

## AEDES FURCIFER AND OTHER MOSQUITOES AS VECTORS OF CHIKUNGUNYA VIRUS AT MICA, NORTHEASTERN TRANSVAAL, SOUTH AFRICA

P. G. JUPP AND B. M. MCINTOSH

*Arbovirus Unit, National Institute for Virology and Department of Virology, University of Witwatersrand, Private Bag X4, Sandringham 2131, South Africa*

**ABSTRACT.** From 1977 to 1981, studies were conducted on a farm at Mica where *Aedes furcifer* had been a vector during an epidemic of chikungunya virus in 1976 to determine whether the virus persisted in this mosquito, the likelihood of vertical transmission, and whether any other *Aedes* species could have been vectors. *Aedes furcifer/cordellieri* was the only prevalent tree hole *Aedes* which fed readily on monkeys and humans and occurred through the summer until the onset of winter. Virus was not isolated from 7,241 females and 4,052 males of this group, which were largely *Ae. furcifer* and which included a sample of the first post-epidemic population. Five additional *Aedes* species were prevalent in bamboo pots, 3 of which (*Ae. aegypti*, *Ae. fulgens* and *Ae. vittatus*) were shown to be competent laboratory vectors. Virus was not isolated from a sample of 13,029 such newly emerged mosquitoes representing the first post-epidemic population. It is concluded that *Ae. furcifer* is an epidemic-epizootic vector which does not maintain the virus at Mica and that no other mosquito species could have been important vectors.

### INTRODUCTION

Chikungunya (CHIK) virus occurs in the tropical region of southern Africa, which in South Africa comprises the eastern Transvaal lowveld and coastal northern Natal. Human outbreaks of the virus have been infrequent and always related to ample rains in wooded savanna. Infections in man have been recognized in the eastern Transvaal in 1956, 1975, 1976 and 1977 (Gear and Reid 1957, McIntosh et al. 1977, Morrison 1979). A large epidemic occurred in the Zimbabwean lowveld in 1962 (McIntosh et al. 1963a) and an epizootic among vervet monkeys (*Cercopithecus aethiops*) in northern Natal in 1964 (McIntosh 1970). Studies done in relation to these outbreaks, especially the outbreak at Mica in the northeastern Transvaal in 1976 (McIntosh et al. 1977), have shown that vervet monkeys and baboons (*Papio ursinus*) are the primary vertebrate hosts, while the primary vector is the *Aedes furcifer/cordellieri*<sup>1</sup> group of mosquitoes.

During the rural epidemic at Mica in March–April 1976, *Aedes furcifer/cordellieri* was by far the most prevalent species collected off human bait and yielded 16 isolations of virus (McIntosh et al. 1977). The identification of male mosquitoes also taken in the catches indicated that *Ae. furcifer/cordellieri* was comprised largely of *Ae. furcifer* (Edwards) and was probably the principal species in the epidemic. In the laboratory

*Ae. furcifer* was highly susceptible to infection with the virus and a moderately efficient transmitter (Jupp et al. 1981).

After the epidemic, field observations were continued at Mica on the farm "Hope," chosen because the highest post-epidemic immune rates were recorded there for both the human and baboon populations. A series of mosquito collections with monkey and baboon baits was carried out in March 1977 during the summer following the outbreak (McIntosh et al. 1977). The *Ae. furcifer/cordellieri* group, composed mainly of *Ae. furcifer*, was the predominant species taken on wild primate bait and 8- to 10-fold greater numbers were collected in the understory than on the ground.

Further observations were made during the summer in 1977, 1978, 1980 and 1981 which were designed to answer 2 questions. The first was whether the virus remained in the local *Ae. furcifer* population, with this species acting as a reservoir vector, or whether it disappeared. Second, could other mosquito species have been involved in the transmission of virus during the outbreak which escaped detection at the time? Answers were sought as follows: 1) the mosquitoes at "Hope" were monitored for virus infection, including post-epidemic survival of virus through the dry winter by vertical transmission, 2) the relative abundance of mosquito species and their feeding preference for man and wild primates were determined, and 3) the vector competence of the 5 most common tree hole breeding aedine mosquitoes apart from *Ae. furcifer* was evaluated. Transmission experiments had been conducted with 3 of these species previously but not with the "Hope" mosquito populations. Furthermore, more tests were needed

<sup>1</sup> Taxonomic examination of the *Ae. furcifer* group in South Africa in the light of Huang's (1986) revision indicates that *Ae. taylori* is not present but *Ae. cordellieri* Huang occurs there.

on these species before a 50% infection threshold could be determined.

## MATERIALS AND METHODS

*Human-baited catches:* In these collections 2 volunteers used test tubes to collect mosquitoes which alighted on their bare legs for 2–3 h after sunset. At night, flashlights shaded with red cloth were used. Forty-nine man-hours of collecting were done for 12 days during March 1977 and February 1978.

*Monkey-baited suction traps:* In the March 1977 collection series (McIntosh et al. 1977), a single anesthetized baboon or monkey was placed on a wire mesh platform with two 12-volt, 8-watt fans suspended below. These fans sucked mosquitoes attracted to the bait downward into organdie cages (Jupp 1978). Subsequently, the traps differed slightly in that 2 unanesthetized vervet monkeys were housed in a wire mesh cage (60 × 45 × 30 cm) under which the 2 suction fans were hung. Traps were suspended 10 m above the ground in the understory of 3 trees for 2 h after sunset during March 1977, February 1978 and January to June 1980.

*Carbon dioxide-baited light traps:* The light traps used had a 5-mm wire mesh filter fitted over the trap entrance and CO<sub>2</sub> was released through 3-mm bore polythene tubing close to the opening (Jupp et al. 1980). The source of the CO<sub>2</sub> was a tin insulated with corrugated cardboard containing about 2 kg of dry ice. The traps were set overnight during February, March and April 1977 and February, March and June 1980 with a total of 63 trap-nights.

*Artificial oviposition sites in trees:* To collect tree hole breeding species unsampled as adults by the 3 collecting methods described above, bamboo oviposition pots were exposed so as to include all or part of 4 summers: November 1976 to April 1977, December 1977 to April 1978, February to June 1980, and February to June 1981. During 1980, 560-ml plastic bottles, painted black on the outside, containing 2 wooden paddles ("tongue depressors") for oviposition were exposed in addition to the pots. Pots and bottles were suspended from trees at varying heights up to 6 m above the ground. In 1980 and 1981, the openings of about half the pots were reduced by closing them partly with board. During each visit to "Hope" a sample of any larvae present in pots or bottles was preserved for subsequent identification and the paddles were changed in the case of the plastic bottles, the exposed ones being returned to the laboratory under humidification. At the end of summer, pots and bottles with their several sets of paddles were returned to the laboratory where

the eggs were hatched and adults reared. The pots were alternately flooded and dried twice so that the majority of eggs were hatched.

Mosquitoes collected as adults were killed with hydrogen cyanide, pooled according to species and stored in liquid nitrogen. Adults reared from pots and bottles were discarded after identification, except those from the 1976–77 summer which were stored according to species in liquid nitrogen and selected species which were kept alive for vector competence studies.

*Attempted virus isolation from mosquitoes:* Mosquito suspensions prepared from pools of each species were inoculated intracerebrally into 1- to 2-day-old mice for attempted virus isolation.

*Vector competence tests:* Specimens of the 5 most prevalent *Aedes* species aged 1–14 days, and reared from the bamboo pots, were used for vector competence tests. Experiments were conducted in an insectary where the mosquitoes were maintained at 75–80% RH 24–26°C. The virus used was the H817 strain of CHIK virus at the third mouse-passage level. Attempts to infect mosquitoes were made by feeding them on vervet monkeys while these monkeys were viremic after inoculation of virus 48 h previously. Monkeys were anesthetized and exposed to mosquitoes held in 10-cm diam canisters with mesh-covered ends strapped to the monkey's chest and abdomen. Immediately before feeding began, a blood sample was collected from each animal to determine the viremia. Virus titrations were done in infant mice inoculated intracerebrally and titers refer to log<sub>10</sub>LD<sub>50</sub>/ml. The infection rate, i.e., the proportion of mosquitoes feeding which became infected, was determined by testing them individually for virus by inoculation of infant mice. These determinations were done 15–16 days after the infective meal and 1–2 days after the transmission feeds. Infected mosquitoes were held for 13 or 14 days before transmissions were attempted. These were done by feeding groups of potentially infected mosquitoes on 1 or 2 Syrian hamsters. Transmission of virus was determined by testing for hemagglutination-inhibition antibodies in the serum of hamsters 21 days after the transmission was attempted. The titer of virus needed to infect 50% of the mosquitoes was estimated from the infection rates.

## RESULTS

*Adult mosquito collections:* At least 15 species were collected off human bait (Table 1) but *Ae. furcifer/cordellieri* represented 84% of the total catch with a high biting rate of 40.2 mosquitoes per man-hour. Other species which occurred in

Table 1. Mosquito collections using 3 different baits.

Species	Human bait at ground level <sup>a</sup>			Monkey-baited suction traps 10 m above ground in trees <sup>b</sup>			Light traps with CO <sub>2</sub> at ground level <sup>c</sup>		
	No.	No. as % of total	No./man-hour	No.	No. as % of total	No./trap hour	No.	No. as % of total	No./trap night
<b>Tree hole <i>Aedes</i> species</b>									
<i>Ae. (Dic.) furcifer/cordellieri</i> females <sup>d</sup>	1,968	83.9	40.2	3,995	43.6	16.6 <sup>e</sup>	174	4.8	2.8
<i>Ae. (Dic.) furcifer</i> males				4,825	52.6	20.1	265	7.4	4.2
<i>Ae. (Dic.) cordellieri</i> males				49	0.5	0.2			
<i>Ae. (Dic.) adersi</i>							2		
<i>Ae. (Dic.) fasciipalpis</i>							11	0.3	0.2
<i>Ae. (Adm.) vittatus</i>							31	0.9	0.5
<i>Ae. (Stg.) aegypti</i>	2			2			13	0.4	0.2
<i>Ae. (Stg.) ledgeri</i>							2		
<i>Ae. (Stg.) metallicus</i>	8	0.3	0.2	2			43	1.2	0.7
<i>Ae. (Stg.) unilineatus</i>							2		
<b>Other species</b>									
<i>Cx. (Cux.) poicilipes</i>	28	1.2	0.6	271	3.0	1.1	334	9.3	5.3
<i>Aedes (Adm.)</i> 6 spp.	1						20	0.6	0.3
<i>Aedes (Neom.)</i> 2 spp.							25	0.7	0.4
<i>Anopheles</i> 11 spp.	334	14.2	6.8				2,498	69.6	39.7
<i>Culex</i> 11 spp.	1			12	0.1		149	4.2	2.4
<i>Mansonia</i> 2 spp.	1						9	0.3	0.1
<i>Uranotaenia mashaensis</i>							12	0.3	0.2
<b>Total</b>	2,345			9,174			3,590		

<sup>a</sup> Mosquitoes collected in 49 man hours over 12 days during 2 summers (March 1977 and February 1978).

<sup>b</sup> Collected in 240.5 trap hours over 36 days in March 1977, February 1978 and January–June 1980.

<sup>c</sup> Collected in light traps baited with CO<sub>2</sub> over 26 nights (63 trap nights) in February–April 1977 and February–June 1980.

<sup>d</sup> Proportion of *Ae. cordellieri* 1.0–3.5% based on males attracted to females in traps.

<sup>e</sup> No./trap hour varied from 0 to 77.5, depending on the month.

significant numbers were *Culex poicilipes* (Theobald) and several species of *Anopheles*.

The use of 2 unanesthetized monkeys as bait in the understory was the most productive method for collecting *Ae. furcifer/cordellieri* as can be seen in Table 1. This modification of the original trap was much easier to use as each pair of monkeys were kept in the same cage for the duration of a visit to "Hope." The identification of males which were drawn into the suction traps because they had been attracted to females on or near the bait showed that the proportion of *Ae. cordellieri* Huang varied from 1.0 to 3.5%. *Aedes furcifer/cordellieri* was consistently the most prevalent species group during the 3 years of study; *Cx. poicilipes* also regularly entered the traps but in rather low numbers. The numbers of *Ae. furcifer/cordellieri* depended on rainfall and in 1980 the group was collected throughout the summer but disappeared by the end of May at the onset of the dry winter.

Carbon dioxide baited light traps were also set to sample other mosquitoes whose presence and abundance might not be revealed by the human and monkey-baited collections (Table 1). Fewer *Ae. furcifer/cordellieri* were taken in these traps than in the other collections but there

were larger numbers of *Cx. poicilipes* and *Anopheles* species. No other aedine species were taken in the light traps in significantly large numbers.

**Artificial oviposition sites:** Table 2 shows the frequency of occurrence of various mosquito species, expressed as percentages, as they occurred in bamboo pots exposed during 4 summers and in plastic bottles in the 1980 summer. A total of 155 pots were exposed, while 134 samples were identified from the bottles. The bottles yielded a high percentage of collections of *Ae. aegypti* (Linn), *Ae. ledgeri* Huang, *Ae. metallicus* Edwards and *Cx. horridus* Edwards, while other species rarely occurred; *Ae. furcifer/cordellieri* occurring only once. A larger number of different species were collected at a higher frequency in the pots. These included the 4 species that were common in the bottles but also *Ae. vittatus* (Bigot) (27%), *Ae. fulgens* (Edwards) (48%) and *Cx. nebulosus* Theobald (39%). The mean frequency for *Ae. furcifer/cordellieri* was 16% but a much higher frequency of 48% occurred in 1981. Similar figures for *Ae. haworthi* (Edwards) were 16% and 31%, respectively. The plastic bottles with their wooden paddles were less bulky and easier to use than the pots although they failed to sample as many different species.

*Mosquitoes tested for virus:* Neither CHIK virus, nor any other virus, was isolated from either *Ae. furcifer/cordellieri* or any of the other species collected at Mica since the 1976 epidemic (Table 3).

*Vector competence tests:* Table 4 shows the results of vector competence tests with 5 species. The 50% infection thresholds were 6.7  $\log_{10}$ LD<sub>50</sub>/ml. (*Ae. aegypti*) and < 6.7  $\log_{10}$ LD<sub>50</sub>/ml (*Ae. fulgens* and *Ae. vittatus*), and small groups of each of these 3 species transmitted the

virus to hamsters. *Aedes ledgeri* and *Ae. metallicus* were poorly susceptible to the virus, with a 50% infection threshold of > 7.2  $\log_{10}$ LD<sub>50</sub>/ml, and large groups of mosquitoes failed to transmit to more than half the hamsters exposed to them indicating a lower transmission efficiency.

## DISCUSSION

The results of the man-baited catches were very similar to those obtained during the epi-

Table 2. Frequencies of occurrence of different species in bamboo pots or plastic bottles expressed as percentages of total collections.

Species	Bamboo pots					Bottles
	1976-77 (47) <sup>a</sup>	1977-78 (30)	1980 (49)	1981 (29)	Mean (155)	1980 (134) <sup>b</sup>
<i>Ae. (Adm.) cumminsi</i>	2	0	0	0	1	0
<i>Ae. (Alb.) haworthi</i>	6	10	18	31	16	0
<i>Ae. (Alb.) marshalli</i>	2	10	4	0	4	0
<i>Ae. (Adm.) vexans</i>	6	0	0	0	2	0
<i>Ae. (Adm.) vittatus</i>	15	17	33	45	27	1
<i>Ae. (Dic.) furcifer/cordellieri</i>	4	3	14	48	16	1
<i>Ae. (Fin.) fulgens</i>	64	37	33	62	48	1
<i>Ae. (Fin.) nyasae</i>	0	3	6	0	3	0
<i>Ae. (Stg.) aegypti</i>	98	93	100	90	96	78
<i>Ae. (Stg.) ledgeri</i>	89	97	100	100	96	87
<i>Ae. (Stg.) metallicus</i>	96	67	71	62	76	46
<i>Ae. (Stg.) simpsoni</i>	2	0	0	0	1	0
<i>Ae. (Stg.) subargenteus</i>	4	0	0	0	3	0
<i>Ae. (Stg.) unilineatus</i>	2	0	6	7	4	2
<i>Cx. (Cui.) cinereus</i>	27	ND	3	ND	17	1
<i>Cx. (Cui.) nebulosus</i>	57	ND	14	ND	39	1
<i>Cx. (Eum.) horridus</i>	21	ND	67	ND	40	23
<i>Cx. (Lut.) tigripes</i>	0	ND	2	ND	1	0
<i>Tx. (Tox.) brevipalpis</i>	0	ND	8	ND	4	1

ND = larval collections of these species not done.

<sup>a</sup> No. of pots exposed.

<sup>b</sup> No. of pairs of wooden paddles removed from bottles and larval samples.

Table 3. Number of mosquitoes<sup>a</sup> tested for virus: wild caught as adults and reared from bamboo pots.

Species	Wild caught						Bamboo pots	
	1977 <sup>b</sup> (28 <sup>c</sup> )		1978 (7)		1980 (31)		1977	
	No. mosq.	No. pools	No. mosq.	No. pools	No. mosq.	No. pools	No. mosq.	No. pools
<i>Ae. furcifer/cordellieri</i> females	3,522	149	2,068	82	1,643	75	8	1
<i>Ae. furcifer/cordellieri</i> males	2,136	89	1,725	9	187	2	8	1
<i>Ae. aegypti</i>	29	4			3	1	5,413 <sup>d</sup>	66
<i>Ae. fulgens</i>			1	1			1,175 <sup>d</sup>	20
Other <i>Aedes</i> spp.	484	26			38	3	6,441 <sup>d</sup>	81
<i>Culex</i> spp.	545	18			434	15		
<i>Anopheles</i> spp.	3,631	74			702	13		
Totals	10,347	360	3,794	92	3,007	109	13,045	169

<sup>a</sup> All mosquitoes were females except where indicated otherwise.

<sup>b</sup> Includes catch in Feb-March after first rains.

<sup>c</sup> Number of collecting days.

<sup>d</sup> Males and females.

Table 4. Results of vector competence tests with chikungunya virus and 5 tree hole breeding mosquitoes collected at Mica.

Species	Mosquito infection			Transmission to hamsters			
	Titer of infective feed ( $\log_{10}LD_{50}/ml$ )	Rate <sup>a</sup>	50% infection threshold <sup>b</sup> ( $\log_{10}LD_{50}/ml$ )	Days after infective meal	No. hamsters exposed	No. mosquitoes feeding	Results
<i>Ae. aegypti</i>	7.2	10/16 (63%)	6.7	14	1	16	+
	6.7	6/12 (50%)		14	1	12	+
<i>Ae. ledgeri</i>	7.2	1/27 (4%)	>7.2	14	2 <sup>c</sup>	79	+;-
	6.7	1/17 (6%)		14	2 <sup>c</sup>	10	+;-
<i>Ae. metallicus</i>	7.2	12/34 (35%)	>7.2	14	2 <sup>c</sup>	37	+;-
	6.7	4/22 (18%)		14	2 <sup>c</sup>	23	-;-
<i>Ae. vittatus</i>	6.7	10/10	<6.7	13	1	12	+
<i>Ae. fulgens</i>	6.7	4/6 (67%)	<6.7 <sup>d</sup>	13	1	4	+

<sup>a</sup> Numerator = no. mosquitoes infected; denominator = no. mosquitoes tested 15 days after their infective meal.

<sup>b</sup> The titer of virus needed to infect 50% of the mosquitoes.

<sup>c</sup> 2 hamsters exposed simultaneously in the same cage of mosquitoes.

<sup>d</sup> In a previous test (Jupp et al. 1981) 29/33 (88%) of this species was infected after a viremic meal of 7.4  $\log_{10}LD_{50}/ml$ .

demic except that *Cx. poicilipes* and *Anopheles* species were more prevalent than in the earlier collections (McIntosh et al. 1977). The most successful method for sampling the *Ae. furcifer/cordellieri* population was the monkey-baited trap in the tree understory, followed by the ground level man-baited catch and light-carbon dioxide suction trap, in that order. All 3 methods, especially the light-carbon dioxide traps, collected significant numbers of *Cx. poicilipes*, but were ineffective in collecting species of *Aedes* other than *Ae. furcifer/cordellieri*. The low frequency at which *Ae. furcifer/cordellieri* usually oviposited in the pots appeared to be due to the wide openings of the pots. In 1980 and 1981 about half the pots exposed had their openings made smaller which may account for the higher frequency (14 and 48%) of this species group ovipositing during those summers. This concurs with the observations of Raymond and coworkers (1976) who found that *Ae. furcifer/cordellieri* in Senegal preferred to oviposit in tree holes with small openings.

Virus isolation studies in Africa as a whole have indicated that tree hole breeding *Aedes* species are the main vectors of CHIK virus to wild primates and humans. No isolations have been made from *Anopheles* species and only occasional isolations from *Culex* species (Jupp and McIntosh 1988). *Culex poicilipes* is almost refractory to infection (McIntosh and Jupp 1970). Thus the tree hole breeding aedine mosquitoes which are prevalent at Mica and which feed on wild primates and humans are the only mosquitoes that qualify as candidate vectors. All 3 types of bait collections failed to collect significant numbers of any aedine species except *Ae. furcifer/cordellieri*. This indicates that these

are the only aedine mosquitoes feeding on man and wild primates at "Hope" to any appreciable extent. It is possible that other potential aedine vectors which were present but not sampled during the actual epidemic were absent during this study although this is considered unlikely. The exposure of bamboo pots and plastic bottles showed that 13 other tree hole *Aedes* species also occurred, with *Ae. aegypti*, *Ae. ledgeri*, *Ae. metallicus*, *Ae. fulgens* and *Ae. vittatus* present at significant frequencies. None of these species appeared to be daytime man-biters since personnel who camped and worked throughout the day on "Hope" were rarely bitten. Three of these 5 species, viz *Ae. aegypti*, *Ae. fulgens* and *Ae. vittatus*, were shown to be competent laboratory vectors of CHIK virus in the present study, while *Ae. aegypti* and *Ae. fulgens* had also been incriminated previously (McIntosh and Jupp 1970, Jupp et al. 1981). Since vervet monkeys circulate the virus at titers of 3.5–7.0  $\log_{10}LD_{50}/ml$  and baboons 4.6–8.2  $\log_{10}LD_{50}/ml$ , respectively (McIntosh et al. 1963b), on the basis of their infection thresholds of 6.7 or < 6.7  $\log_{10}LD_{50}/ml$ , these 3 species could become infected after feeding on wild primates. However, because they failed to feed on humans or monkeys, it is concluded that only *Ae. furcifer/cordellieri* were involved in the epidemic.

Viral assay of mosquitoes indicated that CHIK virus was no longer present in the local *Ae. furcifer* population at "Hope" after the epidemic. Infected mosquitoes were not detected among the first *Ae. furcifer/cordellieri* mosquitoes to emerge after the epidemic following the rains in February/March 1977 nor among the *Ae. furcifer/cordellieri* collected in the 1978 and 1979 summers. Hence it is unlikely that trans-

ovarial transmission occurs in *Ae. furcifer/cordellieri*. Additional evidence supporting this conclusion is our failure to demonstrate vertical transmission experimentally in other studies (Jupp et al. 1981; Jupp and McIntosh, unpublished data). It also seems unlikely that any other tree hole breeding *Aedes* is responsible for vertical transmission as no virus isolation was made from 13,029 male and female mosquitoes which emerged from the bamboo pots exposed on "Hope" from November 1976 to April 1977 immediately following the epidemic.

It is concluded that *Ae. furcifer/cordellieri*, predominantly *Ae. furcifer*, was the principal mosquito vector of CHIK virus at Mica during the 1976 outbreak and that other *Aedes* species would have at least only played a very minor vectorial role. *Aedes furcifer* is not thought to be a reservoir vector for overwintering of the virus unless the virus enters a dormant state in the vector undetectable by usual viral assay methods.

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