THE SUSCEPTIBILITY OF FOUR SOUTH AFRICAN SPECIES OF *CULEX* TO WEST NILE AND SINDBIS VIRUSES BY TWO DIFFERENT INFECTING METHODS[1]

P. G. JUPP

Arbovirus Research Unit, South African Institute for Medical Research, Johannesburg, S. Africa

ABSTRACT. Four species of *Culex* were tested for viral susceptibility by feeding on chicks and pigeons circulating different levels of either West Nile (WN) or Sindbis (SIN) viruses. After 14–19 days mosquitoes were tested individually for virus. The following concentrations of virus were found to infect 10% of the mosquitoes: *C. pipiens* Linnaeus—1.0 logs (WN) and 6.4 logs (SIN), *C. quinquefasciatus Say=fatigans* Wiedemann—1.4 logs (WN) and ca. 5.0 logs (SIN), *C. univittatus* Theobald—<1.0 logs (WN) and 2.5–3.2 logs (SIN) and *C. theileri* Theobald—<2.4 logs (WN) and 2.5–3.2 logs (SIN).

C. pipiens was also fed on suspensions of virus in defibrinated blood which gave a much higher 10% infection threshold for WN virus (ca. 5.2 logs), although the threshold for SIN virus (>5.0 logs) remained essentially the same. Similarly, *C. quinquefasciatus=fatigans* had previously been found rather refractory to WN virus when infected in this manner, although there was no difference with SIN virus. Hence, caution is indicated when using blood-virus mixtures for infecting mosquitoes if information on their natural susceptibility is sought. The vector potential of *C. pipiens* and *C. quinquefasciatus=fatigans* with WN virus on the South African Highveld is reassessed in the light of these findings.

The 10 percent threshold levels of infection previously determined for *C. univittatus* and *C. theileri* and both viruses using blood-virus mixtures were comparable to the thresholds determined when viremic birds were used to infect these mosquitoes.

The susceptibility of the commoner culicine mosquitoes from the Highveld region of South Africa to infection with West Nile (WN) and Sindbis (SIN) viruses and their ability to transmit these viruses has been the subject of laboratory experiments undertaken by our Unit. *Culex univittatus* Theobald was shown by Jupp & McIntosh (1970b) to have a high vector capability with both viruses and *Culex quinquefasciatus* Say=*fatigans* Wiedemann a low capability (Jupp & McIntosh 1970a). *Culex theileri* Theobald was found to have a high susceptibility to both viruses but poor transmission rates (Jupp et al. 1972). In the experiments on these 3 *Culex* species the mosquitoes were fed through a membrane on a blood-virus mixture. When this same infecting method was used to test the susceptibility of *Culex pipiens* Linnaeus to infection with WN virus, this mosquito, the remaining one of the 4 commoner species of *Culex* from the Highveld, proved only slightly susceptible to the virus, whereas if it engorged on viremic chicks it was far more readily infected. These results for *C. pipiens* are presented here, and they led in turn to the assessment of the susceptibility of this mosquito to SIN virus using both methods. Furthermore, it was considered necessary to undertake additional infection tests by feeding the mosquitoes on viremic birds in the case of both viruses and the 3 other species of *Culex* which had already been tested by means of blood-virus mixtures. This paper reports the results of such experiments and compares the level of susceptibility obtained by both methods with each virus for all 4 *Culex* species. Descriptions of other experiments with *C. pipiens* are presented. These experiments were undertaken to try to explain the different susceptibilities to WN virus shown by the 2 infecting methods.

MATERIALS AND METHODS

MOSQUITOES. These were either drawn

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from laboratory colonies or were the progeny reared from wild-caught females except in a single instance—one of the tests with *C. pipiens*—where wild-caught females were used. The category of mosquito used for each test is given in the tables. For all species the original mosquitoes were collected in the Johannesburg area and the age of the insects when given the infective feed varied from 1–30 days.

INFECTIVE MEALS. The viruses used and the membrane-feeding method were described by Jupp & McIntosh (1970a). Mosquitoes were allowed to ingest a blood-virus mixture of estimated virus concentration, through either freshly-harvested chicken skin or a Badruche membrane. This method is referred to as the "membrane method." The actual virus titer was determined by inoculation of serial ten-fold dilutions of the blood-virus mixture with the highest viral dose intracerebrally into infant (SIN) and adult or infant (WN) mice at 0.02 ml per mouse. Since the infectivity titer of WN virus is 1.0 log greater in infant than in adult mice, titers derived from the use of infant mice were reduced by 1 log so as to permit comparison with those determined in adults. Infectivity end points are expressed as MLD 50 (mouse lethal dose 50 percent) and were calculated by the method described by Reed and Muench (1938).

In most of the experiments mosquitoes obtained infective meals from viremic birds; this is referred to as the "viremia method." Birds were exposed to mosquitoes at various intervals after inoculation with virus so that the mosquitoes could ingest different concentrations of virus, as shown below:

Chicks and pigeons were restrained and exposed singly to mosquitoes in 35 cm^3 cages. Feeding was allowed to occur for 2–3 hrs in the dark in an insectary where the temperature was 25–26° C and relative humidity 75–85 percent. Immediately before exposure a blood sample was collected to determine the concentration of virus. The day after the infective feed engorged mosquitoes were placed in separate cages and held thereafter at insectary conditions.

INFECTION RATES AND INFECTION THRESHOLD. Infection rates (the proportion of mosquitoes becoming infected) were determined by intracerebral inoculation of a suspension of individual mosquitoes into groups of 12 infant mice. A 10 percent infection threshold (log ED_{10}) was estimated or calculated for each mosquito species with each virus from the infection rates determined for feedings on birds with different viremia levels and also, in the case of *C. pipiens*, from the rates obtained for feedings on different blood-virus mixtures. The probit method as recommended by Dougherty (1964) was used for this calculation.

RESULTS

Table 1 shows the results of *C. pipiens* tested for susceptibility to each virus by means of the membrane method. This mosquito was almost entirely refractory to infection with WN virus as shown by a 10 percent infection threshold of about 5.2 logs, while with SIN virus it was refractory in the single test carried out with a dose of 5.0 logs.

Included in Tables 2 and 3 are the 10 percent infection thresholds determined

Bird + age at inoculation with virus	Interval between inoculation and mosquito feed	Resulting viral titer in blood (logs)	
		WN	SIN
Wet chicks	24 hrs.	3.3–5.5	>7.5
2-day chicks	"	....	6.3–6.8
" "	2 days	5.5–6.7	....
" "	3 days	2.4–3.7	4.1–5.5
" "	4 days	....	....
Adult pigeons	24 hrs.	1.1–2.6	<1.0–2.3

Table 1. Results of quantitative infection tests with *C. pipiens* and West Nile and Sindbis viruses using blood-virus mixtures and membranes.

Virus	Mosquito Generation	Titer of infective feed*	No. days after infective feed mosquitoes tested	Infection rate[b]	Infection threshold log ED_{10}
West Nile	P	5.2[a]	14–18	3/26(12%)	
	Colony F_9	5.1[a]		0/18	ca. 5.2
	P	3.7[a]		0/23	
	P	3.3[d]		0/29	
	Colony F_{11}	2.7[e]		1/25	
Sindbis	P	5.0	17–19	0/26	>5.0

* = mouse lethal dose 50%/0.02 ml. inoculum.
P = progeny reared from eggs laid by field-collected adults.
[a] = these titers have been corrected—actual titrations were done in infant mice and were 1 log higher.
[b] = numerator = No. mosquitoes infected; denominator = No. mosquitoes tested.
[c] = this is calculated from infection rates and is virus titer needed to infect 10% of mosquitoes.
[d+e]—refer text.

for *C. pipiens* from experiments in which the viremia method was used. The log ED_{10} for WN virus was more than 4 logs lower than the value determined using the membrane method. On the other hand, the threshold of infection with SIN virus appeared to be similar in tests done with each method, although the dose employed with the membrane method was not sufficiently high to eliminate the possibility of infection occurring at high levels of virus, 6.3 logs and more, as used in the other series.

Further experiments were conducted in an attempt to explain the discrepancy between the log ED_{10} values determined for *C. pipiens* and WN virus by the 2 different infecting methods. These were as follows:

1. *C. univittatus* and *C. pipiens*, both from laboratory colonies, were fed on the same blood-virus mixture containing 5.1 logs of virus. Although the *C. pipiens* proved refractory to infection, 20 out of 25 (80 percent) of the *C. univittatus* mosquitoes became infected.

2. Mosquitoes from the same broods of *C. pipiens* were given an infective feed by the 2 different methods on the same evening to eliminate the possibility that previous differences had been due to different strains of *C. pipiens*, unwittingly being employed in the experiments. This was done both in the case of progeny reared from field material and for F_{11} mosquitoes from the laboratory colony—2 pairs of infection tests (d and e) were thus undertaken which are indicated in Tables 1 and 2. In each of these tests those mosquitoes which fed through the membrane were refractory to infection, while numerous individuals in the other group became infected.

3. Mosquitoes belonging to 3 species—*C. pipiens, C. quinquefasciatus=fatigans* and *C. univittatus*—which had engorged by both infecting methods were dissected and the blood-meal was found to fill the midgut in each case. Leakage to the dorsal diverticula and ventral diverticulum only occurred when a very large blood-meal was taken from a bird.

Table 2 lists the infection rates and log ED_{10} values obtained for all 4 *Culex* species with WN virus using the viremia method. Comparable log ED_{10} values were obtained for *C. univittatus* and *C. theileri* with each method. However, a large discrepancy was noted in the case of *C. pipiens*. Also there is a difference of about 2.5 logs with *C. fatigans*. The infection rates determined by both methods for *C. univittatus* indicate that the actual endpoint for the log ED_{10} value is probably below that which can be determined even in infant mice.

Table 2. Results of quantitative infection tests with 4 *Culex* species and West Nile virus according to 2 different infecting methods

		Viremia method				Membrane method
	Mosq. colony generation	Titer of infective feed in logs.	No. days after infective feed mosqs. tested	Infection rate	Infection threshold log ED_{10}	Infection threshold log ED_{10}
C. pipiens	P	5.5	14–18	29/29		
	P	5.2		25/26(96%		
	F_{11}	4.5[d]		24/25(96%)	1.0	ca. 5.2
	P	3.3[e]		25/28(89%)	(±0.2)[b]	(table 1)
	F_{11}	2.2		6/25(24%)		
	F_{11}	1.2		0/16		
C. quinquefasciatus=fatigans	C. 139; F_9	4.5	15 or 16	25/25		
	" " "	3.8		20/25(80%)	1.0	ca. 4.0
	C. 83; F_{72}	2.2		13/27(48%)	(±0.3)	(Jupp & McIntosh, 1970[a])
	" " "	1.2		1/25(4%)		
C. univittatus	F_5	4.1–4.6	15 or 18	37/37		
	F_{12}	2.6		24/24	<1.0	<1.0
	F_6	1.0[a]		21/25(84%)		(Jupp & McIntosh, 1970[b])
	F_6	0.2[a]		12/29(41%)		
C. theileri	P	4.5[a]	14	18/18	<2.4	1.5
	P	2.4		27/27		(±0.1)
						(Jupp et al., 1972)

[a]—These titers have been corrected—actual titrations were done in infant mice and were 1 log higher.
[b]—Standard error.
[d+e]—Refer text.

Table 3. Results of quantitative infection tests with 4 *Culex* species and Sindbis virus according to 2 different infecting methods

		Viremia method				Membrane method
	Mosq. colony generation	Titer of infective feed in logs.	No. days after infective feed mosqs. tested	Infection rate	Infection threshold log ED_{10}	Infection threshold log ED_{10}
C pipiens	W	>7.5	15 or 17	38/54(70%)		
	P	6.5		8/26(31%)	6.4	>5.0
	P	6.3		1/24(4%)	(±0.1)	(table 1)
	F_{12}	2.3		0/25		
C. quinquefasciatus=fatigans	C. 139; F_{11}	6.3	15 or 17	5/26(19%)	ca. 5.0	ca. 5.0
	C. 83; F_{78}	2.3		0/25		(Jupp & McIntosh, 1970[a])
C. univittatus	F_{13}	6.3	14 or 15	23/25(92%)		
	F_{16}	5.5		19/20(95%)		1.6
	F_{16}	3.7		22/25(88%)	ca. 2.9	(±0.3)
	F_{15}	3.2		13/26(50%)		(Jupp & McIntosh, 1970[b])
	F_{16}	2.5		0/18		
C. theileri	P	3.7	14	13/25(52%)		2.0
	P	3.2		14/26(54%)	ca. 2.9	(±0.2)
	P	2.5		0/14		(Jupp et al., 1972)

W—Wild-caught females.

As shown in Table 3, with SIN virus there was a good agreement between the log ED_{10} values determined by each method for all 4 species of mosquito. The small differences in log ED_{10} values recorded for *C. theileri* are not considered significant and the difference of 1.0–1.6 logs determined for *C. univittatus* is due to the refractoriness of the mosquitoes which were fed on the bird with the lowest virus titer, i.e. 2.5 logs. It must be pointed out here that the infection rates obtained for the previous colony of *C. univittatus* using the membrane method (Jupp & McIntosh 1970b) were as follows:

titer inlogs	infection rate
3.9	7/12 (58%)
2.9	2/3 (67%)
1.9	1/16 (6%)
0.9	1/19 (5%)

In comparison to these results the infection rates obtained at 3.7 and 3.2 logs (88 percent and 50 percent) in the present experiments indicate that this second colony of *C. univittatus* is probably as susceptible as the earlier colony and suggest that the degree of susceptibility of the mosquito at 2.5 logs in the present series needs confirmation. Indeed, according to calculation, the presence of a single infected individual within the mosquito group at this titer would reduce the log ED_{10} value to 1.6 ($\pm$0.2) logs which is the same value as determined by the membrane method.

DISCUSSION

The noticeably lower susceptibility of *C. pipiens* and *C. quinquefasciatus=fatigans* to infection with WN virus when fed on blood-virus mixtures through a membrane indicates that this method should be used with caution if information on the natural susceptibility of mosquitoes is required. It is noteworthy that it was with one virus only and with these 2 mosquito species which are closely related that this difference occurred. Similar findings were previously reported by Jupp et al. (1966). It appears as though the susceptibility of *C. quinquefasciatus=fatigans* to WN virus determined in that study by means of feeding on blood-soaked cotton is about the same as determined with membrane-feeding in the present study. This suggests that the refractoriness of *C. fatigans* to infection with WN virus, when infant mouse brain virus in defibrinated rabbit blood is used as the infective feed, is due to the nature of this blood-virus suspension and that the use of the membrane is unimportant.

It would seem advisable therefore not to employ the membrane method for testing the susceptibility of a mosquito species to a particular virus in the first instance. If, however, the susceptibility determined for a mosquito species by feeding on blood-virus mixtures is first compared with that determined by feeding on viremic animals and is found to be of similar magnitude, then it would seem acceptable to use the membrane method.

Several other workers have used a blood-virus mixture in susceptibility and/or transmission experiments but the method does not seem to have been applied to any species of *Culex*. In experiments involving *A. aegypti* and Semliki Forest virus, mosquitoes were allowed to feed on viremic mice and on a suspension of infected mouse brain in blood through a mouse skin (Nye & Bertram 1960). These workers did not find a difference in susceptibility with the 2 methods. The real reason for the differences shown in the present study might be found by undertaking further controlled experiments with the 2 species of mosquito concerned.

C. PIPIENS AS A VECTOR. Experiments in which the viremia method was used show that *C. pipiens* is highly susceptible to WN virus. This is unexpected in view of the very low infectivity rate previously determined for wild populations of the mosquito (McIntosh et al. 1967). Why *C. pipiens* does not become infected from avian species in the feral cycle of WN virus is not clear. As theorized previously for *C. theileri* (Jupp & McIntosh 1967),

the answer may lie in preferential feeding among different species of birds by *C. pipiens* resulting in the selection of birds which are relatively resistant to the virus. However, no field observations have been made in support of this. Even if *C. pipiens* did become infected the likelihood of its transmitting virus to further birds is not great owing to its rather low transmission rate (Jupp, unpublished).

Owing to its high infection threshold with SIN virus, *C. pipiens* may be discounted as being of any vectorial importance to this virus.

C. QUINQUEFASCIATUS=FATIGANS AS A VECTOR. The high susceptibility of *C. quinquefasciatus=fatigans to* WN virus shown by the experiments undertaken using viremic birds means that previous assessment of the vector potential of the species with this virus made with the membrane method (Jupp & McIntosh 1970a) must be revised. This high susceptibility together with the low transmission rate reported previously (10–22 percent) would enable the mosquito to play a minor role in transmitting virus between wild birds or from birds to man. The mosquito has suitable feeding habits for this (Jupp 1973). Samples of wild populations of this domestic species have not been collected and tested to find out the proportion of naturally infected mosquitoes which occurs. Thus it is not known whether they would prove to be uninfected in the same way as populations of the more rural *C. pipiens*.

Previously, using the membrane method, it was shown by Jupp & McIntosh (1970a) that *C. quinquefasciatus=fatigans* was only poorly susceptible to WN virus, with a value of about 4.0 logs for the 10 percent infection thresholds. This degree of vector potential seemed lower than that determined for the same species by workers in India and Algeria who used viremic animals (Varma 1960, Vermeil et al. 1960). This was probably due to the use of the membrane method. The same reason probably accounts for the difference in susceptibility between the colonies tested by Jupp & McIntosh (1970a) and those tested by Donaldson (1960) who determined infection rates of 13 percent and 50 percent for mosquitoes fed on sparrows circulating WN virus at 2.0–2.6 logs. Such a level of susceptibility is in better agreement with the present results obtained by using viremic birds.

The results of the tests with SIN virus and *C. quinquefasciatus=*fatigans undertaken with the viremia method confirm the previous experiments in showing that this species is unlikely to be involved either in the transmission of virus between birds or from birds to man.

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