COMPARISON OF THREE METHODS FOR DETERMINING TRANSMISSION RATES IN VECTOR COMPETENCE STUDIES WITH *CULEX UNIVITTATUS* AND WEST NILE AND SINDBIS VIRUSES

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ABSTRACT. *Culex univittatus* mosquitoes were infected by ingesting West Nile and Sindbis viruses, respectively. After 13–19 days, comparative viral transmission rates were determined using 3 methods: 1) transmission to hamsters by bite, 2) in vitro transmission while feeding on suspensions in capillary tubes and 3) transmission while feeding on droplets of suspension. Transmission rates were 59–100%, except for Sindbis virus by droplet feeding when the rate was 9%. Except for the latter, rates determined by the different methods were very similar in the case of each virus. It was concluded that capillary feeding is an acceptable alternative to feeding on hamsters for determining transmission rates for *Cx. univittatus* with West Nile and Sindbis viruses.

INTRODUCTION

In our laboratory a well established method for determining the transmission rates of mosquito vectors with arboviruses is the feeding of individual infected mosquitoes on individual susceptible animals. The choice of animal depends on the virus and vector in question and includes chickens (Jupp 1974), mice or hamsters (McIntosh et al. 1980) and monkeys (Jupp et al. 1981). For our purposes hamsters have been the most useful laboratory animals as they can be used with a number of viruses including Rift Valley fever (RVF), West Nile (WN), Sindbis (SIN) and chikungunya viruses. Successful transmission is measured by virus isolation from a hamster if the virus is pathogenic, such as RVF and WN viruses, or by the subsequent detection of hemagglutinating antibodies in the animal's blood, as in the case of SIN and chikungunya viruses.

Although the use of animals is thought to give an assessment of transmission that is more comparable to the real situation in the field than that measured by an *in vitro* method, it is nevertheless cumbersome, time-consuming and expensive in a project requiring many transmission tests. Such was our problem when we wished to undertake a study on the effect of temperature on the vector competence of *Cx. univittatus* Theobald with WN and SIN viruses. It was therefore decided to evaluate two *in vitro* transmission methods against our existing hamster method to see whether one or both methods gave comparable results and could therefore be used as an alternative.

The methods chosen were the "capillary" and "droplet" methods. The former was developed by Aitken (1977) and has been used successfully to determine transmission rates for *Aedes aegypti* (Linn.) with yellow fever virus (Beaty and Aitken 1979). The droplet method developed by Gubler and Rosen (1976) was used by them for the transmission of dengue-2 virus by *Aedes albopictus* (Skuse). They found that the percentage of mosquitoes transmitting was related to the extent of the infection in their salivary glands.

METHODS

A laboratory colony of *Cx. univittatus* was used which originated from mosquitoes collected in Johannesburg—F_1, F_2 or F_7 adult females, aged 1–18 days, were given infective feeds. All the experiments were undertaken in an insectary where the mosquitoes were maintained at 75–85% relative humidity and 25–26°C.

The viruses used were the H442 strain of WN virus (Kokernot and McIntosh, 1959) and the AR86 strain of SIN virus (Weinbren 1955). Each virus had been passaged 5 times in mice (intracerebral) and 4 times in Vero cells.

Infective meals of high titer were provided by intramuscular inoculation of chicks. For SIN virus, 1-day-old chicks were inoculated and exposed to the mosquitoes 24 hr later, while for WN virus 2-day-old chicks were inoculated and exposed to the mosquitoes 36 hr later. The mosquitoes were starved for 24 hr preceding each infective meal, and feeding took place for 2–3 hr in the dark in the insectary. Immediately before exposure, a blood sample was collected to determine the concentration of virus present. This was titrated in Vero cells, and the 50% cytopathic dose (CPD_{50}) end points were calculated by the method of Reed and Muench (1938). Viral titers were expressed as $\log_{10}CPD_{50}$/ml of chick blood.

Transmissions were attempted 13–19 days after the infective feed with WN and SIN viruses

respectively, by each of the 3 methods described below. In each experiment the transmission rate was subsequently determined, i.e., the proportion of infected mosquitoes which transmitted virus. Fisher exact tests in an Epistat statistical computer program were used to compare the rates obtained by the different methods to ascertain whether they were significantly different.

In the hamster method, individual infected mosquitoes were fed on individual Syrian hamsters. Successful transmission was demonstrated by the development of hemagglutinating antibodies against SIN virus in the hamsters blood 21 days later. In the case of WN virus, death of a hamster indicated transmission, and the animal's liver was harvested and tested for virus to confirm this.

With the droplet method (Gubler and Rosen 1976), an infected mosquito was placed in a tube covered with fine nylon mesh material so that it could feed from a droplet of fluid placed on the material. In this method, 0.025 ml of suspension consisting of sucrose, fetal calf serum and washed goose erythrocytes were placed on the nylon netting. After the mosquito had engorged, the remainder of the drop was diluted 10-fold in 0.25 ml of Liebovitz's medium before inoculation into cultures of Vero cells to test for virus. Transmission was defined to have occurred if virus was recovered from the unimbibed portion of the droplet.

For the capillary method (Aitken 1977), a tube was employed with inner and outer diameters about 0.5 and 1.0 mm, respectively. It was drawn out to a fine point and filled with 0.004 ml of feeding suspension consisting of equal parts of 10% sucrose and fetal calf serum. The point of the tube was inserted over the proboscis of the mosquito which was then allowed to feed to repletion. In this method 0.0025 ml of the feeding suspension remained in the tube after feeding. Subsequently a 100-fold dilution in 0.25 ml of Liebovitz's medium was necessary to permit inoculation of the specimen into cultures of Vero cells.

After each mosquito had completed its transmission feed, it was stored at −70°C and subsequently tested for virus by inoculation of a group of 8 infant mice. The mosquitoes were judged to have been infected if all 8 inoculated mice died within the expected death time of the respective viruses. The infection rate, i.e., the proportion of mosquitoes which had become infected after feeding on the infective meal, could then be determined for each group of mosquitoes.

RESULTS

All of the mosquitoes became infected after ingesting WN virus (Table 1). Infection rates were also high (87–89%) after ingestion of SIN virus. The transmission rates were slightly higher for both viruses with the hamster method as compared to rates determined with the capillary method. These differences were statistically significant, i.e., $P = 0.1$–0.2 (SIN) and $P = 0.3$ (WN) at the 5% significance level but not at the 10% level. Comparison of the transmission rates obtained with the droplet method to those obtained with hamsters showed that the difference was again significant in the case of WN virus ($P = 0.01$) at the 5% but not at the 10% level; however the rate obtained for SIN virus with the droplet method was very much lower (9%). Some additional tests were therefore carried out to try to explain this low transmission rate. SIN virus-infected mosquitoes were fed on a droplet in the usual way, except that the goose erythrocytes were replaced by sheep erythrocytes. The transmission rate still remained below 10%. Secondly, infected mosquitoes were allowed to feed on a droplet but, when half-engorged, were removed and allowed to complete their meals by the capillary method. Both the droplet and capillary fluids were then titrated for virus in Vero cells. No virus was detected in any of the droplet fluids, but 4.5–5.4 $\log_{10}CPD_{50}$/ml of SIN virus was present in all the capillary suspensions.

DISCUSSION

The results of the transmission tests with the 3 different methods indicate that the capillary tube method would be an acceptable alternative

Table 1. Comparative transmission rates by three different methods.

Virus (Titer of infective meal)	Days postinfection	Hamster method		Capillary method		Droplet method	
		Infection rate	Transmission rate	Infection rate	Transmission rate	Infection rate	Transmission rate
Sindbis (7.0–8.5*)	13–19	47/54 (87%)	34/47 (74%)	66/74 (89%)	39/66 (59%)	17/19 (89%)	3/35 (9%)
West Nile (5.0–5.5)	13–14	20/20 (100%)	20/20 (100%)	28/28 (100%)	22/28 (78%)	17/17 (100%)	12/17 (71%)

* Means $\text{Log}_{10}CPD_{50}$/ml.

to feeding infected *Cx. univittatus* mosquitoes on hamsters in the case of SIN and WN viruses. Although the transmission rates obtained for both viruses with the capillary method were only just significantly lower by statistics than the rates obtained with hamsters, the rates were very similar. Hence our results indicate that this method has valid applications as it can give a good approximation as to what the transmission rate would have been in animals. This finding has enabled us to use this method for a long series of transmission tests to investigate the effects of temperature on the vector competence of *Cx. univittatus* with the 2 viruses (Cornel and Jupp, unpublished data). More recently, we successfully used this artificial method to evaluate the competence of several mosquitoes as vectors of RVF virus (Jupp and Cornel 1988).

The primary disadvantage of the capillary method is that the mosquitoes die after the transmission feed due to the prior removal of some of their legs. This makes them no longer available for subsequent transmission tests. Secondly, it was found necessary to test the capillary fluid for virus immediately after the mosquito had fed since no virus could be isolated after a few days storage at −70°C. Perhaps this problem could be overcome by adding a virus preservative such as dimethyl sulphoxide to the fluids before they are stored or by storing them at a lower temperature in liquid nitrogen. Advantages of the method are that determinations of the transmission rate can be made without the use of animals and can provide transmission data for species of mosquitoes which will not readily refeed on laboratory animals. Furthermore, the method is a convenient one and the capillary fluid can be readily titrated in Vero cells to determine the concentrations of virus secreted by individual mosquitoes.

Gubler and Rosen (1976) used the droplet method successfully for transmission of dengue-2 virus by *Aedes albopictus*, but in view of our very low transmission rate of SIN virus by *Cx. univittatus*, this method should be used with caution. Certainly it would be advisable to evaluate it against an animal method first for the particular virus-vector combination under study.

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