VECTOR COMPETENCE TESTS WITH RIFT VALLEY FEVER VIRUS AND FIVE SOUTH AFRICAN SPECIES OF MOSQUITO

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ABSTRACT. *Aedes juppi* was readily infected by inoculation with virus but failed to transmit either horizontally or vertically. Seventy-five to 90% of the other 4 mosquito species became infected after ingesting 6.8–9.8 $\log_{10}CPD_{50}$/ml of virus. These species all transmitted virus at the following rates on the post-infection days indicated: *Aedes unidentatus* (58%—day 11), *Aedes dentatus* (32%—day 11, 50%—day 18), *Culex poicilipes* (15%—day 15, 80%—day 30) and *Aedes argenteopunctatus* (14% on day 30). On the basis of these results and the relative prevalence of each species, it was concluded that *Ae. unidentatus* and *Ae. dentatus* are potential epizootic and possibly reservoir vectors and *Cx. poicilipes* a potential epizootic vector of Rift Valley fever virus in South Africa.

INTRODUCTION

Rift Valley fever (RVF) virus occurs in 2 ecologically distinct regions in South Africa, i.e., the temperate inland plateau west of the Drakensberg escarpment and the subtropical-tropical coastal lowlands of Natal. Multiple isolations of virus made from mosquitoes collected during epizootics and the results of mosquito vector competence tests have indicated the identity of at least some of the mosquito species which are vectors. Table 1 summarizes this evidence from which it can be seen that on the basis of the number of field isolations, coupled to their degree of susceptibility and moderate transmission rates *Culex (Culex) theileri* Theobald, *Culex (Culex) zombaensis* Theobald and *Aedes (Neomelaniconion) circumluteolus* (Theobald) are the most efficient vectors. Vectors incriminated in this way on the plateau are *Cx. theileri, Aedes (Neomelaniconion) mcintoshi* (Huang) (reported as *Ae. lineatopennis* (Ludlow)) and *Aedes (Ochlerotatus) juppi* McIntosh. Gear et al. (1955) made 6 isolations from a mosquito identified then as *Aedes (Och.) caballus* (Theobald). However, subsequent revision of the subgenus *Ochlerotatus* (McIntosh 1973a) indicates the collections concerned could have comprised *Ae. caballus* sensu stricto, *Ae. juppi*, or a mixture of both species. *Culex zombaensis* and *Ae. circumluteolus* occur in the Natal coastal lowlands. These aedine species

Table 1. Southern African mosquito species yielding multiple isolations of Rift Valley fever virus from 1953–81; number of isolations and results of transmission experiments.

Region	Species	No. isolations	50% infection threshold[1] $\text{Log}_{10}LD_{50}$/ml	Transmission rate[2] (days post-infection)	References
Natal coastal lowlands	*Cx. zombaensis*	7	c. 7.5	3–42% (14–29)	McIntosh et al. 1983
	Ae. circumluteolus	3	c. 7.5	32–38% (16–31)	Kokernot et al. 1957 McIntosh et al. 1983 Jupp et al. 1983
S.A. inland plateau	*Cx. theileri*	22	c. 5.7	13–35% (15–22)	Gear et al. 1955
		22			McIntosh, 1972 McIntosh et al. 1973, 1980 Jupp et al., unpublished data
	Ae. juppi	3	8.7	6% (17)	McIntosh et al. 1980
	Ae. caballus s.l.	6	8.0[3]	0%[3] (12; 13)	Gear et al. 1957 McIntosh et al. 1980
	Ae. mcintoshi[4]	2	8.1	17% (10; 17)	McIntosh et al. 1980
Zimbabwe highlands	*Ae. mcintoshi*[4]	2			McIntosh 1972

[1] The titer of virus needed to infect 50% of the mosquitoes.
[2] The proportion of infected mosquitoes transmitting virus to hamsters.
[3] Experiments done with *Ae. caballus* s.s.
[4] Reported as *Ae. lineatopennis.*

are floodwater mosquitoes and among them Linthicum et al. (1985) have recently provided evidence that a field population of *Ae. mcintoshi (Ae. lineatopennis)* in Kenya transmitted the virus transovarially. This would enable the virus to survive through dry interepizootic periods probably for several years until climatic conditions, notably rainfall, were suitable to trigger the next epizootic. We have so far been unsuccessful in obtaining evidence to show that a species of floodwater *Aedes* in South Africa can transmit the virus transovarially (McIntosh et al. 1980 and Gargan et al., in press).

The present paper reports the results of further vector competence studies. Experiments with *Ae. juppi* were conducted to investigate possible transovarial transmission and assess the horizontal transmission potential of this species. Horizontal transmission studies were also conducted with 3 floodwater *Aedes* species: *Ae. (Neo.) unidentatus* McIntosh and *Ae. (Aedimorphus) dentatus* (Theobald) from the inland plateau and *Ae. (Aed.) argenteopunctatus* (Theobald) which occurs in the Natal coastal lowlands. (McIntosh 1971, 1975). Additionally there was *Culex poicilipes* which occurs on the Natal coast. Only *Ae. argenteopunctatus* is an uncommon species. The specimens of this species and *Cx. poicilipes* used for the tests were collected in the Transvaal lowlands because heavy rain had fallen in that area making mosquitoes available. These experiments were designed to identify epizootic vectors and potential reservoir vectors.

MATERIALS AND METHODS

Mosquitoes. From the inland plateau region, *Ae. juppi* was collected at Bethlehem, Orange Free State Province, while *Ae. unidentatus* and *Ae. dentatus* came from Heidelburg in the southern Transvaal. From the eastern Transvaal lowlands, *Ae. argenteopunctatus* and *Cx. poicilipes* were collected at Hazyview.

Infecting Meals. The mosquitoes were infected by allowing them to feed on hamsters which were viremic 30 hours after intraperitoneal inoculation of the AN 1830 strain of RVF virus. The virus had been passed 6 times in mice and 3 times in Vero cell cultures. The concentration of virus in the blood of each hamster was determined by titration in Vero cells of a sample collected shortly before feeding. For this, 0.1 ml of blood was removed from the heart and diluted with 0.9 ml of Leibovitz's medium. Streptomycin, neomycin, gentamicin and fetal calf serum were incorporated in this medium. Tenfold serial dilutions were then inoculated into monolayers of Vero cells in 96 well plates. Vero cells respond to RVF virus by visually displaying cytopathy, plaques forming after 3 days. The 50% cytopathic dose endpoints were calculated by the method of Reed and Muench (1938) and expressed as $Log_{10}CPD_{50}$/ml.

Infected insects were held at 25–26°C and 75–80% relative humidity for varying periods before individual specimens *(Ae. juppi)* or groups of them were allowed to feed on hamsters, or single mosquitoes were tested by the *in vitro* "capillary" method to determine if they could transmit virus.

Transmission attempts. Transmission of virus to hamsters was established by isolation of virus in infant mice inoculated intracerebrally with extracts of the livers of dead animals.

The capillary method for showing *in vitro* transmission by individual mosquitoes was based on the method of Aitken (1977). With this technique a fine capillary tube containing a mixture of equal parts of fetal and calf serum and 10% sucrose solution was inserted over the proboscis of a mosquito which was then allowed to feed on the mixture. Before feeding the tube contained about 0.004 ml of the mixture which after feeding decreased to about 0.0025 ml. The mosquito injects saliva and also virus if present into the capillary tube while feeding. The remaining 0.0025 ml was added to 0.25 ml of Leibovitz's tissue culture medium and tested for virus by inoculation into cultures of Vero cells. A proportion of these specimens were titrated as already described but these titers were expressed as $Log_{10}CPD_{50}$/mosquito capillary fluid. Development of cytopathy due to the presence of virus indicated transmission had occurred. Only visibly engorged infected mosquitoes were considered in estimating the transmission route which is expressed as the percentage of infected mosquitoes transmitting virus.

Mosquito infectivity. The number of mosquitoes infected was determined by testing them individually for virus by inoculation into Vero cell cultures, usually on the same day as their transmission feed. Each mosquito was titurated in 1 ml of Leibovitz's medium, centrifuged at 900 RPM for 5 mins and the supernatant fluid inoculated into established monolayers of Vero cells in 4 wells of a microtiter plate. Titrations were performed on 12 of the mosquitoes by inoculating 10 fold serial dilutions of the supernatant. Titers were expressed as $Log_{10}CPD_{50}$/mosquito. The infection rate was expressed as the percentage of mosquitoes feeding on a viremic hamster which became infected.

Transovarial transmission. To investigate transovarial transmission, *Ae. juppi* were inoculated with virus, allowed to feed on susceptible hamsters daily from days 4 to 6 postinoculation

and F_1 eggs obtained from days 6 to 20. The subsequently reared F_1 adults were fed on hamsters for attempted transmission of virus. In addition, both the F_1 female and male mosquitoes were tested for virus by intracerebral inoculation of infant mice.

RESULTS

The results of the horizontal transmission experiments, given in Table 2, show that all 4 species of mosquitoes were readily infected at the high titers of virus used, and transmitted the virus into capillary tubes. In the case of *Ae. argenteopunctatus* and *Cx. poicilipes* transmission by bite to hamsters was also attempted with groups of 6–18 mosquitoes which was successful. Capillary tube transmission rates were low for *Ae. argenteopunctatus*, moderately high for *Ae. unidentatus* and *Ae. dentatus* and very high for *Cx. poicilipes*. In the case of *Cx. poicilipes*, however, the maximum transmission rate occurred later (30 days) indicating a longer extrinsic incubation period.

The results of the titration of individual mosquitoes and capillary fluids are shown in Table 3. While titers of capillary fluids were similar for *Ae. unidentatus* and *Ae. dentatus* (1.8–2.5 $Log_{10}CPD_{50}$/mosquito), these titers as well as titers for mosquitoes reached a higher level in the case of *Cx. poicilipes* (2.3–3.0 and 3.3–6.3 $Log_{10}CPD_{50}$/mosquito, respectively).

The *Ae. juppi* females had an infection rate of 93% (50/54) when tested 5–15 days after their inoculation with virus (6.5 $log_{10}CPD_{50}$/ml). However, the horizontal transmission rate determined by feeding single infected mosquitoes on single hamsters on day 4 was 0/36. Larval mortality was high when rearing the F_1 progeny so that only 461 females were successfully reared and fed on hamsters. There were no transmissions to any of 10 hamsters. Subsequently, 656 females and 246 males were tested for virus in 19 pools with no isolations of virus.

DISCUSSION

Aedes juppi was investigated as a possible overwintering reservoir because 3 isolations of virus had been obtained from a large collection of this species made during an epizootic at Tweespruit in 1975 and the species had been shown to transmit the virus horizontally (McIntosh et al. 1980). The failure of 4 of the 54 mosquitoes tested in the present study to become infected after inoculation was probably due to inadequate inoculation in the case of these 4 mosquitoes. The mosquitoes were fed

Table 2. Results of vector competence tests with Rift Valley fever virus and 4 mosquito species infected orally.

Species	Titer of infective feed in $Log_{10}CPD_{50}$ PFU/ml	Mosquito infection: Days after infective meal	Mosquito infection: Rate[a]	Transmission: Days after infective meal	Transmission: Test	Transmission: Rate[b]
Ae. argenteopunctatus	7.8–9.8	14–26	29/38 (76%)	9;11	hamster	+ ; +
				30	capillary	1/7 (14%)
Ae. unidentatus	6.8–7.8	11	12/14 (86%)	11	capillary	7/12 (58%)
Ae. dentatus	6.8–7.8	11	28/31 (90%)	11	capillary	9/28 (32%)
		17	6/8 (75%)	18	capillary	3/6 (50%)
Cx. poicilipes	7.8	15	26/29 (90%)	15	capillary	4/26 (15%)
				30	capillary	24/30 (80%)
				15;15	hamster	+ ; +

[a] = numerator = no. mosquitoes infected; denominator = no. mosquitoes tested.
[b] = numerator = no. mosquitoes transmitting; denominator = no. infected mosquitoes feeding.

Table 3. Titers of Rift Valley fever virus infected mosquitoes and capillary fluids, $Log_{10}CPD_{50}$/mosquito.

Species	Days post-infection of capillary feed	Mosquitoes	Capillary fluid
Ae. argenteopunctatus	30	5.0 5.5	0. 2.3
Ae. unidentatus	11	Not done	2.0 2.0 2.0 2.3 2.5 2.5 2.5
Ae. dentatus	11	Not done	1.8 1.8 1.8 1.8 1.8 1.8 2.3 2.5 2.5
	18	Not done	2.0 2.3 2.5
Cx. poicilipes	15	3.3 4.0 4.5 5.0 5.5	— — — 3.3 2.3
	30	3.3 4.3 4.3 5.3 6.3	2.3[a] 2.5[a] 3.0[a] 3.0[a]

[a] = the mosquitoes corresponding to these capillary fluids were not titrated.

soon after inoculation with virus (4 days) because they were already old insects. As inoculation bypasses the midgut, the establishment of infection in the salivary glands should have occurred within this period so the negative transmission rate indicates that this species has a poor vector potential. This concurs with the transmission rate of one out of 18 mosquitoes determined previously (McIntosh et al. 1980). The difficulty in rearing progeny of *Ae. juppi* meant that only a small sample could be made available for the transovarial transmission test. In other investigations on *Ae. juppi* the same problem prevented the production of much larger numbers of adults in the F_1 generation; this was the testing of adults reared from overwintering eggs collected at Tweespruit in 1984 (Gargan et al., in press), when no viruses were recovered from 5,425 mosquitoes tested in 557 pools. All the available evidence therefore suggests that *Ae. juppi* has a low vector potential and that it is probably only a subsidiary epizootic vector not at all involved in vertical transmission of the virus.

The 4 species of mosquitoes orally infected with virus were allowed to feed on hamsters circulating high titers of virus. Similar titers are reached and sometimes exceeded in lambs and calves (McIntosh, 1973b).

In the previous horizontal transmission tests done with several mosquito species (McIntosh et al. 1980) the transmission rates were determined using hamsters whereas in the present work an *in vitro* method was used for most of the tests. This capillary method has been compared with hamster feeding in the case of *Cx. (Cux.) univittatus* Theobald and West Nile and Sindbis viruses when it was found that although hamsters were slightly more sensitive indicators of transmission, the transmission rates obtained with the two methods were not significantly different (Cornel and Jupp, unpublished work). It is likely that the same would apply to various mosquito species and RVF virus in which case the transmission rates shown in Table 1 would also have been a little higher if determined using hamsters. All 4 species listed are potential vectors according to the results in the tests although no isolations of viruses have yet been made from wild-caught mosquitoes of any of them in southern Africa except one isolation from *Ae. dentatus* collected in Zimbabwe (McIntosh 1972). *Aedes argenteopunctatus* must have a low degree of importance as a vector on account of its low transmission rate and uncommon occurrence in the Natal coastal area. The floodwater mosquitoes *Ae. unidentatus* and *Ae. dentatus* on the other hand, can both be prevalent in the highveld region of the inland plateau and this, taken together with their moderately high transmission rates, make them more significant as potential epizootic and possibly reservoir vectors. *Ae. unidentatus* may well be important as a reservoir vector since it is closely related to *Ae. mcintoshi* (reported as *Ae. lineatopennis*) the species shown to transmit transovarially in Kenya (Linthicum et al. 1985). Comparison of the present results with results of a vector competence test done with *Ae. mcintoshi (Ae. lineatopennis)* by McIntosh et al. (1980), shows that *Ae. unidentatus* is a considerably more efficient vector than *Ae. mcintoshi (Ae. lineatopennis)* from the western Transvaal with both a higher susceptibility and transmissibility with RVF virus. *Culex poicilipes* appears to be a more efficient vector than *Cx. zombaensis* which was the major vector during an epizootic at Mtubatuba in the Natal coastal lowlands (McIntosh et al. 1983) but has a similar long extrinsic incubation period necessary for maximum transmission. This mosquito is fairly common in the woodland savannah regions of the Natal coast although not as abundant as *Cx. zombaensis* and could play the role of epizootic vector in this habitat.

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