beaty et al 1980) and Dengue virus by *Ae. albopictus* and *Ae. aegypti* (Rosen et al 1983).

Preliminary data suggesting transovarial transmission of RR virus by *Ae. vigilax* in Queensland has been reported (Kay 1982), and suggestions of arboviral survival in Australia in drought resistant *Aedes* eggs have been made (Doherty 1977), but there have been no reports of attempts to examine possible transovarial transmission of RR virus in *Ae. normanensis*. Part of this paper documents a preliminary investigation of possible transovarial transmission of RR virus by *Ae. normanensis*.

## MATERIALS AND METHODS

Wild caught females *Ae. normanensis* were collected by EVS dry ice baited light traps (Rohe and Fall 1979) in May 1985 at Mataranka, approximately 380 km south of Darwin in the Northern Territory and transported to Darwin in catching containers held in moist towel covered polystyrene foam boxes. The mosquitoes were sorted to species on refrigerated cold tables, and all obviously dead individuals were discarded. The mosquitoes were counted and transferred to two netting-covered cages (30cm x 30cm x 30cm) in an insectary and maintained at 27-31°C and 85-95% relative humidity. Daily moistened cotton towels were placed over each cage.

The mosquitoes were given access to water and a 10% sucrose solution in cotton wool pads on a daily regime of sugar from 0900-1600 hours, and water for the remainder. An outbred strain of straw mice obtained from the Department of Agriculture in South Australia, and maintained as a closed colony at the Department of Primary Production, Berrimah Farm, Northern Territory was utilised. A 0.02 aliquot of two strains of RR virus (V582) and (V591), isolated from *Ae. normanensis* collected at Mataranka in 1984, were inoculated intracerebrally into two litters of six mice, three to four days old. The litters were exposed to the wild caught *Ae. normanensis* on the third day after inoculation for four one hour periods each day over two days. Four hundred and twenty-six mosquitoes contained in Cage 1 were allowed to feed on Litter 1, inoculated with RR virus strain V582, while the seventy-five mosquitoes contained in Cage 2 were allowed to feed on Litter 2, inoculated with RR virus strain V591.

The litters were restrained in nylon stockings during exposure to mosquitoes, and returned after exposure to their mothers in netting covered mice cages. A number of individuals of Litters 3 and 4 were randomly selected and placed in either Cage 1 or Cage 2 and Litter 6 only in Cage 1.

The mice were monitored fro disease symptoms and killed approximately two to four days after the first symptoms of hind leg paralysis in each litter became evident. The mice were examined for cytopathic effects and the presence of alpha viruses by methods described elsewhere (Whelan and Shorthose unpublished).

Samples of blood fed mosquitoes were taken on the same day and one day after initial exposure to the inoculated mice, and examined individually for the presence and amount of virus by taking a 0.2 aliquot of supernatant from each insect sample and assaying in Vero cells and calculating log 10 PFU/ml.

Moribund or dead mosquitoes from each cage were removed four times each day and held in labelled vials in liquid nitrogen. Mosquitoes were removed for up to nineteen days after

initial exposure to inoculated mice and daily samples from each cage were pooled and examined for the presence of virus as described elsewhere (Whelan and Shorthose unpublished). At Day 15 after initial feeding, the mosquitoes were denied access to blood for 24 hours and samples of 9 or 10 live mosquitoes from the respective cages were sampled and tested individually for the presence and amount of virus.

Some positive plaque assays of the pools of moribund and dead or live individuals were assayed by plaque reduction tests as described by Gorman et al (Gorman et al 1975) to confirm the presence of RR virus.

The caged mosquitoes were allowed to lay eggs on moistened mud from Mataranka, and eggs were removed every second day. The adult mosquitoes reared from each egg batch were held for at least 24 hours after emergence, and then pooled separately for each sex. Each pool was tested for virus by taking a 0.1 ml aliquot of supernatant from each insect pool and inoculating to BHK-21 cells and observed for cytopathic effects. Pools were passaged twice before being discarded as negative.

## RESULTS

### Oral Transmission

The numbers of *Ae. normanensis* present in each age and the number of pools positive for virus are shown in Table 1 and Table 2.

The days of exposure of each litter to the caged mosquitoes and the days of first observation of symptoms and evidence for the presence of virus are also shown in Tables 1 and 2.

The amounts of virus detected in individual mosquitoes are shown in Table 3.

The pools of moribund and dead mosquitoes showed the presence of virus from 3 to 8 days after initial exposure to the cerebrally inoculated mice.

Litters of newborn mice showed classic RR virus hind leg paralysis 5 to 8 days after first exposure to the caged mosquitoes, and these litters exhibited cytopathic effects and a number tested were positive for alpha virus.

The individual samples of blood fed mosquitoes showed amounts of virus ranging from 5PFU/ml to log 10-5.1 PFU/ml.

### Transovarial Transmission

The days of egg production and the number of subsequent progeny reared from each cage of mosquitoes are shown in Table 4 and Table 5. All male and female progeny of the eggs produced by the caged mosquitoes were negative for virus. There were 215 and 31 progeny produced from each respective cage after Day 8 following the initial exposure of the mosquitoes to inoculated mice. This indicates that no transovarial transmission was evident at least at the 0.46% level of individuals examined.

## DISCUSSION

### Oral Transmission

In this preliminary investigation there are a number of problems that become evident, but which could be overcome with improved design. These problems include the unknown number of mosquitoes which fed on the inoculated mice initially, and hence the number of infected mosquitoes in the cages at any one time is unknown. The random sharing of both Litter 3 and Litter 4 between the two cages meant that the presence of more than one strain in each cage was possible. This is not critical, as both strains were isolated from *Ae. normanensis* at Mataranka at the same time. This investigation has shown oral transmission of RR virus in *Ae. normanensis* and that virus levels of up to log 10-5.45PFU/ml were present in the infected mosquitoes. The investigation has also demonstrated that the infected *Ae. normanensis* can produce RR virus symptoms and circulating virus in newborn mice in as little as 5 days. The apparent reduction in the number of days after first exposure to produce virus symptoms in the later litters (ie 5 and 6) may be related to a possible increasing number of infected mosquitoes in each cage. There is evidence that even at Day 15 there are both non-infected mosquitoes and individuals that had possibly taken their first virus infected blood meal (Table 3).

### Transovarial Transmission

The investigation of possible transovarial transmission of RR virus in *Ae. normanensis* cannot be conclusive. The eggs laid and progeny subsequently tested may not have come from infected female mosquitoes. The suggestion that the majority of the progeny were not from infected females does however seem unlikely, when 8 out of 9 individuals in one cage sampled at Day 16 were positive for virus (Table 1) and 7 of these individuals had relatively high amounts of virus. Only the 215 progeny produced after Day 8 (Table 4) were included in the calculation to arrive at the 0.46% level of progeny tested for transovarial transmission. This would increase the probability that the progeny tested were from infected females.

The report by Hardy et al (Hardy et al 1984) of transovarial transmission St. Louis encephalitis virus by *Culex* and *Aedes* mosquitoes at 18°C but not at 27°C indicates that transovarial transmission in some species may only occur at either a lower average temperature or possibly by a low night temperature. Kay (Kay 1982) however, reported evidence of transovarial transmission of RR virus in *Ae. vigilax* with females maintained at 28°C. Hardy et al reported the relatively low minimal infection rates for adult progeny in *Culex tarsalis* of 1:1,989. Freier and Beier (Freier and Beier 1984) reported the relatively high filial infection rates for La Crosse virus in F1 adult progeny of parenterally infected *Aedes atropalpus* and *Aedes triseraitus* females of 13.9% and 10.7% respectively. Kay (Kay 1982) reported the relatively high rates of transovarial transmission of RR virus in two of 51 females of RR virus inoculated females of *Ae. vigilax.*

This investigation does suggest that transovarial transmission of RR virus does not occur at a high frequency in *Ae. normanensis*. If transovarial transmission of RR virus in *Ae. normanensis* does occur at the temperatures tested, it may not occur at the relatively high levels of some other species. Further work with a larger number of known infected individuals is necessary to more fully investigate the probability of transovarial transmission of RR virus in *Ae. normanensis.*

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