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The ecology and epidemiology of arboviruses in South Africa with reference to their arthropod vectors.

Jupp, Peter Graham

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THE ECOLOGY AND EPIDEMIOLOGY OF ARBOVIRUSES IN SOUTH AFRICA WITH REFERENCE TO THEIR ARTHROPOD VECTORS

Peter Graham Jupp

Published work submitted to the Faculty of Medicine, University of the Witwatersrand, Johannesburg, for the degree Doctor of Science in Medicine.

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Publications* . . . . . -

* The first page of each paper bears the publication number as given in the 'List of publications' and the papers appear in the order given in the list. However, their page numbers remain those of the original journals except in the case of the "in press" papers.

DECLARATION IN TERMS OF RULE G28

I, Peter Graham Jupp, hereby make the following declaration in terms of Rule G28.

1. The published work included for this D.Sc. degree is my own original work except where indicated in the 'Analysis of Co-authorship'.

2. None of the publications resulted from work which has been submitted in the past or is being or is to be submitted for a degree in any university with the following exception. The content of paper 4.2 and a part of papers 4.1 and 4.3 will be included in Mr A. Kemp's M.Sc. (Med) which will be submitted to the University of the Witwatersrand.

3. The work was done from 1964 until mid 1992. From 1964 until 1st April, 1976, I was employed in the Arbovirus Research Unit of the South African Institute for Medical Research. From 1st April, 1976 until mid 1992 I was employed in the Arbovirus Unit of the National Institute for Virology and Department of Virology of the University of the Witwatersrand. Papers 1.4.5 and 1.4.6 reflect work which was undertaken partly at the field station of the Imperial College of Science and Technology at Ascot, England. The work of paper 6.8 was done partly at the National Institutes of Health, Bethesda, Maryland, U.S.A. I was a visiting researcher at both these laboratories for short periods.



ANALYSIS OF CO-AUTHORSHIP FOR CANDIDATE'S CONTRIBUTION TO PUBLICATIONS

Numbers used are those of the references given in the publications lists. Paper where I was sole author are omitted.

Section 1

1.1.1, 1.1.2 & 1.1.3: These studies were planned and executed jointly by Dr McIntosh and myself, although Dr McIntosh played the major role in writing the papers. The remaining authors were technicians who conducted the routine serological and virological tests.

1.1.4: This study was planned and executed jointly by Dr McIntosh and myself but Dr McIntosh did most of the writing of the paper.

1.1.5 As per 1.1.4

1.1.6 I planned the work, carried out all the fieldwork and some of the laboratory work and wrote the paper. Dr Blackburn joined our unit when the work was almost complete but helped identify some of the viral isolations. The remaining authors were technical assistants.

1.1.8 My contribution was to supply the virus strains for the other authors to do the neutralization tests on.

1.2.1 I played the major role in planning, execution and writing up. Brown helped as a technician and McIntosh made some suggestions as to how things should be done.

1.2.2 I had the major role in all phases of this paper.

1.2.3 This was a joint effort in all phases of the work.

1.2.4 This was a project I thought up for Miss Wiederhold to undertake. I provided her with infected mosquitoes, showed her how to remove infected salivary glands and wrote up most of the paper. Wiederhold did the E.M. work and Alexander helped interpret the E.M. photographs.

1.3.1 I was responsible for 75% of this study and wrote the paper.

1.3.4 Nevill's contribution was restricted to confirming the identification of *Culicoides* midges, otherwise the work was planned jointly by McIntosh and myself. I executed 2/3 of the work and played the leading role in writing it up.

1.4.2 Fontenille collected the mosquitoes in Madagascar while I studied them taxonomically and made a diagnosis as to their identity.

1.4.3 The bulk of this paper is the crossmating work done by me. Harbach did the morphology. I wrote most of the paper.

Section 2

2.1, 2.2 These 2 pieces of work were carried out jointly by McIntosh and myself, the 3rd author assisted technically. McIntosh played the major role in writing up.

2.4 I played the major role in undertaking the experiments, while McIntosh was responsible for most the writing.

2.6 Dos Santos did virus isolation and titrations, McIntosh assisted during the transmission experiments. Apart from this I was responsible for all phases of the work.

2.7 I was responsible for 80% of this study and wrote the paper.

2.8 A review chapter in which I was responsible for 2/3 of the work.

2.9 Mr Kemp helped collect and rear some of the mosquitoes and McIntosh confirmed some of the identifications. Otherwise this is my work.

Section 3

3.1, 3.2 These 2 studies were planned and executed jointly by Dr McIntosh and myself, the other authors did technical work. Dr Barnard provided the sheep for one small part of the study and helped handle these animals. McIntosh did most of the writing.

3.3 A joint study with Dr McIntosh but he wrote most of the paper up.

3.4, 3.5 & 3.7 I did 75% of this paper in all its phases: planning, execution and writing.

3.6 I checked all the mosquito identifications and wrote the paper

3.8 I was responsible for 40-50% of this study in all its phases.

3.9 This was very largely my study, the reason Dr Smith's name is included is because he suggested I try Mepacrine.

3.10 I did the planning and writing, Mr Cornel assisted me with the experimental work.

3.11 This was a joint study between McIntosh and myself (planning and execution), but I analysed the data and wrote the work up.

Section 4

4.1 This study was planned and carried out jointly with Mr Kemp but he did the greater part of the writing up.

4.2 I planned and undertook the mosquito examinations and measurements and wrote the paper: Frangos advised on the statistical analysis while Mr Kemp helped me

undertake the mosquito collecting and rearing. He also compiled the statistical data which we interpreted together.

4.3 This work was initiated by me but planned and carried out jointly with Mr Kemp. He was in charge of the *Ae. aegypti* experiments while I was in charge of experiments on the other species of mosquito. Mr Kemp analysed the data and I wrote up the paper. Dr Blaxbourn helped us perfect the indirect fluorescent antibody test.

4.4 I was entirely responsible for this paper except Mr Kemp helped rear some of the mosquitoes.

4.5 This paper was planned and executed jointly but Mr Kemp wrote it up.

Section 5

5.1 I was involved in the planning and helped collect mosquitoes in the study from 1964-68 so must be regarded as a subsidiary co-author. Dr McIntosh wrote this paper up.

5.2 This work was planned and undertaken jointly with Dr McIntosh, the other authors rendered technical assistance. Dr McIntosh did most of the writing.

5.3 The execution of this ecology study was largely done by me. Anderson tested mosquito blood-meals. I largely wrote up the paper.

5.4 Thompson identified the virus isolates and Cornel helped collect mosquitoes otherwise the study was all my work.

5.5. 5.6 McIntosh and I were jointly responsible for the data used in this paper but I wrote it up.

5.7 This was a large study with several facets. I planned the field trip to the nature reserve where the victim was bitten by ticks and collected all the questing ticks which yielded most of the virus isolations.

Section 6

6.1 Mr Van Wyk arranged for the collection of bedbugs; Mrs McElligott, a medical technologist, did the HBV tests in the routine HBV laboratory; Prof Prozesky helped interpret the results and advised on methodology. Apart from this, I undertook all phases of the project.

6.2 I did everything except the HBV testing which was done by Mrs McElligott.

6.3 As per 6.2 except Prof Prozesky made some suggestions on methodology.

6.4 Mrs McElligott did the HBV tests and Prof Lacatses the Electron-microscope examination of certain specimens (a small piece of the overall project). Otherwise I undertook all phases of this work.

6.5 Miss Williamson helped undertake the haptoglobin electrophoreses. I planned the experiment, undertook the bug feeds and wrote up the work.

6.6 In this paper my role was restricted to analysing the data and helping to write the paper.

6.7 Prof Prozesky suggested I conduct these experiments: Swanevelder did the routine HBV testing: Cornel assisted during the infective feeds and Joubert collected ticks for the experiments. I planned the experiments, was largely responsible for carrying them out in all their facets, analysed the data and wrote the paper.

6.8 ined the work and set up the joint experiment with the Americans. Purcell, Phillip., Shapiro and Gerin supplied the chimpanzee and/or did the HBV tests. I handled all entomology and worked out the way of feeding bugs and ticks on chimpanzees. I did most of the analysis of the results and wrote the paper.

6.9 Prof Schoub suggested the experiment initially and was a consultant when this letter was wri n up. Lyons provided the cell line in which she did the reverse transcriptase measurements. I did all the entomology.

6.10 In this paper which reported all the experiments undertaken, Lyons undertook the reverse transcriptase measu ments to detect HIV. Apart from this, I was resr nsible for this paper in all its aspects.

PREFACE

By definition an arthropod-borne virus (arbovirus) is a virus transmitted biologically between vertebrate hosts by an arthropod vector. It multiplies in both the vertebrate and arthropod host and requires both types of host for its perpetuation. The vector acquires virus by feeding on a viraemic vertebrate, the virus then multiplies in the vector, including the salivary glands, and after an interval is transmitted to another vertebrate when a second blood meal is taken.

As medical entomologist to the Arbovirus Unit of the National Institute for Virology (formerly a laboratory of the South African Institute for Medical Research), I have undertaken research on the ecology and epidemiology of the arboviruses in South Africa since 1964. I am interested in this topic with particular reference to the arthropod vectors which are responsible for transmitting these viruses, both in maintenance cycles with wild vertebrates and carrying virus over to man to cause outbreaks of disease. The published papers included in the present compilation relate mainly to 5 arboviruses which are of significant medical importance. These viruses are the following:

West Nile (WN) virus:	Flaviviridae, *Flavivirus*
Sindbis (SIN) virus:	Togaviridae, *Alphavirus*
Chikungunya (CHIK) virus:	Togaviridae, *Alphavirus*
Rift Valley fever (RVF) virus:	Bunyaviridae, *Phlebovirus*
Dengue (DEN) virus:	Flaviridae, *Flavivirus*

They are all mosquito-borne viruses and 48 of the total 65 papers included relate to them. There are also 7 papers on miscellaneous arboviruses including tick-borne arboviruses. Hepatitis B virus (HBV) (Hepadnaviridae; *Hepadnavirus*) receives attention and, although it does not fit the definition of an arbovirus, observations on field and laboratory bedbugs and tampan ticks suggested they could be mechanical vectors so this work is included (8 papers). Finally, the possibility that Human Immunodeficiency virus (HIV) (Retroviridae, *Retrovirus*) might be transmitted by insects has also been tested experimentally (2 papers).

The largest number of papers (22) relate to vectors of WN and SIN viruses (section 1). The papers in this section are divided into 4 subsections: field epidemiology, laboratory vector competence experiments, mosquito ecology and lastly mosquito taxonomy and distribution. The papers in the other 5 sections are not so divided, although on the whole the same topics are considered. Information is needed on all these topics if a full picture of the ecology and epidemiology of the virus in question is to be built up. The "coordinating document" which follows below give a summary of the scope of the work represented by the 65 papers and includes a summary of the main results and conclusions for each publication or group of publications.

The title of the proposed D.Sc. limits the study to South Africa. There are, however, 3 papers relating to work on vectors that occur outside South Africa which are included. These are one on the taxonomy and distribution of the *Culex univittatus* group of mosquitoes in Madagascar and 2 papers on the tampan tick as a vector of HBV in Namibia. I consider it important to include these 2 pieces of work as they make interesting comparisons with the work on the *Culex univittatus* group and the project on *Cimex lectularius* and HBV in South Africa respectively.

COORDINATING DOCUMENT

Introduction

In order to undertake research on arthropod vectors of arboviruses, it is essential to be able to identify the arthropods concerned correctly. In the case of mosquito vectors, these sometimes belong to a group of closely related species which are difficult to distinguish, or one vector species may show intraspecific variation. This has necessitated taxonomic work on several mosquitoes which are vectors or potential vectors.

To incriminate an arthropod as either a maintenance or reservoir vector or an epizootic-epidemic v[illegible], evidence has to be sought along various lines. Such an arthropod will be prevalent an[illegible] distributed in the areas where activity of the particular virus occurs. It will have appropriate ecological characteristics, notably feeding preferences which bring it into contact with its vertebrate hosts. Multiple isolations of the virus in question will be made from wild populations of the arthropod and it should prove an efficient or moderately efficient vector in laboratory vector competence experiments. The evidence collected along these different avenues for different arthropods can be weighed up so as to show the identity or probable identity of the vector (s) which participate in natural transmission cycles and/or which transfer virus to humans. Observations during epidemics provide further valuable evidence to indicate which arthropods are carrying infection to man. This pattern of investigation has been largely followed in the publications that have been included in this compilation. These papers are referred to by numbers in parenthesis.

Section 1. West Nile (WN) and Sindbis (SIN) viruses

The title of my Ph.D thesis was 'A study of the principal mosquito species in the highveld region of South Africa to assess their relative vectorial importance in the transmission of WN and SIN viruses'. This work was also published as 11 papers which are excluded from the D.Sc. It consisted mainly of field studies on the feeding habits and winter biology of highveld mosquitoes and laboratory studies to measure the vector competence of various mosquito species. This study helped incriminate *Culex univittatus* as the principal mosquito vector for the transmission of both viruses in maintenance cycles with wild birds and as the vector transferring infection to human beings. Observations on the overwintering biology of *Culex* species did not support the possibility that the viruses could survive the winter in overwintering *Cx. univittatus*, although they were not completely conclusive. Previous to the Ph.D degree, I did an M.Sc degree on the taxonomic status of *Cx. univittatus* in South Africa in which I distinguished 2 very similar and closely related mosquito species: *Cx. univittatus* on the Transvaal highveld and *Cx. neavei* on the Natal coastal plane. *Cx. neavei* had earlier been regarded as a variety of *Cx. univittatus*. Three papers arose out of the M.Sc., 2 on the taxonomy and one describing the method which was established for the laboratory colonization of *Cx. univittatus*. These are also excluded from the D.Sc.

The ecological studies on WN virus done between 1965 and 1980 were reviewed in 1982 (1.1.5). Virus isolations from field collected mosquitoes and detection of virus activity by serological conversions, through the exposure of chickens or pigeons as sentinel animals, has shown that the bird-feeding mosquito *Cx. univittatus* is the only important vector of both viruses on the Inland Plateau (highveld and Karoo) in South Africa (1.1.1, 1.1.3, 1.1.4). An extensive epidemic of both viruses occurred in the Karoo in 1974 and a study during the epidemic incriminated *Cx. univittatus* as the principal epidemic vector responsible for human infection and *Cx. theileri* as a secondary much less important vector (1.1.2). This study also showed that far more human infections were due to WN than SIN virus.

In the 1983-84 summer, an epidemic of SIN virus and cases of WN virus occurred in the Witwatersrand-Pretoria region and studies again showed that *Cx. univittatus* was the

vector. It was concluded that an epizootic of both viruses had occurred which was manifested by a high level of viral activity in the feral *Cx. univittatus* - bird transmission cycle. *Cx. univittatus* efficiently transferred infection of SIN virus from this cycle to man to cause the epidemic with that virus but it was unclear why there were apparently only a few cases of WN virus infection in this outbreak (1.1.6). In order to collect mosquitoes during this epidemic, a portable light trap was designed which was used with carbon dioxide bait (1.1.7). This trap has also proved effective for collecting other groups of biting Nematocera including sand flies. Ten of the WN virus strains isolated during the outbreak were compared by cross-neutralization tests with each other and with the prototype South African strain and strains from India and Egypt to show the extent of strain variation (1.1.8). The 10 South African strains differed from the Egyptian and Indian strains.

Most of the vector competence tests carried out on various mosquito species with WN and SIN viruses formed part of the Ph.D thesis. They complemented the field epidemiological observations in confirming the identity of *Cx. univittatus* as primary vector. The species proved an efficient laboratory vector of both viruses, although a superior vector with WN virus. An early paper reported some experiments with WN virus which indicated that intrathoracic inoculation or feeding upon cotton wads soaked in a blood-virus mixture were unsatisfactory methods to use for infecting mosquitoes with virus when information on their natural susceptibility was required (1.2.1). This lead to the choice of feeding on a blood-virus mixture through a membrane and feeding on viraemic animals for determination of the infection thresholds of mosquitoes. The comparison between feeding on hamsters, capillary feeding and droplet feeding as a method for determining transmission rates with WN and SIN viruses showed that the *in vitro* capillary feeding technique could be used as an alternative to the use of hamsters (1.2.3). The capillary feeding method has been used successfully for experiments with DEN virus (4.3).

An electron microscope study on salivary glands of *Cx. univittatus* infected with each virus gave a possible explanation for the superior vector ability with WN virus (1.2.4). It appeared that particles of SIN virus have difficulty being secreted into the salivary

gland ducts because of their mass accumulation in the cytoplasm without ready access to the apical cavities. This secretion probably only occurs once a certain threshold concentration had been reached in the cytoplasm. On the other hand, particles of WN virus occur first in the cytoplasm and were never seen to build up to such large numbers as the SIN virus particles. Rather, it seems that they are being continually secreted.

WN and SIN viruses cause only a low level of human infection in the Natal coastal lowlands compared to a much higher level on the Inland Plateau. In the Natal lowlands *Cx. univittatus* is replaced by the closely related *Cx. neavei* from which far less isolations of either virus have been made (1.1.5). A quantitative vector competence assessment of *Cx. neavei* with both viruses showed that this mosquito had a 50% infection threshold of only 2.1 logs/ml compared to 4.9 logs/ml in *Cx. univittatus* which, it is concluded, accounts for the lower viral activity including the lower incidence of human infection in the Natal lowlands (1.2.2).

Four papers (1.3.1 - 1.3.4) reported studies on prevalence, distribution and ecology of mosquitoes on the highveld and in the Karoo. These observations formed a line of evidence helping to incriminate *Cx. univittatus* as main vector and *Cx. theileri* as a minor vector in both areas. On grounds of prevalence and ecology, various other species were candidates for vector but the absence or near absence of virus isolations from field mosquitoes plus poor vector capability in transmission tests eventually caused them to be discounted. Various facets of the ecology of *Cx. univittatus* are important including its type of larval habitat and bird-feeding habit particularly in the canopy of trees, as well as its poor feeding rate on man. The latter is considered one factor which prevents a more regular occurrence of epidemics of these 2 viruses. Human infection occurs annually but is usually sporadic.

During the early years of the studies on WN and SIN viruses it was believed that the 2 mosquitoes in the *Culex pipiens* complex could be important. The mosquito *Cx. quinquefasciatus*, it was postulated, might be the link vector carrying infection from the *Cx. univittatus* - bird maintenance cycle to humans. This seemed plausible because *Cx. quinquefasciatus* was both ornithophilic as well as strongly anthropophilic. Furthermore,

it entered houses at night to feed on man. The other member of the complex, the feral *Cx. pipiens*, had yielded 3 SIN isolations near Johannesburg where it was very abundant so was initially considered as a vector in the avian-mosquito cycle.

Owing to these factors, a project was started in the mid 1960's to elucidate the taxonomic status of these 2 members of the *Culex pipiens* complex in South Africa. A comprehensive study showed that the 2 South African representatives were indeed 2 species and not subspecies (1.4.4). This study included comparative morphology, ecology and cross-mating of the 2 taxa. Because this mosquito complex is cosmopolitan in its distribution, South African *Cx. pipiens* was also compared to English *Cx. pipiens* to clarify its status further (1.4.6). While working with English *Cx. pipiens* the opportunity arose to compare this mosquito with another English mosquito *Cx. torrentium* which is almost indistinguishable in the female from *Cx. pipiens*. A method was developed for distinguishing females on the basis of the number of pre-alar scales (1.4.5).

A morphological study of specimens of the *Cx. univittatus* group from various African countries showed that 3 species existed: *Cx. univittatus*, *Cx. neavei* and *Cx. perexiguus* (1.4.1). A further detailed morphological and cross-mating study between *Cx. neavei* and *Cx. perexiguus* revealed that they were separate species (1.4.3). This was important to know as *Cx. perexiguus* is the vector of WN and SIN in both Egypt and Israel. Examination of specimens of the *Cx. univittatus* group from Madagascar showed that both *Cx. univittatus* and *Cx. neavei* were present in that country. This has implications for the distribution of both WN and Babanki virus in Madagascar. Babanki is closely related to SIN virus (1.4.2).

Section 2. Chikungunya (CHIK) virus

Most of the author's work relating to CHIK virus has been incorporated into one review article published in 1988 (2.8) except a short paper recently accepted for publication. This deals with the identification and occurrence of the mosquitoes *Aedes furcifer* and *Ae. cordellieri* in tropical and subtropical southern Africa (2.9).

Earlier serological studies at Ndumu in northern Natal and in Zimbabwe incriminated wild primates as vertebrate hosts of CHIK virus during epizootics in monkeys or baboons and epidemics in man. Circumstantial evidence collected in Zimbabwe in 1962 pointed to mosquitoes of the *Ae. furcifer* group as vector(s), probably *Ae. furcifer*. From observations on mosquitoes attracted to monkey, human or avian bait on the ground and in the canopy of gallery forest in Ndumu, it was concluded that *Ae. furcifer* and/or *Ae. taylori* and/or *Mansonia africana* seemed most likely to have been involved as vectors during a previous epizootic among vervet monkeys at the same site (2.1).

Studies during a rural outbreak of CHIK at Mica in the tropical thornveld savanna of the north-eastern Transvaal strongly incriminated the *Ae. furcifer-taylori* group as vector of CHIK virus in transmission cycles with baboons in the tree canopy and with the same mosquito carrying infection to man to cause a rural epidemic (2.2). Vector competence studies confirmed that *Ae. furcifer* was an efficient vector of the virus although transovarial transmission was not demonstrated (2.6). These studies also showed that *Ae. fulgens* and *Ma. africana* were potential vectors. Further investigations at the same farm at Mica showed which were the prevalent *Aedes* species other than *Ae. furcifer* and which mosquitoes were laboratory vectors of the virus (2.7). *Aedes aegypti*, *Ae. vittatus* and *Ae. fulgens* were laboratory vectors but their absence from monkey baited traps in the follow-up study and their failure to bite man either during the outbreak (2.2) or in the subsequent study (2.7) suggested they were unimportant as vectors.

Observations during the 1977-1981 study (2.7) did not support persistence of virus on the farm by transovarial transmission after the epidemic. In earlier vector competence tests, 5 other species of mosquito failed to transmit although *Ae. aegypti* transmitted the virus (2.4). Laboratory colonization of *Ae. furcifer* was necessary before vector competence

experiments could be carried out (2.5). Descriptions of 2 kinds of baboon or monkey-baited traps developed in the project for collection of *Ae. furcifer* group mosquitoes both on the ground and in the tree canopy are given in 2 papers (2.3 and 2.7).

The paper in press (2.9) on *Ae. furcifer* and *Ae. cordellieri* in South Africa shows that what was previously named *Ae. taylori* is *Ae. cordellieri* that *Ae. taylori* probably does not occur in our country. *Ae. cordellieri* seems to be a much rarer species than *Ae. furcifer*.

Section 3. Rift Valley fever (RVF) virus

The work on RVF virus up to 1980 was reviewed in 1981 (3.3) and the vector aspects were reviewed up to date in the chapter of a book in press (3.12). On several occasions field observations were made during epizootics on farms when both livestock and human beings had become infected. In tandem with field studies during such an outbreak in 1970 on the inland plateau, vector competence tests were done on 7 mosquito species, 4 of which transmitted virus in the laboratory including *Culex theileri* and *Culex zombaensis* (3.1). Subsequent field studies during epizootics in 1974-75 on the plateau and in 1981 in the Natal coastal lowlands (Mtubatuba) incriminated *Cx. theileri* and *Cx. zombaensis* respectively as the epizootic vectors among sheep and cattle (3.2, 3.4). These 2 papers (3.2, 3.4) also described the results of improved quantitative vector competence tests on *Cx. theileri* and *Cx. zombaensis*, which indicated that both species had a significant vector capacity. Evidence from viral isolation and vector capability tests also implicated the flood water mosquito, *Aedes circumluteolus* as a vector at Mtubatuba on the Natal coast (3.4, 3.5). This mosquito is thought to provide the mechanism by which the virus overwinters on the Natal coast i.e. transovarial (vertical) transmission. A bionomic study carried out on *Ae. circumluteolus* earlier at Ndumu is therefore important, especially as it showed how dependent the species is on river or pan flooding (3.11). A survey of mosquitoes occuring at Richards Bay recorded the presence there of *Ae. circumluteolus, Cx. zombaensis* and *Cx. theileri* (3.6).

Observations during epizootics among sheep on the inland plateau had suggested viral transmission might also occur mechanically. Laboratory experiments showed that mosquitoes could transmit mechanically between hamsters (3.7). This suggested that mosquitoes and probably several other kinds of biting flies might participate in this kind of transmission.

Field observations by other workers in Kenya showed that RVF virus could survive the dry season there within the eggs of flood water *Aedes* mosquitoes during transovarial transmission. Our attempts to demonstrate that this occurred on the edges of pans in the highveld of the Orange Free State have so far been unsuccessful. Virus was not detected in mosquitoes, mainly *Aedes juppi*, reared from eggs recovered from the soil. However,

it was evident that *Ae. juppi* and *Ae. unidentus* could survive in the egg stage in the soil for at least 4 years during times of drought (3.8). During the rearing of these floodwater *Aedes*, a technique was developed to use Mepacrine to kill *Vorticella* infestation which caused larval mortality (3.9). The most recent paper (3.10) on vector competence tests showed that 4 further species were potential vectors, including the floodwater *Aedes Ae. unidentatus* and *Ae. dentatus*. The results suggested that these 2 species are potential epizootic and possible reservoir vectors in the highveld of the inland plateau (3.10).

Section 4. Dengue (DEN) virus

A large epidemic of DEN occurred in Durban in 1926/27 and recently in 1985 several people returned from India infected with the virus. This development and the recent outbreaks of DEN in nearby countries including Mocambique, Kenya and the Seychelles have caused concern that the virus might be re-introduced into South Africa. Other factors which favour the possible re-introduction of the virus are an increase in air traffic between South Africa and the Far East and the appearance of the mosquito *Aedes albopictus* in consignments of imported used tyres landed at Cape Town and Durban for the tyre retreading industry. *Ae. albopictus* is an efficient vector of DEN, superior even to *Ae. aegypti*, is ecologically very adaptable to varying climates and habitats and has established itself in several countries including the United States where it is now present in 18 states.

In view of the above, a project was initiated to investigate tree-hole and container breeding *Aedes* species, principally members of the subgenera *Stegomyia* and *Diceromyia* in eastern South Africa. The objective was to assess these mosquitoes as potential vectors of DEN virus which could participate in epidemic transmission should DEN be reintroduced. The overall study, which is still in progress, has various aspects. One paper has been published on a study of the ecology of potential vectors (4.1) and on ecological grounds the following mosquitoes were regarded as potential vectors particularly on the Natal coast: *Ae. aegypti*, *Ae. demeilloni*, *Ae. simpsoni*, *Ae. strelitziae*, *Ae. furcifer*, *Ae. cordellieri* and *Eretmapodites quinquevittatus*. These potential vectors have since been assessed in vector competence tests: only *Ae. aegypti*, *Ae. strelitzae* and *Ae. furcifer* transmitted DEN 1 and/or DEN 2 serotypes in the laboratory (4.3). It was concluded that the primary potential vector for an epidemic of DEN 1 or 2 in South Africa would be *Ae. aegypti* and the secondary such vector would be *Ae. strelitziae*. *Ae. furcifer* would seem only a poor potential vector confined to the rural areas of Northern Natal-KwaZulu and the eastern Transvaal.

Because of its known importance as an epidemic vector outside South Africa, different populations of *Ae. aegypti* were the subject of a morphological study to elucidate their taxonomic status. Examination of populations from 18 localities in South Africa,

including both anthropophilic and non-anthropophilic populations, showed that in South Africa *Ae. aegypti* is a single polymorphic species displaying plasticity in its man-biting behaviour (4.2). This finding is important as *Ae. aegypti*, a cosmopolitan species, has long been separated into 3 subspecies on the basis of morphological characters which are shown in our study to vary within the same families. Populations of *Ae. aegypti* from different localties have also been assessed to determine their degree of variation in vector capability. The population at Durban has been shown the most efficient vector of both DEN 1 and 2 viruses. The vector competence indices were highest for Durban with both viruses while indices for the other populations were considerably lower (4.3).

Some work has been done in Durban to monitor the importation of exotic mosquitoes in used tyres (4.4). Among other species identified, *Ae. albopictus* was located in one tyre. The environs of the tyre company concerned, together with those of a second tyre company, were chosen as sites for *Aedes* surveillance by ovitraps (4.5). Six species occurred in the ovitraps including 4 *Aedes* species but not *Ae. albopictus*. Noteworthy was the presence of *Ae. furcifer* in one bamboo pot indicating that the southerly limit of this important vector mosquito is much further south than originally supposed. These results suggested that the asian *Ae. albopictus* has not yet become established in the Durban area (4.5).

Section 5. Other Arboviruses

Over a period of 8,5 years 52 viral isolations, including strains of SIN, Middelburg, Ndumu, Wesselsbron, Spondweni, Bunyamwera, Shokwe, Germiston, Mossuril, Lebombo, Ingwavuma and Simbu viruses were made from 5 species of mosquito collected in the Ndumu game reserve in northern Natal. The most frequently infected species, *Ae. circumluteolus*, yielded 37 strains which included 11 arboviruses (5.1). Noteworthy was the failure to isolate WN virus and the isolation of only one strain of SIN virus from *Cx. neavei* (see also Section 1).

Another study was undertaken in Zimbabwe, at Port Shepstone on the Natal south coast and at several localties along the northern Natal coast. From 19,226 *Culex rubinotus* mosquitoes 80 strains of Banzi virus (Flavivirus), 74 of Germiston virus (Bunyavirus) and 67 of Witwatersrand virus (Bunyavirus-like) were isolated. The regularity of infection in different geographical areas over this period and its frequency among individuals of several different natural mosquito populations indicated that *Cx. rubinotus* is a maintenance vector of all 3 viruses. Germiston virus infects man causing a mild illness, Banzi virus infects man although whether illness results is unknown (5.2). In a companion paper, a study was reported on aspects of the biology of *Cx. rubinotus*. This showed that the mosquito occurred in dense marshland and adjoining sugar cane fields in the Natal coastal plane. The mosquito was shown to feed mainly on wild rodents and also on man to a varying extent. According to this study, *Cx. rubinotus* has biological characteristics consistent with the viewpoint that it is the maintenance vector of the 3 viruses among wild rodents (5.3).

Seventeen *Alphavirus* isolates (16 Middelburg and 1 Sindbis) were obtained from 3 937 *Aedes juppi* mosquitoes collected at Tweespruit in the Orange Free State highveld. As collections were made only one month after the first significant rain of the usually dry summer, these mosquitoes were thought to be the first brood of the season and the appearance of Middelburg virus due to the presence of transovarially infected mosquitoes. This could indicate that *Ae. juppi* serves as a reservoir vector to carry this virus through dry inter-epizootic periods (5.4).

In 1981 a review on 'Ticks and arboviruses in South Africa' was published (5.5). This included details of 4 viruses which were isolated by the Arbovirus Unit from bird-feeding *Argas* or *Ornithodoros* ticks. These were as follows:

Chenuda virus:	Reoviridae, *Orbivirus*
Soldado virus:	Bunyaviridae, *Nairovirus*
Nyamanini virus:	Unclassified
Quaranfil virus:	Arenaviridae

Quaranfil virus can cause illness in humans. Vector competence tests with the sand tampan tick, *Ornithodoros savignyi* and 3 medically important arboviruses were also reported for the first time in this paper (5.5). They showed that this species was refractory to RVF and CHIK viruses while 22% of 2nd instar ticks became infected with Wesselsbron virus. These tests were undertaken to collect evidence as to whether *Ornithodoros* ticks might be reservoir vectors of any of these viruses.

Crimean-Congo haemorrhagic fever (CCHF) virus (Bunyaviridae, *Nairovirus*) was isolated for the first time in South Africa in February, 1981 from the blood of a 13 year-old boy who died in Johannesburg after attending a camp in a nature reserve at Bloemhof, western Transvaal. CCHF virus was isolated from 21/120 pools of questing ticks from the nature reserve, the infected ticks being *Hyalomma marginatum rufipes* and *H. truncatum*. Virus was also isolated from these 2 species and *Rhipicephalus evertsi* collected off animal hosts. Antibodies were found in humans, sheep, cattle and wild vertebrates including hares. It was concluded in this 1983 publication that the virus was widely prevalent in South Africa but that the full medical and veterinary significance of CCHF virus had yet to be determined (5.7). The writer has not done any further studies on this virus.

Section 6. Mechanical Transmission of non-arboviruses by arthropods

Two viruses which do not fit the definition of an arbovirus were investigated to determine whether they could be transmitted mechanically by arthropods. These were hepatitis B virus (HBV) and human immunodeficiency virus (HIV). Mechanical transmission is a passive transfer of virus between vertebrates without any viral replication occurring in the arthropod.

Section 6.1 Hepatitis virus

Bedbugs (*Cimex lectularius*) were collected from various localities in the northern Transvaal and tested for the presence of hepatitis B surface antigen (HBsAg). Bugs from most localities had a high positivity rate and it was concluded that *C. lectularius* could be a vector of HBV in the Transvaal and that the varying degrees of infestation could explain the markedly different HBsAg-positive frequencies previously shown in human sera collected from different population groups in the Province (6.1). Subsequently, HBsAg-positive blood-meals were fed to bedbugs in vector competence experiments. Antigen persisted in the bugs for at least 7½ weeks and was successfully transmitted by adult bugs into HBsAg-negative blood. Furthermore, antibody of HBsAg was detected in a rabbit and guinea pigs on which HBsAg-positive bugs had previously fed. It was concluded that although biological multiplication and biological transmission do not occur, mechanical transmission had been demonstrated. It was considered that this was probably an important means of HBV transmission among humans in South Africa (6.2). The following year a short letter was published reporting an HBV-passage experiment which demonstrated that biological multiplication of HBV definitely did not occur in bedbugs (6.3).

In 1983, a paper appeared which reported the results of tests done on bugs for both HBsAg and HBeAg (6.4). These tests were done on both wild-caught and laboratory colonized bugs, the latter after HBV-positive blood meals. Positivity for both antigens was regarded as an indication of HBV infectivity. Such was demonstrated in most of the bugs collected from the northern Transvaal. In bugs tested upto 31 days after an infective blood-meal, both HB_sAg (98%) and HB_eAg (17%) were present in carcases but only HB_sAg (20%) in salivary glands. The bugs excreted HB_sAg in their faeces for 42

days and both antigens for 3 days. HB_sAg particles were detected by electron microscopy in faeces harvested on day 10. It was again concluded that no biological multiplication of HBV occurred in *C. lectularius* but that mechanical transmission from insects to man could occur by: (i) contamination of a person when crushing infective bugs; (ii) contamination from infected bug faeces; and (iii) infection by bite due to regurgitation or interrupted feeding (6.4).

Arising from the third conclusion given above, it became important to try to demonstrate whether interrupted feeding occurred naturally in bedbugs. A technique was used successfully in the laboratory to detect multiple feeding by means of the identification of 2 different haptoglobin serum proteins in the same insect blood-meal (6.5). However, it has not yet been possible to try this procedure out on field-collected bedbugs because of an intensive spraying programme inside the huts in the study area.

Almost the same work was undertaken on the hut tampan tick, *Ornithodoros moubata* which occurs in the huts of the Kavango tribe in Namibia. It was done because of the extremely high positivity of both children and adults for HBV in this region which could not be explained except by the high prevalence of tampan ticks. A high HBsAg positivity but lower HB_eAg positivity was shown for wild-caught ticks (6.6) and vector competence tests led to very similar conclusions as to those from the experiments with bedbugs. The only difference was that instead of faeces being a means of transferring infection, the coxal fluid secreted by the tick on feeding was thought important. It was considered likely that contamination could occur through coxal fluid being inoculated when scratching bites (6.7).

When some chimpanzees became available for this research, some further vector competence tests were undertaken (6.8). Chimpanzees are the only wholly suitable animal model for HBV work became the virus multiplies in these animals. Bedbugs (*C. lectularius*) were fed on an infective blood-HBV mixture. Further bedbugs and tampan ticks (*O. moubata*) were fed on HBV-carrier chimpanzees. After a 10-13 day interval for oviposition, tests done on samples of individual arthropods showed that 53-85% of the bugs were HB_sAg-positive and none HB_eAg-positive, while 100% of the ticks were

HB_sAg-positive and 88% HB_eAg-positive. The remaining arthropods were fed on 3 susceptible chimpanzees which failed to develop HBV infection after 11 months, indicating no transmission had occurreed. Subsequently, the presence of viable virus in the original infective meals was confirmed by inoculation of the relevant donor sera directly into the 3 still susceptible chimpanzees. HBV infections quickly followed in each animal. It was concluded that, while mechanical transmission of HBV is most unlikely after a 10-13 day interval between feedings in bedbugs and tampans, it is still possible that mechanical transmission between humans might occur during interrupted feeds.

Section 6.2. Human immunodeficiency virus (HIV)

In 1986 a letter was published in the Lancet reporting initial experiments designed to test whether HIV could perhaps be mechanically transmitted by bedbugs (*Cimex lectularius*) (6.9). Insect transmission would, it was thought, account for the 15-22% of AIDS cases reported for children in Africa in contrast to the 1-4% reported for children in the U.S.A. (Clumeck, Int symposium on African AIDS, Brussels, 1985). The survival of HIV for 1 hour in *C. lectularius* following a feed on a blood-virus mixture suggested that mechanical transmission of the virus between human beings might be carried out by bedbugs (6.9).

In a subsequent more comprehensive series of experiments both the common and tropical bedbugs (*C. lectularius* and *C. hemipterus*) and mosquitoes (*Ae. aegypti formosus*) were assessed as potential vectors of HIV (6.10). The amount of virus present in the blood-meals of replete female and male bedbugs was calculated. It was shown that female bedbugs were larger than males and took larger blood-meals with more virus. Reverse transcriptase activity was assayed after culture of insect extracts in H9 cells: this showed survival of virus in *C. lectularius* for up to 4 hours, in *C. hemipterus* for up to 1, possibly 2 hours, but no survival in *Ae. aegypti formosus*. Four attempts to transmit the virus by interrupted feeding by *C. lectularius* from a blood-virus mixture to uninfected blood failed. It was concluded that *Ae. aegypti formosus* and probably other mosquitoes are not mechanical vectors of HIV and that such transmission is also unlikely to occur in bedbugs under natural conditions.

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ECOLOGICAL STUDIES ON SINDBIS AND WEST NILE VIRUSES IN SOUTH AFRICA. I. VIRAL ACTIVITY AS REVEALED BY INFECTION OF MOSQUITOES AND SENTINEL FOWLS*

by

B. M. McINTOSH, P. G. JUPP, D. B. DICKINSON, G. M. McGILLIVRAY, J. SWEETNAM
Arbovirus Research Unit, South African Institute for Medical Research, Johannesburg.
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This paper is the first of a series reporting the results of an ecological study on Sindbis (Sd) and West Nile (WN) viruses in South Africa. Investigations were instituted late in 1961 after earlier work had incriminated these viruses as the two arboviruses most frequently infecting man in South Africa. These viruses were implicated in this role mainly by the outcome of extensive antibody surveys, although the existence of both viruses in the country had also been established by isolation at that time.

The antibody surveys, which had included most of South Africa [Weinbren, 1955; Kokernot *et al.*, 1956; McIntosh *et al.*, 1962a, b], had shown that human infections with both viruses occurred most frequently in the inland plateau region, the so-called "highveld." This suggested the existence in the highveld of more favourable conditions for the propagation of these viruses, or at least, for the occurrence of human infection, and it seemed that the highveld region would be the most suitable area for field studies. Another factor which had become apparent from the antibody surveys was the close correlation in the distribution and prevalence of these two viruses, thus intimating a similarity in ecology which suggested that the same methods of field study would be equally applicable to both viruses.

Prior to the commencement of the present study there had been seven isolations of Sd virus and two of WN virus in South Africa. Sd virus had been isolated four times from *Culex (Culex) univittatus* Theobald, twice from pools of *Culex* species, one of which included *C. univittatus* [Weinbren *et al.*, 1956; Worth *et al.*, 1961] and once from *Mansonia (Mansonioides) africana* (Theobald). Three of these isolations had been accomplished in the highveld and four in the tropical lowlands in northern Natal. The two isolations of WN virus were made simultaneously from man and a wild bird in northern Natal in 1958 [Kokernot and McIntosh, 1959].

When the present study started there was little definite evidence on the extent of human illness in South Africa caused by either virus, Sd virus not then being known to be pathogenic for man. It was, in fact, not until 1961 that it was recovered for the first time from man when its isolation from blood specimens taken from five ill Africans in Uganda was reported [Haddow, 1962; Woodall *et al.*, 1962]. In 1963 this virus was shown to cause an illness in man in South Africa somewhat similar to WN virus [Malherbe *et al.*, 1963; McIntosh *et al.*, 1964] and in subsequent years clinical cases due to both viruses have been encountered annually during the summer in the highveld. In retrospect it seems likely that certain undiagnosed outbreaks of fever with rash and joint pain which occurred in the highveld in 1941 (Prof. J. H. S. Gear, personal communication) and in 1954 and 1956 [Cochrane and Loewenthal, 1955; Rudolf, 1955] were due to either one or both viruses.

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The objectives of the present study, which are still being pursued, are intended to be comprehensive, including all aspects of the feral transmission cycles as well as the circumstances favouring human infection. At the outset it was important that the mosquito species carrying virus be identified. It was hoped that the testing of large numbers of wild-caught mosquitoes for the presence of virus would go a long way towards revealing the main vector(s) and that collections of mosquitoes throughout the summer and at various localities would supply relevant information on incidence and geographical distribution of viral activity. An unknown factor was whether these viruses were securely established in the temperate highveld or whether their presence there was of infrequent occurrence and the result of fortuitous introductions from other, seemingly more favourable, environments. From what was already known of these two viruses it seemed likely that the main participants in the feral cycles would be found among wild birds and aviophilic mosquitoes.

A sewage effluent disposal area, Olifantsvlei, five miles from the outskirts of Johannesburg, was selected as the main study area. The area is accessible to the laboratory and carries high mosquito and bird populations. As representative of a more undisturbed highveld habitat a sheep and cattle farm at Lake Chrissie, 130 miles south east of Johannesburg, was chosen as a subsidiary study area. In addition to these permanent study areas small outbreaks of human infection with Sd and WN viruses at the highveld towns of Boksburg and Welkom respectively, provided opportunities for short-term observations to be made under circumstances associated with very recent human infection.

The observations at Olifantsvlei were conducted over four consecutive summers, viz., from the spring of 1961 to the autumn of 1965. Studies during the first summer were limited, being concerned mainly with the testing of field techniques and assessment of the locality as a suitable study area. In subsequent years the studies were intensified and observations were fairly continuous during the summer months. Work there consisted mainly of collection of mosquitoes for attempted virus isolation, observations on mosquito bionomics, exposure of sentinel fowls and attempted virus isolation and antibody surveys on the bird, rodent and human populations. At Lake Chrissie, observations started in March 1962 and included the exposure of sentinel fowls throughout the summer months and short, intermittent visits when mosquitoes were collected for attempted virus isolation.

Aspects of the overall ecological study dealt with in this paper concern the mosquito species found to be infected and the incidence of viral activity in relation to time and geographical distribution. Evidence on these points was obtained from the isolation of virus from wild-caught mosquitoes and from antibody conversions to these viruses in fowls exposed as sentinels.

THE STUDY AREAS

Olifantsvlei

This is about 10 square miles in extent located in a shallow valley at an altitude of about 5,000 feet with low-lying surrounding hills some 200 feet higher. Processed sewage effluent is used to irrigate artificial and natural grass pasture on which cattle graze. The quantities of effluent are large in relation to disposal area, thus leading to the formation of semi-permanent to permanent water in furrows and on grazing lands which provides suitable mosquito-breeding sites and attracts large numbers of birds. A prominent feature is the small, perennial Klip river into which surplus effluent is discharged. In the river-bed are extensive reeds which provide shelter as well as roosting and nesting sites for numerous birds. Along part of the northern boundary are several

small agricultural holdings where dairying is practised. Adjoining these holdings and outside the study area is the densely populated area of Nancefield, a suburb of Johannesburg. Olifantsvlei has a fairly large human population consisting of about 100 labourers living in compounds as well as several farm overseers and other employees living with their families either in isolated dwellings or in semi-built-up areas. The study area is therefore partly suburban and partly rural although most of the observations were made in the rural part. Due to effluent disposal it is much wetter than natural highveld and the large number of exotic trees provide an unusually densely wooded landscape for this region.

The highveld has a temperate climate with summer rains with an annual precipitation at Olifantsvlei of 30.3 ins. The winters are cold and dry during which all mosquito activity ceases. At Olifantsvlei mosquitoes first appeared in baited traps in September or October when mean daily temperatures rise to about 15–17°C (Fig. 1) and just before the summer rains commence. From about the beginning of May when mean temperatures fall to about 13–14°C adult mosquito activity falls to minimum levels, and ceases entirely thereafter. A mosquito "season" when collecting was feasible and profitable extended therefore from November to April.

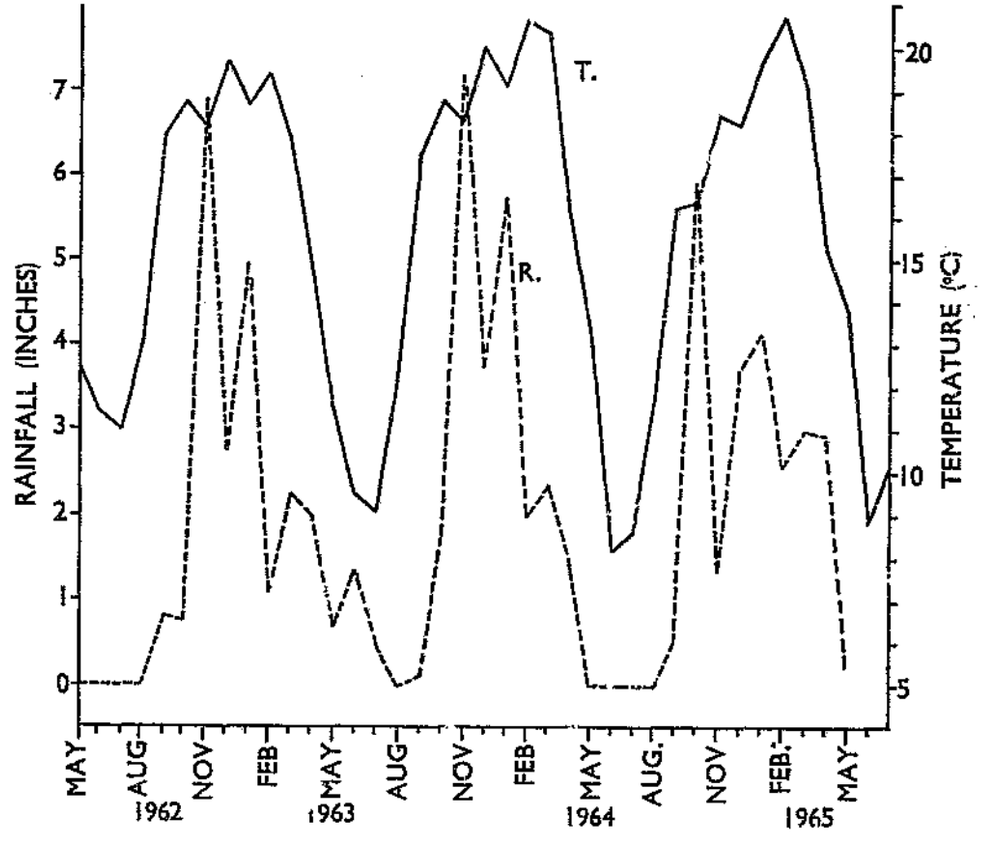

Fig. 1. Monthly temperature (T.) and rainfall (R.) of Johannesburg area from 1962 to 1965. Temperature values derived from monthly mean of daily (max.+min.)/2.

Lake Chrissie

This is fairly typical grassland country of the eastern highveld, at an altitude of about 5,500 feet and with an annual summer rainfall of 29.7 ins. The farm where observations were made is exceptionally well-watered for highveld, with several pans, some very large and permanent. On account of the pans the avifauna is richer in species and numbers than highveld generally, but densities were never as high as at Olifantsvlei. Weather records at Ermelo, some 20 miles away, show very similar temperatures to those at Olifantsvlei and as at the latter locality all mosquito activity ceases during the winter.

Welkom and Boksburg

Boksburg is a medium-sized town 15 miles from Johannesburg. Some mosquito collections were made at the houses where human cases of Sd virus infection had occurred but the majority of mosquitoes were collected in an open marshy area fed by rainwater furrows a few hundred yards from these houses.

Welkom is of similar size and is some 200 miles south of Johannesburg in the Orange Free State province. In the vicinity of Welkom are several gold mines from which large quantities of brackish water are pumped. This water is stored in dams on the surface for disposal by evaporation and seepage, for its high saline content makes it unsuitable for industrial or agricultural usage. The dams however support a rich avifauna and favourable conditions for mosquito breeding exist during the summer. Mosquito collections were made at dams as well as in residential areas where human WN cases had occurred.

METHODS AND MATERIAL

Mosquito collecting

Collecting methods are described in detail in part II of this series [Jupp and McIntosh, 1967]. Mosquitoes for virus isolation were collected mainly in lard-can traps baited with solid carbon dioxide. Mosquito fowl-pen traps baited with a flock of from 6 to 12 sentinel fowls were also used fairly extensively, while most aedine mosquitoes were collected in net-traps. Traps were usually set to operate overnight on two nights a week throughout the summer. At Olifantsvlei several wild and peridomestic collecting sites were exploited and two sites were used fairly regularly. Both were peri-domestic, i.e., within yards of isolated farm homesteads, one being some 300 yards from the Klip river and the other on higher ground about two miles from the river.

Mosquito processing

Mosquitoes were killed with chloroform vapour, then identified, tubed and stored on solid CO_2, all procedures normally being completed on the day of collection. Mosquitoes were pooled according to species, collecting site, and calendar month of collection. Pool sizes were limited to a maximum of 300 mosquitoes for all species except *C. univittatus* in which case pools did not exceed 100 during the last three years of study. Many pools of this mosquito were inoculated on the day of collection thus eliminating freezing and storage.

Virus isolation and identification

Mosquito suspensions were inoculated intracerebrally (IC) into two litters of mice 2–3 days old, each litter consisting of 6 mice. Brain from sick, or in a few instances, dead mice, was used for passage which was continued in infant mice for 3 to 4 passages. In one of these passages 12 adult mice were inoculated IC and another 12 intraperitoneally. If these adult mice survived they were re-inoculated with the same viral isolate on about the 14th day and exsanguinated two weeks later. The sera were used in complement-fixation (CF) tests for identification of the isolate. In these tests

crude saline or sucrose-acetone extracted antigens made from infected infant mouse brain were used [Clarke and Casals, 1958] and antigens of unknown viral isolates were tested against hyperimmune antisera to known arboviruses prepared in adult mice. For confirmatory identification tests, neutralization (N) and haemagglutination-inhibition (HI) tests were used. N tests were conducted in infant (Sd) or adult (WN) mice inoculated IC and antisera were "single-inoculation" sera prepared in guinea-pigs inoculated IC. HI tests were done according to the method of Casals [1964] in which serial two-fold dilutions of antisera were tested, at the same pH, against a series of 6 antigen dilutions containing from 8 to 256 units of antigen. Reactions were read at 0.5, 2 and 18 hours. To simplify tabulation only the 2-hour reading is given in the tables. In these tests "single-inoculation" guinea-pig antisera were used. For identification purposes viral isolates were compared with the AR86 strain of Sd virus [Weinbren *et al.*, 1956] and the H442 strain of WN virus [Kokernot and McIntosh, 1959].

Sentinel fowls

Fowls were used as sentinels to detect activity by Sd and WN viruses only after the suitability of fowls for this purpose had been demonstrated by laboratory studies. This was shown by antibody tests on fowls which had been inoculated with these viruses. At two, and sometimes three mosquito collecting sites at Olifantsvlei and one at Lake Chrissie flocks of from 6 to 12 adult fowls were exposed during summer in the same fowl-pens used for collecting mosquitoes. Fowls were numbered and bled before exposure and at approximately monthly intervals in the field. Bloods were diluted 1/5 in a heparine-saline mixture at the time of collection. The plasmas were tested for Sd and WN antibodies in an HI test in which two-fold plasma dilutions were tested against 8 units of antigen.

C. univittatus infectivity rates

Virus infection rate in *univittatus* populations at Olifantsvlei is expressed as the number of isolations per 1,000 mosquitoes tested and they are calculated on the basis of all mosquitoes collected during single monthly periods at all mosquito-collecting sites.

RESULTS

Identification of viral isolates

The pathogenicity of viral isolates for adult mice gave early indication of their probable identity, as Sd virus is invariably non-pathogenic for adult mice in early mouse passage whereas WN virus at the same passage level is usually lethal to adult mice by both IC and IP routes. This proved a useful guide as all but two isolates were either Sd or WN viruses. The exceptions were an apparently undescribed virus (AR5133) of the Bunyamwera group and a Group B virus (AR5189) closely related to WN virus.

Identification of the Sd virus isolates was made using the CF test and this was considered adequate because in this test with our antisera there was no cross-reaction with other Group A viruses known to occur in South Africa, viz., Middelburg, Ndumu and chikungunya viruses. Furthermore, of these three, only Middelburg virus is suspected of occurring in the highveld. In confirmatory N tests, involving 6 randomly selected viral isolates from Olifantsvlei and one each from Lake Chrissie and Boksburg, AR86 antiserum neutralized at least 4.0 logs of each of the viral isolates. In more comprehensive identification tests two Sd isolates from Olifantsvlei were randomly selected and reciprocal N and HI tests done with the AR86 strain, using techniques for the HI test described by Casals [1964]. From the results shown in table I it is apparent that the two isolates are immunologically indistinguishable from AR86.

TABLE I

Reciprocal N and HI tests between two Sd isolates (AR 5850, AR 6232) from Olifantsvlei and the AR 86 strain of Sd virus.

Viruses	Antisera					
	AR 5850		AR 6232		AR 86	
	N	HI	N	HI	N	HI
AR 5850	>5.3*	32†	>5.3	32	4.9	32
AR 6232	>4.7	32	>4.7	32	4.1	32
AR 86	>3.7	32	3.5	64	3.6	16

* – Neutralization index.
† – Units antigen inhibited by the same dilution of each antiserum after 2 hours contact.

In a few cases CF antigens of Sd isolates were of low titre or even failed to react. This was attributed to inadequate viral multiplication in the brain in early mouse passage as in these cases satisfactory antigens were obtained after one or two further mouse passsages.

For the identification of WN virus isolates the CF test was also mainly relied upon. However, with these isolates the test was not as satisactory as was the case with Sd isolates as some WN isolates reacted to some extent with other Group B viral antisera, viz., H336, Wesselsbron, Spondweni and Usutu. The identity of these isolates was nowever firmly established with the N test, and 13 isolates from Olifantsvlei and one from Welkom were identified with this test. Furthermore, in reciprocal N and HI tests with two randomly selected WN isolates from Olifantsvlei and the H442 strain of WN virus no immunological differences between these strains could be detected as shown in table II.

TABLE II

Reciprocal N and HI tests between two WN isolates (AR 5995, AR 6618) from Olifantsvlei and the H 442 strain of WN virus.

Virus	Antisera					
	AR 5995		AR 6618		H 442	
	N	HI	N	HI	N	HI
AR 5995	4.0*	64†	3.9	32	4.3	32
AR 6618	>4.2	64	4.3	32	3.9	32
H 442	>3.5	32	>3.5	32	5.8	32

*, † – refer Table I.

Mosquito infection at different study areas

During the four years of study 31 strains of Sd virus and 21 strains of WN virus were isolated from the total of 203,424 mosquitoes of all species tested. In table III

TABLE III

Number of viruses isolated from all mosquitoes tested from each study locality.

Mosquito	Olifansvlei			Lake Chrissie			Welkom			Boksburg			Totals		
	No. mosq. tested	Isol.		No. mosq. tested	Isol.		No. mosq. tested	Isol.		No. mosq. tested	Isol.		No. mosq. tested	Isol.	
		Sd.	WN.		Sd.	WN.		Sd.	WN.		Sd.	WN.		Sd.	WN.
C. pipiens	104,439	3		215			270			1,600			106,524	3	
C. theileri	38,530		1	6,304	1		828			1,144			46,806	1	1
C. univittatus	35,965	21	17	3,274	5	2	51		1	527	1		39,817	27	20
A. lineatopennis	4,367			216						67			4,650		
A. mixtus	1,582			14						14			1,610		
A. dentatus	909			368						2			1,279		
A. caballus	97			37						74			208		
Other species	1,311			910			3			306			2,530		

TABLE IV

Number of Sd and WN strains isolated from three Culex species collected over four seasons at Olifantsvlei.

Mosquito	1961/62			1962/63			1963/64			1964/65		
	No. mosq. tested	Isol.		No. mosq. tested	Isol.		No. mosq. tested	Isol.		No. mosq. tested	Isol.	
		Sd.	WN.		Sd.	WN.		Sd.	WN.		Sd.	WN
C. pipiens	5,416	1		16,391	1		57,228	1		25,404		
C. theileri	702		1	4,739			23,615			9,474		
C. univirtatus	1,679	1		5,974	6	6	15,426	9	8	12,886	5	3

these isolations are analysed according to mosquito species and locality. The importance of *C. univittatus* as viral carrier and by inference on this account as vector, is clearly demonstrated since 47 isolates originated from this mosquito which was only the third most prevalent species tested. *Culex (Culex) theileri* Theobald produced one strain of each virus while from *Culex (Culex) pipiens* Linnaeus, the most prevalent mosquito at Olifantsvlei, three strains of Sd virus were obtained. The aedine species of which *Aedes (Neomelaniconion) lineatopennis* (Ludlow) was the most prevalent were negative for virus, but, as they were tested in considerably smaller numbers, no conclusions as to their possible role as vectors can really be made from this observation.

Both viruses were isolated at Olifantsvlei and Lake Chrissie, the two main study areas, with Sd virus showing a slightly higher incidence at both localities. At Welkom where human infections with WN virus had occurred [McIntosh *et al.*, 1964] one strain of this virus was isolated from *C. univittatus*, and at Boksburg where Sd virus infections were diagnosed the latter virus was isolated from the same mosquito.

Mosquito infection at Olifantsvlei over four years

From table IV it can be seen that during the four years of study at Olifantsvlei both Sd and WN viruses were isolated from mosquitoes each summer. Furthermore, there was a similarity in the number of isolations of each virus made during particular seasons. For instance during the summer 1962/63 six isolations of each virus were made from nearly 6,000 *C. univittatus* giving an infectivity rate for each virus of about 1.0. During the following two summers, the *univittatus* infectivity rates for Sd and WN viruses respectively dropped to 0.6 and 0.5 in 1963/64 and to 0.4 and 0.2 in 1964/65. On the basis of these figures it would seem therefore that over the last three years of study a steady and rather similar decline in viral activity occurred, with both viruses reaching their highest and lowest levels during the same seasons.

Incidence of viral activity at Olifantsvlei on a monthly basis

In table V the incidence of viral activity at Olifantsvlei, determined from isolation of virus from mosquitoes and sentinel fowl infections, is shown on a monthly basis for the last three summers of study. Included in the virus isolations are two isolations of Sd virus from *C. pipiens*, one in February 1963 and the other in December 1963. However, the figures for infectivity rates are calculated only from isolations made from *C. univittatus*. The number of sentinel fowls which had converted in antibody status at each bleeding is also shown in this table. As these fowls were only tested monthly it was not possible to be certain that each infection was allocated to its correct month.

It can be seen from table V that Sd virus was first isolated each season during November or December, and was usually isolated during all subsequent summer months, and was consistently active during December, January and February. Failure to isolate this virus during March 1963 and 1965 could perhaps be accounted for by the small numbers of *univittatus* tested during these months. In spite of this continuous activity there was no evidence that the incidence of mosquito infection increased as summer progressed. This is evident from the virtually constant monthly infectivity rates. The relatively higher rates recorded in November 1962 and April 1965 could probably be accounted for by a chance isolation occurring among the small numbers of mosquitoes tested during these months of low population densities. It would therefore seem that there was a failure of Sd virus to build up to what might be considered epizootic levels, even during the 1962/63 season when the highest annual infectivity rate was recorded (table IV).

As shown in table V multiple isolations of WN virus were also made over several months during each season, although with this virus isolations were accomplished

TABLE V

Viral activity at Olifantsvlei shown by isolation of virus from mosquitoes, infectivity of C. univittatus and sentinel fowl infections.

Virus		1962/63						1963/64						1964/65						Totals
		Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	
Sd.	V.I.	1	2	1	3*				4*	2	3	1			2	1	1		1	22
	I.R.	4.3	1.2	0.4	1.4				0.7	1.3	0.8	0.6			0.4	0.2	0.5		8.1	
	S.F.I.		2		1	4	1		2			1							3	14
WN	V.I.	1	2	1	2				1		5		2				1	1	1	17
	I.R.	4.3	1.2	0.4	1.4				0.2		1.1		8.9				0.5	2.1	8.1	
	S.F.I.		6	2	5	4					6						3	7	5	38
Total *C. univit.* tested monthly		235	1,692	2,320	1,470	217	19	2,154	5,402	1,507	4,455	1,620	223	1,001	4,907	4,484	1,891	169	123	44,189

V.I. Number viral isolations.
S.F.I. Number sentinel fowl infections.

I.R. Infectivity rate, i.e. number isolations per 1000 *univittatis* tested.
* One isolation during each of these months was from *C. pipiens*, all other isolations were from *univittatus*.

slightly more irregularly than with Sd virus. For instance, WN virus was not isolated during January and March of the 1963/64 season, and in the last season initial isolations were not made until as late as February. With WN virus also there was an apparent failure of viral activity to build up to epizootic levels, if allowance be made for the abnormally high rates at the end of the last two seasons when samples of *univittatus* tested were small and therefore not really adequate for estimation of an infectivity rate.

Infections among the sentinel fowls over the 18 months of observations in the last three seasons (table V) were recorded during seven months with Sd virus and during eight with WN virus, with a total of 14 and 38 infections for each virus respectively. Virus was isolated from *univittatus* in the same months as fowl infections were recorded on five occasions with Sd virus and on seven with WN virus. In view of the practical difficulties involved in detecting activity of arboviruses, especially at the lower levels, and the variety of mosquito collection methods and sites used at Olifantsvlei, it was to be expected that activity would at times be detected by one method but not with the other. Nevertheless, a fairly close agreement in the time of occurrence of fowl infection and virus isolation was evident. This was more apparent with WN virus; for example in February 1964 and during several months in the 1962/63 and 1964/65 seasons. From this it would seem likely that if more *univittatus* had been tested for virus in March 1963 when several fowl infections occurred more isolations of both viruses would have been effected. In January and February 1964 and from December to February in the following season several isolations of Sd virus were made without fowl infections being recorded. This discrepancy is possibly partly a reflection of a relatively lower transmission: infectivity ratio of Sd virus in *univittatus* in comparison with WN virus, in which case it would require more Sd than WN infected *univittatus* to cause the same number of fowl infections. This is an aspect dealt with again below.

Mosquito collections from the fowl-pen traps which house the sentinel fowls showed that only *pipiens, univittatus* and *theileri* fed significantly on these fowls [Jupp and McIntosh, 1967]. Of these only *univittatus* showed a significant infectivity rate. From these observations as well as the close association in time of occurrence between fowl infections and isolations of virus from *univittatus* it may be concluded that *univittatus* was the main vector involved in these fowl infections.

As mosquitoes for virus isolation were collected only intermittently from the fowl-pens whereas the sentinel fowls were under continuous exposure, a valid comparison of transmission and infectivity rates with the same virus is of course not permissible. Since the same primary vector however is apparently involved with both viruses, a comparison of the proportion of fowl infections to isolations between the two viruses would seem justified. It is shown in table V that for WN virus a total of 38 fowl infections as against 17 isolations were recorded whereas for Sd virus the proportion was 14 to 22. The fact that two Sd isolations came from *pipiens* would not materially alter these proportions. Hence, many more infections than isolations were recorded with WN virus, while with Sd virus there were more isolations than infections. From this it does seem likely that the transmission by *univittatus* of WN virus is more efficient than its transmission of Sd virus.

Observations at Lake Chrissie

Although only limited and irregular observations were made at Lake Chrissie on account of its distance from the laboratory as well as the extent of resources available to us, the results there are of interest because of the relatively undisturbed nature of the Lake Chrissie habitat. In table VI the number of viral isolations and sentinel fowl

infections over the three seasons between 1962 and 1965 are shown. In comparing these results with those from Olifantsvlei it should be emphasized that relatively few mosquitoes were tested and only one flock of fowls was exposed annually at Lake Chrissie.

TABLE VI

Virus isolations from mosquitoes and sentinel fowl infections at Lake Chrissie over three seasons.

Virus		Summer season			Totals
		1962/63	1963/64	1964/65	
Sd.	V.I.	1	0	5	6
	S.F.I.	0	0	3	3
WN	V.I.	0	2	0	2
	S.F.I.	7	3	3	13

For explanation of abbreviations refer to table V.

From the table it can be seen that, apart from a negative record for Sd virus in 1963/64, activity by each virus was recorded during each season, either by isolation or fowl infection, or both. Table III shows that out of the eight isolations, five Sd and two WN isolations came from *univittatus* and one of Sd from *theileri*. The overall *univittatus* infectivity rate for the whole study period for both viruses combined was 2.1 compared with a rate of 1.1 for Olifantsvlei.

It is also apparent from Table VI that with WN virus there were considerably more fowl infections than isolations whereas with Sd virus the proportions were reversed, thus duplicating in this respect the results obtained at Olifantsvlei. Here again, these results seem to indicate a relatively more efficient transmission of WN virus by *univittatus*.

DISCUSSION

The isolation of 27 strains of Sd and 20 strains of WN virus from *C. univittatus* out of a total of 52 isolations from all mosquito species tested seems to have established within reasonable limits of certainty that *univittatus* is the primary feral vector of both viruses in the highveld region of South Africa. The fact that these isolations were made over a period of several years and in different highveld localities gives added assurance on this point. Furthermore, in the related papers on mosquito bionomics [Jupp and McIntosh, 1967; Anderson, 1967] evidence is produced on the prevalence and feeding habits of this mosquito which supports this claim, for this widespread and prevalent mosquito feeds mainly on birds which are apparently the primary vertebrate host of both viruses. Although isolation of virus from a wild-caught mosquito only identifies infected, but not necessarily transmitting mosquitoes, additional evidence is available implicating this species as vector. Firstly, preliminary laboratory-conducted viral transmission trials, with specimens of *univittatus* collected from highveld populations of this mosquito, have indicated that this form of the species is able to transmit both viruses. Secondly, it seems beyond question that the main mosquito infecting the sentinel fowls exposed at Olifantsvlei and Lake Chrissie was *univittatus*. Besides this mosquito only *C. pipiens* and *C. theileri* fed on these fowls in sufficient numbers to justify consideration as vectors and the low viral isolation

frequency from *pipiens* and *theileri* in relation to the number of infections recorded in these fowls would seem to preclude these two species as being concerned significantly in these infections. The implication of *univittatus* as primary vector is also in agreement with observations on this mosquito and these viruses in Egypt [Taylor *et al.*, 1955, 1956].

There is so far no definite evidence on the identity of the main vector infecting man. Observations on highveld populations of *univittatus* have shown that this form feeds rarely on man [Jupp and McIntosh, 1967; Anderson, 1967] and therefore it cannot at this stage be implicated as the cause of the majority of human infections. On the only two occasions when mosquitoes were tested for virus in relation to recent human infections, viz., at Boksburg and Welkom, Sd and WN viruses were only isolated from *univittatus*. This, however, allows no firm conclusion on what infected man in these outbreaks because it is likely that man would usually be infected when viral activity in the feral transmission cycles was fairly high. This is a time when these viruses could be expected to be isolated from *univittatus* at a reasonably high frequency. On present knowledge a more likely cause of most human infections is the existence of a link vector which feeds fairly frequently on both birds and man. *C. theileri* and *Aedes lineatopennis* are species with the requisite feeding habits and prevalence which may fulfil this role. The isolation of one strain of each virus from *theileri* and none from *lineatopennis* would favour the former species as a link vector, but more extensive observations at the time of human infections are necessary before worthwhile conclusions on this point can be attempted.

Because of the very low Sd infection rate observed in *pipiens* and the failure to isolate WN virus in spite of the very large numbers of this species tested, it must be concluded that *pipiens* plays a minor role in the transmission of these viruses. The fact that it feeds mainly on birds [Jupp and McIntosh, 1967; Anderson, 1967] suggests that it possesses a very low viral susceptibility, since such feeding habits should expose it to viraemic birds, from which one would expect a much higher isolation rate should it possess a reasonable level of susceptibility.

From the regular annual appearance of these viruses at Olifantsvlei and Lake Chrissie it can be inferred that both viruses are widespread and prevalent in the highveld, although allowance must be made for the fact that these two localities were selected on account of the anticipated favourable environments existing there. It would seem probable that every summer a certain amount of activity by both viruses will occur at some localities in the highveld region. Experience at Lake Chrissie indicates that in favourable localities this would occur even in the presence of low population densities of *univittatus*. It is to be expected that certain localities with more favourable conditions would act as permanent viral-maintaining foci from where virus would spread to the less favourable localities. Olifantsvlei and Lake Chrissie, among others, may perhaps be such permanent foci. The regular appearance of these viruses raises the question of their over-wintering since there can be no possibility of transmission by mosquitoes during the highveld winters. This factor, together with the utilization of *Culex* mosquitoes and wild birds as hosts, brings to mind the similarities in ecology between these viruses and western equine encephalitis virus in North America [Reeves and Hammon, 1962].

A puzzling feature of the pattern of viral activity at Olifantsvlei was the failure of viral activity to increase in intensity to high epizootic levels as summer progressed in the presence of seemingly adequate susceptible wild bird and *univittatus* populations. This is clearly apparent from the nearly constant monthly infectivity rates of *univittatus*. Apart from relatively high rates in November and April, due probably to the small

sample sizes of *univittatus* tested during these months of low mosquito population densities, the highest Sd virus rate, calculated on a monthly basis, was 1.4 and for WN virus 2.1. These rates may be compared with rates of from 10 to 20 at times encountered with western equine encephalitis virus in California [Reeves and Hammon, 1962] and of 20 to 500 with Japanese encephalitis virus in Japan [Buescher *et al.*, 1959]. At Olifantsvlei the *univittatus* infectivity rate was calculated on the basis of the total number of *univitattus* tested from all collecting sites over full monthly periods. This procedure has naturally given a somewhat low infectivity rate for it has included collecting sites where no viral activity was present. Nevertheless it is clear that high peaks of viral activity failed to appear at Olifantsvlei during the period of observations. Calculation of infectivity rates on a single site basis still gave low rates. The highest rate on this basis was 12.8. Analysis of viral activity at individual sites showed that viral activity there was frequently short-lived, i.e. recorded only during an isolated month, with little evidence that it spread to adjoining sites. These transient and usually localized episodes may have been more apparent than real due to failure to detect activity at low levels, but the fairly close correlation between viral isolations and sentinel fowl infections at the same site suggests that little viral activity passed undetected. In view of the large number of factors involved and on which there is at the moment meagre or no information available, the reason for the failure of the epizootic type of activity to appear at Olifantsvlei is not clear, but it does seem possible that the low summer temperatures prevailing on the highveld may be one factor through its adverse influence on viral multiplication in the mosquito. Whatever factors might have been limiting viral activity, it seems from the pattern of viral activity observed that both viruses are firmly established and well-adapted to the highveld environment. This possibly means that their existence there is of long standing.

SUMMARY

Over a period of four summers, from 1961 to 1965, viral activity by Sindbis and West Nile viruses was studied at several localities in the highveld region of South Africa, by isolation of virus from mosquitoes and the exposure of fowls as sentinels. From a total of 52 strains of virus isolated, 27 strains of Sindbis virus and 20 strains of West Nile virus were isolated from 39,817 *Culex univittatus* Theobald. One strain of each virus was isolated from 46,806 *Culex theileri* Theobald and three strains of Sindbis virus were isolated from 106,524 *Culex pipiens* Linnaeus. Fifty-one infections due to West Nile virus and 17 due to Sindbis virus were recorded among sentinel fowls. It was concluded that *Culex univittatus* is the primary vector of both viruses in the feral transmission cycles and was the main vector causing the sentinel fowl infections. On two occasions when human infections with either Sindbis or West Nile virus had recently occurred the causal virus was isolated from *Culex univittatus* collected in the localities where these infections had occurred. The pattern of viral activity at the two main study areas showed the appearance of both viruses to be a regular summer phenomenon, usually occurring during December to March. The incidence of infection in *Culex univittatus* remained at low levels throughout summer with both viruses reaching their highest and lowest levels in the same summers. There was circumstantial evidence that the transmission of West Nile virus by *Culex univittatus* was more efficient than its transmission of Sindbis virus.

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1-1-2

Epidemics of West Nile and Sindbis Viruses in South Africa with *Culex (Culex) univittatus* Theobald as Vector

B. M. McINTOSH, P. G. JUPP, I. DOS SANTOS and G. M. MEENEHAN

Arbovirus Research Unit, National Institute for Virology, Department of Health, Private Bag X4, Sandringham 2131.

During the first half of 1974, after exceptionally heavy rains in what is normally an arid region, an epidemic characterized by fever, rash, myalgia and arthralgia, occurred over extensive areas of the Karoo and northern Cape Province. A mosquito-borne virus was suspected and field and laboratory investigations implicated West Nile (WN) virus, but also indicated that another mosquito-borne virus, Sindbis (SIN), infected man simultaneously but to a lesser extent. This was the largest epidemic of mosquito-borne virus disease ever recorded in South Africa and we describe here the epidemiological findings on this unusual episode.

Epidemiology of the infections

Since the epidemiologies of WN and SIN virus infections are complex and not fully understood, it is convenient first to review their salient features, particularly in respect of South African conditions.

Both viruses are normally transmitted among wild birds by bird-feeding mosquitoes and the viruses are apparently maintained in this manner. In South Africa, *Culex* (*Culex*) *univittatus* Theobald has been identified as the vector concerned in avian infection on the entire temperate inland plateau, including the Transvaal highveld and Orange Free State, the Karoo and northern Cape Province. In the case of WN virus, man is certainly involved secondarily to avian infection and most human infections are, evidently, largely 'blind-alleys' in the virus transmission chain because virus concentrations in human blood during acute illness are so low that only an insignificant number of mosquitoes would transmit virus after becoming infected from man.[1,2] Although there is less information available on viraemia levels in humans infected with SIN virus, the indications are that here too, viraemia levels are low and feedback of virus from man to the vector has no significant consequence for further transmission. We believe, therefore, that most human infections with both viruses result from mosquitoes which acquired their infection from viraemic birds. It is probable that a large epidemic could only eventuate if a concurrent epizootic occurred among wild birds in the same area.

Both viruses have been responsible for sporadic annual infection in man during the summer in South Africa.[3] As a cause of illness these infections have largely been recorded in the moister regions of the Transvaal and Orange Free State, but this localization may be merely a reflection of proximity to the diagnostic facilities available in Johannesburg. Even so, antibody surveys in man and animals[4,5] showed that both viruses occur extensively in Southern Africa. Field observations from 1968 to 1971 at Bethulie and Lückhoff, in the southern Orange Free State showed that even in arid areas avian infection by *C. univittatus* is a regular summer phenomenon. Antibody surveys among the human populations in the Karoo and along the Orange river showed that human infection by both viruses had also occurred but its annual incidence is not known. For example, among 218 adults at 5 Orange river localities in 1961, 12% were positive for neutralizing antibody to SIN virus and 13% to WN, although at one locality (Upington) immune rates were 27 and 21% respectively. A survey among 125 adults in several arid localities in the Cape Province showed 19% positive to SIN and 24% to WN, and among 408 children rates were 3 and 5% respectively. The highest rates recorded were at Clanwilliam where 45% of the 20 children sampled were positive to SIN and 50% to WN but these rates were exceptional. These earlier studies had therefore revealed the potential of a future epidemic in this arid area.

Noteworthy in these antibody surveys has been the remarkable parity in immune rates between the two viruses at the various localities, apparently a result of their ecological similarity, especially since both viruses utilize the same mosquito species as vector and the same vertebrate host. As a result it seems that factors favouring one virus would also favour the other and consequently it is likely that increased human infection by both viruses might frequently occur simultaneously.

Because of the sporadic nature of human infection in the past in South Africa, it has been difficult to obtain reliable information on the identity of the main vector transmitting infection to man. As observed among wild populations in the Transvaal, *C. univittatus* has a low feeding rate on man and it seems likely that this has been at least one reason for the absence of large-scale human infection in this region. It has been postulated[6] that other mosquito species, particularly *Culex* (*Culex*) *theileri* Theobald, feeding more frequently on man and to some extent on birds, could be more frequent causes of human infection. More recently it became evident that *C. theileri* was a poor vector[7] and, furthermore, this species has shown a low natural infection rate. Similar objections to the importance of these mosquitoes as vectors were evident in respect of other species with a high feeding rate on man. These facts, together with recent information that *C. univittatus* probably fed on man more frequently than originally believed[8], tended to implicate *C. univittatus* as the main cause of human infection. The epidemic in 1974 therefore gave expectations of providing a unique opportunity for assessing the relative merits of *C. univittatus* and other mosquitoes as vectors of human infection.

When the existence of the epidemic came to our notice, in April 1974 a visit was made to Upington, where the epidemic was severe, to collect human blood samples for diagnosis and mosquitoes for attempted isolation of virus. The onset of cold weather shortly after our arrival put an end to the epidemic and curtailed the entomological observations which were consequently not as comprehensive as they might have been. More particularly, it was not possible at the time to determine relative feeding rates on man by *C. univittatus* and *C. theileri* in Upington, the two species found to be infected, and it seemed unwise to extrapolate too readily observations on Transvaal populations of these species to the Upington populations since it is well known that habits of different geographical populations of the same species can differ. Fortunately, observations in this regard became possible in Upington in 1976 when another exceptionally wet summer occurred. It seemed that population densities of these two mosquitoes in 1976 would be comparable with those existing during the epidemic and the feeding rates

determined in 1976 would be a valid reflection of those during the epidemic as relative population densities and absolute abundance strongly influence host biting rates.

Methods

Mosquitoes and sera were stored either in liquid nitrogen or in solid dioxide until tested for virus in the laboratory in Johannesburg. Mosquitoes were identified on the day of collection and pooled according to species before storage. Pool sizes were 25 for *C. univittatus*, 100 for *C. theileri* and *Culex* (*Culex*) *pipiens* Linnaeus, and variable numbers for other species. In an attempt to isolate virus, sera and mosquito suspensions were inoculated intracerebrally into suckling mice, 1–3 days old. Mosquito suspensions yielding SIN virus were re-inoculated into mice after the addition of SIN immune mouse ascitic fluid to block SIN virus in an attempt to isolate WN virus, as SIN virus will outgrow WN virus in pools containing both viruses. In this way an additional 4 isolations of WN virus were made. For identification of virus isolates complement-fixing antigens were prepared from the brains of sick suckling mice, either by sucrose-acetone extraction of the brains or from a lightly centrifuged 20% buffered saline suspension. These antigens were tested in complement-fixation tests against hyperimmune mouse ascitic fluids. In the case of some virus isolates confirmatory neutralization (N) tests were done in mice.

For the purpose of post-epidemic antibody assays, blood specimens were collected 2–4 months after the epidemic from humans in Upington and other Orange river localities and from wild birds in Upington 6 weeks after the epidemic.

All human sera were tested for antibodies in micro-haemagglutination-inhibition (HI) tests against antigens of SIN, WN, chikungunya, Spondweni, Banzi, Wesselsbron, dengue 1, dengue 11, yellow fever, Bunyamwera, Germiston, and Rift Valley fever viruses. Ten-fold dilutions of serum over the range 1/20-1/2560 were tested against 8 units of viral antigen. Apart from a few sera of which insufficient quantities were available, all these sera were also tested in N-tests in mice against 50-100 LD_{50} of SIN and WN viruses. Sera were tested undiluted and those which protected half or more of the mice inoculated were recorded as positive.

Since the main purpose of the antibody tests on human sera collected after the epidemic was to determine the attack rate in man, differentiation between the antibody reactions of sera from donors with recent, as distinct from old infections, was necessary. Recent infections, either in immunological virgins or in persons previously infected and re-infected during the epidemic, were assessed as those in which the serum was positive in the N and HI test, and which gave an HI titre of 1/160 or greater. Conversely, old infections were assessed as those in which the serum was positive, either in only one kind of test or, if positive in both tests, the HI antibody titre was less than 1/160.

Bird sera were tested for antibodies against SIN and WN viruses in HI and N tests. Sera were recorded as positive if reacting positively in at least one kind of test.

In April 1974, during the epidemic, mosquitoes intended for virus isolation tests, were collected in net and 'lard-can' traps, set overnight and baited with solid carbon dioxide. These traps were set in the gardens and verandahs of private houses, the town square, a park in the town and on Olyfenhoudts Island in the Orange river opposite the town. 'Lard-can' traps were suspended 1 metre above the ground or at 2-3 metres in trees. In March 1976, when observations on mosquito biting rates on man were made in Upington, mosquitoes were collected in test-tubes off volunteer human bait in the open, for 90 minutes after sunset for 7 nights. For these collections torches were used shaded with red cloth. Control collections intended to determine the relative population densities of different mosquito species were made nearby in carbon dioxide-baited net traps. Both kinds of collections were near reed beds but within the built-up areas of the town or on Olyfenhoudts Island.

After the epidemic a questionnaire was sent to district surgeons in the Cape Province to obtain information on clinical aspects and the geographical distribution of clinical cases.

Results

Viral and antibody studies on sick and convalescing humans

While the epidemic was still active, 59 blood specimens from suspected cases were obtained from medical practitioners in Upington. According to the recorded date of onset of illness 31 of these specimens were regarded as having been collected during the acute illness and were tested for virus. A further 12 specimens in which the date of onset was uncertain were handled similarly. The remaining 16 sera were regarded as having been collected too late for isolation of virus and these were tested only for antibodies.

WN virus was isolated from 6 of the 43 sera tested for virus. In 3 cases in which date of onset was recorded, virus was isolated on the 2nd, 3rd and 5th days after onset. Virus concentrations in the 6 positive sera were low because among each group of 12 mice inoculated at least some mice failed to sicken, indicating that virus titres were less than 1.0 logs.

When all 59 sera were tested for antibodies to several arboviruses, there were 24 (41%) positive to WN virus in the HI test, 13 of them at high titres suggesting recent infection, and 33 (57%) positive for N antibody. None of the sera from which WN virus was isolated contained antibody to this virus. Against SIN virus were positive in the HI test and 18 in the N test, but HI titres were generally low, and as a result no definite conclusion with regard to recent infection by this virus could be made at the time.

The antibody results excluded the other arboviruses included in the tests. While there were some reactions in the HI test to some Group B viruses these reactions were clearly the result of immunological overlap with WN virus. Negative results were recorded with chikungunya, Bunyamwera, Germiston and Rift Valley fever viruses.

Changes in antibody status during convalescence

The causal role of WN virus became more evident when, some 7 weeks after the epidemic, a second blood sample was obtained from 8 patients, of whom 3 had yielded WN virus. The

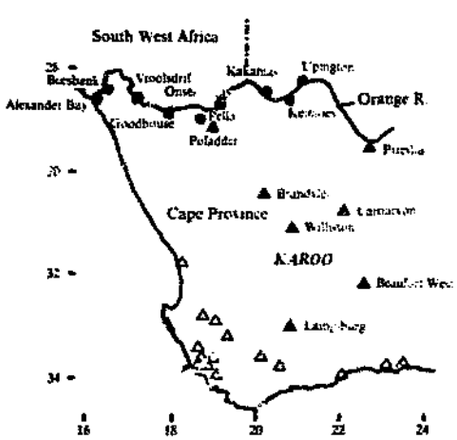

Fig. 1. The area where the epidemic occurred. ▲ towns where cases were reported; ● towns where antibody studies on the human population were made; Δ towns in the winter rainfall area where no cases were seen.

Table 1. Results of virus isolation attempts from acute phase sera and antibody tests on paired sera from 8 patients in Upington

Case No.	Days after onset	Virus isolated	Antibody test SIN HI[a]	SIN N[b]	WN HI	WN N
1	3	+	–	–	–	–
	51		–	–	2560	+
2	?	+	–	–	–	–
	52		–	–	2560	+
3	5	+	–	–	–	–
	53		–	–	2560	+
4	4	–	160	+	–	+
	52		80	+	2560	+
5	3	–	–	–	–	+
	52		–	–	1280	+
6	8	–	–	–	80	+
	55	–	–	–	2560	+
7	?	–	40	+	–	+
	50		40	+	2560	+
8	3	–	40	–	–	–
	52		160	+	2560	+

[a]HI titres expressed as reciprocal of serum dilution: [b]N test results expressed qualitatively.

results of antibody tests with WN and SIN viruses on the paired sera from these cases are shown in Table 1. It can be seen that all individuals showed a rise in HI antibody levels against WN virus in the period between bleedings and 4 (cases 1, 2, 3, 8) also developed N antibody to this virus. Cases 4, 5, 6 and 7 had N antibody to WN virus in both specimens but this is presumably the result of early appearance of this antibody as the same sera were negative for HI antibody. Cases 4 and 7 had antibody to SIN virus at equivalent levels in both sera while case 8 showed a significant rise in HI antibody. The appearance of N antibody indicates that this latter individual had also recently been infected by SIN virus.

Geographical extent of outbreak

According to information received from 11 medical practitioners in the affected area, cases had occurred in or in the neighbourhood of the towns of Williston, Pofadder, Lutzingsburg, Carnarvon, Beaufort West, Prieska, Brandvlei, Upington, Kakamas and Keimoes (Fig. 1). The number of cases seen by individual practitioners averaged about 300 and varied from 14 at Keimoes to 1 700 at Upington. From all accounts a high proportion of the inhabitants of Upington had been infected. Over the entire area, cases were first seen towards the end of January and continued in some areas until June with most infections occurring in February, March and April. Some practitioners reported a higher incidence of cases among adults, which is in accord with previous experience that WN virus infection is mild in juveniles.

Reports from 32 medical practitioners in the winter rainfall area of the Cape Province showed that this region had remained free from infection, apart from a few imported cases which had apparently been infected in the Karoo.

It seems, therefore, that the outbreak had occurred over an area of some 2 500 km² of the summer rainfall area of the central and northern Cape Province. Precipitations of up to six times the average rainfall occurred in some localities in this area as is evident from the following records for the period from November 1973 to April 1974: Brandvlei 376 mm (average 80 mm); Pofadder 277 mm (73 mm); Prieska 558 mm (185 mm); Upington 296 mm (126 mm); Williston 388 mm (61 mm). At Orange river localities the effects of local rainfall were compounded by the extensive flooding of this river.

Table 2. Number of mosquitoes collected in Upington, April 1974, with the number of virus isolations made from them

Species	Total mosq.	No. pools	No. isolations WN	No. isolations SIN	Infection rate[a] WN	Infection rate[a] SIN
An. squamosus	41	1				
An. coustani	14	1				
An. listeri	41	1				
Ae. ?uppi	1	1				
Cu. poicilipes	3	1				
Cu. pipiens	1200	12				
Cu. theileri	4889	53	4	4	0.83	0.83
Cu. univittatus	1325	52	33	8	39.0	6.48

[a]Calculated according to Chiang and Reese[8] and is the number of infected mosquitoes per 1000.
An. = *Anopheles*; *Ae.* = *Aedes*; *Cu.* = *Culex*.

Clinical aspects

The symptoms and signs reported by medical practitioners in the affected area were as follows: Those frequently seen were fever, rash, muscular pain, backache, joint pain, headache, orbital pain, diarrhoea, nausea, and those less frequently seen were sore throat, enlarged lymph glands, vomiting, insomnia, encephalitis (apparently mild) orchitis, enlarged and tender liver.

In many cases recovery was slow with continued listlessness and weakness. No deaths were reported.

Isolation of virus from mosquitoes

Table 2 shows the numbers of the various mosquito species collected in Upington in April 1974, during the epidemic, and the number of isolations of WN and SIN viruses made from them. This shows that virus was isolated from 2 species only, namely *C. univittatus* and *C. theileri*. Infection rates for *C. univittatus* of 1 infected mosquito per 26 for WN and 1 per 154 for SIN virus were found, the rate for WN virus being one of the highest ever recorded for mosquitoes in South Africa. For *C. theileri* a low infection rate for both viruses of 1 infected mosquito per 1204 of the wild population was found.

Post-epidemic immune rates in man

The results of the HI and N tests with the sera collected from humans in Upington and at other localities along the Orange river after the epidemic, showed that the only noteworthy positive reactions occurred with WN and SIN viruses. Most of the sera reacting positively to WN virus in the HI test were also positive to Banzi and Wesselsbron viruses, but frequently at a lower titre. Some of those positive to Wesselsbron virus at high titre were negative to this virus in the N tests, whereas 91% of the sera positive to WN virus in the HI test were also positive to this virus in the N test. Similarly, with SIN virus a high

Table 3. Summary of results of antibody tests with WN and SIN viruses on 558 human sera, collected after the epidemic

Virus	Total positive No.	Total positive %	Old infections[a] No.	Old infections[a] %	Recent infections[a] No.	Recent infections[a] %
WN	383	69	74	13	309	55
SIN	130	23	46	8	84	15

[a]For differentiation of old and recent infections see text.

Table 4. Percentage of human sera, collected after the epidemic from various Orange river localities, reacting in antibody tests with patterns indicative of recent infections of WN and SIN viruses

Locality	Donor category	Age	Total tested	Positive sera (%) WN	SIN
Upington	Civilian	Juvenile	94	71	16
Upington	Civilian	Adult	86	66	17
Upington	Soldier	Adult	99	44	8
Onseepkans	Scholar	Juvenile	48	77	25
Pella	Scholar	Juvenile	49	80	12
Goodhouse	Scholar	Juvenile	20	85	25
Kotzeshoop	Scholar	Juvenile	49	53	27
Vioolsdrif	Scholar	Juvenile	16	56	31
Beesbank	Scholar	Juvenile	49	23	2
Alexander Bay	Scholar	Juvenile	48	4	4
			558	55	16

proportion (75%) of the sera positive in the HI tests were also positive in the N test. Correlation between the HI and N tests with both viruses might have been even higher had not a lack of sufficient quantities of some sera precluded their inclusion in the N test.

When the antibody reactions were differentiated on the basis of either old or recent infections the number and percentage of the total of 558 sera tested giving antibody reactions indicative of old or recent infections by WN and SIN viruses, are shown in Table 3.

In Table 4 recent infections are analysed according to locality, age and category of donor. It can be seen that among the civilians at Upington a very high rate of recent infection by WN virus was recorded and rates were about the same in juveniles and adults. Recent infections by SIN virus were much lower and again similar in the two age groups. The combined infection rate of 52% to both viruses among the soldiers in Upington correlates reasonably well with the estimate of 60% that reported sick during the epidemic.

The results on the sera from school children at the first 5 localities along the Orange river below Upington show a similar infection pattern with high infection rates by WN virus and lower rates by SIN virus. Much lower rates were recorded at Beesbank and Alexander Bay near the mouth of the Orange river, a reflection probably of the extreme aridness of this region, and an indication that even during the epidemic infection there was limited.

Table 5. Percentage of wild bird sera, collected after the epidemic in Upington, reacting positively in antibody tests with WN and SIN viruses

Species	Total tested	% Positive WN	SIN
Turdus olivaceus (olive thrush)	24	92	94
Streptopelia senegalensis (laughing dove)	72	86	45
Passer domesticus (house sparrow)	48	50	8
Euplectes orix (red bishop)	153	40	0.7
Pycnonotus nigricans (red-eyed bulbul)	9	22	33
Quelea quelea (red-billed quelea)	5	60	0
Ploceus velatus (masked weaver)	9	33	0
Streptopelia capicola (turtle dove)	2	100	50
	322	53	17

Immune rates in wild birds in Upington after the epidemic

The results of antibody tests on sera from wild birds are shown in Table 5. Blood specimens from 4 species, all of which are very prevalent in the town, were obtained in fair numbers while specimens from a further 4 species were obtained in small numbers. All species gave evidence of infection by WN virus and in those species collected in fair numbers immune rates were at very high levels. The average immune rate of 53% for this virus among all species is significantly higher than that previously recorded among wild birds in an enzootic situation near Johannesburg.[10] That immune rates were high shortly after the epidemic is consistent with the occurrence of an avian epizootic at about the same time the epidemic occurred. The immune rate of 50% in house sparrows is noteworthy because extremely large numbers of this species were observed roosting in trees in the town park during our visit in April when *C. univittatus* was collected in traps set in the same trees.

It is more difficult to interpret the results with SIN virus because it has been shown experimentally that some avian species do not always carry demonstrable antibody after infection with this virus. This doubt is applicable particularly to house sparrows, red bishops and masked weavers in which the antibody response was shown to be weak and transient.[11] The low immune rates recorded in these species do not therefore necessarily exclude high infection rates. However, from the high immune rates recorded in thrushes and doves it is probable that avian infection with SIN virus was also at abnormally high rates during the epidemic.

Mosquito studies

Despite the onset of inclement weather shortly after our arrival in Upington during the epidemic in April 1974, fairly large numbers of *C. theileri* and *C. univittatus* were collected in moist situations with a dense cover of vegetation, i.e. at a stream with reed beds in the town park and on Olyfenhoudts Island. Both species, in fewer numbers, were also collected in gardens and on verandahs of private dwellings in the town. The conclusion made from these collections in the light of previous collections in the same area during years with normal rainfall was that these 2 species had been unusually abundant during the epidemic.

Table 6 shows the numbers of mosquito species collected by various means in March 1976. From the net-trap collections it can be seen that 3 species, *C. univittatus*, *C. theileri* and *C. pipiens*, in descending order of prevalence, were collected in numbers far in excess of other species and it seems likely that *C. univittatus* was the most abundant species because it ranked first in these collections. The inference is that it was also the most abundant species during the epidemic. In collections off human bait *C. theileri* predominated, which is in accord with previous experience. On the other hand, somewhat contrary to experience with highveld populations in the Transvaal and Orange Free State, *C. univittatus* was also collected in large numbers off man, the total being slightly more than half that of *C. theileri*. *Ae. juppi* was apparently rare at the time collections were made during the epidemic, in contrast to its comparative abundance two years later.

Discussion

Several aspects of the investigation suggest that WN virus was the main cause of the epidemic. The isolation of WN virus

Table 6. Results of mosquito collections off human bait and CO_2-baited net-traps in Upington, March 1976

	Human bait (56 man-hours)			Net-traps (11 trap-nights)	
	Total	%[a]	No. per man-hour	Total	%[a]
An. coustani	105	7	2	9	
An. listeri	3			25	1
An squamosus	48	3		43	2
Ae. caballus	19	1		3	
Ae. juppi	240	17	4	138	5
Ae. dentatus	2			2	
Ae. durbanensis	16	1		59	2
Ae. lineatopennis	8			7	
Ae. mixtus/microstictus	36	3		11	
Ae. contiguus	2			6	
Cu. quinquefasciatus	13	1		2	
Cu. pipiens	35	2		534	21
Cu. poicilipes	2			1	
Cu. rubinotus	3			0	
Cu. theileri	567	40	10	661	26
Cu. univittatus	334	23	6	1071	42

An. = *Anopheles*; *Ae.* = *Aedes*; *Cu.* = *Culex*.
[a] % of total catch.

from 6 ill persons, a rise in levels of serum antibody to this virus in 8 patients during convalescence, and the presence of this antibody at levels indicative of recent infection at high rates in the human population after the epidemic, are consistent with this diagnosis. Also the presence of this antibody at equivalent rates in juveniles and adults is indicative of recent infection. Similarly, the clinical features are in accord with a diagnosis of West Nile infection. Furthermore, the fact that a very high infection rate by this virus existed among *C. univittatus* in Upington, a species abnormally abundant at the time and apparently feeding readily on man, and that WN antibody was present at high rates in wild birds, may be interpreted as supportive circumstantial evidence. The outbreak was typically arbovirus-like in that it was restricted to a distinct ecological zone, occurred after heavy rains and ended with the onset of cold weather.

If credibility is to be given to the results of the post-epidemic antibody assays on the human population it may be inferred that SIN virus also infected the human population in the epidemic area at the same time but to a lesser extent. The antibody surveys indicated that 15% of the 558 persons sampled had been infected recently with SIN virus. The failure to isolate SIN virus and the demonstration of a rise in antibody to this virus in only one person could be explained by the difficulty experienced in isolating this virus from the blood of man. The small number of acutely ill persons sampled and the even fewer numbers from which paired sera were obtained may also have played a role.

SIN virus was, moreover, shown to be present in *C. univittatus* at a moderately high infection rate and if this species is to be implicated as the main epidemic vector, as we believe it should, the conclusion that SIN virus also infected man is unavoidable. Because of the similarity in the clinical features of WN and SIN virus infection in man, it would not have been possible to differentiate the infections clinically and the impression would have been that a single aetiological entity was involved.

It is not possible to estimate the total number of infections by either virus but the post-epidemic immune rates and reports we received of the number ill indicate that infection was widespread and at a higher incidence than previously observed in South Africa. In Upington 60% of the 279 persons assayed for antibody had, according to our criteria, been infected recently by WN virus and 14% by SIN virus. Extrapolation of these rates to the population of 30 000 indicates that about 18 000 WN and 4 000 SIN infections occurred in the town and environs. The post-epidemic antibody surveys indicate that even higher infection rates are probable at some other Orange river localities.

To what extent infections were subclinical is not clear. Taylor *et al.*[17] reported that WN infection is mild or even largely inapparent in children, hence it is likely that many infections among children were of this nature and reports from some practitioners agree with this viewpoint. Previous antibody surveys in the affected area indicated that some 10–20% of the population was probably immune to each virus before the epidemic. As these individuals, if infected, would have presumably developed a further immune response and therefore been classified among those recently infected, it is likely that 10–20% of the infections were sublinical because of prior immunity. One consequence of the belief that those mosquitoes actively transmitting virus to man become infected almost exclusively from birds is that the human immune rate would have no influence on the incidence of human infection but only on the incidence of overt disease. High population immunity would not therefore decrease the incidence of disease among the susceptible part of the population as would occur with those arboviruses producing high viraemia levels in man making a man-mosquito-man cycle possible.

As both *C. univittatus* and *C. theileri* are capable of transmitting both WN and SIN viruses, though with widely differing efficiency, it would seem that both species did infect man. It is necessary, nevertheless, that conversion of the observed natural infection rates in these 2 species to the relative number of human infections caused by each be attempted for any meaningful conclusion on the identity of the main epidemic vector to emerge. For this purpose at least two crucial parameters, vector capability and human biting rates, can be evaluated with what we believe is acceptable accuracy. Transmission rates, i.e. the proportion of infected mosquitoes successfully transmitting virus, have been estimated from previous experimental studies of ours in which transmission rates were as follows:

Virus	Mosquito	Infecting dose (logs)	Transmission rate (%)
WN	*univittatus*	5.0	89
WN	*univittatus*	2.6	33
WN	*theileri*	5.4	25
WN	*theileri*	4.5	0
SIN	*univittatus*	5.1	57
SIN	*theileri*	5.5	9

The transmission rates decided upon are shown in Table 7 in which the natural infection rates during the epidemic are converted to the relative number of human infections. These estimates are based on an average infecting dose of 3.0–4.0 logs of virus.

For estimated human biting rates it was assumed that on average each person in Upington was bitten by 3 *C. theileri* nightly, and that the biting rate for *C. univittatus* was 20% of that for *C. theileri*.

From Table 7 it can be seen that *C. univittatus* was almost certainly the main vector of both WN and SIN viruses in human infections.

Further evidence tending to decrease the importance of *C. theileri* as vector of both viruses is the widely divergent observed natural infection rates in the 2 species. The much lower rate in *C. theileri* strongly suggests that this species fed insignificantly on viraemic birds because, had it fed even moderately, its infection rate should have been closer to that of *C. univittatus*, considering

Table 7. Natural mosquito infection rates transposed to human infections in Upington (population 30 000)

Virus	Species	Infection rate per 1000 mosq. (*b*)	Transmission rate (%)[a] (*c*)	No. trans. per 1000 mosq. (*d*)	No. mosq. feeding nightly[a] (*e*)	No. human infections nightly (*f*)
WN	*univittatus*	39	50	20	20 000	400
WN	*theileri*	0.83	5	0.04	100 000	4
SIN	*univittatus*	6.48	35	2.26	20 000	45
SIN	*theileri*	0.83	1	0.01	100 000	1

[a] See discussion for methods of estimation: $d = b \times c$; $f = d \times e/1000$.

that experimental studies have shown that it is nearly as susceptible as *C. univittatus* to infection. With both viruses it seems likely therefore that *C. theileri* might have been infected largely from man, in which event it probably caused negligible human infection as experimental evidence indicates that were *C. theileri* to ingest virus at the low viraemic levels prevailing in man it would not be able to transmit either virus.

WN virus was also isolated once from either *Ae. juppi* or *Ae. caballus* at Bethulie in 1969. However, the ability of these species to transmit WN and SIN viruses is still unknown and there is no evidence at present to suggest they were vectors in the epidemic.

An interesting question is why the epidemic occurred where it did. Such a large epidemic has never broken out on the Transvaal highveld or Orange Free State, which periodically have good summer rains and, in fact, received above average falls in many localities in 1974. No obvious answer is available. A possible cause is the higher prevailing summer temperatures in the epidemic area, which increased the abundance of *C. univittatus* and promoted multiplication and dissemination of virus in the tissues of the vector. In this regard it was shown experimentally[2] that when *C. univittatus* was infected with WN virus and held at a temperature of 26°C the transmission rate was 97% whereas in a comparable group at 18°C the rate was 48%. A further possible factor is the apparent higher man-biting rate of *C. univittatus* in Upington compared to the low rate observed among highveld populations.[8] There might well be an intrinsic behavioural difference between the two geographical populations of the species, or it could be the result of the extremely high population levels existing in Upington at the time observations were made. Abnormally abundant *C. univittatus* would also tend to cause increased resistance to mosquito attack by the natural host, and the mosquitoes would also interfere with each other, both effects resulting in a divergence of feeding to alternative hosts, including man. It seems likely therefore that several factors acting jointly towards the same end made the epidemic possible.

Summary

The epidemiological findings on the largest epidemics caused by West Nile and Sindbis viruses ever recorded in South Africa are described. The epidemics occurred simultaneously in 1974 in an arid region of some 2500 km² of the Cape Province following heavy rains. West Nile virus was isolated from 6 ill persons. Post-epidemic antibody surveys on the affected human population indicated that 55% had been infected by West Nile virus and 16% by Sindbis virus. Multiple isolations of both viruses were made from *Culex univittatus* and *Culex theileri* collected in the town of Upington. Infection rates in *C. univittatus* were 1 infected mosquito per 26 for West Nile virus and 1 per 154 for Sindbis virus. In *C. theileri* the infection rate for each virus was 1 infected mosquito per 1204. Observations on the mosquitoes in Upington indicated that *C. univittatus*, which is mainly ornithophilic, also fed readily on man, at least when abundant. It is concluded that *C. univittatus* was the main epidemic vector of both viruses. An antibody survey of the wild birds in Upington after the epidemic showed high immune rates for both viruses in several avian species. It is postulated that an avian epizootic also occurred and that viraemic birds were the main source of infection for *C. univittatus*.

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Infection by Sindbis and West Nile viruses in wild populations of *Culex (Culex) univittatus* Theobald (Diptera: Culicidae) in South Africa

by

B. M. MCINTOSH, P. G. JUPP and I. DOS SANTOS

National Institute for Virology, Department of Health,
Private Bag X4, Sandringham, 2131.

Adult blood-sucking insects were collected in a variety of traps from 1966 to 1976 at 9 localities on the inland plateau region of South Africa and tested for virus. The insects in the catches were taxonomically segregated into groups i.e. pooled. Sindbis virus was isolated from 57 of 998 pools (30 220 insects) of *Culex univittatus*, from 10 of 1 227 pools (77 999) of other Culicidae and from 1 of 322 pools (42 584) of *Culicoides* spp. West Nile virus was isolated from 108 of the pools of *Cx. univittatus* and from 7 of those of other Culicidae. There were no isolations of virus from 64 pools (7 096) of Simuliidae. It is concluded that *Cx. univittatus* is the only important vector of these viruses on the plateau.

INTRODUCTION

As a result of field studies in South Africa from 1961 to 1965 it was concluded that *Culex (Culex) univittatus* Theobald is the probable maintenance vector of Sindbis (SIN) and West Nile (WN) viruses in the Highveld region (McIntosh *et al.* 1967, Jupp & McIntosh 1967). Since that time, in the course of further field studies, blood-sucking Diptera (Culicidae, Ceratopogonidae and Simuliidae) have been collected in the Highveld region and other parts of the inland plateau and tested for infection by arboviruses. SIN and WN viruses were isolated from *Cx. univittatus* on numerous occasions. The results of these studies are reported here. They confirm the earlier conclusion that *Cx. univittatus* is the only important vector of the viruses in the Highveld region and show that it plays the same role in other regions of the inland plateau.

METHODS

Adult insects were collected in a variety of traps, with light, carbon dioxide, pigeons or volunteer human beings as the attractant. They were identified and segregated into groups (i.e. pooled) on the day of collection, and stored in vessels cooled by solid carbon dioxide or liquid nitrogen. Pool sizes for mosquitoes varied, rarely exceeding 100 specimens, and for *Cx. univittatus* were usually 25 or 50. Mosquitoes containing visible ingested blood were usually pooled separately from 'empty' specimens. Pool sizes for *Culicoides* spp. and Simuliidae varied, with a maximum of 400.

In the laboratory, suspensions of the pooled insects were inoculated intracerebrally into suckling mice 2 days old in attempts to isolate virus. Brain from sick mice was used for passage of virus in mice and as a source of virus for identification. Virus isolates were identified by complement-fixation tests in which hyperimmune mouse ascitic fluids were used as a source of known antibody. The identification of some isolates was confirmed by mouse neutralization tests.

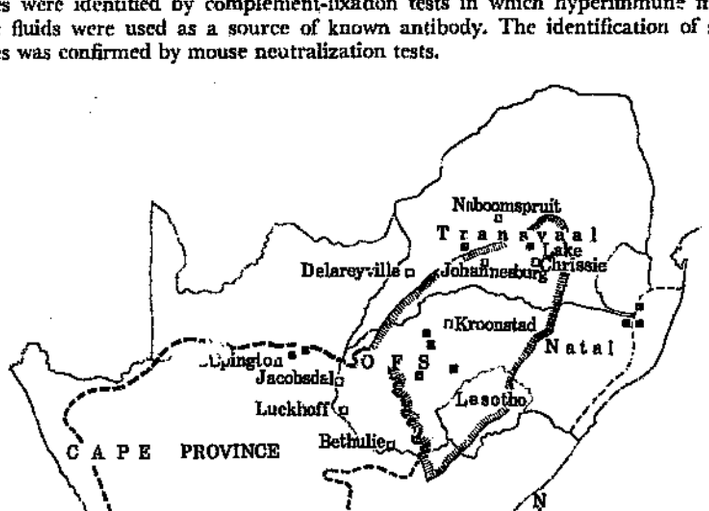

Fig. 1. Collecting sites in South Africa. Fringed line – boundary of Highveld; heavy interrupted line – boundary of Karoo; faint interrupted line – boundary of Natal coastal plain; open squares – viruses isolated from *Cx. univittatus* and other insects; solid squares – *Cx. univittatus* collected but not tested for virus.

As shown in fig. 1, insects were collected and tested for virus at 9 localities widely distributed over the inland plateau region of South Africa: Lake Chrissie, Johannesburg and Kroonstad in the Highveld; Jacobsdal, Luckhoff, Bethulie and Upington in the Karoo; Naboomspruit and Delareyville in the region of scrub acacia thorn-veld bordering on the Highveld and at somewhat lower altitudes than the Highveld localities. Except at Upington, collections were in rural environments, but for ease of identification the locality recorded is that of the nearest town. Collections recorded for Johannesburg were made at Olifantsvlei, a sewage disposal area on the outskirts of the city and one of the localities utilized in the earlier studies from 1961 to 1965 mentioned above. The observations here extend over a 12-year period from 1961 to 1972, with breaks in 1968 and 1971 when no observations were made.

In the current study, if all localities are lumped together, collections were made each year from 1966 to April 1976, except for 1973 when collections were

suspended. With a few exceptions, collections were made during the 4 wet summer months of January to April. They took place during a total of 205 days distributed over the 11-year period.

The collections at Upington in 1974 took place during epidemics of WN and SIN viruses in the human population (McIntosh *et al.* 1976). All other collections were made at times when no human infections from these viruses were evident.

TABLE 1. Numbers of blood-sucking Diptera tested for arboviruses, 1966–1976, and number of isolations of SIN and WN viruses.

Family and Species	No. of insects	No. of pools (see text)	Virus Isolations	
			SIN	WN
CULICIDAE				
Anopheles coustani	219	18		
Anopheles tenebrosus	258	12		
Anopheles imbelvus	1	1		
Anopheles [illegible]	138	21	1	
Anopheles [illegible]*sis*	17	4		
Anopheles [illegible]*nsis*	3	1		
Anopheles [illegible]*zi*	375	14		
Anopheles squamosus	1 467	52		
Anopheles spp. (indet.)	46	3		
Coquillettidia fuscopennata	37	9		
Coquillettidia microannulata	2 123	35		
Aedes caballus	1 046	12		
Aedes juppi	1 775	49		
Aedes caballus/juppi	1 809	66		1
Aedes aegypti	1	1		
Aedes dentatus	1 575	45		
Aedes durbanensis	30	1		
Aedes hirsutus	5	1		
Aedes mixtus	756	21		
Aedes mixtus/microstictus	9	3		
Aedes lineatopennis	1 097	30		
Aedes luridus	34	3		
Aedes lineatopennis/luridus	4 035	43		
Aedes unidentatus	1 122	35		
Culiseta longiareolata	278	29		
Culex rubinotus	126	6		
Culex tigripes	1	1		
Culex annulioris	1	1		
Culex antennatus	583	6		
Culex pipiens	22 443	156	1	1
Culex poicilipes	341	6		
Culex quinquefasciatus	53	4		
Culex theileri	36 195	538	8	5
Culex univittatus	30 220	998	57	108
CERATOPOGONIDAE				
Culicoides spp.	42 584	322	1	
SIMULIIDAE	7 096	64		
TOTAL ISOLATIONS			68	115

RESULTS

Table 1 gives details of the insects that were tested for virus from all localities. Over 32 mosquito species, totalling 108 219 specimens, were tested. *Culex theileri*, *Cx. univittatus* and *Cx. pipiens* were the 3 most prevalent species. Also shown is the number of isolations of SIN and WN viruses from these insects. Noteworthy is the high isolation rate from *Cx. univittatus*.

Table 2 lists the isolations from *Cx. univittatus* according to locality and year. Although small collections were made in 1968 no *Cx. univittatus* were caught. It can be seen that one or other of the viruses was isolated from rather small numbers of mosquitoes at every locality, both were isolated at most localities, and both were isolated virtually every year at localities where collections were repeated annually. The infection rates fluctuated, and it seems that the viruses were enzootic and epizootic at different places and times.

TABLE 2. Isolations of SIN and WN viruses from *Cx. univittatus* collected at 9 localities.

Locality	*Year*	No. of *Cx. univittatus*	No. of pools (see text)	SIN		WN	
				Isolations	Infection rate[a]	Isolations	Infection rate[a]
Johannesburg	1966	3 522	164	1	0,3	3	0,9
Johannesburg	1967	2 875	114	7	2,4	11	3,8
Johannesburg	1969	1 670	51	1	0,6		
Johannesburg	1970	6 943	221	2	2,9	7	1,0
Johannesburg	1972	4 259	177	3	0,7	10[b]	2,3
Bethulie	1969	1 389	48	1	0,7	2	1,4
Bethulie	1970	461	15	1	2,2	5	10,9
Bethulie	1971	2 966	42	24	8,1	3	1,0
Kroonstad	1970	113	10	1	8,9	3	26,6
Luckhoff	1971	800	21	1	1,3		
Lake Chrissie	1971	862	15	1	1,2		
Upington	1974	1 325	52	8	6,0	33	24,9
Upington	1976	1 794	37	5	2,8	27[b]	15,1
Delareyville	1975	377	10	1	2,7	1	2,7
Naboomspruit	1975	292	12			2	6,8
Jacobsdal	1976	572	9			1	1,7
Totals		30 220	998	57		108	

a – infection rate is average number infected per 1 000 tested. *b* – 1 isolate from recently blood-engorged mosquitoes

DISCUSSION

The results show that infection of *Cx. univittatus* by SIN and WN viruses was demonstrated frequently over widely separated localities on the inland plateau. The regularity in space and time of infection in *Cx. univittatus*, and its virtual absence in other potential vectors, convincingly implicated *Cx. univittatus* as the maintenance vector of both viruses in the area sampled. There is also adequate evidence concerning the vector capability of *Cx. univittatus* (Jupp & McIntosh 1970), its marked feeding

preference for birds (Jupp & McIntosh 1967) and the prevalence of avian infections (McIntosh *et al.* 1968, 1976) to support this conclusion. Furthermore, studies during the epidemic in Upington in 1974, (McIntosh *et al.* 1976) indicated that *Cx. univittatus* was also the main epidemic vector. It is of interest that, whereas many species of birds serve as hosts for both viruses (McIntosh *et al.* 1969), only one species of mosquito is of any importance as vector.

It is pertinent to mention the failure during other arbovirus studies, despite considerable effort, to identify the main vector of SIN and WN viruses in the subtropical coastal lowlands of Natal Province (fig. 1) where both viruses occur (Worth *et al.* 1961, McIntosh *et al.* 1972) but *Cx. univittatus* is absent. *Cx. neavei*, a closely related species, is probably implicated, and both viruses have been isolated from it, but not so frequently or consistently as from *Cx. univittatus* on the plateau. The facts suggest that the ecology of the viruses is not the same in the temperate inland plateau as in the subtropical coastal lowlands of South Africa.

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INFECTIONS IN SENTINEL PIGEONS BY SINDBIS AND WEST NILE VIRUSES IN SOUTH AFRICA, WITH OBSERVATIONS ON *CULEX (CULEX) UNIVITTATUS* (DIPTERA: CULICIDAE) ATTRACTED TO THESE BIRDS

By B. M. McIntosh and P. G. Jupp[1]

Abstract. Pigeons were exposed over several years in the field in 2 ecologically distinct regions in the inland plateau region of South Africa, viz, moist Highveld and arid Karoo, and infections by Sindbis and West Nile viruses were recorded in these pigeons by periodic tests for viral antibodies. In both regions, infections by both viruses occurred during most years. The monthly incidence fluctuated and was usually low. Infections were recorded in the Highveld from December to May and in the Karoo from January to June inclusive, i.e., from the middle of the warm, wet season through the early part of the cold, dry season. During the remainder of the year, no infections were recorded. Infections by both viruses occurred at a higher overall rate in the Karoo. West Nile virus caused a higher overall rate of infection than Sindbis virus in both regions. *Culex univittatus*, the primary vector of both viruses on the plateau, was trapped in significant numbers inside the pigeon cages at both study areas, and its presence in these cages over a 12-month period at the Highveld locality correlated with the seasonal prevalence of pigeon infections.

Observations of the past 2 decades have identified Sindbis (SIN) and West Nile (WN) viruses as the most common mosquito-borne viral infections of man on the inland plateau region of South Africa (McIntosh et al. 1964, 1976). Evidence obtained from the isolation of these viruses from wild mosquito populations (McIntosh et al. 1967, 1978) and in laboratory transmission tests (Jupp & McIntosh 1970) has implicated *Culex (Culex) univittatus* Theobald as the only important vector of both viruses on the plateau. This mosquito is predominantly ornithophilic (Jupp 1973), and studies in South Africa (McIntosh et al. 1968, 1969), as well as other countries (Taylor et al. 1955, 1956, Shah et al. 1960), have strongly incriminated wild birds as the primary vertebrate host of both viruses.

For surveillance of viral transmission in the field, domestic pigeons (*Columba livea*) were exposed in the field over long periods and tested for serological evidence of infection. The main objectives were to obtain evidence on field transmission rates in 2 ecologically distinct areas, the relative rates between the viruses, the regularity of infection annually and to determine the time of the year that transmission occurred. Insect traps were periodically operated within the pigeon cages to obtain information on the species and numbers of hematophagous insects attracted to these pigeons. More particularly, it was considered necessary to demonstrate that *Cx. univittatus* was one of the numerically dominant species entering the cages and that this species was present there during the time of the year when infections were recorded in the pigeons. In this way it was hoped to obtain additional evidence on the involvement of *Cx. univittatus* as maintenance vector of these viruses among wild birds.

These studies, reported in this paper, were made at 2 climatically different localities where contemporaneous studies on other aspects of the ecology of these viruses, including periodic infectivity surveys of the mosquito fauna, were in progress. One study was in moist Highveld, at Olifantsvlei sewage farm on the outskirts of the city of Johannesburg, at an altitude of 1753 m and with an average rainfall of 769 mm. The other was located about 600 km south of Johannesburg, in arid Karoo, in and around the small town of Bethulie, with an altitude of 1274 m and an average rainfall of 441 mm.

MATERIALS AND METHODS

Pigeons, known to be free of antibodies against SIN and WN viruses, were exposed in pairs in wire cages with a mesh of 5 × 5 cm. Cages were placed on the top of a pole 2 m high.

At Olifantsvlei, 1 to 4 pairs were exposed from January 1967 through September 1971, with 3 periods, viz, July–October 1967, July–October 1968, and May–June 1971, when no pigeons were exposed. Pigeons were exposed over a total of 47 months, of which 28 were within the virus transmission season. Numbers exposed varied from time to time as follows: 1 pair for 3 months; 2 pairs

[1]National Institute for Virology, Private Bag X4, Sandringham 2131, South Africa.

TABLE 1. Effective exposure times, in pigeon-days, of pigeons to Sindbis (SIN) and West Nile (WN) viruses at 2 localities.

LOCALITY	PERIOD OF YEAR	NO. MONTHS**	NO. PIGEON-DAYS	
			SIN	WN
Olifantsvlei	In season*	28	3981	3878
	Out of season	19	3716	3634
Total		47	7697	7512
Bethulie	In season	16	4882	4156
	Out of season	13	3927	3765
Total		29	8809	7921

* Refers to virus transmission season.
** Months when pigeons were exposed.

for 7 months; 3 pairs for 7 months and 4 pairs for 30 months. Pigeons were placed in an area of about 50 ha. This consisted of marshy grassland and a large riverine reed bed. Studies here, which had started in 1961, had indicated that the area was an enzootic focus of both viruses. The area carried a large and diverse avian population throughout the year, and during the summer *Cx. univittatus* was also plentiful.

At Bethulie, 8 pairs of pigeons were exposed from November 1968 through September 1971, with no exposures from April to November 1969, inclusive. Birds were exposed for a total of 29 months, of which 16 were during the transmission season. Two pairs were located in Bethulie and the others on farms within a radius of 10 km of the town. On farms the sites were close to streams, dams or homesteads, which in this arid area provided the most favorable habitats for most avian and mosquito species. Elsewhere, the habitat was virtually devoid of surface water and supported tufted grasses and stunted scrub.

At both localities, loss of exposure time resulted from predation, theft and subtraction of time that pigeons remained in the field after infection, resulting in a decrease in effective exposure time. TABLE 1 shows the effective exposure times at both localities, in and out of the transmission season, for each virus. While fewer birds were exposed at Olifantsvlei at any one time, they were exposed there over more months, with the result that the total effective exposure periods were much the same at both localities.

Exposed pigeons were bled weekly or monthly during the summer and late autumn, i.e., November to June, when infections were likely, and at longer intervals during the winter and spring when they were not. In estimating exposure periods and times of infection, infection was assumed to have occurred at a time midway between the

TABLE 2. No. of infections by Sindbis (SIN) and West Nile (WN) viruses in pigeons, with infection rates according to year and locality.

LOCALITY	YEAR	SIN			WN		
		No. infections	Exposure pigeon-days	Infect. rate*	No. infections	Exposure pigeon-days	Infect. rate*
Olifantsvlei	1967	4	747	5.4	5	768	6.5
	1968	2	1357	1.5	5	1247	4.0
	1969	0	1651	0	1	1607	0.6
	1970	4	2760	1.4	9	2532	3.6
	1971	4	1172	3.4	0	1358	0
Total		14	7697	1.8	20	7512	2.7
Bethulie	1968	0	541	0	0	541	0
	1969	2	1284	1.6	3	1174	2.6
	1970	9	4766	1.9	25	3865	6.5
	1971	10	2218	4.5	13	2341	5.6
Total		21	8809	2.4	41	7921	5.2

* Infection rate = no. of infections per 1000 pigeon-days of exposure.

TABLE 3. Infections, according to month, of Sindbis (SIN) and West Nile (WN) viruses in sentinel pigeons at 2 localities.

LOCALITY EXPOSURE PERIOD	MONTH	SIN			WN		
		Exposure pigeon-days	No. infections	% pigeons infected	Exposure pigeon-days	No. infections	% pigeons infected
	Jan.	589	2	7	565	4	14
	Feb.	526	5	18	536	2	7
	Mar.	721	4	17	804	6	17
	Apr.	722	2	8	612	6	18
Olifantsvlei	May	757			733	1	4
Jan. 1967	Jun.	585			555		
to	Jul.	54			633		
Sept. 1971	Aug.	701			680		
	Sept.	678			678		
	Oct.	496			496		
	Nov.	592			592		
	Dec.	666	1	4	626	1	4
Total		7697	14		7512	20	
	[illegible]	1297	1	2	1209	5	11
	Feb.	956	13	29	853	12	28
	Mar.	909	3	8	784	5	15
	Apr.	398	3	14	178	14	31
Bethulie	May	619			485	1	6
Nov. 1968	Jun.	703	1	4	652	4	17
to	Jul.	663			549		
Sept. 1971	Aug.	496			465		
	Sept.	543			526		
	Oct.	425			425		
	Nov.	642			642		
	Dec.	1158			1158		
Total		8809	21		7921	41	

detection of antibody and the previous negative test. Pigeons developing antibody were replaced with susceptible birds during the next visit, and the period they were in the field after infection was subtracted from their exposure times. In the determination of the significance of various infections, a Poisson distribution was assumed. As numbers were sufficiently large, the normal approximation could be used to estimate by χ^2 test the significance of the differences in rates between the 2 areas and between the 2 viruses.

Pigeon sera were tested for antibody in microhemagglutination-inhibition tests. Sera were diluted 2-fold, over the range $^1/_{10}$ to $^1/_{1280}$, and tested against 8 units of viral hemagglutinin. Hemagglutinins were prepared by sucrose-acetone extraction of infected infant mouse brain. Sera inhibiting agglutination at a dilution of $^1/_{10}$ or greater were recorded as positive.

For the collection of insects, a portable suction trap, powered by a 12-V car battery, was suspended from the floor of a pigeon cage and run overnight. A hole 15 cm in diameter in the floor of the cage accommodated the trap intake, and insects were sucked downwards into a collecting bag. Mosquitoes were identified to species, *Culicoides* to genus, and Simuliidae to family.

At Olifantsvlei, these traps were set at approximately weekly intervals over a 12-month period, i.e., from November 1969 through October 1970. At Bethulie, a total of 19 traps was set during January and March 1971. A series of unbaited control traps was set at both localities.

RESULTS

Pigeon infections

The number of infections in the pigeons with exposure times in pigeon/days and infection rates is given in TABLE 2. The figures are expressed on a yearly basis to show the pattern of infection over the various years of observations at both localities. Infections by both viruses at each locality were recorded during most years. At Bethulie, 1968 is excluded since exposure in that year was limited to November and December, infections by both

TABLE 4. Hematophagous insects collected in suction traps placed inside pigeon cages at Olifantsvlei (Highveld) during 44 trap-nights from November 1969 to October 1970 inclusive, and at Bethulie (Karoo) during 19 trap-nights in January and March 1971.

	TOTAL INSECTS	
	Olifantsvlei	Bethulie
Culicidae		
Culex pipiens	16,572	1554
Cx. univittatus	6478	2927
Cx. theileri	1224	197
Cx. quinquefasciatus		28
Aedes unidentatus	332	
Ae. dentatus	218	
Ae. mixtus	72	
Ae. juppiteaballus	19	5
Coquillettidia microannulata	761	
Other Culicidae spp.	35	311
Culicoides spp.	1258	3769
Simuliidae	1580	ca 3000

viruses were recorded annually. Despite the fact that 1970 was an exceptionally dry year at Bethulie, infections by both viruses were recorded there during that year, with WN infections at a relatively high rate. The overall infection rates with both viruses were higher at Bethulie than at Olifantsvlei. In the case of SIN virus there is no significant difference in the rates between the 2 localities ($\chi^2 = 0.382$; $0.5 < P < 0.6$), whereas for WN virus the difference is significant at $P < 0.025$ ($\chi^2 = 5.613$).

In TABLE 3 the number of infections recorded and the percentage of pigeons infected each month are shown, the values for each month over the duration of each study being combined so as to show infections in relation to the seasons of the year. Infection by each virus at both localities was seasonal, being recorded for only 5 to 6 months of the year, from midsummer to early winter. Infection at Bethulie started and ended a month later than at Olifantsvlei; probably a reflection of the later onset of the rainy season at Bethulie.

There were fluctuations in the monthly infection rate, which was generally low. The highest rate was in April at Bethulie with WN virus. This resulted from 7 out of 17 pigeons becoming infected during April 1970, and the same number again in April 1971. The highest monthly rate with SIN virus was also at Bethulie. This occurred in February 1971, when 8 out of 15 pigeons were infected.

At both localities, WN virus showed a higher overall infection rate than SIN virus. If infections at both localities during the 6 months when virus transmissions were recorded, i.e., from December to May at Olifantsvlei and January to June at Bethulie, are considered together with respect to each virus, i.e., 35 infections by SIN virus during 8863 pigeon/days (1 infection per 253 days) compared to 61 WN infections during 8034 pigeon/days (1 infection per 132 days), the difference is highly significant at $P < 0.001$ ($\chi^2 = 18.89$).

Insect collections

TABLE 4 shows the number of Culicidae, *Culicoides* spp., and Simuliidae collected inside pigeon cages at Olifantsvlei and Bethulie. At both localities *Cx. univittatus* was one of the numerically dominant mosquito species. *Cx. pipiens* was by far the most prevalent at Olifantsvlei and was almost as prevalent as *Cx. univittatus* at Bethulie.

TABLE 5 shows the average number of *Cx. univittatus* collected per trap-night in pigeon cages on a monthly basis at Olifantsvlei. It can be seen that this species was collected in the cages from November through May. It is apparent therefore that, with the exception of November, at the beginning of the rainy season, its presence in the cages corresponded with the time of the year when infections were recorded in the pigeons.

Only insignificant numbers of *Cx. univittatus* were caught in cages without pigeons.

DISCUSSION

The demonstration of infection in sentinel pigeons with high seasonal regularity but low monthly incidence at both localities is indicative of enzootic infection and suggestive that both viruses exist permanently in the Highveld and Karoo re-

TABLE 5. Average no. of *Cx. univittatus* collected monthly per trap-night inside pigeon cages at Olifantsvlei (Highveld).

	1969		1970									
	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sept.	Oct.
No. traps	5	5	5	3	4	4	3	3	3	4	4	1
Avg. no.	61	227	682	183	252	14	2	–	–	–	–	–

gions of South Africa. This is also in accord with the pattern of infection found in *Cx. univittatus* and in humans living in these regions. During surveillance for infection in *Cx. univittatus* populations on the inland plateau over the 15-year period from 1962 through 1976, both viruses were isolated from this mosquito during 13 of these years. Of the remaining 2 years, no *Cx. univittatus* were collected in one and in the other only very few were sampled (McIntosh et al. 1967, 1978). Endemic human infection has also been evident on the plateau; in every year since 1963 when surveillance of human infection was started, serological evidence of infections by both viruses was obtained (unpubl. studies). The sentinel pigeon studies demonstrated yearly avian infection by both viruses from 1967 through 1971, suggesting that wild avian infection is also an annual occurrence on the plateau.

As both viruses produce only low viremia in man (McIntosh et al. 1976), it is probable that most human infection results from vectors that acquire their infection from wild birds. Human infection with these viruses is consequently more temporally dependent upon avian infection than might be the case were a mosquito-man-mosquito transmission cycle more frequent. The sentinel pigeon studies, in identifying those months of the year when avian infection occurs, have therefore also indicated the time of the year when human infection is most likely.

Collections of insects inside the pigeon cages showed *Cx. univittatus* as one of the numerically dominant bloodsucking species and its presence there at one locality correlated with the time of the year when infections were recorded in the pigeons. *Cx. pipiens* was the most prevalent species inside the cages at Olifantsvlei and ranked 2nd at Bethulie, but this prevalence is exceptional for most plateau habitats where *Cx. pipiens* is usually a rather rare species in habitats more natural than those sampled in the present collections (Jupp 1973). *Culicoides* spp. and Simuliidae were also prevalent in the cages but low natural infection rates exclude these insects from any significant vector role (McIntosh et al. 1978). Infectivity surveys among mosquitoes on the inland plateau have produced overwhelming evidence that *Cx. univittatus* is the only important vector in this region. For example, in the surveys made from 1962 through 1976 mentioned above, which included surveys at Olifantsvlei and Bethulie during the sentinel pigeon studies, a total of 84 isolations of SIN virus and 128 of WN were made from 70,057 *Cx. univittatus*. In comparison, only 2 other mosquito species yielded multiple isolations. Nine isolations of SIN and 6 of WN were made from 83,001 *Cx. theileri* and only 4 isolations of SIN were made from 128,967 *Cx. pipiens*. Laboratory transmission tests also showed *Cx. univittatus* as an efficient vector of both viruses and *Cx. theileri* and *Cx. pipiens* as rather poor vectors (Jupp & McIntosh 1970, Jupp et al. 1972, Jupp 1976). There is little question, therefore, that *Cx. univittatus* was the vector of most sentinel pigeon infections and that these infections were an artificial counterpart of natural avian infection with this mosquito as vector.

Doubt remains whether failure to record infection during the winter and spring was due to the exposure of inadequate numbers of pigeons or whether transmission ceased during this period. If transmission does occur, which is possible since *Cx. univittatus* has been collected in small numbers during the winter (Jupp 1969), it is rare, which suggests that transmission among birds during this time of the year carries little significance for the persistence of these viruses on the plateau.

The reason for a higher rate of infection by both viruses in the arid Karoo, compared to the moister Highveld, even during an abnormally dry year, is not clear. It is possible that the higher summer temperatures prevailing in the Karoo, by enhancing virus multiplication in the vector and thereby increasing vector efficiency, are the cause. The relatively high rate of transmission recorded at Bethulie suggests that at the lower rates of transmission, i.e., enzootic infection, the aridity of the Karoo imposes little significant restraint on the transmission of these viruses in the avian population.

The reason for a higher overall transmission rate by WN virus compared to SIN virus is also not clear. A similar observation was made at Olifantsvlei in an earlier study with sentinel fowls over 3 consecutive years, in which only 14 infections of SIN virus were recorded as against 38 by WN virus (McIntosh et al. 1967). In this regard it is pertinent to mention that laboratory transmission experiments showed *Cx. univittatus* to be a more efficient vector of WN virus. When this species was infected by blood meals containing ±5.0 log of either virus, transmission rates of 89% with WN virus and 57% with SIN virus were obtained (Jupp & McIntosh 1970). Because of its apparent higher rate of transmission among wild birds, it seems that WN virus is more likely to produce infection in man than is SIN virus.

Relevant to these sentinel pigeon studies, it should be mentioned that the largest epidemic ever recorded in man due to these viruses in South Africa was caused by WN virus in the Karoo in 1974 (McIntosh et al. 1976). An abnormally high rate of human infection by SIN virus also occurred at the same time, but this was at a much lower level than that by WN virus.

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ARBOVIRUS RESEARCH IN AUSTRALIA

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ECOLOGICAL STUDIES ON WEST NILE VIRUS IN SOUTHERN AFRICA, 1965-1980

B. M. McIntosh and P. G. Jupp

National Institute for Virology,
Private Bag X4, Sandringham, 2131, South Afica

Southern Africa carries a wide range of temperatures, determined by both latitude and altitude. It encompasses an area extending over 17°S of latitude, from to 34°S. Coastal plain is somewhat limited, the bulk of the area consisting of an extensive, inland plateau, varying from 400 to 2000 m above sea level. By utilising the 18 and 13°C midwinter isotherms Southern Africa may be demarcated into tropical, subtropical and temperate thermal zones. The subtropical and temperate zones may be subdivided into moist and arid zones by the 500 mm isohyet which bisects the southern and central regions into a moist eastern part, and an arid western part. In Zimbabwe, this isohyet takes a direct westerly course, thus creating a broad band of moist territory along the northern limits. Almost the entire western shoreline is desert.

Apart from antibody surveys in adjoining countries, most of the work has been in South Africa, either on the temperate, inland plateau, or on the moist, coastal plain of Natal Province, which is partly tropical and partly subtropical. It is on the plateau that West Nile (WN) virus has been most active and it is there that the emphasis of the work has been located. Study sites there have been in both the Highveld, a high, cool and moist grassland, and in the Karoo, a lower-lying, warmer and arid region of the plateau.

West Nile virus was found to be widely distributed in Southern Africa. It was isolated at 11 localities on the South African plateau, at two localities on the Natal coast and at one locality on the coastal plain of central Mocambique. In addition, the presence of virus in Botswana was established, when a strain of virus, isolated by the local veterinary authorities from a dog, was identified as WN virus. Neutralising antibodies were found in man throughout South Africa, also widely in Mocambique, as well as along the northern borders of Namibia and Botswana. It seems therefore that the virus persists throughout Southern Africa, wherever suitable vector habitats and sufficient vertebrate hosts are present.

Antibody rates in man have conformed to a distinct geographical pattern; high on the plateau, varying from 15 to 40%, and low on the coastal plain (0.5 to 5%). This has correlated positively with viral activity determined by other techniques, viz., by infection rates in wild mosquito populations, in observed human illness, and in avian infection. This has been particularly evident between plateau and coastal lowland in South Africa. The reason for this rather paradoxical situation is not clear, but is probably related to the presence on the plateau of a particularly efficient vector - Culex univittatus.

During the 25 year period under review 158 isolations of WN virus were obtained. These were made up of 139 mosquitoes, 14 from human blood, two from sentinel hamsters, and one each from a wild bird, a sentinel pigeon and a dog.

One hundred and twenty eight isolations came from Cx univittatus (Table 1), clearly implicating this mosquito as a vector on the plateau, as well as directing attention to birds as vertebrate host since this mosquito is primarily bird-feeding (McIntosh et al. 1978; Jupp, 1973; Jupp et al., 1980). In coastal Natal, where nearly twice as many mosquitoes were tested only three isolations were made, and these from three species, one of which was Cx neavei, closely related taxonomically to Cx univittatus, and replacing that species in the coastal lowlands (Jupp, 1971).

Table 1. West Nile virus isolations from mosquitoes in South Africa 1956-80.

Region	No. mosquitoes	Infected species	No. tested	No. isolations
Plateau	250,000	Cx univittatus	70,037	128
		Cx theileri	82,995	6
		Cx pipiens	128,967	1
		Ae caballus	1,809	1
Lowlands (Natal coast)	420,000	Cx neavei	57,559	1
		Cq microannulata	254	1
		Ae circumluteolus	201,427	1

On the plateau there were 58 isolations from 56,564 Cx univittatus in the Highveld (infection rate, 1.0 per 1,000 mosquitoes) and 70 from 7,935 of the same species in the Karoo (8.8/1,000). The higher rate in the arid Karoo is noteworthy since this conforms with the higher activity found there during sentinel pigeon studies described below. The reason for this apparent higher viral activity in the arid Karoo is not clear, but it is possibly related to the higher summer temperatures prevailing in the Karoo enhancing vector capability. Surveillance over a 10 year period for infection in Cx univittatus at the Olifantsvlei study site near Johannesburg, yielded isolations in each of the seven years that observations were made and a similar pattern of annual summer infection was recorded at certain other plateau localities. There can be little question that Cx univittatus is the viral maintenance vector on the plateau.

Vector capability tests concurred with this belief as they showed Cx univittatus as an efficient vector (Table 2) (Jubb and McIntosh, 1970; Jupp, 1974). Furthermore, they eliminated Cx theileri and other possible vectors as important vectors, thereby strengthening the evidence against Cx univittatus (Jupp et al., 1972). Tests with Cx neavei indicated moderate vectorship but suggested that this was below that of Cx univittatus because of a higher infection threshold. This may well account for the relatively lower viral activity in the coastal lowlands.

Table 2. Infection and transmission tests with West Nile virus and Culex univittatus.

Experiment	Infective dose Logs/ml	Temp. °C	Infection rate*	Transmission rate†
1	6.4	26	14/15 (93)	4/4
	5.4	26	15/16 (94)	8/9 (89)
	4.4	26	20/25 (80)	Not done
	3.4	26	6/15 (40)	Not done
2	4.3	26	15/35 (43)	5/15 (33)
3	6.0	26	37/37	35/36 (97)
	6.0	18	30/30	13/27 (48)

* Numerator, number infected; denominator, number tested.

† Numerator, number transmitting; denominator, number infected mosquitoes that fed; each hamster was exposed to a single mosquito.

When 13 common avian species from the plateau fauna were inoculated with WN virus, all species became viraemic, some at high concentrations, although none showed ill effects (McIntosh et al., 1969). Evidence of natural avian infection was obtained by the isolation of the virus from a warbler and a sentinel pigeon, and the finding of antibodies in avian populations. An antibody survey among birds at Olifantsvlei showed 20% of 405 domestic fowls and 13% of 2,022 wild birds to have antibodies (McIntosh et al., 1978). A higher rate was recorded in wild birds, shortly after an outbreak of WN fever in man in the Karoo, when 53% of 322 birds had antibodies.

Two sentinel pigeon studies were carried out over three years, one at Olifantsvlei, the other at Bethulie in the Karoo (McIntosh and Jupp, 1979). At both localities enzootic, annual infection, from midsummer through autumn was recorded. Despite similar exposure periods at each locality there were only 20 infections at Olifantsvlei compared to 41 at Bethulie, the drier locality. Insect collections from inside the pigeon cages demonstrated the prsence of Cx univittatus throughout the transmission season. The studies suggested that natural avian infection was a yearly summer phenomenon on the plateau and strengthened the evidence against Cx univittatus as the vector of avian infection.

For many years an arbovirus diagnostic service has been provided at our laboratory in Johannesburg. Through this service, based largely on voluntarily submitted blood specimens from suspected human cases, it has become evident that WN virus is responsible for endemic infection on the plateau (McIntosh et 1., 1964). Single isolations were made in 1958, 1964, 1968 and 1970 while during 1974 there were nine isolations. During the same period nearly 600 presumptive infections were diagnosed serologically either in paired sera or from high, predominently monotypic, HI titres in a single convalescent serum. Except in 1974, infections have been sporadic and scattered throughout the plateau. Under conditions of average population levels, Cx univittatus has shown a low biting frequency on man, and this seems to be the most likely factor limiting human infection.

There occurred one notable exception to this relatively tranquil state of affairs. The summer of 1974 was unusually wet throughout the South African plateau. An epidemic of WN fever, characterised by fever, rash, myalgia and arthralgia, confined almost exclusively to adults, appeared over a large area of the Karoo (McIntosh et al., 1976). Investigations showed Cx univittatus to be unusually prevalent, significantly anthropophilic and heavily infected. Tests for virus in mosquitoes resulted in 33 isolations of WN virus from only 1,325 Cx univittatus.

As already mentioned high antibody rates were recorded in birds after this epidemic suggesting that an avian epizootic had occurred and this had been crucial in generating the epidemic. Antibody tests on 558 human sera from the affected area indicated that 55% had been recently infected. The indications were that human infection had been heavy and thousands of clinical cases had occurred, although no mortality had been reported. In the town of Upington, with a population of 30,000, some 1,700 persons had consulted practitioners.

In summary, the studies indicated that WN is widespread in Southern Africa, where it is the cause of one of the most common arbovirus infections of man. Although most active on the temperate plateau, where it utilises a most efficient vector, it has adapted to diverse climates. It evidently enjoys a variety of avian species as vertebrate host, which presumably provides the virus with the potential to move from locality to locality, perhaps even from region to region, although there were no suggestions that this facility was the means which enabled the virus to flourish each summer in

temperate South Africa. On the contrary, migrant birds seem not to be involved in the annual introduction of virus into the South African plateau, since they arrive in September and October, before the appearance of the spring Cx univittatus populations.

Because of low viraemia in man, human infection is totally dependent, spatially and temporally, on avian infection. There is no evidence of an amplifying, non-avian vertebrate host, which would promote human infection, nor is there a likelihood of an alternative, more anthropophilic and equally efficient vector, superceding Cx univittatus on the plateau. Because of these factors, the past pattern of endemic human infection, with intermittant epidemics, is likely to prevail, in this region at least in the future.

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Sindbis and West Nile virus infections in the Witwatersrand-Pretoria region

P. G. JUPP, N. K. BLACKBURN, D. L. THOMPSON, G. M. MEENEHAN

Summary

From mid-December 1983 until mid-April 1984, there was an epidemic of Sindbis (SIN) virus infection in the Witwatersrand-Pretoria region in which hundreds of human cases were diagnosed clinically. Twenty-eight of these diagnoses were confirmed in the laboratory by seroconversion as being infections with SIN virus, and 5 cases of infection with West Nile (WN) virus were also found. Attempts to isolate virus from 66 patients, mainly from serum specimens, were unsuccessful. Infection rates for the mosquito vector *Culex univittatus*, collected at localities on the Witwatersrand in February and March, were mostly higher for both SIN and WN viruses than in previous years. The highest rates determined were 5,4 (SIN) and 9,6 (WN) per 1 000 mosquitoes. It is concluded that an epizootic of both viruses occurred which was manifested by a high level of viral activity in the feral *Cx. univittatus*-bird transmission cycle. *Cx. univittatus* efficiently transferred infection of SIN virus from this cycle to man to cause the epidemic of infection with that virus but it is unclear why there were apparently only a few cases of WN virus infection.

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Arbovirus Research Unit, National Institute for Virology and Department of Virology, University of the Witwatersrand, Johannesburg

P. G. JUPP, M.SC., PH.D.SCI.NAT.
N. K. BLACKBURN, F.I.M.L.T., M.PHIL., D.PHIL.
D. L. THOMPSON, B.SC. HONS
G. M. MEENEHAN, M.T.

In South Africa during the summer Sindbis (SIN) and West Nile (WN) viruses have been responsible for sporadic annual infection in man.[1] Up to 1974 the cases reported were largely from the moister regions of the Transvaal and the Orange Free State, although antibody surveys in man and animals[2,3] showed that both viruses occur extensively in both the Highveld and Karoo regions. In early 1974, however, the largest epidemics of both virus infections ever recorded in South Africa occurred over extensive areas of the Karoo and northern Cape Province, with thousands of human infections estimated.[4] More recently, during the summer from mid-December 1983 until mid-April 1984, hundreds of human infections due to SIN virus were diagnosed by general practitioners and hospitals in the Witwatersrand-Pretoria region; these constituted the largest epidemic of SIN virus infections ever recorded on the Highveld and the largest occurring in the country since the 1974 Karoo epidemic.

Our Unit received sera from some of the acutely ill and convalescent patients so that infections with SIN virus could be confirmed serologically. Sera received here are routinely tested for 5 viral antigens including both SIN and WN so that the incidence of WN virus was also determined. This was important, since the symptoms of WN virus infection are indistinguishable from those of SIN virus.[1] Attempts were also made to isolate virus from the sera of acutely-ill patients and from skin lesions.

When the results of laboratory tests began to confirm the presence of SIN virus activity in the human population at a much higher level than in past years for the region, it was decided to undertake contemporaneous studies on the mosquito vector. The vector of both SIN and WN viruses, *Culex (Culex) univittatus* theobald, maintains the viruses in a feral cycle with wild birds. This same mosquito also transfers infection of both viruses from the feral cycle to man to cause human infections.[4] Man-to-man transmission of either virus by *Cx. univittatus* can be discounted because of the extremely low titres which are reached in the human peripheral bloodstream.[4]

The population of *Cx. univittatus* was sampled at several localities on the Witwatersrand to determine the vector infection

1647

25×10

rates for both viruses during the epidemic. This was done to investigate whether the high frequency of human cases of SIN virus infection was also expressed in the feral cycle by a higher infection rate in *Cx. univittatus*.

The human infection and *Cx.* [illegible] infection with both viruses is reported.

Methods

Virology

On arrival in the laboratory blood from acute human cases was centrifuged and the sera stored at −70°C, while sera from blood of convalescents were stored at −20°C. In an attempt to isolate the virus, sera and mosquito suspensions were inoculated into suckling mice, 1 - 3 days old, intracerebrally. A few suspensions prepared from skin vesicle scrapings were also inoculated. For identification of virus isolates complement-fixing antigens were prepared from the brains of sick suckling mice, either by sucrose-acetone extraction of the brains or from a lightly centrifuged 20% buffered saline suspension. These antigens were tested in complement-fixation tests against hyperimmune mouse ascitic fluids.

All human sera were tested for antibodies in microhaemagglutination-inhibition (HI) tests against antigens of SIN, WN, Chikungunya, Wesselsbron and Rift Valley fever viruses. Ten-fold dilutions of serum over the range 1/20 - 1/2560 were tested against 8 units of viral antigen. Those sera which reacted against both WN and Wesselsbron antigens were also tested for neutralizing antibodies by microneutralization tests using vero cells.

Mosquitoes

Up to 20 portable battery-operated light traps baited with solid carbon dioxide were set overnight for 15 nights during February and March 1984. The trap used was a National Institute for Virology light suction trap designed and made by the senior author (P. G. J.).[5] Collections were made at four localities on the Witwatersrand, chosen because of the presence of permanent water for mosquito breeding and for their concentrations of birds. These localities were Germiston (Rondebult Lake), Rietfontein (Jukskei River), Modderfontein (several dams) and Florida (Florida Lake).

In order to collect as many *Cx. univittatus* as possible the large number of traps was necessary, because *Cx. univittatus* was invariably greatly outnumbered by *Culex pipiens* and *Culex theileri*. The *Cx. univittatus* were either extracted from the catches on the day of collection, pooled in 25s and stored at −70°C, or all mosquito species were stored at −70°C and subsequently the *Cx. univittatus* were extracted and stored again at −70°C.

Results

There were 28 cases of infection with SIN virus and 5 with WN virus shown by seroconversion in the HI test. These may be compared with only 2 such seroconversions for SIN virus and none for WN virus among paired sera received and tested the previous summer (1982/1983). All WN virus infections were confirmed by the neutralization test. No viruses were isolated from 63 sera from acutely-ill humans or from the skin lesions of 5 patients.

The numbers of isolations of SIN and WN viruses obtained from *Cx. univittatus* collected at four Witwatersrand localities in 1984 are shown in Table I. As a comparison the same data obtained during five previous summers at Olifantsvlei in the southern Witwatersrand[6] are included.

Discussion

The significant increase in the number of human infections with SIN virus in the 1983/1984 summer compared with 1982/1983 confirmed clinical reports which indicated that an epidemic of this virus had occurred. The increase in the number of human infections with WN virus over the previous season was only slight. More of the SIN cases were not confirmed in the laboratory because suitable specimens were not obtained. In infection by either SIN or WN virus the viraemia is of a low level and transient, the patient usually seeking medical attention only when the maculopapular rash appears. The rash indicates the commencement of antibody formation which usually precludes successful virus isolation. Therefore the demonstration of a rise in antibody titre — seroconversion — is the only practical method of laboratory diagnosis and it depends on paired sera — acute and convalescent — being available from each patient. The first or 'acute' serum should be obtained as early in the illness as possible and the second or 'convalescent' serum 2 - 3 weeks or longer afterwards. From 50 or more patients in this study with antibodies to one or both of the viruses only 1 serum sample was collected. Therefore, any rise in antibody level indicative of a recent infection could not be demonstrated.

Malherbe *et al.*[7] managed to isolate virus from the skin lesions of 1 patient but McIntosh *et al.*[1] in a later study failed

TABLE I. ISOLATIONS OF SIN AND WN VIRUSES FROM *CX. UNIVITTATUS* COLLECTED AT FOUR WITWATERSRAND LOCALITIES IN 1984 COMPARED WITH PREVIOUS RESULTS FOR OLIFANTSVLEI (SOUTHERN WITWATERSRAND)

				SIN		WN	
Locality	Yr	No. *Cx. univittatus*	No. pools	Isolations	Infection rate	Isolations	Infection rate†
Germiston	1984	1 980	79	5	2,5	19	9,6
Rietfontein	1984	817	33	2	2,4	3	3.7
Modderfontein	1984	553	23	3	5,4	1	1,8
Florida/ Rietfontein	1984	22	1	—	—	1	—
Olifantsvlei*	1972	4 259	177	3	0,7	10	2,3
Olifantsvlei*	1970	6 943	221	2	0,3	7	1,0
Olifantsvlei*	1969	1 570	51	1	0,6	—	—
Olifantsvlei*	1967	2 875	114	7	2,4	11	3,8
Olifantsvlei*	1966	3 522	184	1	0,3	3	0,9

*From McIntosh *et al.*[6]
†Infection rate is number infected per 1 000 tested.

TABLE II. MONTHLY RAINFALL AND TEMPERATURES FOR 1983/1984 COMPARED WITH NORMAL MEANS FOR THE SAME MONTHS CALCULATED FROM 20 YEARS OF PREVIOUS RECORDINGS

	Rainfall (mm)		Temperature (°C)			
			Normal		Current month	
Month	Normal mean	Current month	Mean max.	Mean min.	Mean max.	Mean min.
October	55,0	90,4	24,2	11,2	23,3	12,1
November	120,2	131,9	24,4	12,8	25,2	14,8
December	113,4	167,3	24,6	13,6	24,6	14,9
January	129,2	80,5	25,1	14,3	26,4	15,2
February	91,1	13,1	24,8	14,0	27,9	14,8
March	88,7	77,5	23,7	12,9	25,3	14,8

to do this with several patients. Similarly, no virus was isolated from skin vesicle scrapings in our study.

The infection rates (IRs) for *Cx. univittatus* determined in 1984 were compared with those determined in previous years at Olifantsvlei. The IR for SIN virus at Modderfontein and for WN virus at Germiston were much higher, while IRs for SIN virus at Germiston and for both viruses at Rietfontein equalled the highest rate previously recorded at Olifantsvlei.[6] These high IRs indicate that an epizootic of both viruses occurred which was manifested by a high level of viral activity in the feral maintenance cycle between birds and *Cx. univittatus*. The unusually high rainfall early in the summer together with higher than normal temperatures throughout the mosquito season were probably important. These environmental factors probably contributed towards the occurrence of these epizootics and the subsequent epidemic of SIN virus infection. Table II shows the rainfall and temperatures from October to March 1984, compared with the normal averages usually expected during the same months. The rainfall pattern favoured mosquito breeding early in the summer so that densities of *Cx. univittatus* would have been high by December. The high temperatures are thought to have favoured viral infection in the mosquito and also to have enhanced the subsequent transmission of virus: Jupp[8] has demonstrated how increased temperature can enhance the transmission of WN virus by *Cx. univittatus*.

Although *Cx. univittatus* evidently successfully transferred infection of SIN virus from the feral cycle over to man, hence causing the epidemic of infection with that virus, it is unclear why such transfer only occurred to a limited extent with WN virus.

A grant from the South African Medical Research Council towards this work is acknowledged. We are grateful to Mrs N. Aldridge and Miss L. Ben Joseph for technical assistance and to the following for allowing us to set mosquito traps in areas under their jurisdiction: the Director of Parks, Germiston and Florida Municipalities; the Senior Administrative Officer, Rietfontein Hospital; and African Explosives and Chemical Industries, Modderfontein. We thank the Director-General of National Health for permission to publish.

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Linognathus africanus is host-specific and occurs on sheep and goats (Ledger, J. 1980. *The arthropod parasites of vertebrates in Africa south of the Sahara*, vol. 5. Phthiraptera (Insecta). Publication of the South African Institute for Medical Research No. 56 Johannesburg).

Although with a definite preference for and abundantly found on Cape buffalo, the blood-sucking *Haematobia thirouxi potans* has also been collected in low numbers from giraffe, white rhinoceros and blue wildebeest in the K.N.P., and it is known that sheep are attacked by this fly where their distribution ranges overlap (Zumpt, F. 1973. *The Stomoxyine biting flies of the world*. Gustav Verlag, Stuttgart). It seems highly improbable that the louse had infested any of the wild animal species in the K.N.P. Since no sheep or goats exist anywhere in this conservation area, the fly must have settled on lice-infested sheep or goats in adjacent areas. Goats in rural settlements in Mocambique are likely to have been the source of the louse. The closest Mocambican settlement to the collection site is 10 km to the northeast, with intervening harsh country not supporting any livestock.

This record confirms that sucking lice do utilize biting flies for transportation to new hosts, and suggests that they cling to the transporting species for considerable periods before dropping off (the fly must have travelled several kilometres with the louse, periodically resting on vegetation, without the louse dropping off). It also suggests that *H. thirouxi potans* cover considerable distances in their search for blood-meals or suitable breeding substrates.

We thank Chief Ranger Bruce Bryden (Kruger National Park) for the information on human settlements and activities in the area of Mocambique under discussion – L. E. O. Braack, Nature Conservation, Skukuza 1350, South Africa and K. C. Emerson, 560 Boulder Drive, Sanibel, Florida 33957, United States of America.

The NIV Light Trap for collecting biting Nematocera (Diptera). – The need arose in the Arbovirus Unit for a compact, lightweight portable light-suction trap for collecting biting Nematoceran flies during our field research programmes. It was necessary for such a trap to collect large samples of these insects as representative as possible of the fauna present at the locality under study. Such catches would be made for ecological studies on insect vectors and to provide specimens for virus isolation attempts. Previously we had made and used a light suction-trap with a 12 volt, 17 watt Lienlun motor and 15 candle power globe which worked well. However, this trap was cumbersome to use in the field owing to the heavy 12 volt wet motorcar battery required as its power source. For this reason, various imported portable traps were tried out but their performance was not found wholly satisfactory. Additionally there was the problem of high prices and the unavailability of spares in South Africa for these traps. Hence it was decided to design a trap which could be made from locally available materials and which could be set in large numbers to collect biting flies frequenting inaccessible habitats. This paper gives the design for the NIV light-suction trap and reports its successful use both with and without CO_2 supplement. Some comparative catches with the larger Lienlun trap are also given.

The design is based on that of the CDC miniature light trap (Sudia, W. D. & Chamberlain, P. W. 1962. *Mosquito News 22:* 126–129) but simplified. Fig. 1 is a lateral diagrammatic view of the complete trap except for its support pole and battery, while

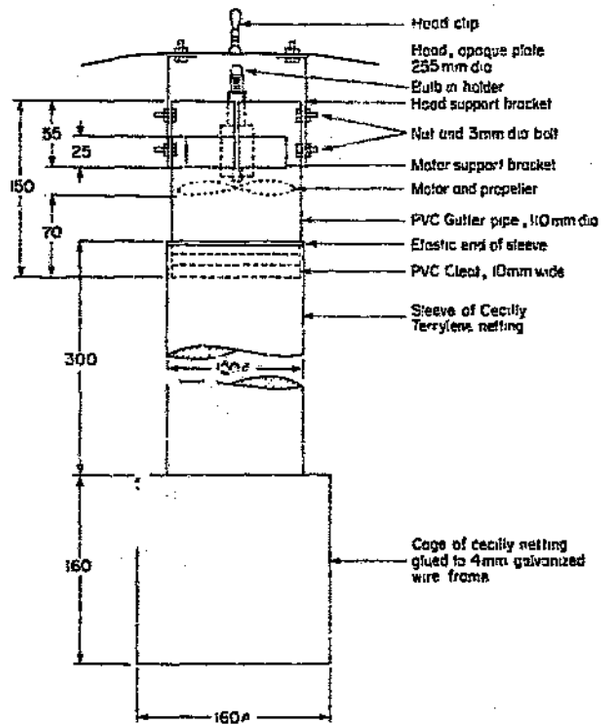

Fig. 1. Diagrammatic lateral view of trap complete with collecting cage.

Fig. 2 is a top view of the suction unit after removal of the hood. All measurements are given in millimetres.

The body of the suction unit consists of a cylinder provided by a piece of grey PVC gutter pipe 150 mm long and 110 mm outer diameter. A slot is cut from the top 55 mm down each side for insertion of the motor support brackets. These are made from two strips of 0,53 mm thick galvanized sheet iron measuring 200 mm long and 25 mm wide which are bent to fit the motor, while their ends are bent to conform to the outside of the cylinder (Fig. 2). A 130 mm polythene three-blade model aeroplane propeller, cut down to a diameter of 96 mm, is connected to the bottom of the motor by a custom-made brass bush. The distance from the lowest edges of the propeller to the bottom of the cylinder is then approximately 70 mm. The motor is a 6 volt, maximum 2 amp, Marx Monoperm sold by hobbies shops for model boats and cars. It is connected to two power input terminals fitted to the side of the cylinder by Ripcord twin flex separated into two cords. On the top of the motor an 'E.10' globe holder with a 6,3 volt 150 mA or 250 mA bulb [Electro-Quip (Pty) Ltd] is connected by short lengths of 1 mm gauge copper wire in insulation sleeves.

The hood or lid of the trap is an inverted 3 mm thick unbreakable polycarb plastic plate, 255 mm in diameter, painted with white enamel on the inside. It is bolted to the cylinder by two strips of 0,53 mm thick, 20 mm wide, galvanized sheet iron. These strips are 130 mm long before bending. Bolts used throughout are galvanized

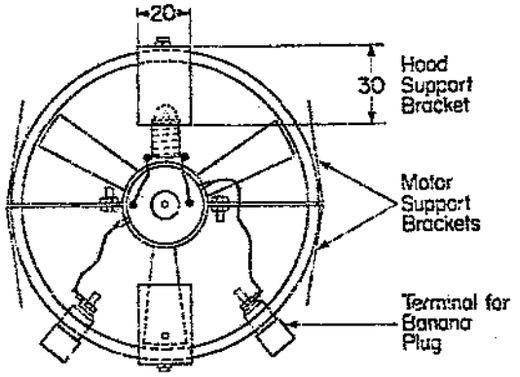

Fig. 2. Top view of suction unit with hood removed.

and measure 12 × 3 mm. The hood is fitted so as to leave a 45 mm space above the top of the cylinder where the bulb is situated. A clip designed for a dog's lead is fastened to the centre of the hood to permit the trap to be suspended from either a metal pole or the branch of a tree.

The support pole consists of a 1200 mm length of 20 mm galvanized conduit bent at right angles 220 mm from one end: the trap is suspended from the arm thus provided. A cast iron tent peg, 300 mm long and 12,5 mm diameter, is welded to the base of the pole so that it can be hammered into the ground.

The power source is a lead-acid 6 volt, 10 amp-hour rechargeable battery (GS Portalac PE10–6R or Chloride Powerstore F6) which is connected to the terminals on the cylinder by a length of Ripcord double flex bearing banana plugs. The other ends of the flex are connected to the battery terminals by crocodile clips: the correct polarity is essential to ensure the motor sucks air downwards.

If desired a wire mesh can be fitted to the top end of the cylinder to prevent large insects entering the trap. This is made of 22 gauge galvanized wire with a mesh measuring 4,76 × 4,76 mm ($\frac{3}{16}$" ins).

The cuboid collecting cage is fastened to the lower end of the suction cylinder by an elasticized sleeve which slips over the cleats provided. The two cleats are easily made by cutting a 10 mm piece off a gutter pipe, cutting the resultant ring into half circles and gluing these either side on the exterior of the suction cylinder 10 mm from its bottom. The cage itself is made of fine-meshed (sand fly-proof) 'Cecilly' Terylene netting glued by means of Pliobond adhesive to a 16 × 16 × 16 mm frame constructed from 4 mm galvanized wire. The access sleeve joined to one face of the cage is 120 mm in diameter and 300 mm long.

An insulated holding box was designed for the cage so the catch could be kept alive in the field. The box is constructed of 12,5 mm polystyrene sheets joined to give internal dimensions 170 × 180 × 185 mm. The lid has a second layer of polystyrene glued on its lower surface so that it fits tightly into the box. The box is painted on the outside only with a coat of white PVA followed by a coat of white enamel which makes it much stronger.

An alternative cage, cylindrical in shape, measuring 355 mm in diameter, 250 mm high and with a sleeve extending 320 mm from the top centre of the cylinder

can also be used. This cage is fitted with a zip fastener along half its lower perimeter which facilitates removal of dead insects.

The traps are set at sundown, if possible under trees for protection against wind, and are left until the following morning when it is preferable to remove the cages with the catch shortly after sunrise. A trap depends on the downward suction which draws flies reaching the space between the hood and the top of the cylinder downwards through the cylinder into the collecting cage. However, there is a choice from three attractants: (1) light only, (2) CO_2 only, (3) light and CO_2. The use of CO_2 without light is effective when the aim is to collect host-seeking mosquitoes only without positively phototaxic flies such as Chironomids.

A flow of CO_2 is obtained by placing about 2 kg of chopped dry ice into one of 2 kinds of container. The simplest container is a padded envelope, measuring 310 × 250 mm, which is filled with the ice, closed with masking tape and suspended with wire from the arm of the support pole near the trap's entrance. The other container is a coffee tin, 125 mm diameter and 145 mm high, which is filled with the ice. The tin is insulated by a surrounding tube of corrugated cardboard and from the plastic lid a 5 mm diameter Polythene tube delivers the CO_2 to a spot just above the trap's entrance, the tube being taped to the support pole. The tin rests on the ground below the trap.

The NIV trap has been used successfully to collect Phlebotomini at Mtubatuba, Natal, and Culicidae, Simuliidae and *Culicoides* at localities on the Witwatersrand and at Tweespruit, Orange Free State. At Mtubatuba its performance was compared with that of our Lienlun light trap.

When the trap was being developed a variety of small motors were tested. Most were rejected as unsuitable because of their poor suction but the Monoperm motor was chosen because the suction it provided in the trap approached that of the bigger Lienlun trap. Catches obtained in the NIV trap compared favourably, as regards the number and variety of flies, with the Lienlun trap and were superior to catches collected in the CDC miniature light-trap.

It was found that two operators could set up to 20 traps per evening. Initially a disposable battery was employed (Eveready sealed hand lamp and general purpose battery No. 991) but this was subsequently replaced by the rechargeable lead-acid battery. A trap runs for 14–15 hours with this battery which is sufficient time for overnight trapping. The battery can be recharged in 5–6 hours by means of a 4 amp solid state battery charger.

Mosquitoes survived well when kept for 10 days or longer in a collecting cage within the insulated holding box. A pad of cotton wool, measuring 100 × 100 × 20 mm, was soaked in 5% sucrose solution and placed on top of the cage to provide both nourishment and a high relative humidity. However, if insect densities are extremely high and the catch is not required alive, the more spacious cylindrical cage is more suitable.

At Mtubatuba, 1078 Phlebotomines, mainly *Sergentomyia* species, were collected in 88 trap-nights using NIV traps without CO_2 supplement and with a 250 mA light bulb. The results of comparative catches in NIV and Lienlun traps are given in Table 1. They show firstly that the CO_2 supplement did not significantly increase the size of the catches in the case of either trap. Secondly, the size of catch taken by the NIV trap was only slightly less than that taken by the Lienlun trap. Table 2 gives the numbers of mosquitoes (Culicidae), black flies (Simuliidae) and *Culicoides* biting midges (Ceratopogonidae) collected per trap-night at Roudebult dam, Germiston, and at

TABLE 1. Number of Phlebotomini collected per trap-night at Mtubatuba, Natal, in March, 1983.

	Lienlun Light Trap		NIV Light Trap	
	Light	Light + CO_2	Light	Light + CO_2
Females	9,3	11,3	8,7	11,8
Males	6,3	7,3	4,0	4,0
Total	15,6	18,6	12,7	15,8

TABLE 2. Numbers of Nematocera collected per trap-night in March, 1985.

	Culex spp.	Aedes spp.	Simuliidae	Culicoides spp.
Rondebult	522,3	3,4	526,0	61,0
Tweespruit	72	340	1	48

Tweespruit, Orange Free State during the 1985 summer; the NIV traps used were fitted with 150 mA bulbs. Poor rainfall explains the paucity of *Aedes* species at Rondebult. However, the trap set for one night at Tweepsruit where there had been soft rain, collected a substantial number of *Aedes* mosquitoes – mainly *Aedes juppi*.

From the above results it would appear that the NIV light trap is a useful method for collecting biting Nematocera of medical and veterinary importance particularly as its portability enables it to be utilized in inaccessible habitats. Furthermore, it has the added advantage of being constructed from locally available components and materials.

Acknowledgements: I am grateful to the Medical Research Council for a grant which assisted this work and to the Director-General of the Department of National Health and Population Development for permission to publish. – P. G. Jupp, Arbovirus Research Unit, National Institute for Virology, Private Bag X4, Sandringham, Johannesburg, 2131, and Department of Virology, University of the Witwatersrand, Johannesburg, South Africa.

The Pupae and Final Instar Larvae of *Aslauga atrophifurca* Cottrell (Lepidoptera; Lycaenidae). – A recent paper by Cottrell (1980, *Systematic Entomology* 6: 5–45) proposed that southern African specimens of Lycaenids previously referred to the species *Aslauga purpurascens* Holland in fact comprise a complex of species. The material on which Jackson (1938, *Transactions of the Royal Entomological Society of London* 86(12): 201–238.) based his description of the early stage of *A. purpurascens* has been assigned to the taxon *A. latifurca* Cottrell while material described by Boulard (1968, *Cahiers de la Maboke (Paris)* 6: 117–126.) has been confirmed to be of *A. purpurascens* (Cottrell ibid.). The early stages of the four remaining members of the complex have not been described, although Lamborn apparently reared imagoes of *A. orientalis* from larvae (Cottrell ibid.). Pupae and final instar larvae collected in Zimbabwe in 1975 and 1977 yielded adults which were recently identified as specimens of *A. atrophifurca* Cottrell. Two pupae and two larvae were preserved, and are illustrated in Fig. 1 and described here.

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Antigenic relationship of West Nile strains by titre ratios calculated from cross-neutralization test results

By N. K. BLACKBURN, D. L. THOMPSON and P. G. JUPP

Department of Virology, University of the Witwatersrand and Arbovirus Unit, National Institute for Virology, Sandringham 2131, Johannesburg

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SUMMARY

The antigenic relationship of ten South African West Nile isolates, the South African prototype virus H442, the Egyptian strain EG101 and the Indian strain G2266 were compared using titre ratios. The titre ratios or 'R' values were calculated from heterologous and homologous neutralization titres and expressed as a percentage.

Substantial antigenic differences were demonstrated between the South African prototype strain and the majority of the recently obtained South African isolates, seven of which were fairly closely related and possibly form a distinct antigenic sub-set. The recent isolates also differed from the Egyptian and Indian West Nile isolates. The heterologous results between the South African West Nile strains and the Indian strain G2266 suggest that prior infection with an Indian West Nile virus would give poor protection against the South African viruses, whereas the reverse would not be so.

INTRODUCTION

Studies on the antigenic relationship of West Nile (WN) virus strains from three geographic regions suggested the existence of two main antigenic groups, African-Middle-Eastern and Indian (Hammam, Clarke & Price, 1965). Of the three South African strains included in that study two appeared to belong to the African-Middle-Eastern group and the third was antigenically distinct from either group, suggesting the existing of at least one further group. Antigenic variation amongst isolates within these main groups has been described by Nir *et al.* (1968), Umrigar & Pavri (1977) and Odelola & Fabiyi (1976).

This paper presents the results of a study of the antigenic relationship of several South African WN virus isolates collected from one site during one season. The method of assessing the relationships of these isolates to each other, and to members of the two main WN antigenic sub-sets, was by calculating the titre ratios or 'R' values from heterologous and homologous neutralization titres. Jordan & Gaylin (1953) studied the antigenic relationship of influenza B strains using 'R' values. Rweyemamu *et al.* (1977) applied this method to evaluate the antigenic relationship between foot and mouth disease vaccine strains.

MATERIALS AND METHODS

Virus strains

Ten WN virus isolates from pools of *Culex univittatus* mosquitoes caught at the Rondebult bird sanctuary, Germiston, in 1984 (Jupp *et al.* 1986) were included in the study. The isolates, all at mouse passage 3 and tissue culture passage 3 (M3TC3) were AR20724, AR20730, AR20735, AR20739, AR20758, AR20760, AR20761, AR20763, AR20769, AR20778. The South African WN prototype strain H442, at passage level M4TC3 was also tested, together with the Indian strain G2266 (M5TC3) and the Egyptian WN strain EG101 (M13TC3), which were obtained from the Yale Arbovirus Research Unit, New Haven, Connecticut, USA.

Antisera

Hyperimmune ascitic fluids (HAF) were prepared against all the WN strains using the method described by Tikasingh, Spence & Downs (1966).

Neutralization test (NT)

The NT test was based on that used by Blackburn & Swanepoel (1980). Each virus strain was adapted to Vero cells (Yasamura & Kawakita, 1963) and stored in aliquots as third passage material at −70 °C in 10% dimethyl sulphoxide. The viruses were titrated in quadruplicate just prior to use in the test. The HAF's were inactivated at 50 °C for 30 min and dilutions were made from 1 in 8 to 1 in 8192. A volume containing a calculated 100 $TCID_{50}$ of virus was added to each well and the serum-virus mixture was incubated for 1 h at 22 °C. The microtiter wells were seeded with 8×10^4 Vero cells per well. The virus was titrated in quadruplicate and the end point calculated by the method of Kärber (1931). If the titre of the virus used in the test was outside the range of 100 $TCID_{50} \pm Log_{10}0{\cdot}25$, the test was repeated. The tests were read after 6 days incubation and endpoints recorded.

The first group of cross-NT tests consisted of the ten local WN isolates and the South African prototype WN virus H442. These tests were carried out in duplicate and the neutralization titre was the dilution at which neutralization occurred in both replicates.

The second group of cross-NT tests included selected local isolates from the first group, H442, the Egyptian strain EG101 and Indian strain G2266. The HAF's of this group were tested in quadruplicate, the neutralization titre of each replicate was recorded and the average NT titre calculated.

Titre ratios

The 'R' values were calculated as described by Jordan & Gaylin (1953) where two virus strains were compared using homologous and heterologous antibody titres.

$$R = 100 \sqrt{r_1 \times r_2},$$

where

$$r_1 = \frac{\text{Heterologous titre (Virus 2)}}{\text{Homologous titre (Virus 1)}}.$$

and

$$r_2 = \frac{\text{Heterologous titre (Virus 1)}}{\text{Homologous titre (Virus 2)}}.$$

Using this formula 'R' is expressed as a percentage. A value of 50% or greater represents an up to fourfold total difference in heterologous and homologous cross NT titres, 24–49% represents a four to eightfold difference and 0–24% a greater than eightfold difference.

RESULTS

The cross neutralization test results for the first group of WN strains are shown in Table 1, and the 'R' values are in Table 2. The South African prototype strain H442 had a less than 25% relationship to six of the local strains and a 50% relationship to only one strain, AR20735.

Five of the local strains were selected to be tested against the strains G2266 and EG101, these were the prototype H442, AR20735 because of its fairly close relationship to H442, and representatives of the remaining local strains AR20730, AR20739 and AR20778. The cross neutralization results of the second group are shown in Table 3, the r_1 and r_2 results in Table 4 and the 'R' results in Table 5. The relationship of the local isolates to H442 was confirmed and with the exception of AR20735, they showed a closer relationship to the strain EG101 than to H442 or G2266.

DISCUSSION

The use of the 'R' value to assess the relationship between virus strains is discussed by Jordan & Gaylin (1953). This calculation reduces the four figures taken from heterologous and homologous titres to one figure expressed as a percentage, taking into account variation in r_1 and r_2 titre ratios.

The titre of virus used in the NT test is probably the most important factor for accurate reproducible results. In cross-neutralization studies wide variation in virus titre used will result in incorrect NT titres, therefore the 'R' value calculations will be invalid. Rweyemamu *et al.* (1977) took great care to ensure that 100 $TCID_{50}$ virus was used in all the tests. In this study the test results were only accepted if the virus dosage was within $\log_{10}0{\cdot}25$ of 100 $TCID_{50}$. The first group of viruses studied consisted of the South African WN prototype virus and ten local isolates. The majority of the isolates were significantly different to the prototype strain, only one strain, AR20735, having an 'R' value of over 35% against H442. Seven of the local isolates were fairly closely related, with the majority having 'R' values of 50% or greater to each other, these isolates were AR20724, AR20730, AR20758, AR20761, AR20763, AR20769 and AR20778. These results would suggest that these strains form one group distinct from AR20735 which is antigenically closer to H442 and AR20760 and AR20739 which give a range of 'R' values from 18% to 100 per cent against the seven isolates above.

None of the local isolates included in the second group of tests were significantly

Table 1. *Titres of cross-neutralizing antibody among 11 South African strains of West Nile virus*

	Hyperimmune ascitic fluid											
West Nile virus strains	H 442	AR 20735	AR 20761	AR 20763	AR 20769	AR 20724	AR 20730	AR 20739	AR 20758	AR 20760	AR 20778	$TCID_{50}$ used in test
H 442	*1024*	64	64	64	16	16	16	32	32	8	64	178
AR 20735	256	*64*	64	32	64	16	256	128	32	16	128	100
20761	128	8	*128*	32	32	64	128	64	32	32	128	56
20763	512	32	256	*256*	128	256	512	128	64	64	512	100
20769	512	128	128	128	*128*	256	512	128	128	32	128	178
20724	256	8	128	64	64	*256*	256	32	32	32	128	178
20730	128	16	256	256	128	64	*512*	64	128	32	128	178
20739	256	16	256	64	64	64	256	*256*	256	16	128	100
20758	128	32	256	128	64	256	512	256	*128*	32	256	178
20760	128	8	128	128	32	128	256	64	128	*128*	128	100
20778	128	16	256	64	32	128	512	256	256	64	*256*	56

Table 2. *Titre ratio or 'R' value between the 11 West Nile strains expressed as a percentage using the 'R' value formula (see Materials and Methods)*

	West Nile virus strains										
West Nile virus strains	H 442	AR 20735	AR 20761	AR 20763	AR 20769	AR 20724	AR 20730	AR 20739	AR 20758	AR 20760	AR 20778
H 442	100										
AR 20735	50	100									
20761	25	25	100								
20763	35	25	50	100							
20769	25	100	50	71	100						
20724	12	9	50	50	71	100					
20730	6	35	71	100	100	35	100				
20739	18	35	71	35	50	18	35	100			
20758	18	35	71	50	71	50	100	100	100		
20760	9	12	50	50	25	35	35	18	50	100	
20778	18	35	100	71	35	50	71	71	100	50	100

Table 3. *Titres of cross-neutralizing antibody among South African Indian and Egyptian West Nile strains*

West Nile virus strains	Hyperimmune ascitic fluid against: EG 101	G 2266	H 442	AR 20735	AR 20730	AR 20739	AR 20778	$TCID_{50}$ used in test
EG101	*596*	178	106	45	354	64	150	100
G2266	256	*1024*	708	16	224	64	128	100
H442	150	89	*1024*	64	16	32	64	100
AR20735	89	16	256	*64*	298	64	89	178
AR20730	149	16	256	16	*355*	89	178	100
AR20739	89	106	128	8	64	*89*	64	178
AR20778	53	53	128	16	128	34	*150*	178

Table 4. *Cross-neutralization activity ('r' values) between the South African, Indian and Egyptian West Nile strains*

West Nile virus strains	Hyperimmune ascitic fluid against: EG 101	G 2266	H 442	AR 20735	AR 20730	AR 20739	AR 20778
EG101	1	0, 17	0, 1	0, 7	1	0, 72	1
G2266	0, 43	1	0. 7	0, 25	0, 63	0, 72	0, 85
H442	0, 25	0, 09	1	1	0, 05	0. 36	0, 43
AR20735	0, 15	0, 02	0, 25	1	0, 84	0. 72	0, 59
AR20730	0, 25	0, 02	0, 25	0, 25	1	1	1, 19
AR20739	0, 15	0, 1	0, 125	0, 125	0, 18	1	0, 43
AR20778	0, 09	0, 05	0, 125	0, 125	0. 36	0. 72	1

Table 5. *Titre ratio or 'R' value between the South African, Indian and Egyptian West Nile strains expressed as a percentage using the 'R' value formula (see Materials and Methods)*

West Nile virus strains	West Nile virus strains: EG 101	G 2266	H 442	AR 20735	AR 20730	AR 20739	AR 20778
EG101	100						
G2266	27	100					
H442	16	25	100				
AR20735	32	7	50	100			
AR20730	50	10	11	46	100		
AR20739	33	27	21	30	42	100	
AR20778	30	21	23	38	65	56	100

closely related to either EG101 or G2266, although AR20735 and AR20730 were much closer to the Egyptian strain than the Indian. Contrary to the results of Hammam, Clarke & Price (1965) the South African prototype H442 was shown to be antigenically distinct from the Egyptian strain with an 'R' value of 16%. This is possibly due to the NT test being more specific than the modified haemagglutination inhibition (HI) test used in that study. Rweyemamu *et al.* (1977) found that 'R' values from NT results showed much wider differences between strains

than those calculated from the less specific complement fixation test. The differences between the specificity of the HI, CF and NT in flavivirus serology is clearly demonstrated by Blackburn & Swanepoel (1978).

Strain H442 had a 25% relationship to the Indian strain G2266. this was interesting in that the r_1 and r_2 values were so different. The r_1 value representing the ratio of H442 antibody titre against H442 virus and the heterologous G2266 virus was 0·7, whereas the r_2 value was 0·09. These results indicate that H442 antibodies have a significant neutralizing effect on G2266 virus, whereas the reverse would not be so. This also applied to three of the four local isolates included in the study group.

G2266 is antigenically fairly representative of the Indian strains, according to the results of Umrigar & Pavri (1977), although there must be some reservations about these results as they were based on the erroneous assumption that the calculation of the 'R' values eliminated discrepancies of different virus dosage. The possibility is that a prior infection with an Indian strain of WN virus will give poor protection against the introduction of South African strains.

The minor antigenic differences between the isolates could be due to minimal changes in the glycoprotein during the bird-mosquito transmission cycle. Mah Lee & Westaway (1983) observed that Kunjin virus, closely related to West Nile, underwent alteration in the properties of the envelope protein on passage in *Aedes albopictus* cells. The major antigenic differences demonstrated between the H442 strain and the local isolates, and these viruses against EG101 and G2266 would appear to place these viruses into separate groups. It needs to be determined if the more specific cross NT test is detecting subgroups of the two main antigenic groups, or the number of main groups is larger than previously suspected.

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AN ASSESSMENT OF TWO METHODS FOR ARTIFICIALLY INFECTING MOSQUITOES WITH ARBOVIRUSES*

by

P. G. JUPP, R. G. BROWN, and B. M. McINTOSH

Arbovirus Research Unit, South African Institute for Medical Research, Johannesburg

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In testing the susceptibility of mosquitoes to infection by arboviruses intra-thoracic inoculation and feeding upon infected blood-soaked cotton-wads are among methods which have been used by various workers, mainly on account of their simplicity and ease of application. However, these two methods possess obvious disadvantages should any conclusions be derived as to the natural susceptibility of the mosquito in question. These dangers are emphasized by the results presented in this paper. It became necessary to test the validity of these two infection routes when it was desired to attempt infection of mosquitoes with known graded doses of virus, a method which precludes the use of viraemic vertebrates as a means of infection.

MATERIALS AND METHODS

Mosquitoes. The mosquitoes used were *Aedes (Stegomyia) aegypti Linnaeus* and *Culex (Culex) fatigans* Wiedemann from laboratory colonies which originated from material collected at Durban and Johannesburg, South Africa, respectively. The age of the adult females was 1-5 days in the case of *A. aegypti* and 5-30 days in the case of *C. fatigans*. The mosquitoes were held in an insectary at 80 per cent relative humidity and 25°C.

Virus strain used. The virus used was the H442 strain of West Nile virus [Kokernot and McIntosh, 1959] at either its second or third intracerebral mouse-passage level.

Dilution of virus. For infecting the mosquitoes five ten-fold graded doses of the virus were prepared by serial dilution of virus stock with bovine plasma albumen (B.P.A.) for inoculation and with B.P.A.-defibrinated rabbit blood in the proportions 1:3 for membrane feeding. In the case of cotton-wad feeding and infection through tarsal contact only the most concentrated doses of virus made up in B.P.A.-defibrinated blood was used. The amount of virus in the highest dose was determined by titration in adult mice either just before or during attempted infection of the mosquitoes.

Methods of infection.

(a) Inoculation. Intra-thoracic or parenteral inoculation was carried out in a manner similar to that described by Aitken and Anderson [1959]. The mosquitoes were pricked in the mesothoracic pleurae, usually the post-spiracular area, with a mounting pin dipped in the virus suspension after they had been anaesthetized with carbon dioxide.

(b) Membrane feeding. For membrane feeding the mosquitoes were placed in two in. diameter perspex canisters, the tops of which were closed by mosquito netting and the bottoms by shaved, freshly-prepared mouse skins. The canisters were supported on glass beads in beakers containing the blood-virus suspension to allow adequate

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contact between membrane and suspension. The beakers were held in a 30°C water-bath during feeding.

(c) Cotton-wad feeding. The method employed for infecting mosquitoes by feeding on a cotton-wad saturated with blood-virus suspension was similar to that employed by Muspratt *et al.*, [1957]. The wad, saturated with blood-virus suspension, was placed on top of the cage housing the mosquitoes.

(d) Tarsal contact. Mosquitoes were anaesthetized individually with carbon dioxide and then brushed momentarily on a saturated wad of blood-virus suspension so that the distal segments of the tarsi came into contact with the suspension.

Determination of infectivity rates. The infectivity of the mosquitoes was determined by inoculation of a suspension of each mosquito into six adult (Experiment 1) or 12 infant mice (Experiments 2 and 3). The mosquitoes tested were those found dead from the 7th to 14th day after infection as well as survivors killed on the 14th day (Experiment 1) or on the 20th to 25th day (Experiments 2 and 3).

Estimation of infectivity threshold. In experiment 1 the infectivity thresholds were calculated from the infectivity rates following the method of Reed and Muench [1938], and were the virus concentrations required to infect 10% of the mosquitoes.

Experiments. In experiment 1, to evaluate intra-thoracic inoculation, the susceptibility of *A. aegypti* obtained for this method was compared with that obtained using membrane feeding. Five batches of *A. aegypti* were 'infected' with the graded viral doses using both methods. Membrane feeding lasted for two hours in the light.

Experiments 2 and 3 were designed to assess cotton-wad feeding. When a mosquito feeds on a blood-soaked wad it cannot avoid making tarsal contact with the wad. Therefore, the infectivity rates obtained with cotton-wad feeding were compared with those of controls in which infection was attempted by brushing legs (tarsi) on a similar infected wad. The mosquitoes, *C. fatigans,* were divided into two batches, the first being allowed to feed on a wad for three hours in darkness while mosquitoes in the second batch were 'infected' through tarsal contact only.

RESULTS AND DISCUSSION

The results of the experiments are summarised in Table I. In experiment 1, while with membrane feeding *A. aegypti* was completely refractory to infection at the highest concentration of virus used, viz., 4.9 logs, with the inoculation method a threshold of infectivity of 2.9 logs was obtained. In experiments 2 and 3, 75% and 70% infectivity rates resulted through feeding *C. fatigans* on the cotton-wads, while about half these rates, 31% and 36%, were recorded in mosquitoes exposed to tarsal contact alone.

It appears then that neither intra-thoracic inoculation nor cotton-wad feeding should be used for determining the vector capability of mosquitoes. In the first method virus entering the haemocoel of the mosquito via inoculation has apparently a much greater chance of infecting the mosquito than would virus ingested naturally with a blood-meal. This method would thus give fictitiously high susceptibility levels bearing no relation to what might occur in nature. To explain the reason for this would require further study. In the second method virus must gain access into the mosquito through the cuticle of the tarsus itself or through that of one or more of the appendages of the 5th tarsus, namely, the claws, pulvilli and empodia. However, since much lower infectivity rates were obtained by infection through the tarsi it would seem unlikely that a mosquito feeding on cotton-wads would become infected via the tarsi without also becoming infected from ingested virus. This method, therefore,

TABLE I

Showing the infectivity of mosquitoes infected by different artificial methods.

Expt. No.	Mosquito	Infection method	Titre of infective feed in logs	Mosquito infectivity		
				Period in days when mosquitoes tested for virus	Infectivity rate*	Infectivity threshold †
1	A. aegypti	Inoculation	4.9	7–14	11/11	2.9
			3.9		6/7	
			2.9		1/10	
			1.9		0/8	
			0.9		0/7	
	A. aegypti	Membrane feeding	4.9	7–14	0/11	>4.9
			3.9		0/18	
			2.9		0/12	
			1.9		0/16	
			0.9		0/18	
2	C. fatigans	Cotton-wad feeding	6.8	25	18/24 (75%)	
	C. fatigans	Tarsal contact	6.8	23	5/16 (31%)	
3	C. fatigans	Cotton-wad feeding	5.5	20	14/20 (70%)	
	C. fatigans	Tarsal contact	5.5	20	4/11 (36%)	

* Numerator=No. mosquitoes infected: denominator=No. mosquitoes tested.
† This is calculated from infectivity rates and is the titre of virus required to infect 10% of mosquitoes.

would seem unlikely to give a fallaciously high susceptibility, and on that account would seem permissible when other methods could not be applied.

SUMMARY

The susceptibility of mosquitoes to viral infection by intra-thoracic inoculation and by feeding upon blood-soaked cotton-wads was tested. It was possible to infect *A. aegypti* with West Nile virus by inoculation but not by ingestion. It was also found that while *Culex fatigans* was infected by this virus after feeding on cotton-wads, it was also infected through tarsal contact with such wads, although susceptibility by the latter route was much lower than by ingestion. It is suggested that mosquitoes should not be infected by inoculation or by feeding on blood-soaked cotton-wads when information on their natural susceptibility is required.

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Experimental assessment of the vector competence of *Culex (Culex) neavei* Theobald with West Nile and Sindbis viruses in South Africa

P. G. Jupp, B. M. McIntosh and N. K. Blackburn

Arbovirus Research Unit, National Institute for Virology, Private Bag X4, Sandringham, Johannesburg 2131, and Dept. of Virology, University of the Witwatersrand, Johannesburg, South Africa

Abstract

Culex univittatus is the maintenance vector of West Nile (WN) and Sindbis (SIN) viruses on the inland plateau of South Africa and also transfers infection to man. Both viruses have frequently been isolated from this mosquito and human immunity is high on the plateau. In the Natal lowlands where *Cx univittatus* is replaced by *Cx neavei*, apparently the main vector there, immune rates are low and few isolations have been made from this species. Vector competence experiments were carried out with *Cx neavei* to compare with those previously done with *Cx univittatus*. It was thought that a difference in such competence might explain the contrasting epidemiological situation in the two regions. After infective feeds on chicks with high viraemias of each virus, *Cx neavei* showed a high transmission rate. However, the 50% infection thresholds determined by exposing birds with differing viraemias to mosquitoes of this species, were 4·4 logs per ml (WN) and 5·8 logs (SIN). These thresholds are both higher than those for *Cx univittatus*. It is concluded that *Cx neavei* is a poorer vector of both viruses which accounts for the lower viral activity, including the lower incidence of human infection, in the Natal lowlands.

Introduction

The Arbovirus Research Unit in Johannesburg has made a long term study of the ecology and epidemiology of West Nile (WN) and Sindbis (SIN) viruses in South Africa. These investigations have shown a higher level of activity by both these viruses on the temperate high inland plateau than in the tropical and subtropical coastal lowlands of Natal (Fig. 1). The large inland plateau region consists of the Karoo, a semi-desert area and the moister highveld, an area of grassland, while the Natal lowlands are more wooded, stretching along the coast from Port Shepstone in the south of the Mozambique border in the north.

The mosquito *Culex (Culex) univittatus* Theobald is the primary vector of both viruses on the inland plateau where it is important both in the maintenance of these viruses in transmission cycles with wild birds and in causing human infection (McIntosh *et al.*, 1967; Jupp & McIntosh, *et al.*, 1967, 1970; McIntosh *et al.*, 1976, 1978). The number of virus isolations obtained from 70,037 *Cx univittatus* between 1956 and 1976 were 128 (WN) and 85 (SIN) and significant immune rates have been shown against both viruses in people living on the plateau. On the other hand, in the Natal coastal lowlands where *Cx univittatus* is replaced allopatrically by the closely related *Culex (Culex) neavei* Theobald (Jupp, 1971), which is apparently the main vector in this region, there were only one (WN) and four (SIN) isolations obtained (McIntosh *et al.*, 1972; McIntosh & Jupp, 1980; Worth *et al.*, 1961) and immune rates were low.

Table I shows the immune rates in people living in surveys (Kokernot *et al.*, 1956; Kokernot & McIntosh, 1959; McIntosh *et al.*, 1962a&b; Dickinson *et al.*, 1965) and unpublished surveys made since 1962 at Bethulie and Olifantsvlei in the plateau region (Arbovirus Research Unit, Johannesburg). The proportion of the population immune to WN and SIN viruses in samples [illegible] at 23 localities on the plateau and at 24 localities in the Natal coastal lowlands is shown based on antibodies detected by the neutralization test. For WN virus there were immune rates of 17·1% in the Karoo and 8·0% in the highveld areas as compared to only 0·2% in the coastal lowlands. Similarly, with SIN virus there were rates of 9·6% and 5·5% for the Karoo and highveld respectively as against only 1·1% in the Natal coast.

These differences in the viral isolation rates from mosquitoes together with the differences in human immune rates between the two regions prompted the experimental study reported below. It was thought that the two contrasting epidemiological situations found in the two regions may be accounted for by differences in vector competence between the two mosquito species. Previous experiments had shown *Cx univittatus* to be an efficient vector of both viruses (Jupp & McIntosh, 1970; Jupp, 1974, 1976). The present paper reports similar experiments done with *Cx neavei* to make a quantitative assessment of its vector competence for comparison with that already determined for *Cx univittatus*.

Materials and Methods

Mosquitoes

These were either drawn from established laboratory colonies or were the progeny reared from wild-caught females which were being used to start colonies. The category of mosquitoes used for each test is given in the tables. *Cx neavei* originated from Ndumu, Mkani's Drift or Mtubatuba in northern Natal, while *Cx univittatus* used in the previously published experiments came from the Johannesburg area. The age of the mosquitoes was 6 to 25 days.

Infective meals

The viruses used were the AR86 strain of SIN virus (Weinbren *et al.*, 1956) and the H442 strain of WN virus (Kokernot & McIntosh, 1959) at their fifth and sixth intracerebral mouse-passage level respectively. For most of the tests with SIN virus three additional passages were made in vero cells. Mosquitoes obtained infective meals from viraemic chickens or pigeons as described previously (Jupp, 1976). A sample of blood was taken from each bird just

the 2 regions based on published serological

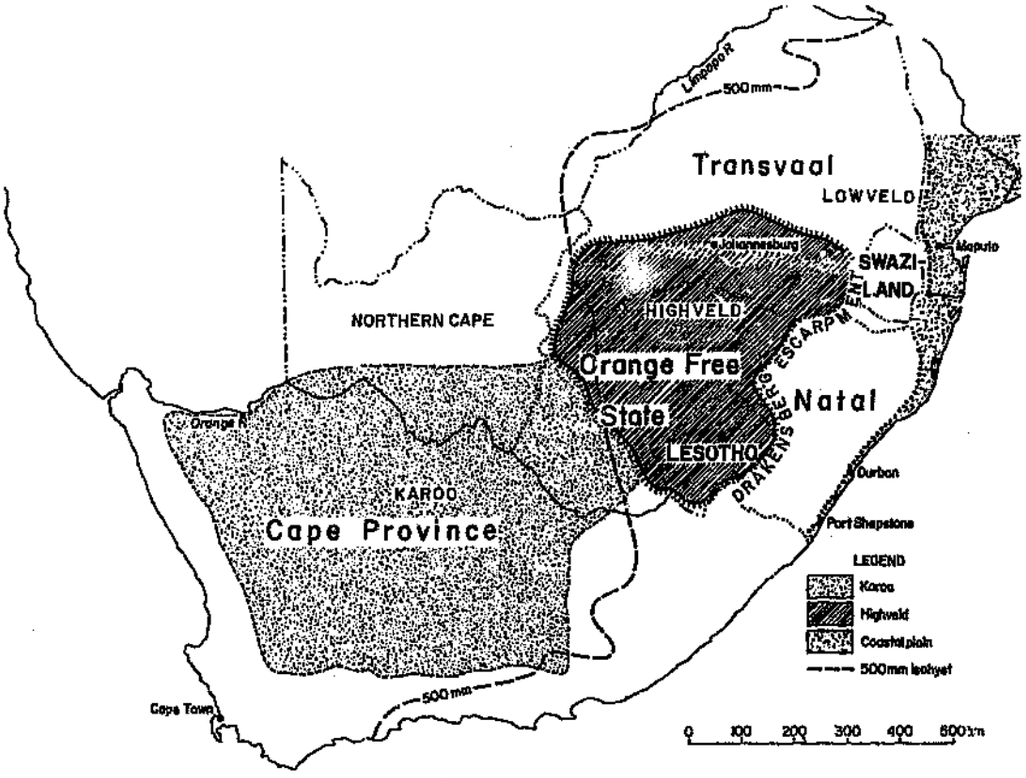

Fig. 1. South Africa, showing provinces, the Karoo, highveld and Natal coastal plain (lowlands). The 500mm isohyet bisects the country into eastern moist and western arid parts.

Table I—Antibodies to West Nile and Sindbis viruses in man in South Africa by the neutralization test

	Inland Plateau		Natal Coastal Lowlands
	Karoo; 14 localities	Highveld; 9 localities	24 localities
West Nile	169/987[a] (17·1%)	26/326 (8·0%)	1/533 (0·2%)
Sindbis	92/956 (9·6%)	18/326 (5·5%)	6/569 (1·1%)

a — numerator = number of sera positive, denominator = number of sera tested

before exposure to the mosquitoes so that the concentration of virus could be determined. This was done by intracerebral inoculation of infant mice and the titre expressed as the $\log_{10}$ LD_{50} per ml of inoculum.

Transmission tests

The transmission rate, i.e., the percentage of infected mosquitoes which successfully transmitted virus, was determined for each virus using the group of *Cx neavei* which fed on the chick circulating the highest level of virus. Between 17 and 23 days after attempted infection each mosquito was allowed to engorge on a different susceptible Syrian hamster. Successful transmission was assessed by testing the sera of these hamsters three weeks later for antibodies against WN or SIN virus by the haemagglutination inhibition test.

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 8 to 12 infant mice. Using the probit method as recommended by DOUGHERTY (1964), a 50% infection threshold was calculated for each virus from the infection rates determined for feedings on birds with different viraemia levels. Since the infectivity titre of WN virus is consistently 1·0 log greater in infant than in adult mice (JUPP, 1974), titres derived from the use of infant mice were reduced by 1 log so as to permit comparison with those determined in adults in some of the previous experiments with *Cx univittatus*.

Results

The transmission rates determined in the experiments with *Cx neavei* are shown in Table II where they are also compared with the rates previously determined with *Cx univittatus* (see JUPP & MCIN-

Table II—Mosquito transmission rates to hamsters by *Cx neavei* and to chickens by *Cx univittatus*

	West Nile virus				Sindbis virus			
	Mosq. colony generation	Titre infective feed (logs per ml.)	No. days after infective feed	Trans. rate	Mosq. colony generation	Titre of infective feed (logs per ml.)	No. days after infective feed	Trans. rate
Cx neavei	P	5·7	21	29/29	P	6·7	23	24/31 (77%)
Cx univittatus	F^{5+6}	5·8–6·3	17	35/36[a] (97%)	F^{14+15}	6·8	18	12/21[b] (57%)

[a] Jupp (1974)
[b] Jupp & McIntosh (1970)
P = progeny of wild-caught females

TOSH, 1970; JUPP 1974). It can be seen that when mosquitoes of both species were infected by feeding on birds circulating high titres of WN virus, the subsequent transmission rates were 100% (*neavei*) and 97% (*univittatus*). In the case of SIN virus transmission rates were 77% (*neavei*) and 57% (*univittatus*).

Table III shows the infection rates obtained for *Cx neavei* over a range of viral doses with WN virus. The 50% infection threshold calculated from these rates was 4·4 logs for *Cx neavei* as compared to only 2·1 logs for *Cx univittatus*. Table IV shows the results for similar infection tests with SIN virus. The 50% infection threshold was 5·8 logs for *Cx neavei* as compared to 4·9 logs for *Cx univittatus*.

Discussion

It might be concluded from the high transmission rates that both species of mosquito would be efficient vectors of both viruses. However, it has previously been shown by our laboratory that the maximum titre of virus reached in the blood of different species of wild birds, the vertebrate maintenance hosts of both viruses, does not exceed 6·7 logs/ml (McINTOSH *et al.*, 1969). Hence it would be only those mosquitoes which are susceptible to infection at viral titres lower than this which would be expected to act as efficient vectors. This was the reason we determined 50% infection thresholds for *Cx neavei* so as to compare the relative susceptibilities of the two species to the viruses. The thresholds determined indicate that *Cx univittatus* is significantly more susceptible to WN virus than *Cx neavei*. *Cx univittatus* is also more susceptible to SIN virus than the other species but in this case the difference between the two thresholds was only 1 log of virus as compared to the more than 2 logs with WN virus. Nevertheless, the infection threshold of 5·8 logs would severely limit the number of *Cx neavei* mosquitoes which could become infected after feeding on viraemic birds.

It does not appear that there are other species of mosquito which are more efficient vectors of either virus in the Natal coastal lowlands judging from the pattern of virus isolations from all species collected there. There have been single isolations of WN virus from *Cx neavei*, *Aedes circumluteolus* and *Coquilletidia microannulata* and the following isolations of SIN virus: *Cx neavei* (four), *Mansonia africana* (one), *Ae. circumluteolus* (two), *Ae. cumminsi* (one) and *Cx tigripes* (one) (WORTH *et al.*, 1961; McINTOSH *et al.*, 1972, and Arbovirus Unit, Johannesburg, unpublished).

It is concluded therefore, that the lower susceptibility of *Cx neavei* to infection with both WN and SIN viruses shown in the experiments accounts for its poorer vector capability with these viruses. This affords an explanation as to why there is a far lower natural infection rate by both viruses in *Cx neavei* in the Natal coastal lowlands and a far lower incidence of human infection there as compared to that on the inland plateau where the closely related mosquito *Cx univittatus* is a more efficient vector. *Cx neavei* is considerably more anthropophilic than *Cx univittatus* and if this attribute were coupled to a high vector competence, one would expect not only a higher incidence of both viruses in natural populations of this mosquito but also a much higher level of human infection in the Natal coastal lowlands than occurs.

Table III—Quantitative mosquito infection tests with West Nile virus

	Cx neavei				*Cx univittatus*
Mosq. colony + generation	Titre of infective feed (logs per ml.)	No. days after infective feed mosqs. tested	Infection rate	50% infection threshold (logs)	50% infection threshold
C.158; P	5·7	12–28	31/32 (97%)		
C.158; p	4·0	13–29	8/33 (24%)	4·4	2·1 (JUPP, 1976)
C.125; P	3·7	15–18	1/25 (4%)		
C.125; P	3·2	15–18	2/24 (8%)		

Table IV—Quantitative mosquito infection tests with Sindbis virus

	Cx neavei				*Cx univittatus*
Mosq. colony + generation	Titre infective feed (logs per ml.)	No. days after infective feed mosqs. tested	Infection rate	50% infection threshold (logs)	50% infection threshold
C. 158; p	6·7	23–35	39/47 (83%)		
C. 169; F_{13+14}	6·3	15	20/30 (67%)	5·8	4·9 (JUPP, 1976)
C. 169; F_{13+14}	5·1	15	10/30 (33%)		
C. 169; F_{13+14}	4·6	15	2/27 (7%)		

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Book Review

The pyrethroid insecticides. J. P. Leahey (Editor). Taylor & Francis, 1985. 440 pp., illus. Price: £65·00. ISBN: 0-85066-283-4.

Solvent extraction of the flower heads of certain species of chrysanthemum yields pyrethrum extract, the insecticidally-active constituents of which are known as pyrethrins. The synthetic analogues of the constituent esters of these pyrethrins are referred to as pyrethroids. This complex group of chemicals forms the subject of this collection of six papers, covering important aspects of the subject, by expert authors from England, France and the USA. The cut-off date for the growing amount of information has been set at the beginning of 1984.

The perfect insecticide is one which will kill insects effectively for a considerable period without adversely affecting the environment, particularly non-target organisms. The claim has been made, perhaps rashly, that pyrethroids are the most perfect group of insecticides yet available. The first paper in this book details the history and development of pyrethrum, introduced into Europe in the middle of the last century and still an effective and acceptable knock-down insecticide, and goes on to describe the synthesis of the pyrethroid group, the first member of which, allethrin, appeared in 1950. It has many advantages over other insecticides but was unstable in light. The first light-stable pyrethroids came on the market in 1970. These had effective residual insecticidal properties, a rapid breakdown and low mobility in soil and were quickly metabolized and excreted by animals. This and much else is covered by two papers, one on the mode of action and another on mammalian toxicity. It is pointed out that, while pyrethrins are accepted as safe, more by usage over a long period than by factual data, there is more data available on pyrethroids than any other insecticide group.

The effects of pyrethroids on non-target organisms are covered in a separate chapter. Toxicity for mammals is low (indeed I have several times witnessed my commercial colleagues expressing their faith in this by drinking glasses of the insecticide without any apparent ill effects!). The toxic effect on birds is even less, but most pyrethroids are toxic to aquatic invertebrates and fish, and particularly dangerous to honey bees. A paper on the metabolism and environmental degradation of the group points out that they break down readily in aerobic soil, rather more slowly in anaerobic conditions, with a half-life of from one to 16 weeks. They are very unlikely to move from soil into other systems.

The final chapter on the uses of pyrethroids in agriculture, public health and animal husbandry highlights the enormous increase in usage, from US$10 million in 1976, US$220 million in 1978, to US$630 million in 1983, one quarter of the total world market for insecticides.

The six papers of this book, each with a good list of references, and a sensible index, make this an erudite and comprehensive work on the subject, informative and easy to read by anyone with a special interest in insecticides.

N. R. H. BURGESS

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

A. J. CORNEL AND P. G. JUPP

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

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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Sindbis and West Nile Viruses: an electron microscope study of salivary gland infection in the vector mosquito *Culex univittatus*

by

A.H. WIEDERHOLD[1], P.G. JUPP[2] and J.J. ALEXANDER[1]

[1]Department of Microbiology, University of the Witwatersrand, and [2]National Institute for Virology, Department of Virology, University of the Witwatersrand, Private Bag X4, Sandringham 2131

Culex univittatus Theobald was infected by intrathoracic inoculation with Sindbis virus and West Nile virus respectively. Salivary glands were dissected out after 7 and 20 days extrinsic incubation and studied by transmission electron microscopy. Sindbis virus particles were always observed either within cytoplasmic vacuoles or accumulated in the cisternae of the endoplasmic reticulum where crystalline arrays were sometimes seen. Particles of West Nile virus were far fewer, not compartmentalized, and formed viroplasms without crystalline arrays. Vesicular structures were present in cells infected with this virus. No cytopathogenic changes occurred except possibly the distension of the endoplasmic reticulum by Sindbis virus particles. How the superior vector capability of *Cx. univittatus* with West Nile virus over Sindbis virus could be explained by these ultrastructural observations is discussed.

INTRODUCTION

Culex (Culex) univittatus Theobald is the primary vector of West Nile (WN) and Sindbis (SIN) viruses on the temperate inland plateau in South Africa (Jupp & McIntosh 1970; McIntosh *et al.* 1978). This mosquito is a more efficient vector of WN virus than SIN virus according to vector competence experiments which showed a lower infection threshold and higher transmission rate with WN virus (Jupp & McIntosh 1970; Jupp 1974, 1976).

Several electron microscope studies have been carried out on the ultrastructure of arbovirus-infected mosquito salivary glands (Janzen *et al.* 1970; Scott & Burrage 1984; Sriurairatna & Bhamarapravati 1977; Takahashi & Suzuki 1979; Withfield *et al.* 1971, 1973). However, no studies have been reported on infected salivary glands of *Cx. univittatus* or on SIN virus or WN virus infected mosquito salivary glands. We therefore decided to undertake a study of the salivary glands of *Cx. univittatus* infected with WN and SIN viruses respectively. We hoped that such an examination of the infection pattern with each virus would reveal differences that might explain the different vector capabilities of the vector. Furthermore, we wished to look for any evidence of ultrastructural pathogenic changes associated with either of these viruses.

The anatomy of uninfected salivary glands has been determined by means of both light and electron microscopy for *Anopheles stephensi* (Wright 1969) and *Aedes aegypti* (Janzen and Wright 1971). Mosquitoes have 2 glands, each of which consists of a median and 2 lateral lobes. Each lobe is composed of different regions. The lateral lobe has proximal, intermediate and distal regions, while the shorter median lobe consists of an intermediate region where the 3 lobes join and a distal region. Each lobe consists of a single layer of tubular epithelial cells surrounding the chitinized cuticle-lined duct. This duct is continuous with the salivary gland duct extending to the mouthparts. The cell type of each region is distinctive and in the distal region apical cavities store the secretary material made by the glandular epithelial cells.

In a competent mosquito vector infected with an arbovirus, viral multiplication first occurs in the epithelial lining of the midgut, from which infection spreads to the haemolymph which carries virus to the salivary glands. Transmission by bite to another vertebrate host will not occur until the salivary glands have become infected. Viral replication occurs in the epithelial cells and virus particles are ultimately secreted into the duct along with the normal secretory products.

MATERIALS and METHODS

Viruses

The viruses used were the H442 strain of WN virus (Kokernot & McIntosh 1959) and the AR88 strain of SIN virus (Weinbren *et al.* 1956). Both had been passaged 5 times intracerebrally in infant mice followed by 5 times in Vero cells.

Mosquitoes

The F_2 generation of a laboratory colony, which originated from adult females collected at Bethulie, Orange Free State Province, South Africa, were inoculated with either virus at 1–10 days of age. The mosquitoes were housed in an insectary where the temperature was maintained at 24–26 °C and the relative humidity at 75–80%. The daily light cycle was provided by controlled artificial lighting and consisted of a day period of 12 hours, a night period of 10 hours and twilight periods of 60 minutes each when the light intensity was either gradually increased (dawn) or decreased (dusk).

Infection of mosquitoes

The intrathoracic inoculation technique of Rosen & Gubler (1974) was used to infect more than 100 female mosquitoes with either virus. Seven days later infectivity was confirmed in a sample of 6 mosquitoes by inoculating homogenates of each insect intracerebrally into 8 infant mice. A mosquito was judged to have been infected if all 8 mice died within the expected death time of the respective virus, and if brain material from these mice was positive by the fluorescent antibody test (Shope and Sather 1979).

Transmission electron microscopy

Mosquitoes were killed with carbon dioxide and their salivary glands were immediately dissected directly into 0,1 M sodium cacodylate buffer and fixed on the slide in buffered 2,5% gluteraldehyde for 2 hours at room temperature. The glands were then rinsed in buffer and transferred to a molten drop of 1,5% noble agar and immediately

chilled on a bed of ice. The trimmed blocks of agar containing the specimen were postfixed for 30 min in 1,0% buffered osmium tetroxide and dehydrated in a graded ethanol series containing uranyl acetate. The ethanol was then replaced by propylene oxide and the specimen embedded in Araldite resin. Specimens were sectioned on a Reichert Om U3 ultramicrotome. Thin sections were stained with lead citrate (Venable & Coggeshall 1965) and examined with a JEOL JEM-100S electron microscope.

In the case of each virus, salivary glands were dissected from 30 infected and 15 uninfected mosquitoes on both day 7 and day 20 after inoculation. For each virus, several sections were successfully prepared from salivary glands from 12 infected mosquitoes and 6 uninfected control mosquitoes killed on each of the two dates.

RESULTS

The anatomical structure of the salivary glands of female *Cx. univittatus* appeared essentially the same as those of other mosquito species previously described (Wright 1969; Janzen & Wright 1971). The infection pattern in the glands did not differ at day 7 or at day 20 post-inoculation in the case of either virus. Apart from the distension of the endoplasmic reticulum by particles of SIN virus, no ultrastructural damage to the cell membranes or organelles was observed that could be attributed to WN or SIN virus infection.

In the uninfected controls, vacuoles without virus particles were observed in the epithelial cells. Similar vacuoles containing virus particles were shown in SIN

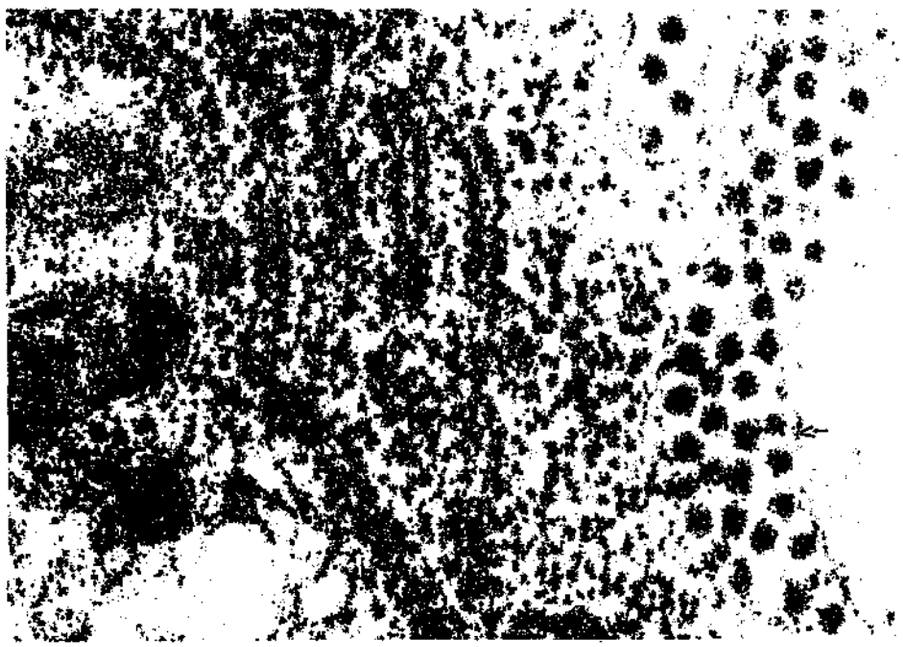

Fig. 1. Elongated vacuole (arrow) distended by enveloped SIN virus particles associated with endoplasmic reticulum (ER). (× 75 000)

virus-infected cells but it appeared that infection with this virus did not cause an increased vacuole formation. The nuclei and mitochondrial cristae of infected cells were similar in the case or both viruses and did not differ from those of uninfected cells. Cells from similar regions of the salivary glands exhibited a similar degree of granularity in the case of both types of infected glands and controls. No particles of either virus were detected in the intermediate region of the lateral lobes but particles of SIN virus were often found in the apical cavities of the proximal region in association with micro-villi-like structures. Particles of each virus were recorded in the distal portion of the lateral lobes which are regions of electron dense secretory material and rich in convoluted endoplasmic reticulum. However, because the number of WN viral particles was lower than that of SIN virus, particles of WN virus may have gone undetected in the proximal region.

Intracellular SIN virus particles were spherical with diameters of 50–70 nm. High magnifications showed this virus to have an electron dense core of 30–45 nm, surrounded by a less dense envelope layer (Figs. 1 & 2). This structure is in agreement

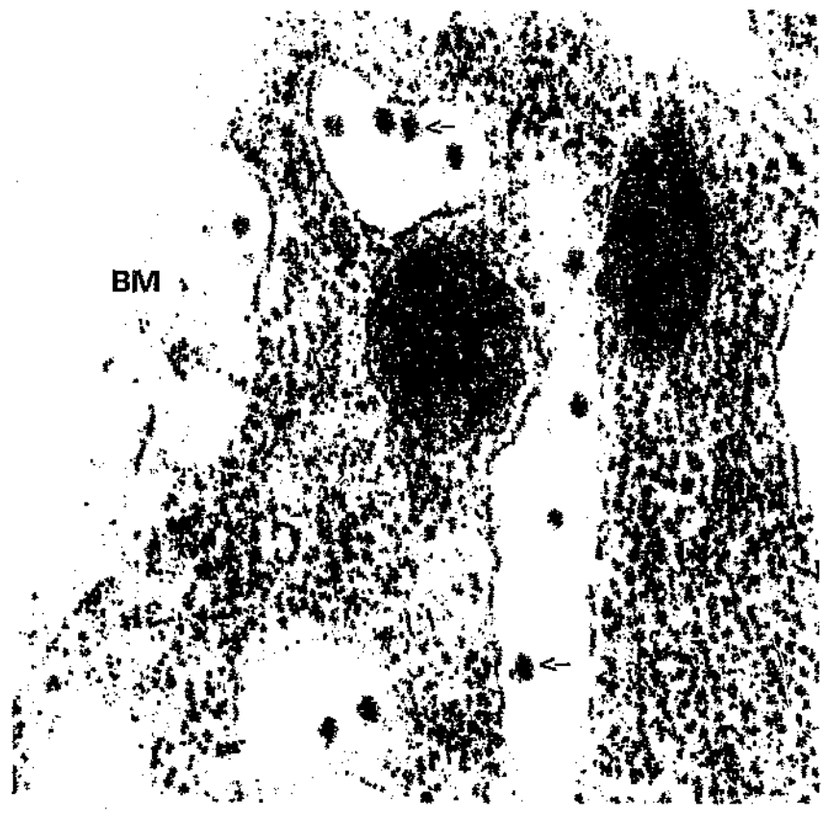

Fig. 2. SIN virus particles in the lumen of vacuoles (arrow) adjacent to the basal membrane (BM) of a salivary gland cell. (× 60 000)

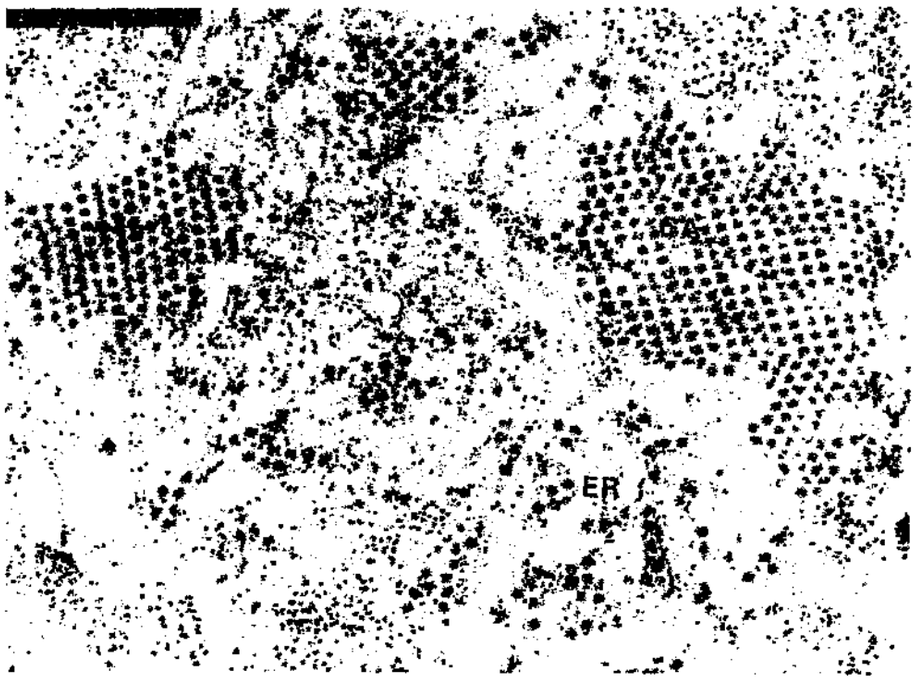

Fig. 3. Crystalline arrays (CA) of virus particles associated with distended endoplasmic reticulum (ER) 7 days after SIN virus was inoculated into the haemocoel of a *Cx. univittatus* female. (× 31 400)

with the observations of other workers (Harrison 1986). WN virus particles had a similar morphology and were 40–50 nm in diameter (Fig. 5) which is in agreement with Brinton (1986).

The infection patterns caused by SIN virus and WN virus differed distinctly. SIN virus particles were always compartmentalized within the cytoplasm, often in vacuoles (Fig. 2), but more frequently associated with the endoplasmic reticulum (Figs. 1 & 3). Particles of WN virus, on the other hand, were not compartmentalized although vacuoles did occur in the cytoplasm. WN virus infection was, however, associated with the presence of vesicular structures (Figs 4 & 5). These vesicles were about 60 nm in diameter and many had a dense thread-like region (Fig. 5). Not all glandular cells examined contained virus particles, but those that did had an abundance of particles in the case of SIN virus while there were far fewer particles in the case of WN virus. SIN virus particles were seen in some parts of the glands as large aggregations in the form of crystalline arrays (Fig. 3) while the WN particles were observed individually or in small viroplasmic aggregations without crystalline arrays (Fig. 4). There was no evidence of capsids budding through cellular membrane systems for either virus.

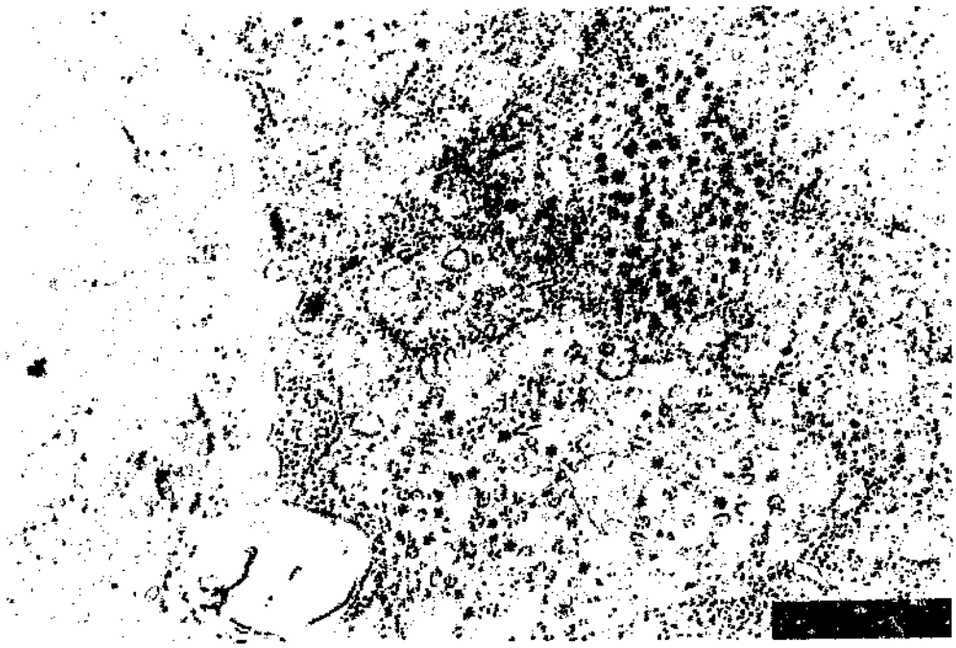

Fig. 4. An aggregation of WN virus particles (A) and scattered individual virus particles (arrow) with associated vesicles. (× 30 600)

DISCUSSION

It is well known that arboviruses multiply within the salivary glands of their mosquito vectors. Although we failed to observe SIN or WN virus nucleocapsids budding through membranes in *Cx. univittatus* salivary glands, each virus must multiply within the glands since viral transmission continues throughout the mosquito's life as shown by vector competence experiments (Cornel 1987).

SIN virus particles were compartmentalized within membranous cytoplasmic structures such as vacuoles and endoplasmic reticulum. In many cases the endoplasmic reticulum was extremely distended by viral particles which sometimes formed crystalline arrays within its cisternae. This is very similar to the infection pattern of the alphavirus eastern equine encephalitis (EEE) in salivary gland cells of *Aedes triseriatus* (Whitfield *et al.* 1971). Viral particles seemed to accumulate without ready access to the secretory material of the apical cavity

WN virus particles and vesicles associated with WN viral infection were observed in the cytoplasm, possibly representing nascent capsid protein. These vesicles may be characteristic of flavivirus infections of salivary glands as they have been described previously for dengue-2, Japanese B encephalitis and St Louis encephalitis viruses (Sriurairatana & Bhamarapravati 1977; Takahashi & Suzuki 1979; Whitfield *et al.* 1973). In the previous studies, however, the flavivirus particles were always compartmentalized within the cytoplasm and a preference for membranes of the endoplasmic reticulum was noted. This was not the case for WN virus.

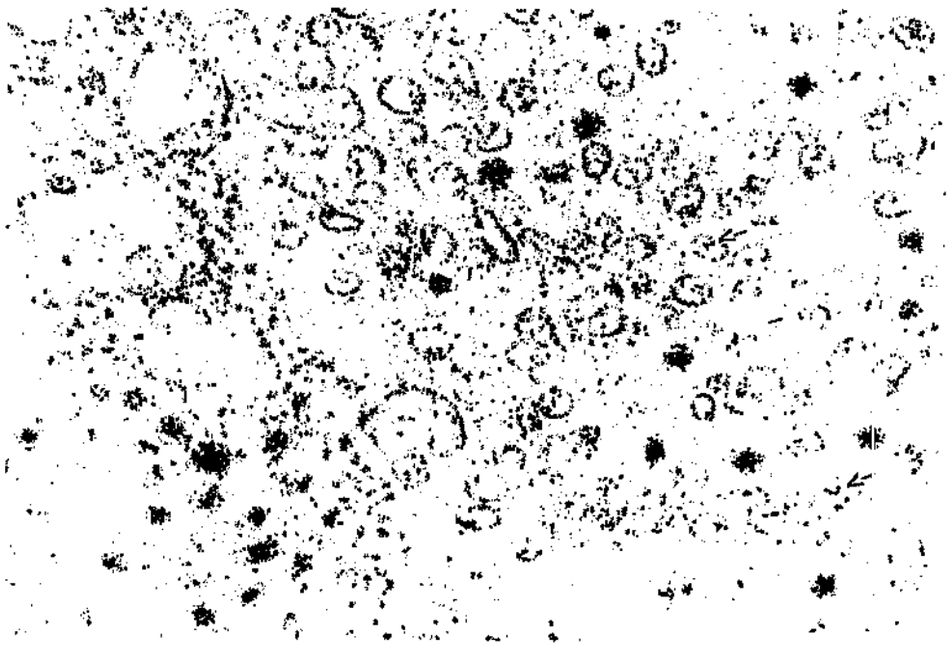

Fig. 5. Higher magnification of characteristic vesicles with a threadlike dense region (arrows) associated with WN virus infected salivary glands (× 75 700)

It appears that particles of SIN virus have difficulty being secreted into the salivary gland ducts because of their mass accumulation in t[illegible] cytoplasm without ready access to the apical cavities. Their secretion probably only occurs once a certain threshold concentration has been reached in the cytoplasm. On the other hand, particles of WN virus occur free in the cytoplasm and were never seen to build up to such large numbers as the SIN virus particles. Rather, it seems that they are being continually secreted. Such a more enhanced and consistent secretion of WN virus would explain the higher vector capability shown for this virus in vector competence tests (Jupp & McIntosh 1970; Jupp 1974, 1976). It would also explain why transmission rates fluctuate with time much more in mosquitoes infected with SIN virus (Cornel 1987).

The only morphological change in response to infection which might be evidence of cytopathogenesis was the distension of the endoplasmic reticulum by particles of SIN virus. Apart from this, cell organization in glands infected with either virus remained the same as in uninfected glands over the 20 days of the experiment.

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ECOLOGICAL STUDIES ON SINDBIS AND WEST NILE VIRUSES IN SOUTH AFRICA. II. — MOSQUITO BIONOMICS*

P. G. JUPP AND B. M. McINTOSH
Arbovirus Research Unit, South African Institute for Medical Research, Johannesburg
[RECEIVED 24TH OCTOBER, 1966]

In the first of this series of papers McIntosh *et al.*, [1967] reported on the infectivity of wild-caught mosquitoes with Sindbis (Sd) and West Nile (WN) viruses in the highveld region of South Africa.

From these virus studies and corroborative evidence it was concluded that *C. univittatus* is the primary vector of both viruses.

Concurrently with these infectivity studies observations were also made on mosquito bionomics. In this study particular emphasis was paid to prevalence and feeding preferences with a view to more firmly identifying the vector or vectors involved in the wild transmission cycle as well as those causing human infection. Most mosquito collections were, however, designed primarily for the collection of large numbers of mosquitoes for attempted virus isolation and information on prevalence and feeding preferences has necessarily been obtained largely from routine collections. The procedure adopted in these collections has been described [McIntosh *et al.*, 1967].

The major part of these studies was conducted at Olifantsvlei, a sewage effluent disposal area five miles from the outskirts of the city of Johannesburg. Minor studies were also made at a subsidiary study area on a farm at Lake Chrissie in the Eastern Transvaal province. Small mosquito collections were also made at the following highveld localities: — Welkom, Boksburg, Vereeniging, Germiston, Standerton, Deneysville, Vaal Barrage and Bloemhof. Those at Welkom and Boksburg were made shortly after human infections with WN and Sd viruses respectively had occurred at these towns. The studies at Olifantsvlei and Lake Chrissie dealt with in this paper extended over a period of three consecutive mosquito seasons, from the spring of 1962 until the autumn of 1965. Topographical and climatic features of the main study areas are described in the companion paper [McIntosh *et al.*, 1967].

METHODS

Mosquito collecting

1. *Lard-can trap.* Portable lard-can traps of the type designed by Bellamy and Reeves [1962] were used with solid carbon dioxide as an attractant in the manner described by Hayes *et al.*, [1958], except that about 5 lb. of broken dry ice was used instead of a 2½ lb. block and this was contained in a cardboard box instead of the kraft paper wrapping. On occasions, this trap was baited with a fowl secured in a nylon stocking [Reeves *et al.*, 1961]. The traps were suspended, usually from trees, about 4–6 ft. above ground level. The carbon dioxide baited lard-can was the most frequently used trap.

2. *Fowl-pen trap.* This trap, based on the design of Rainey *et al.*, [1962], was baited with 6 to 12 fowls and had an east-facing window trap on the side opposite to the inlet.

3. *Net-trap.* This is a modified version of the trap used by Moorhouse and Wharton [1965]. It consisted of a rectangular mosquito net, measuring 7 ft. long, 5½ ft. wide and 6 ft. high, supported on poles and having a zip at one end to permit access. When set, the sides were rolled up to provide an 8-inch opening all round to allow entry of

* The studies and observations on which this paper is based were financed jointly by the South African Institute for Medical Research, the Poliomyelitis Research Foundation and the South African Council for Scientific and Industrial Research.

mosquitoes. For collection of the catch the sides were dropped to the ground to form a mosquito-proof seal. Animal bait was secured in a cage of 2-inch wire-mesh placed on the ground in the centre of the net. The mosquitoes were either collected individually in test tubes off the net or a knock-down aerosol was employed. In the latter case, the bait was removed and a white sheet was placed on the ground before spraying.

4. *No. 2 trap.* This trap is made of galvanized sheet-iron with a diameter of 13 inches and a length of 12 inches. One end has an invaginated mosquito-gauze cone with a hole 1 inch in diameter for entry of mosquitoes and the other end an outwardly projecting "Perspex" cone. When in operation the end with the gauze cone faces downwards, the mosquitoes entering from below. When mosquitoes attempt to leave the trap they fly upwards, through the "Perspex" cone and into a collecting cage resting over the apex of this cone. The bait rests on a wide mesh screen placed horizontally just above the mosquito entrance hole.

5. *Human-bait catches.* In these collections the bait was exposed in the open and mosquitoes alighting on the bait were collected in test-tubes by hand. Usually the collectors operated in pairs, the one acting as bait and the other catching. At night shaded torches were used.

6. *Room catches.* Mosquitoes were collected by hand in test-tubes while resting in an empty room, the door and window of which were left open. The room remained in darkness except while collecting was in progress. Collections were made irregularly during the first half of the night.

Host preference studies with net-traps

For this purpose variously baited net-traps were set simultaneously on five consecutive nights at the same site at Olifantsvlei. The traps were set from 1800 to 0600 hours, except the human-baited one which was operated from 1800 to 2100 hours. The traps were placed to form a hollow square, each trap being 50 yards away from its neighbours. In addition to an unbaited control trap, traps were baited either with 8 fowls, a goat or man. The animal baits were confined within the wire cage as described above but when man-baited the cage was not used. In this case the individual merely sat within the net and collected mosquitoes alighting on himself. With fowl- and goat-baited traps the proportion of fed to unfed mosquitoes was recorded. In man-baited catches mosquitoes collected alighting on the bait were recorded as fed and the remainder within the trap as unfed.

Analysis of results

For host preference and population studies, Williams' modification of the geometric mean (Mw) as recommended by Haddow [1960] for mosquito collections was used. To facilitate comparisons these means were converted to a percentage basis in the table. In cases where a population index was calculated, this was based on the Mw obtained from numbers of mosquitoes collected in all dry-ice baited lard-can traps set during any one month. The proportion of engorged mosquitoes is expressed in the tables as the arithmetic mean of the percentages engorged from all catches of a series.

Mosquito identification

The mosquito nomenclature used follows that of Stone *et al.*, [1959], with the exception that their Culex *(Culex) pipiens quinquefasciatus* Say is referred to as *Culex (Culex) fatigans* Wiedemann.

Both *C. pipiens* and *C. fatigans* occur in the highveld but in our routine identification of females of this complex no attempt was made to differentiate between the two, all specimens being identified as *pipiens*. From a practical viewpoint, there is reason to believe that this was, in fact, the actual composition of the majority of the collections at the rural habitats of Olifantsvlei and Lake Chrissie. This opinion is based on

identifications made from male terminalia of adults bred out from females collected at both localities. For instance, at Olifantsvlei, males from 288 different sources were all identified as *pipiens*. At Lake Chrissie, fewer males were examined but *fatigans* was not found there either. Less assurance exists in respect of collections in the suburban habitats at Welkom, Boksburg and Vereeniging where no determinations from male terminalia were made, but even here the collecting methods used strongly favoured *pipiens* for it does seem from trapping in Johannesburg where *fatigans* occurs that this species does not enter the lard-can trap readily.

Although females of *Aedes (Aedimorphus) mixtus* Edwards and *Aedes (Aedimorphus) microstictus* Edwards are difficult to distinguish and though both may occur in the highveld [Muspratt, 1955] only *mixtus* was identified from male terminalia as occurring at Olifantsvlei. For this reason, as well as the apparent rarity of *microstictus*, all female specimens of this group collected were probably *mixtus* and were so named.

RESULTS

Efficacy of traps

At the outset of this study it was not known what kinds of mosquito trap would be most suitable. This unfortunately has resulted in some irregularity in collecting methods as various traps were introduced during the course of the study and either retained or discarded, depending upon their suitability.

The lard-can and fowl-pen traps have proved extremely successful in collecting *pipiens, theileri* and *univittatus*. Lard-cans baited with dry-ice were particularly useful, catches per trap of over 3,000 mosquitoes per night being frequently recorded at Olifantsvlei. Such large catches severely limited the number of observations that could be made owing to the time required for their identification. This factor was of particular significance in attempting to estimate population densities from lard-can collections. The lard-cans and fowl-pens collected few aedine mosquitoes. In an attempt to overcome this deficiency the net-trap was introduced. Experience with this trap showed that most species entered it readily and on this account it is probably the most useful single method for sampling the highveld fauna, although it failed to collect *Mansonia (Coquillettidia)* species adequately. However, it is cumbersome and collection of large catches from this trap is time-consuming. The No. 2 trap was designed primarily to overcome the difficulty in removing captured mosquitoes from lard-cans, particularly when these were required alive for rearing or other purposes. In this it has been successful, but only *pipiens* and *univittatus* entered it in significant numbers.

Prevalence of species in different highveld localities

Although mosquito collecting methods in different areas varied to a certain extent and consequently precise comparison of different areas is not possible, it is important that some analysis be attempted. This is especially necessary, since Olifantsvlei, the main study area, is an artificial habitat, thus raising the question of the applicability of results obtained there to the natural highveld habitat. It was because of concern on this point that Lake Chrissie was included as a subsidiary study area.

In table I the results of animal-baited net-traps and dry-ice baited lard-cans set at Olifantsvlei and Lake Chrissie are shown. The fact that different baits were used in the net-traps at the two localities is believed not to have significantly influenced these results. In assessing these results it should be borne in mind that lard-cans sample only *pipiens, theileri* and *univittatus* adequately, and as only few observations were made with the net-traps, collections with the latter traps have not truly reflected the seasonal nature of the *Aedes* species.

TABLE I

Relative prevalence of mosquitoes at Olifantsvlei and Lake Chrissie

Locality	Olifantsvlei						Lake Chrissie					
	Net-trap & goat* (11)†			Lard-can & CO_2 (218)			Net-trap & sheep (6)			Lard-can & CO_2 (21)		
Species	Total mosqs. collected	Mw‡	Mw as %	Total mosqs. collected	Mw	Mw as %	Total mosqs. collected	Mw	Mw as %	Total mosqs. collected	Mw	Mw as %
An. coustani	5	<1	<1	4	<1	<1	—	—	—	0	—	—
An. tenebrosus	98	5	1	79	<1	<1	1	<1	<1	0	—	—
An. squamosus	5	<1	<1	1	<1	<1	67	8	6	2	<1	<1
A. caballus	26	1	<1	51	<1	<1	16	2	1	10	<1	<1
A. dentatus	182	5	1	581	1	1	44	4	3	83	2	3
A. lineatopennis	334	15	3	932	2	2	82	8	6	99	2	3
A. mixtus	138	4	1	450	1	1	1	<1	<1	11	<1	<1
C. pipiens	2,475	233	53	59,630	42	41	1	<1	<1	12	<1	<1
C. theileri	2,209	121	28	36,312	33	33	698	102	75	2,183	48	82
C. univittatus	718	55	12	18,397	22	22	89	9	7	342	6	10
M. fuscopennata	—	—	—	3	<1	<1	1	<1	<1	7	<1	<1
M. microannulata	—	—	—	2	<1	<1	14	2	1	12	<1	<1

*—bait †—number of traps set ‡—Williams' modification of geometric mean

As far as the relative abundance of the three *Culex* species is concerned, the results of the two collecting methods are in close agreement at both localities. At Olifantsvlei *pipiens* was the most abundant mosquito followed by *theileri* and then *univittatus*. At Lake Chrissie *theileri* was by far the most prevalent, followed by *univittatus*, while *pipiens* was exceedingly rare. Reference to the results of the net-trap collections shows the aedine species at Olifantsvlei to be rarer than the three Culex species, although at Lake Chrissie *Aedes (Neomelaniconion) lineatopennis* (Ludlow) was recorded to be almost as prevalent as *univittatus*. At both localities *lineatopennis* was the most prevalent *Aedes*, while *Aedes (Ochlerotatus) caballus* (Theobald) was rare, being exceeded by *Aedes (Aedimorphus) dentatus* (Theobald) at both localities and by *mixtus* at Olifantsvlei. While the figures for aedine mosquitoes may be questioned on account of the small number of observations on which they are based, experience with a variety of collecting methods indicates that they are, nevertheless, probably a true reflection of the situation usually existing at the two localities.

What the figures in table I do not reveal is the exceedingly high biting activity which *lineatopennis* and *caballus* may periodically exhibit in the highveld. For instance, on one occasion at Olifantsvlei, 698 *lineatopennis* were collected at a single locality by four human collectors, off vegetation as well as alighting on man, between 0700 and 0800 hours. Other collections to be dealt with below will further emphasise this feature.

Other species differences reflected in the net-trap collections at Olifantsvlei and Lake Chrissie were the greater prevalence of *Anopheles (Anopheles) coustani* var. *tenebrosus* Dönitz at Olifantsvlei, and that of *Anopheles (Cellia) squamosus* Theobald at Lake Chrissie, and the occurrence of *Mansonia (Coquillettidia) fuscopennata* (Theobald) and *Mansonia (Coquillettidia) microannulata* (Theobald) at the latter locality. Although the latter two species, in addition to *Culex (Culex) annulioris* Theobald, were also recorded from Olifantsvlei in collections not shown in table I, they were definitely more prevalent at Lake Chrissie where, at times, fairly large numbers of all these species were collected in fowl-baited lard-cans.

Other species collected in small numbers at both localities but not shown in table I include: *Anopheles (Cellia) walravensi* var. *schwetzi* Evans, *Culiseta (Theobaldia) longiareolata* (Macquart), *Culex (Lutzia) tigripes* Grandpre and Charmoy, and *Culex (Culex) poicilipes* (Theobald).

Numbers of various species collected at other localities in the highveld are shown in table II. As mentioned earlier, the Boksburg and Welkom collections were made soon after outbreaks of either Sd or WN virus had occurred in the human populations at these localities. These collections as well as those at Vereeniging were made in residential and rural areas on the outskirts of these towns. Those at Germiston were made at a sewage farm. The remaining collections shown in table I were made largely off human bait during daylight at five widely scattered localities in the highveld.

At the time of the Boksburg collections, the net-trap was available and at this locality comparison between collections with this trap and lard-cans, both baited with CO_2, was possible. It can be seen that both methods showed *pipiens* to be the most prevalent species. The net-traps showed *theileri* to be more prevalent than *univittatus* whereas the lard-cans revealed the reverse. This difference conflicts with the results obtained with goat-baited net-traps and CO_2-baited lard-cans at Olifantsvlei where both traps gave similar results, so that it is difficult to decide which of the collecting methods at Boksburg revealed the true situation more closely. Nevertheless, it would seem that both species were fairly abundant. Net-trap collections showed the *Aedes* species to be rarer than the *Culex* species and noteworthy was the abundance of

TABLE II

Mosquitoes collected by different methods at various highveld localities

Localities	Boksburg				Welkom		Vereeniging		Germiston		Various*	
Species	Net-trap $+CO_2$	% of collect:	Lard-can $+CO_2$	% of collect:	Lard-can $+CO_2$	% of collect:	Lard-can $+CO_2$	% of collect:	Lard-can $+CO_2$	% of collect:	By hand off man	% of collect:
An. coustani	43	2	—	—	—	—	—	—	2	<1	—	—
An. squamosus	178	9	21	2	3	<1	—	—	—	—	—	—
A. caballus	50	3	3	<1	—	—	—	—	—	—	461	67
A. dentatus	1	<1	1	<1	—	—	5	2	—	—	60	9
A. lineatopennis	46	2	4	<1	—	—	1	<1	2	<1	109	16
A. mixtus	4	<1	1	<1	—	—	—	—	—	—	—	—
C. pipiens	777	40	768	63	270	23	208	73	9,137	87	51	7
C. theileri	616	32	130	11	829	72	51	18	962	9	—	—
C. univittatus	206	11	284	23	51	4	17	6	361	3	4	<1

*—Pooled collections made from Lake Chrissie, Standerton, Deneysville, Vaal Barrage and Bloemhof, made mainly during daylight.

caballus, coustani and *squamosus*. At Welkom, Vereeniging and Germiston only lard-cans were used so that information at these localities is only available on the three normally prevalent *Culex* species. At Welkom the dominance of *theileri* in these collections is evident while at Vereeniging and Germiston *pipiens* was by far the most prevalent, with *theileri* and *univittatus* following on in the same sequence at Olifantsvlei. In the pooled collections from Lake Chrissie, Standerton, Deneysville, Vaal Barrage and Bloemhof, relatively large numbers of *caballus, lineatopennis* and *dentatus* were collected. Failure to collect *theileri* at these sites does not necessarily mean its absence but is probably the result of these collections being made during daylight. These collections are of interest in that they reveal an abundance of *caballus* not encountered at the two main study areas.

Fluctuations in population levels of C. pipiens, C. theileri and C. univittatus at Olifantsvlei.

To determine variations in population levels of these *Culex* species a population index for each species was calculated from numbers collected in CO_2-baited lard-cans. Although pooling of collections on a monthly basis for these calculations would tend to obscure minor fluctuations, it was considered inadvisable to reduce this to shorter periods on account of the small number of observations on which such calculations would be based. The monthly fluctuations for the three seasons of study are shown in figure I, and the number of traps set each month from which the population index was calculated is also shown in this figure.

All three species first entered the traps in September or October, with *theileri* the most prevalent in the early part of the season. Build-up of all three species proceeded steadily, with *pipiens* at a slightly slower rate, and all had reached fairly high levels in December which was the peak month for *univittatus* each season. During January *univittatus* populations fell slightly and during the last two seasons, there being no observations during February 1963, there was a slight rise in *univittatus* levels during February producing a bimodal curve. This curve is possibly related to fluctuations in rainfall which showed peaks in November and January during the 1963/64 season and in October and December/January during the 1964/65 season [McIntosh *et al.*, 1967]. The other two species only reached their peaks, which far surpassed those of *univittatus*, in February or March. The higher levels attained by these species would appear to be attributed to the greater utilization of effluent waters by these two species, particularly by *pipiens*, and hence their relatively greater independence of rainfall. By March *univittatus* populations were low and during the following two months all species decreased rapidly in numbers.

While generally there was a fairly consistent pattern during different seasons among all three species, minor variations were apparent. For instance, there was a slight delay in growth rates in the early part of the 1964/65 season in comparison with the previous season, in that December populations of all three species were lower in 1964 than in the same month of 1963. This may possibly be the result of the average higher temperatures recorded in September and October 1963 as compared with the same months the following year. Another difference was the low level of the *univittatus* population during the 1962/63 season in comparison with the following two seasons. This may be a reflection of the dependence of *univittatus* upon rainfall and may have been due to the late onset of the spring rains in 1962 as in this year heavy rains did not fall until December [McIntosh *et al.*, 1967].

The results as a whole seem to suggest that in sewage effluent disposal areas where suitable breeding water in the form of effluent is constantly available to them, populations of *theileri* and *pipiens* are regulated almost entirely by temperature, whereas those of *univittatus* are influenced by both temperature and rainfall as this species prefers

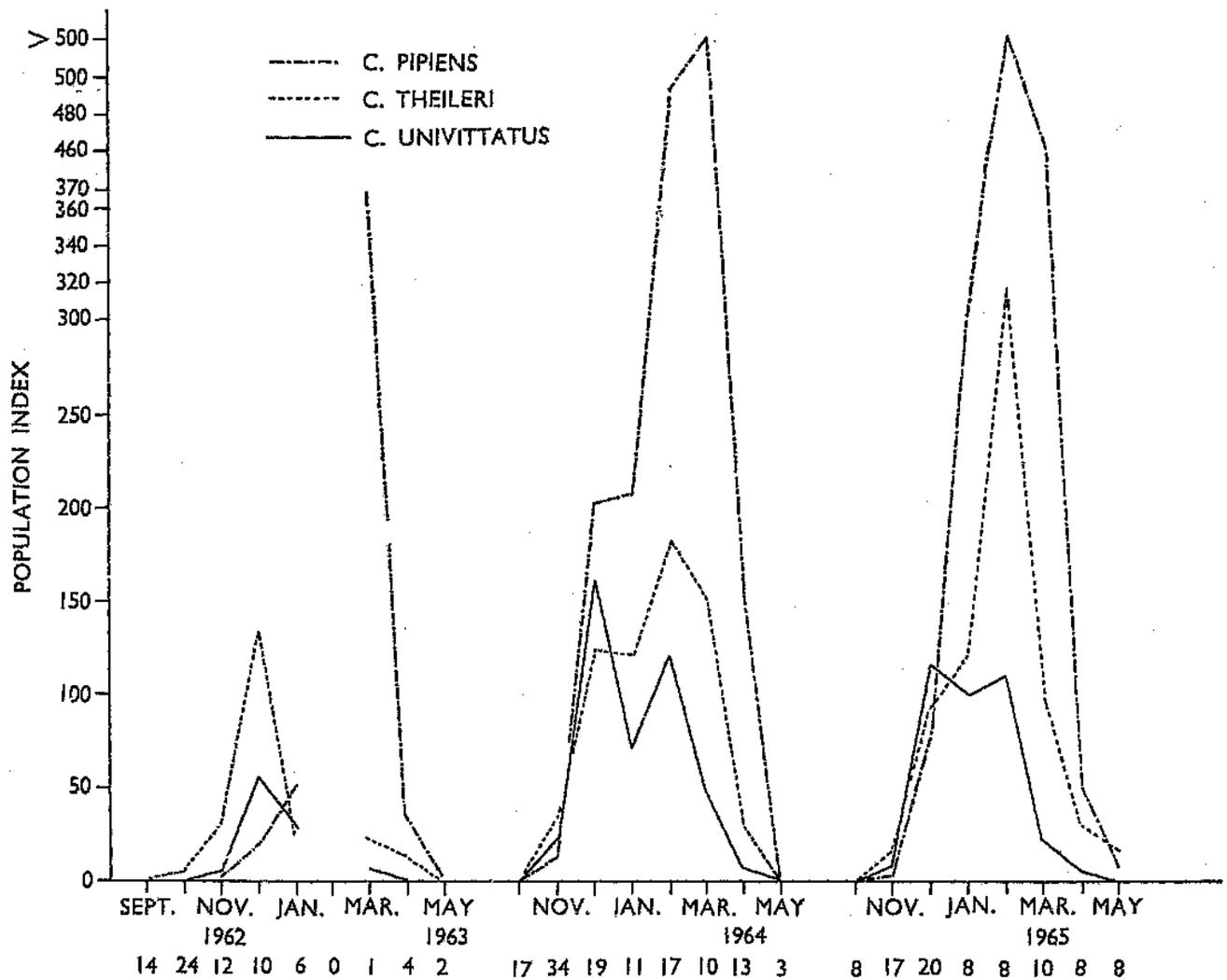

Fig. 1. Monthly fluctuations in population levels of *C. pipiens*, *C. theileri* and *C. univittatus* at Olifantsvlei between 1962 and 1965. The figures below the dates indicate the number of observations made at these times.

the less contaminated waters and consequently is more dependent upon rainfall for breeding sites.

Mosquito feeding preferences as determined by relative attraction to different baits

For convenience of exposition and [illegible] account of the bias inherent in different collecting methods, these results are best [illegible]cribed in relation to the kind of trap used. To differentiate mere attraction from a distance from actual feeding acceptability, where possible more reliance was attached to the proportion of mosquitoes actually feeding or alighting on the bait as against those entering the trap but not feeding or alighting. However, even this approach is not without bias. For example, confinement of a fowl in a nylon stocking gave considerably higher feeding rates than if the fowl was not so confined. while the use of wire-mesh cages to confine baits reduced feeding rates.

(a) *Net-trap.* As most species enter this trap readily it is believed that a good deal of reliance can be placed on the series of collections with this trap in which fowl, goat or human baits were used, the results of which are shown in table III. In evaluating the percentages of mosquitoes feeding on various baits it should be remembered that the fowls and goat were confined in a wire-mesh case, whereas man was not and the "fed" mosquitoes in the latter case refers to all mosquitoes alighting on the bait. From the table it can be seen that *tenebrosus, theileri* and the four *Aedes* species fed readily on goat and man while *pipiens* and *univittatus* showed little or no preference for these hosts, despite the entrance in fair numbers of *pipiens* into goat- and man-baited traps, and *univittatus* into goat-baited traps. Towards fowls preferences were somewhat reversed with *pipiens* and *univittatus* showing high feeding rates, while *tenebrosus, dentatus, mixtus* and *caballus* showed low rates. Noteworthy exceptions were a high feeding rate by *lineatopennis* and a moderate rate by *theileri* on fowls. *A. lineatopennis* actually showed a higher rate than *univittatus* on fowls but it would seem from other observations that the wire mesh was acting as a deterrent to *univittatus* in these collections. *A. lineatopennis* showed three ti[illegible]s the feeding rate of *theileri* on fowls, but due to the greater prevalence of *theileri* more individuals of the latter species actually fed on this bait.

(b) *Hand-collections off human bait.* In a series of five collections in which human bait was exposed in the open at Olifantsvlei, from 1800 to 2400 hours, the result of which are shown in table IV, a very similar picture of preferential feeding on man was obtained. It will be noted that *theileri* and *lineatopennis* together composed 89 per cent of the total mean catch off human bait, whereas *pipiens* and *univittatus* together accounted for only 6 per cent, despite their high population densities in the locality at the time, as catches of these last two species in the dry-ice baited control lard-cans revealed.

(c) *Fowl-pens.* In table V which shows the results of collections from a series of 48 fowl-pen trappings at Olifantsvlei, further evidence can be seen of the relative attraction of fowls for *pipiens, theileri* and *univittatus,* the only species which entered these traps in significant numbers. The high preference of *pipiens* and *univittatus* for the avian host is again evident as is also a moderate feeding rate by *theileri.* The feeding rate of *univittatus* (41 per cent) in this series was higher than that obtained in net-traps (25 per cent). As mentioned before, the lower rate in the latter collections was probably due to the wire-mesh cage confining the fowls having acted as a deterrent to feeding. In lard-cans baited with a fowl in a nylon stocking feeding rates for *pipiens* and *univittatus* were usually about 90 per cent.

TABLE III

Total numbers, with their geometric means (Mw) and percentage fed, of mosquitoes of various species collected in net-traps with different kinds of bait at Olifantsvlei.

Species	Fowls (5)*			Goat (5)			Man (5)			Control (empty)
	Total mosquitoes collected	Mw	Mean† % fed	Total mosquitoes collected	Mw	Mean % fed	Total mosquitoes collected	Mw	Mean‡ % fed	No.
An. tenebrosus	61	9	4	53	4	46	40	5	60	—
A. caballus	14	1	3	7	1	43	40	7	73	—
A. dentatus	80	13	6	106	3	25	66	5	78	—
A. lineatopennis	225	24	33	256	7	58	244	29	72	1
A. mixtus	34	3	1	60	2	67	49	5	74	—
C. pipiens	4,106	751	37	2,465	454	<1	319	46	<1	3
C. theileri	1,638	184	10	1,272	130	38	1,025	146	52	1
C. univittatus	891	132	25	357	59	0	87	13	3	—

*—Number of traps set.
†—Arithmetic mean of percentage feeding in each collection.
‡—"Fed" for man-baited traps refers to mosquitoes collected alighting on bait while for the other traps it refers to engorged mosquitoes.

TABLE IV

Relative attraction of mosquitoes for man at Olifantsvlei. As a comparison, results of CO_2-baited lard-cans are shown.

Species	Human-bait (5)*			Lard-can+CO_2 (5)†		
	Total mosq. collected	Mw	Mw as %	Total mosq. collected	Mw	Mw as %
An. tenebrosus	15	2	1	0	—	—
A. caballus	9	1	1	0	—	—
A. dentatus	25	4	2	0	—	—
A. lineatopennis	462	73	38	0	—	—
A. mixtus	12	2	1	0	—	—
C. pipiens	30	5	3	1,136	207	46
C. theileri	564	98	51	1,110	125	28
C. univittatus	32	6	3	826	115	26

* Number of collections
† Lard-can traps set overnight

TABLE V

Mosquitoes collected in fowl-pen traps at Olifantsvlei

	Total mosqs. collected	Mw	Mw as %	Mean* % fed
C. pipiens	19,116	47	46	44
C. theileri	2,297	16	16	14
C. univittatus	6,192	38	38	41

*—given in methods

(d) *No. 2 traps.* Although it is probably largely true that mosquitoes feeding on fowls would also feed to a somewhat similar degree on wild birds, the studies of Buescher *et al.* [1959] and Dow *et al.* [1957] emphasise that caution is indicated in such deductions. The latter authors differentiate between attraction and acceptability (feeding attraction) of host and showed that acceptability varied for bird species and even for different individuals of the same species, while mere attraction, on the other hand, was proportional to the size of bird, irrespective of species. A small amount of trapping with No. 2 traps, using pigeons, laughing doves and two species of passerines, the red bishop and red-billed queleas, has been done at Olifantsvlei and the results of these collections are shown in table VI. In these collections, the pigeons and doves were confined in nylon stockings, each species in a separate trap, whereas the red bishops and queleas were used in the same trap and confined in a 1 inch wire-mesh cage. From the results of the pigeon- and dove-baited traps it is clear that *univittatus* and *pipiens* feed readily on these birds. Feeding rates for the two passerines species are not entirely reliable as it appears likely that the wire-mesh cages acted as a deterrent to feeding. Of interest in these collections is the attraction of *C. annulioris* and *C. tigripes*, rare species at Olifantsvlei, to birds.

TABLE VI

Numbers of mosquitoes, with percentages fed, entering No. 2 traps, baited with wild birds

Mosquitoes	Total number mosquitoes fed and unfed, with mean percentage feeding.								
	Pigeon (4)*			Laughing dove (4)			Red bishop and quelea (2)		
	unfed	fed	Mean % fed	unfed	fed	Mean % fed	unfed	fed	Mean % fed
C. univittatus	619	699	55	118	652	77	120	0	0
C. pipiens	1,107	3,140	78	462	2,051	80	2,405	418	15
C. annulioris	3	3	—	1	2	—	0	0	—
C. tigripes	0	0	—	0	0	—	6	0	0

*—number of traps set

(e) *Miscellaneous observations.* Lard-can and net-traps baited with a fowl and sheep respectively, yielded further information on feeding preferences of some rare mosquitoes. These collections showed *C. annulioris, M. fuscopennata,* and *M. microannulata* to feed on fowls, with indications that *M. microannulata* occasionally bites sheep. *An. squamosus* fed readily on sheep and occasionally on fowls.

Mosquito-biting cycles

No extensive study was made of mosquito biting activity during daylight as it was clearly obvious that biting at this time was usually minimal. There were, however, notable exceptions which may have important epidemiological consequences. As mentioned earlier, *lineatopennis* and, to the extent that its rarity permitted observations, *caballus* also, on occasions, fed readily on man during daylight (table II). Reports by Bedford [1928] and Steyn and Schulz [1955] also mention the diurnal biting habits of *caballus.* With *lineatopennis,* however, the impression gained was that there was little active seeking of hosts during daylight, as distinct from the crepuscular period, and that biting at this time was a chance phenomenon being dependent upon the bait approaching close to the daylight resting haunts of this mosquito, or even actual disturbance of the sheltering vegetation being necessary.

To determine man-biting activity at night five collections were made off human bait from 1800 to 0600 hours at Olifantsvlei. Apart from *lineatopennis* and *theileri* the numbers of other species collected were too few to allow analysis of the results (see table IV) but those obtained for the former two species are shown in figure 2. Catches were recorded hourly, and to enable comparisons between the two species to be made, were converted to percentages. The general biting pattern shown by each species was somewhat similar although minor differences were apparent. Both species showed peak activity shortly after sunset with the peak for *lineatopennis* occurring during the first hour after sunset and that for *theileri* during the second hour, although considerable biting by this species also occurred during the first hour. *A. lineatopennis* showed definite bimodal activity with a later but minor peak occurring during the hour before sunrise. After the initial post-sunset biting peak feeding by both species fell steadily to reach their lowest levels between 0300 and 0400 hours.

Endophilism

As a means of further assessing the likelihood of various species feeding on man a series of three collections were made at night in a room at one of the regular collecting

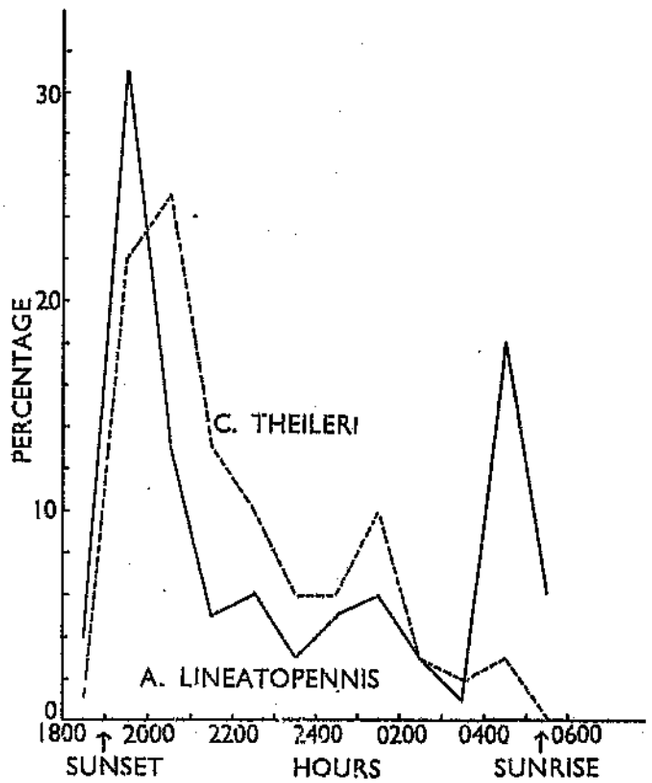

Fig. 2. Nocturnal biting activity of *C. theileri* and *A. lineatopennis* at Olifantsvlei as shown by human-baited catches. Catches were recorded hourly. Geometric means have been adjusted to percentages to facilitate comparison.

sites at Olifantsvlei. From the results shown in table VII, it can be seen that nearly all the species present at Olifantsvlei were obtained in these collections but only *tenebrosus, lineatopennis* and *theileri* were collected in reasonable numbers.

TABLE VII

Numbers of mosquitoes of various species collected indoors on three nights at Olifantsvlei. As controls, the results of CO_2-baited lard-can traps set outside are shown.

	Number mosquitoes collected	
	Room	Lard-can $+CO_2$
An. tenebrosus	94	0
A. caballus	5	0
A. dentatus	6	0
A. lineatopennis	200	0
A. mixtus	4	0
C. pipiens	11	2,246
C. theileri	98	1,878
C. univittatus	7	390

TABLE VIII

Numbers, with their geometric means of three Culex species collected in No. 2 traps with avian and dry-ice baits at ground level and in a tree canopy at Olifantsvlei.

	CO_2				Pigeons				Doves			
	Ground level (4)*		25 ft. (3)		Ground level (2)		25 ft. (2)		Ground level (2)		25 ft. (2)	
	No.	Mw	No.	Mw	No.	Mw	No.	Mw	No.	Mw	No.	Mw
C. pipiens	665	147	2,859	583	1,346	669	2,901	1,138	973	486	1,540	758
C. theileri	41	7	3	1	1	<1	0	0	0	0	0	0
C. univittatus	15	2	137	42	492	234	826	397	142	70	628	278

*—Number of traps set

Vertical distribution of C. pipiens, C. univittatus and C. theileri

A few exploratory trappings were done comparing mosquito densities within 3 ft. of the ground and at 25 ft. in the canopy of a tree. For these collections, either dry-ice, pigeons or doves were used as baits in No. 2 traps. The results are shown in table VIII. It is clear that *pipiens* and *univittatus* are more active in the canopy than at ground level, again suggesting their predilection for the avian host. Although *theileri* was not collected in sufficient numbers to warrant definite conclusions, the results with the dry-ice baited traps suggest that this species was more active at ground level.

Overwintering of mosquitoes

On account of the severity of climatic conditions there can, of course, be no question of normal viral transmission by mosquitoes occurring anywhere in the South African highveld during the winter. This naturally raises the question of overwintering of the vectors, at least in so far as the *Culex* species are concerned, which are usually presumed to overwinter in the adult stage.

During the winter a search was made at Olifantsvlei for hibernating adults and, to a lesser degree, for larvae. Adults were looked for in rank grass and other vegetation in moist situations, in rodent holes, and in a disused mine shaft, but no adult specimen of any species was found. The search for larvae was also negative.

DISCUSSION

The work described here was intended as a complementary study to that on mosquito infectivity described in the related paper [McIntosh *et al.*, 1967]. It was hoped that a synthesis of the data of these contemporaneous studies would materially assist in leading to an understanding of the ecology of Sd and WN viruses. The nature of the study dictated that priority be given to observations on mosquito infectivity rather than to bionomics. This meant that a large part of the latter information has necessarily been derived from mosquito collections designed primarily for virus studies.

In view of the difficulties in determining mosquito abundance and the complexity of host-seeking behaviour, further work will be necessary for a more accurate determination of these factors. The present study should be considered as mainly exploratory. However, though observations were limited on some aspects, the same general trends were evident from a variety of experimental methods and it is believed that the data obtained in the present study is sufficiently reliable to enable a tentative assessment to be made of those mosquito factors relevant to virus-vector relationships. As only meagre information is so far available on vector capability, this aspect must for the time being be ignored in the assessment of vector species. Accordingly, on grounds of prevalence alone, only five species seem to warrant consideration as vectors of epidemiological importance in the highveld. They are dealt with below in what is believed to be their order of importance.

Culex univittatus. When suitable collecting methods were used they showed this species to be widely distributed throughout the highveld which is in agreement with the observations of others [Muspratt, 1955]. However, only rarely were excessively large numbers encountered and at times Lake Chrissie's numbers were at low levels. Its numbers were almost invariably exceeded by those of *theileri* and in sewage farms and the environs of towns, *pipiens* also was far more plentiful. At Olifantsvlei, *univittatus* seemed to be at reasonably high population levels from December until March, the period when viral activity was usually detected. At Lake Chrissie, Boksburg and Welkom, this mosquito also seemed reasonably prevalent when viral activity was detected. It is mainly a bird-feeder, an attribute which strongly implicates it in viral

transmission since, with good reason, birds are believed to be the main vertebrate host of both viruses. There is thus strong circumstantial evidence in favour of this species fulfilling the role of arthropod maintenance host [Audy, 1958] in the wild cycle of both viruses ascribed to it from the infectivity studies. This agrees with the observations of Taylor *et al.*, [1955, 1956] on this mosquito in Egypt.

The extent to which the highveld form of *univittatus* was shown to feed on man in the present study seems inadequate to account for the majority of human cases, even if allowance be made for the sporadic or non-epidemic forms of infection which usually seem to occur in the highveld. There was evidence, however, that a certain amount of feeding on man did occur and tests on blood meals of *univittatus* at Olifantsvlei showed a significant feeding rate for cattle, dogs and horses [Anderson, 1967] and it seems prudent, until other species can be incriminated with more assurance than at present, to regard *univittatus* as the cause of at least some human infection with both viruses.

The low feeding rates recorded on mammals for the highveld populations of *univittatus* in the present study conflict with those obtained for *univittatus* from other geographical areas. For instance, in Egypt, precipitin tests on blood meals of *univittatus* showed 91.2 per cent as having come from mammals, including 11.3 per cent from man, with 8.5 per cent from birds, although the mosquitoes concerned were collected in rural villages which may have biased the samples in favour of mammals [Harlbut and Weitz, 1956]. Even more significant are observations on *univittatus* from further south in Africa. With the same test, Paterson *et al.* [1964] found that blood meals of *univittatus* collected in the subtropical lowlands of South Africa were 60 per cent of avian origin with the balance from a variety of mammals, including man. In the same locality our own recent studies have also shown that the local form of *univittatus* is readily attracted to human bait [Arbovirus Research Unit, Johannesburg: Annual Report, 1964/65]. In Bechuanaland de Meillon [1947] found *univittatus* bit man and monkey avidly. Morphological differences between the lowland and highveld forms of *univittatus* in South Africa are apparent, and Mattingly [1952] has also drawn attention to morphological variation of this species in Africa.

Culex theileri. Our results indicate that this species is the most consistently prevalent mosquito generally throughout the highveld although on sewage farms its numbers are probably usually exceeded by those of *pipiens*. It is primarily a mammal feeder. It feeds avidly on man and is also endophilic. It also, to a certain extent, feeds on fowls from which it may be inferred that it will also feed on wild birds. It therefore apparently possesses the necessary attributes which would enable it to act as a link vector between the avian wild cycles and man. Suspicion in this regard is further enhanced by the isolation of Sd and WN viruses from this species. From all the available evidence it seems likely that *theileri* would be more likely to infect man than any other mosquito, even including *univittatus*. It is perhaps noteworthy that at Boksburg and Welkom, two localities where collections were made shortly after human infections had occurred, this species was plentiful.

Aedes lineatopennis. Little has been published on the prevalence of this species in South Africa [Muspratt, 1955]. This mosquito was clearly the most prevalent *Aedes* at the two main study areas and at times showed extremely high biting frequencies on man, thus showing the highveld populations of this species to be as anthropophilic as those elsewhere in Africa [Kerr, 1933; Haddow, 1960]. *A. lineatopennis* is the grassland counterpart to the bush-dwelling *Aedes (Neomelaniconion) circumluteolus* Theobald, the important viral vector in the subtropical lowlands of South Africa [Smithburn,

1958; Worth *et al.*, 1961]. Apart from its high anthrophilism *lineatopennis* is apparently a strong flier [Teesdale, 1950; Haddow, 1960], attributes which have obvious epidemiological implications. The preference of *lineatopennis* for fowls, and hence presumably for wild birds, seems even greater than that of *theileri* and *lineatopennis* must therefore also be regarded as a potential link vector between wild cycles and man. Its habit of feeding on man during daylight in addition to the more usual nocturnal feeding, at which time it also enters houses readily, would further implicate this species in human infections. This habit of daylight feeding may have other important consequences as it may expose this species to viraemic birds which otherwise may not occur. For instance, this could occur when species of birds, which at night roost in trees where they could be exposed to *univittatus* attacks, during the day feed on the ground in among vegetation where *lineatopennis* is present.

Aedes caballus. Although this species was rare at the two main study areas, our collections at other highveld localities and studies by other workers [Bedford, 1928; Steyn and Schulz, 1955; Muspratt, 1955; Gear *et al.*, 1955] show this mosquito to be prevalent and highly anthropophilic in the highveld. It has already been incriminated as vector of Wesselsbron, Middelburg and Rift Valley Fever viruses in the highveld [Gear *et al.*, 1955; Kokernot *et al.*, 1957, 1958]. Like *lineatopennis* this species feeds readily on man during daylight which would further tend to emphasise its potential importance as a cause of human infection. Further implicating this species is the circumstantial evidence that it may have infected a staff member with WN virus during daylight at Olifantsvlei. There is an obvious need for more observations on this species in relation to Sd and WN viruses.

Culex pipiens. Apart from a reference by de Meillon [1928] that *pipiens* larvae were common in a suburb of Johannesburg, there is no evidence that earlier workers had observed the extremely high population densities of this species encountered by us. This may be due partly to the traps used by us, but on the other hand it would seem more likely that this is a recent phenomenon as a result of increasing modern urbanisation. In fact, the rarity of this species at Lake Chrissie is very likely a reflection of the situation which existed over the highveld generally before urbanisation. If this were true, it would mean that this species could be expected to increase in numbers in future. However, this may not result in untoward consequences as it seems likely that this species can only have a minor vectorial role in the transmission of Sd and WN viruses. The isolation of only three strains of Sd virus after extremely large numbers of *pipiens* were tested tends to rule out this species entirely as a vector of WN virus and to suggest a role of only minor importance in the transmission of Sd virus. The low infectivity rate is indeed surprising in view of the strongly aviophilic feeding habits of this mosquito. The answer may lie in a certain amount of preferential feeding among different species of birds by *pipiens*, resulting in the selection of birds which are relatively resistant to the viruses, or in low vectorial capability. The low feeding rate on man of this mosquito further emphasises the minor role it is likely to play in human infection.

Other species. The study failed to throw light on the part *C. fatigans* may have in Sd or WN virus infections in the highveld. Apart from the difficulty in differentiating this mosquito from *pipiens* in routine collections, this failure was mainly due to the scarcity or even absence of *fatigans* in the main study area.

Although the remaining species have been excluded from consideration as vectors on account of low abundance, it is possible that, on feeding preferences alone, *An.*

coustani tenebrosus, An. squamosus, A. dentatus, A. mixtus, M. fuscopennata and *M. microannulata* may still play some part, even though it be a minor one, in the transmission of Sd and WN viruses.

Mention should also be made of the absence of *Culex (Culex) rubinotus* Theobald from the collections, especially as this species has been collected before at Olifantsvlei and Germiston at which time H336 and Germiston viruses were isolated from it [Smithburn *et al.*, 1959; Kokernot *et al.*, 1960]. At that time this mosquito was collected in human-baited catches. That this type of collecting method was not much used in our study may explain our failure to record it. Even so, it would seem that *rubinotus* is a relatively rare species in the highveld. Previous records of it in South Africa confirm this opinion [Muspratt, 1955].

SUMMARY

The bionomics of mosquitoes in the highveld region of South Africa in relation to the ecology of Sindbis and West Nile viruses are described. Data on prevalence, including, in some cases, observations of seasonal fluctuation, feeding preferences and other host-seeking behaviour obtained in a three-year study were used, in conjunction with evidence on viral infectivity of mosquitoes, to assess the likely vectorial roles of various mosquitoes.

The conclusion arrived at was that only five species are sufficiently prevalent to warrant consideration as vectors of epidemiological significance. These are: *Culex (Culex) univittatus* Theobald, *Culex (Culex) theileri* Theobald, *Aedes (Neomelaniconion) lineatopennis* (Ludlow), *Aedes (Ochlerotatus) caballus* Theobald and *Culex (Culex) pipiens* Linnaeus.

Culex univittatus is apparently the primary wild cycle vector of both viruses. It feeds mainly on wild birds and domestic fowls but, with a low feeding preference for man, seems unlikely to be the main vector causing human infection. Some, if not most, human infection is probably caused by *Culex theileri* and/or *Aedes lineatopennis*, both of which feed significantly on birds and man, as well as being endophilic. *Aedes caballus* might also cause human infection but due to scarcity in the main study areas few observations on this species were possible. *Culex pipiens* is almost exclusively aviophilic and on this account could only be involved in the wild cycles, or in infection among domestic fowls.

Data on prevalence and feeding preferences of several other mosquito species is also given.

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Larval habitats of culicine mosquitoes (Diptera: Culicidae) in a sewage effluent disposal area in the South African Highveld

by

P. G. JUPP
Arbovirus Research Unit, South African Institute for Medical Research, Johannesburg

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P. G. JUPP

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

INTRODUCTION

This paper describes the results of a study of habitat associations of larvae of culicine mosquitoes in the South African highveld region. This study is part of a comprehensive investigation on the ecology of the mosquito-borne viruses known as Sindbis and West Nile, the results of which are at present being published (McIntosh *et al.*, Jupp and McIntosh, in press).

The larval study was carried out at Olifantsvlei, near Johannesburg, which was also the main study area for the other aspects of the general investigation. Olifantsvlei is a sewage effluent disposal area and cattle farm owned by the Johannesburg Municipality. It seemed particularly important that the extent to which sewage effluent was being utilized by potential viral vectors be determined as it appeared likely that increasing urbanization in the future would lead to increased effluent disposal over large areas of the highveld.

METHODS

Olifantsvlei, the study area, is situated 5 miles from the outskirts of Johannesburg, and is predominantly grassland country but traversed from West to East by a large vlei with reed beds. The vlei is fed by the Klip river.

The area is also continually irrigated with sewage effluent from nearby purification works which causes additional larval breeding sites, not present in more typical highveld country, to be made available.

Larval collections were made during the four summer months, December to March, when mosquito populations are at their highest on the highveld. This

* The studies and observations on which this paper is based were financed jointly by the South African Institute for Medical Research, the Poliomyelitis Research Foundation and the South African Council for Scientific and Industrial Research.

was done during three summers from December 1963 to March 1966. Dipping was carried out with a white, one-pint, enamel dipper. The number of dips varied between one and 25 for each collection and larger expanses of water were examined at several spots.

For the purpose of the study, the larval breeding sites present in the area were classified into six habitat types which accounted for nearly all these sites. In addition, water occurring in each habitat type, except "rainwater over grass", was sampled and submitted to certain chemical analysis. For each habitat five or six samples were drawn from representative sites, nearly always sites from which larvae had been collected.

Classification of habitat types

1. Sewage effluent

(a) *Furrows.* A network of furrows carries effluent through the grazing lands. Usually the water is slow flowing but sometimes a furrow is blocked off, its contents becoming stagnant. Water is semi-permanent to permanent, depending upon how long a furrow is left in communication with the irrigation network, but is usually permanent. A typical furrow has a muddy bottom and grass sides where mosquito breeding occurs in the stiller water.

(b) *Over grass.* Pasture is periodically flooded from the furrows so that the long grass is partly submerged. Water from a single flooding may remain standing for 1-2 weeks (temporary water), or a further irrigation may result in water being present for a longer period (semi-permanent). Usually the latter case applies.

2. Rainwater

(a) *Over grass.* Water is temporary to semi-permanent, usually temporary. It is standing water which is usually clear and may contain emergent vegetation or algae (flooded grassland, grass-lined ditches or pools).

(b) *Over soil.* Water is as in (a) except that it lies over soil, which may be partially covered by dead leaves. Algae are absent and emergent vegetation rarely occurs.

(c) *Polluted ground pools.* These are large pools in cattle camps containing water of sufficient depth to remain for several weeks without further rain (semi-permanent water). Water is stagnant, usually containing algal mats but no emergent vegetation, and is polluted with organic waste from cattle.

3. Vlei margins

These consist of permanent standing water with emergent vegetation (reeds etc.) and often contain aquatic plants and algae.

Chemical analysis of water

In addition to pH measurement, chemical analysis was undertaken, using standard analytical methods, to determine:

(a) Settleable solids. This is a measure of the degree of "muddiness" of the sample.

(b) Suspended solids. This is a measure of the suspended colloidal particles which consist mainly of organic matter.

(c) Permanganate value. The British method was employed in which oxidation is carried out using acid permanganate for four hours. The empirical values obtained in this way are a measure of the more readily oxidizable material in the sample and give an indication of the total dissolved organic matter present.

(d) Nitrate as N, free and saline ammonia as N and albuminoidal ammonia as N. These measurements together are an estimate of the inorganic nitrogen present in the sample, a large portion of which is derived from a breakdown of nitrogenous organic material, so that the three measurements are correlated with the amount of dissolved organic material present in the sample.

The above analysis was undertaken in an attempt to obtain data which would permit a more complete and objective description of the different habitat types, thus allowing a better understanding of the habitat associations of the different species. In interpreting results of the analysis the figures obtained for the permanganate value and the nitrogen determinations were used together to assess the amount of dissolved organic matter present in a sample.

RESULTS OF CHEMICAL ANALYSIS OF WATER

The results of the analysis of water from different habitat types are shown in Table I. It can be seen that samples from polluted rainwater ground

TABLE I. Partial chemical analysis of water samples from five habitat types. [The figures cited are mean values in mg/l except settleable solids (ml/l).]

	Effluent		Rain		Vlei margins
	Over grass	Furrows	Over soil	Polluted ground pools	
Settleable solids	0.3	0.7	0.3	2.0	0.4
Suspended solids	56	52	35	1,784	52
Permanganate value *	26.6	18.9	14.2	501	7.6
Nitrate as N.	2	9	2	Trace	1.5
Free and saline ammonia as N.	21.6	8.9	3.7	27.2	2.2
Albuminoidal ammonia as N.	2.5	1.4	0.5	23.1	0.5
pH	7.3	7.5	7.3	7.7	7.2
No samples taken	5	5	6	5	6

* = British method: amount of acid permanganate reduced in four hours by the sample.

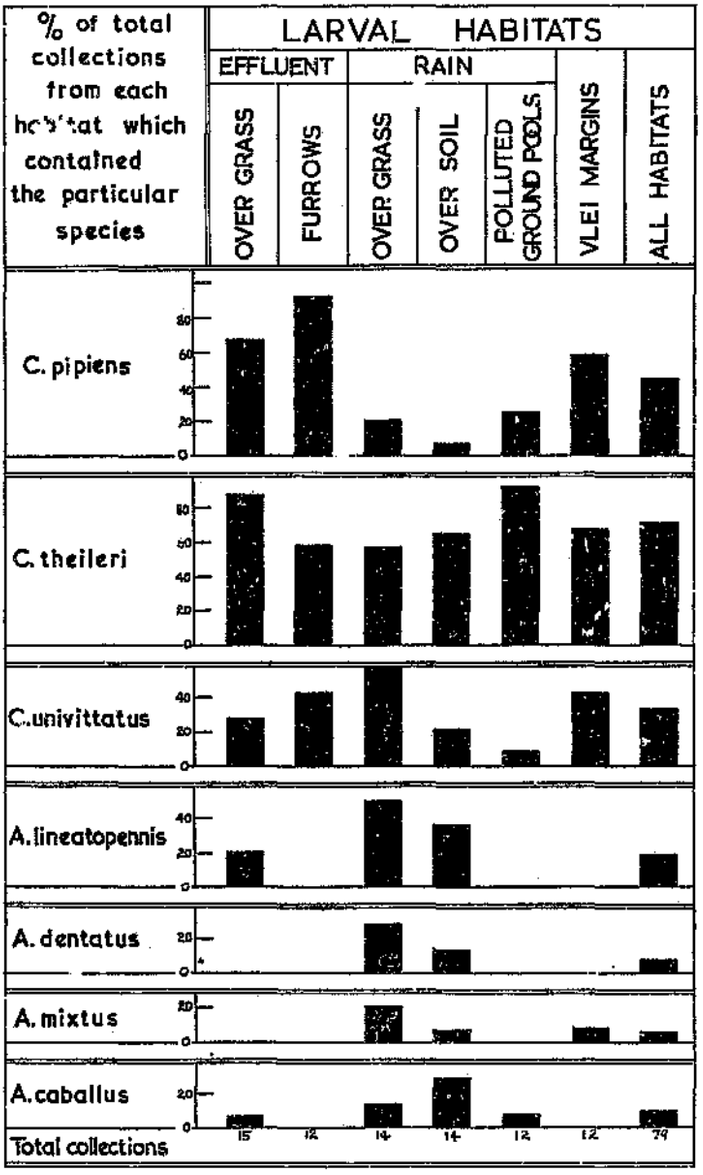

Fig. 1. Habitat associations of th larvae of seven culicine species at Olifantsvlei, Tvl.

pools were the muddiest (2 ml. settleable solids per litre) and also possessed by far the largest amounts of both suspended and dissolved organic matter. This was to be expected as these pools were heavily polluted by cattle. Effluent carried by furrows was next muddiest, containing more settleable solids than effluent lying on pasture, rainwater and water from the vlei. Both types of effluent and vlei water contained about the same amounts of suspended organic matter (52-66 mg. per litre). However, examination of the permanganate values and the nitrogen analysis indicates that these three habitats differed regarding the amount of dissolved organic material. Effluent on pasture showed the largest amount probably partly due to pollution by grazing cattle, while effluent from furrows had a somewhat lower quantity and vlei water showed the smallest amount of all the habitats. Examination of the figures for rain over soil reveal that rainwater was the clearest water (smallest amounts of settleable and suspended solids) and contained the lowest proportion of dissolved organic material of samples from all habitats except the vlei. All the water samples were slightly alkaline, pH 7.3-7.7.

HABITAT ASSOCIATION

For the study period the total number of collections containing larvae made from each habitat type are given in fig. 1. Seven species were studied and the frequency at which each one occurred in each habitat type was calculated on a percentage basis. This was done by calculating the percentage of the collections at a habitat which contained the species concerned. Percentages calculated in this way for three *Culex* species and four aedine species are shown in fig. 1.

Culex (Culex) pipiens Linnaeus

Although *C. pipiens* occurred in all the habitats, it was much more frequent in effluent furrows (92%) and in effluent-flooded pasture and vlei margins (67% and 58%) than in the other habitats. Thus, this species utilized mainly permanent water, both polluted and unpolluted, for breeding, although it demonstrated a marked preference for effluent. In fact, it was the species which exploited the habitats provided by effluent to the greatest extent, occurring a little more frequently in these than *Culex theileri*. Effluent contained considerable suspended and dissolved organic material which may be the reason why it was chosen. However, *pipiens* avoided the polluted rainwater ground pools, favoured by *theileri*, which contained even more organic matter.

These observations are generally in agreement with those of Hopkins (1952) who mentions that *pipiens* is not deterred by water with a high organic content and that it was perhaps more common in dirty and unshaded water. He also describes the breeding places of *pipiens* in Africa as consisting of an immense range of sites including temporary pools besides permanent water. However, the present study showed that temporary water was only used to a small degree.

Culex (Culex) theileri Theobald

In contrast to *C. pipiens*, *C. theileri* was found to exploit all six habitats with either temporary, semi-permanent or permanent water to a large degree, although it showed a definite preference for two of them, polluted rainwater ground pools (92%) and effluent-flooded pasture (87%). It is probably important to this mosquito that these two habitats both contained the largest amounts of suspended and dissolved organic matter, and it is interesting that *theileri* is able to utilize water for breeding which is contaminated to the extent of 1,784 mgms. suspended solids per litre.

The studies of other workers have also demonstrated that *theileri* shows great diversity in its choice of larval breeding places (Ingram and de Meillon, 1927-8, and Bedford, 1928). Nieschulz *et al.*, (1934) confirmed this and mentioned that larvae were found in both temporary and permanent water.

Culex (Culex) univittatus Theobald

C. univittatus was collected most frequently in rainwater accumulating over grass (57%), usually in pools and ditches. To a somewhat lesser extent (42%), it was found in effluent furrows and vlei margins. Thus, this species preferred certain temporary and permanent habitats.

Unfortunately, it was not possible to arrange for analysis of freshly collected samples from the habitat 'rainwater over grass' and analytical figures for this habitat are missing. However, water from this habitat would be expected to have the low proportions of suspended and dissolved organic matter found in rainwater lying over soil, and would differ mainly from the latter habitat in possessing emergent vegetation. If this were the case, it would appear that *univittatus* showed a certain preference for clearer water with emergent vegetation - such water also being without a high proportion of dissolved organic matter. This species would seem to avoid heavily polluted water since effluent from furrows and water from the vlei contained less dissolved organic matter than samples from the other permanent habitats, particularly vlei water which contained the smallest proportion. However, even though the frequency with which *univittatus* was collected in effluent-flooded grassland was low, the extent to which it utilized this habitat is significant and it is likely that when effluent becomes diluted by rainfall, the species would breed even more frequently in both effluent habitats.

According to Hopkins (1952), the breeding places of *univittatus* are usually stagnant pools of various kinds, particularly those in marshy land and at the edge of swamps. He mentions that vegetation is usually present. This is in general accord with the present findings, bearing in mind that Olifantsvlei contains the artificial habitat-furrows carrying effluent. Hopkins does not, however, give any record of the proportion of organic matter in the water of the breeding places utilized by this species.

Aedes (Neomelaniconion) lineatopennis (Ludlow)

This mosquito was shown to breed only in temporary accumulations of rainwater and the temporary to semi-permanent effluent flooding grassland. It was never encountered in any of the habitats with permanent water. Of the habitats it exploited, *lineatopennis* preferred rain water lying over grass. These results confirm the observations of Nieschulz *et al.* (1934) who also found that this species depends upon the drying out and reflooding of a breeding place for the development of more than one generation.

Aedes (Aedimorphus) dentatus (Theobald), *Aedes (Aedimorphus) mixtus* Edwards and *Aedes (Ochlerotatus) caballus* (Theobald)

These three species occurred at only low frequencies. They were found predominantly in temporary sites provided by rain, *dentatus* never being found breeding outside such habitats. *A. dentatus* and *mixtus* preferred rainwater lying over grass to that lying over soil, while *caballus* was collected most frequently in the latter habitat. On a few occasions only, larvae of *mixtus* and *caballus* were found in habitats with semi-permanent or permanent water.

Nieschulz *et al.* (1934) found that *caballus* used the same breeding places as *lineatopennis* and that the two species showed the same type of biology. These findings are different from the present ones in that at Olifantsvlei *caballus* occurred more frequently in rainwater lying over soil than in that over grass.

Hopkins's records (1952) for *dentatus* agree with the present ones but those of Nieschulz *et al.* (1934) differ in as far as these workers found that the mosquito preferred those rainwater pools which have bottoms of mud and leaves but no vegetation. The Olifantsvlei study showed that the species prefers rainwater over vegetation.

With regard to the larvae of *mixtus*, the only previous collection record appears to be of that of Muspratt (1955) who found the species in a swamp pool.

GENERAL CONCLUSIONS

The results for the habitat associations show that disposal of sewage effluent at Olifantsvlei causes an increased rate of breeding of the majority of the culicine species occurring in the area, particularly certain ones which favour the larval habitats that the effluent introduces. Species which particularly favour these habitats are *C. pipiens* and *C. theileri* in that order, while *A. dentatus* and *A. mixtus* were not found to breed at all in such habitats. Although the larval sites provided by effluent would generally not appear to be ideally suited to *C. univittatus* and *A. lineatopennis*, they are nevertheless used to some extent by these species and so would cause higher populations of these mosquitoes in an area supplied with sewage effluent. In this connection it is noteworthy that the popul-

ations of *univittatus* are lower on the highveld at Lake Chrissie (Eastern Transvaal), another study area, where there is no effluent, than at Olifantsvlei.

The great diversity of breeding places used by *theileri* would seem to partly explain why it is the most consistently prevalent mosquito generally throughout the highveld. Since this mosquito is highly anthropophilic, its utilization of effluent is of importance when considering the problem of sewage disposal.

Effluent disposal might seem also to have a bearing on the transmisson of Sindbis and West Nile viruses since populations of *univittatus*, the main vector of these viruses, are increased by effluent disposal to a significant degree. The fact that the habitat it uses most is rainwater over grass would explain why the seasonal fluctuation of the *univittatus* adult population at Olifantsvlei shows a relationship to rainfall.

The study showed that while the aedine species, *lineatopennis*, *dentatus*, *mixtus* and *caballus*, relied heavyly upon rainfall for their larval breeding sites, *lineatopennis* and *caballus* also used effluent to a small extent. Although *lineatopennis* was found in significant numbers, the other three species were rare at Olifantsvlei.

Of interest in the results is the paucity of *caballus*, a highly anthropophilic species and a known vector of several viruses (Gear *et al.*, 1955; Kokernot *et al.*, 1957, 1958), which is normally a fairly prevalent and widespread species on the highveld (Bedford, 1928; Steyn and Schulz, 1955; Muspratt, 1955; Gear *et al*, 1955). The reason for the small numbers may perhaps be attributable to the absence of temporary and semi-permanent rainwater pans in the area.

The larval study was not designed to measure the densities of culicine species at Olifantsvlei and only the frequency with which each species occurred in the habitats was determined (see fig. 1), and on this account no measurement of absolute numbers was made. However, it may be mentioned that the differing frequencies tend, on the whole, to follow the relative density measurements found by collecting the adults in various kinds of baited traps at the same locality, except that the larval collections indicate that *lineatopennis* and *caballus* may be more abundant than has been shown by trapping adults.

SUMMARY

A study was made of the larval habitat associations of seven culicine species at a locality in the highveld region of South Africa which is a sewage effluent disposal area and cattle farm. It formed part of a comprehensive investigation on the ecology of the mosquito-borne viruses, Sindbis and West Nile. Breeding places in the area were classified into six habitat types, with three main divisions consisting of effluent, rain and vlei waters.

It was found that *Culex pipiens* utilized mainly permanent water, particularly effluent, while *Culex theileri* exploited all habitats with some preference for polluted rainwater ground pools and effluent-flooded pasture, both semi-permanent habitats. *Culex univittatus* was relatively selective in its choice of breeding sites, preferring temporary to semi-permanent accumulations of rainwater over grass

as well as permanent vlei margins and effluent furrows. Emergent vegetation was important to this species.

Aedes lineatopennis, A. dentatus, A. mixtus and *A. caballus* were shown to depend largely upon temporary rainwater sites, usually rain over grass, although *lineatopennis* and *caballus* were found rarely in effluent water.

Water samples from most habitats were submitted to a partial chemical analysis in order to define the different habitats more clearly. The habitat associations of the different species are discussed in the light of the results of this analysis. Mention is also made of the similarity between the frequencies of the different species occurring in the larval collections and the relative densities of these species found in adult collections.

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CULEX QUINQUEFASCIATUS, CULEX PIPIENS AND OTHER CULICINES OVIPOSITING IN CONTAINERS IN THE KAROO REGION OF SOUTH AFRICA

P. G. JUPP

Arbovirus Research Unit,
National Institute for Virology,
Private Bag X4,
Sandringham 2131, South Africa.

Over a 15-month period from Feb. 1, 1970, plastic containers were exposed as mosquito oviposition sites at Bethulie in the southern part of the Orange Free State Province. This was carried out as part of a survey of the mosquito fauna at this locality and particularly to determine the distribution of *Culex quinquefasciatus* and *Cx. pipiens*. Bethulie is situated in the semi-desert Karoo region of South Africa which has very hot summers with low rainfall and cold winters. Over the period of the study there was a drought until the 2nd summer when 321 mm of rain fell (December 1970 through March 1971).

The containers were plastic, 40 cm long and 25 cm square in cross-section and were filled with grass infusion to attract gravid mosquitoes. Each container was fitted with a withdrawable sleve for easy removal of larvae and was protected by a covering of 4 cm wire mesh. Five containers were sited in the environs of buildings in the town of Bethulie itself (urban) while 6 others were sited on 3 farms (rural) within 10 km of Bethulie. These oviposition sites were examined 10 times during the exposure period, usually monthly but sometimes after a 2-month interval. Samples of larvae were taken for identification and many of the determinations were confirmed by rearing these through to adults. Periodically the containers were cleaned out and filled with a fresh infusion.

Larvae collected are shown in table 1. *Culiseta longiareolata* and 5 different *Culex* species utilized the oviposition sites. *Cx. quinquefasciatus* occurred most frequently and except for 1 collection was confined to the urban area. Nearly as prevalent were *Cs. longiareolata*, which occurred to about the same extent in rural and urban environments, and *Cx. pipiens* which was commoner in the urban area. The remaining 3 species were much rarer.

On 10 occasions larvae of *Cx. quinquefasciatus* and *Cx. pipiens* were found co-existing. Samples of male mosquitoes were reared from such collections and the genitalia of 117 individuals were examined without, however, finding any evidence of hybridization between these 2 taxa. This supports the view that these 2 members of the *Cx. pipiens* complex are distinct species in South Africa.

Third or fourth instar larvae were present in 3 containers in the urban area during midwinter on July 22. There were collections of *Cs. longiareolata*, *Cx. quinquefasciatus* and in the 3rd container *Cx. quinquefasciatus* and *Cx. pipiens* together which were probably larvae overwintering by quiescence. Such larvae have been recorded previously in the highveld region of South Africa in the case of 2 of these species-*Cx. pipiens* and *Cx. quinquefasciatus* (Jupp, 1969 and 1975) but this is a new observation in the case of *Cx. longiareolata*.

The overall total of 83 collections for larvae of all species taken from the containers in the 15 month period may be compared with only 15 similar collections made from the same number of identical containers exposed for the same duration in another project at Olifantsvlei near Johannesburg in the Highveld region (Jupp, unpublished). The difference in the number of collections at these 2 localities may be attributed to the relative abundance of other aquatic breeding sites at Olifantsvlei available to mosquitoes as compared to a shortage of such sites in the Bethulie area. Species of *Culex* and *Culiseta* in the arid environment of Bethulie tend, out of necessity, to utilize whatever oviposition site is made available to them. Furthermore, the much larger number of collections from containers in the urban area of Bethulie compared with the rural area may reflect a shortage of other breeding sites in the town as compared to the farms where there are dams and streams.

Table 1. Number of occurrences of mosquito species in larval collections made from containers exposed for oviposition in rural and urban environments at Bethulie, February 1970 through March 1971.

	Rural	Urban
Cs. longiareolata	10	13
Cx. pipiens	5	14
Cx. quinquefasciatus	1	24
Cx. theileri	2	6
Cx. tigripes	1	0
Cx. univittatus	5	2
Totals	24	59

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A SURVEY OF THE MOSQUITO AND *CULICOIDES* FAUNAS AT TWO LOCALITIES IN THE KAROO REGION OF SOUTH AFRICA WITH SOME OBSERVATIONS ON BIONOMICS

P. G. J[illegible] B. M. McINTOSH(1) and E. M. NEVILL(2)

ABSTRACT

JUPP, P. G., McINTOSH, B. M. & NEVILL, E. M., 1980. A survey of the mosquito and *Culicoides* faunas at two localities in the Karoo region of South Africa with some observations on bionomics. *Onderstepoort Journal of Veterinary Research*, 47, 1–6 (1980).

The mosquito and *Culicoides* faunas were surveyed at Bethulie and Luckhoff in the arid Karoo region, southern Orange Free State, to determine which species occurred, their relative prevalence and the effects of rainfall. The feeding preferences of these insects were also investigated by means of baited catches.

Twenty-three mosquito species and 16 *Culicoides* species were collected. The commonest mosquito species, with their feeding preferences, if known, were as follows: *Culex* (*Culex*) *univittatus* Theo and *Culex* (*Culex*) *pipiens* Linnaeus, which are strongly ornithophilic and poorly anthropophilic; *Culex* (*Culex*) *theileri* Theo, which feeds on sheep and man avidly but is only moderately ornithophilic; *Aedes* (*Neomelaniconion*) *luridus* McIntosh, *Aedes* (*Neomelaniconion*) *lineatopennis* (Ludlow), *Aedes* (*Ochlerotatus*) *caballus* (Theo) and *Aedes* (*Ochlerotatus*) *juppi* McIntosh, all of which feed on sheep and man readily and which can aestivate as eggs for up to 20 months but only appear in numbers after rain; *Anopheles* (*Cellia*) *listeri* De Meillon, *Anopheles* (*Cellia*) *squamosus* Theo, *Culex* (*Culex*) *quinquefasciatus* Say and *Culiseta* (*Allotheobaldia*) *longiareolata* (Macquart). By far the commonest *Culicoides* at both localities was *Culicoides pycnostictus* Ingram & Macfie, which is strongly ornithophilic and also feeds on sheep. The following 5 species were also prevalent: *Culicoides similis* Carter, Ingram & Macfie, *Culicoides* spec. nov. 1., *Culicoides schultzei* (Enderlein), *Culicoides onderstepoortensis* Fiedler and *Culicoides nivosus* De Meillon. The last species is strongly ornithophilic.

Résumé

UNE ENQUÊTE DES FAUNES DE MOUSTIQUES ET DE CULICOIDES *DANS DEUX LOCALITÉS DE LA RÉGION DU KAROO EN AFRIQUE DU SUD AVEC CERTAINES OBSERVATIONS ÉCOLOGIQUES*

Des faunes de moustiques et de Culicoides *ont été recherchées à Bethulie et à Luckhoff dans la région aride du Karoo dans le sud de l'Etat Libre d'Orange pour déterminer quelles espèces survenaient, leur predominance relative et les effets des chutes de pluie. Les préférences alimentaires de ces insectes furent également investiguées au moyen de pièges à amorce. Vingt-trois espèces de moustiques et* 16 *espèces de* Culicoides *ont été rassemblées. Les espèces de moustiques les plus communes, avec leur préférences alimentaires, quand elles étaient connues, furent les suivantes:* Culex (Culex) univittatus *Theo et* Culex (Culex) pipiens *Linnaeus, qui sont fortement ornithophiliques et faiblement anthropophiliques;* Culex (Culex) theileri *Theo, qui s'alimente aviidement sur le mouton et sur l'homme mais qui est seulement modérément ornithophilique;* Aedes (Neomelaniconion) luridus *McIntosh,* Aedes (Neomelaniconion) lineatopennis (*Ludlow*), Aedes (Ochlerotatus) caballus (*Theo*) *et* Aedes (Ochlerotatus) juppi *McIntosh, qui tous se nourrissent principalement sur le mouton et sur l'homme et qui peuvent estiver sous forme d'oeufs jusqu'à 20 mois mais apparaissent seulement en nombres après la pluie;* Anopheles (Cellia) listeri *De Meillon,* Anopheles (Cellia) squamosus *Theo,* Culex (Culex) quinquefasciatus *Say et* Culiseta (Allotheobaldia) longiareolata (*Macquart*). *De loin le plus, commun des* Culicoides *dans les deux localités fut* Culicoides pycnostictus *Ingram & Macfie, qui est fortement ornithophilique et qui s'alimente aussi sur le mouton. Les cinq espèces suivantes furent aussi prédominantes:* Culicoides similis *Carter, Ingram & Macfie,* Culicoides *spec. nov.* 1., Culicoides schultzei (*Enderlein*), Culicoides onderstepoortensis *Fiedler et* Culicoides nivosus *De Meillon. La dernière espèce est fortement ornithophilique.*

INTRODUCTION

In the past the mosquito and *Culicoides* faunas of the Karoo region have received little attention. However, it was feared that after the construction of dams and irrigation works on or along the Orange River in the arid Karoo region of the Orange Free State, there might be an increase in the populations of arbovirus vectors in this area. This in turn would probably increase virus transmissions and thus endanger the health of both humans and livestock. Studies were therefore conducted on the prevalence of these viruses in insects and sentinel avian hosts at 2 representative localities, Bethulie and Luckhoff (McIntosh, Jupp & Dos Santos, 1978; McIntosh & Jupp, 1979).

Mosquitoes, *Culicoides* and, to a lesser degree, Simuliidae were collected and assayed for virus over 4 summers, at Bethulie during 3 summers between December 1968 and March 1971 and at Luckoff during the summers of 1971 and 1976. The collection methods used were aimed primarily at securing large numbers of insects for virus assay and they tended to be somewhat biased towards the collection of *Culex* (*Culex*) *univittatus* Theo because of this mosquito's known importance as a vector of West Nile and Sindbis viruses. However, efforts were made to employ methods which would also show which species of mosquito and *Culicoides* were present in the 2 localities, their relative prevalence and the feeding preferences of the commoner species. One aspect of this survey, that is, on mosquitoes ovipositing in containers, has already been reported (Jupp, 1978). This paper reports the findings on the collections of adult insects.

STUDY LOCALITIES

Bethulie and Luckhoff, which are located about 150 km apart near the Orange River in the southern part of the Orange Free State, both have an altitude of 1 289 m and are situated in the semi-desert Karoo region where the vegetation is mainly xerophytic dwarf shrub. Both localities are in sheepfarming areas and insect collections were made on several such farms in the vicinity and, in the case of Bethulie, also near the dam in the town itself. Insect traps were usually set near dams, vleis or perennial streams on the farms.

(1) Arbovirus Unit, National Institute for Virology, Private Bag X4, Sandringham, Johannesburg 2131
(2) Veterinary Research Institute, Onderstepoort 0110

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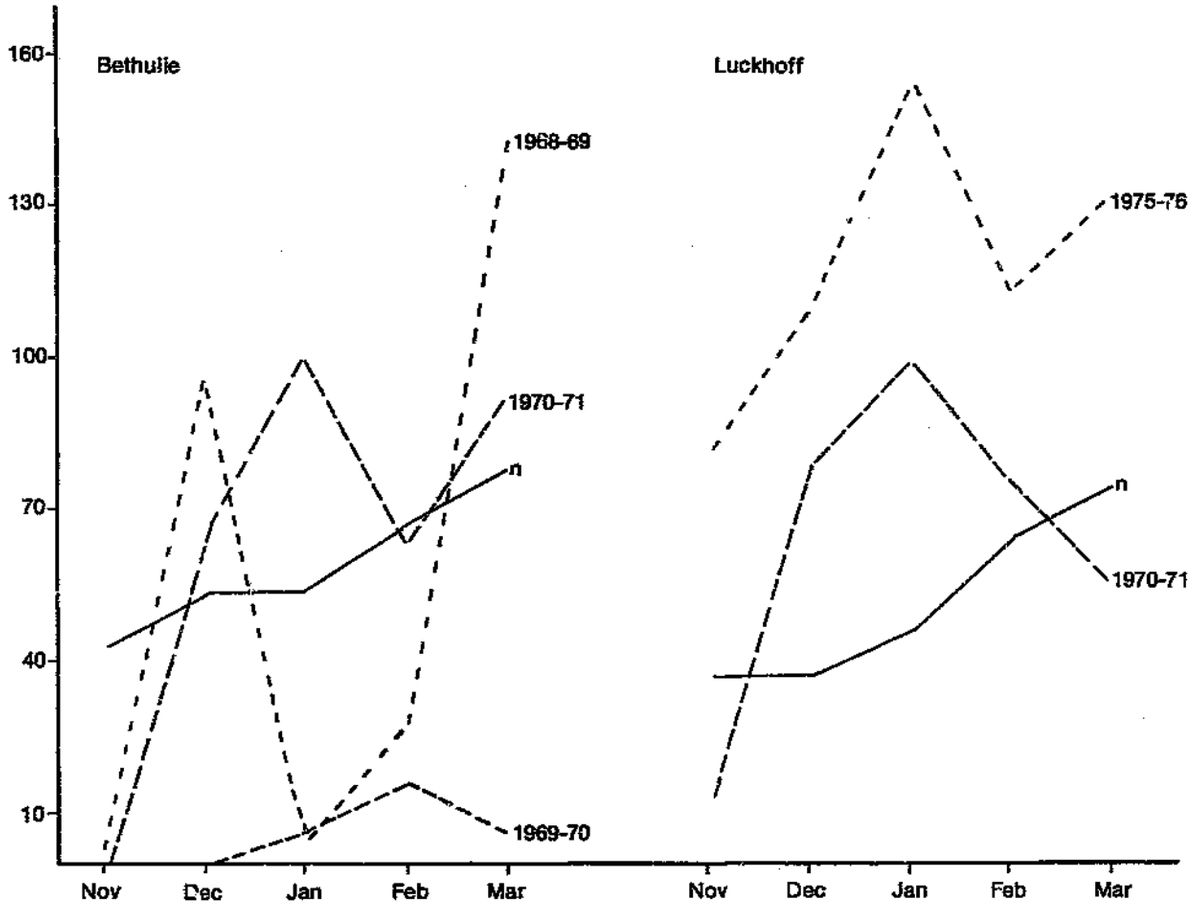

FIG. 1 Monthly rainfall (mm) at Bethulie and Luckhoff during summers when insects were collected. The normal (n) rainfall is shown for comparison.

The Karoo climate has very hot summers with a low rainfall and dry, cold winters. The mean annual rainfall for Bethulie is 439 mm and Luckhoff is somewhat drier with 379 mm. From November to March, when collecting was undertaken, the mean daily temperature varies from about 20–23 °C, the mean daily minimum being 11–15 °C while the mean daily maximum soars to about 28–31 °C. The normal monthly rainfall in summer at the 2 localities given in Fig. 1 shows that it is lower at Luckhoff for each of the 5 months. Monthly precipitation during the summers when the study took place is also shown. During the 1968–69 summer at Bethulie, rainfall was negligible during November, January and February but well above average in December and March, particularly in March, when it reached 143 mm. In 1969–70 there was a severe drought when very little rain fell the whole summer at Bethulie, while in 1971 precipitation was consistently above average at both study localities. Figures shown for the 1975–76 summer at Luckhoff reveal the abnormally high rainfall of that season.

Collection of mosquitoes and Culicoides

Five different collecting methods were used with traps operated overnight. In 3 of these methods the type of bait varied as follows:

(1) *Light trap:* This had an 8 watt suction motor and a 3 candle-power incandescent bulb powered by a 12 volt car battery. It was hung with its entrance about 1 m above the ground and insects were sucked downwards into a collecting cage made of organdie. The filter fitted over the entrance was either 5 mm wire mesh or nylon mosquito screen. The former prevented insects larger than mosquitoes from entering the trap while the latter prevented the entrance of these as well as mosquitoes, so that only *Culicoides* and similar small-sized insects were collected. In the summer of 1976 a few traps with the coarse filter were used with carbon dioxide as an additional attractant, as described previously (Jupp, McIntosh & Anderson, 1976).

(2) *Suction trap:* This consisted of the same motor unit as the light trap, without the light and filter, and was used in conjunction with either pigeon or sheep bait. When pigeons (*Columba itvea*) were used the open end of the cylinder containing the motor unit was inserted into the bottom of a cage containing 2 sentinel birds about 2 m above ground level. Diptera attracted to the birds were sucked downwards into the collecting cage before they could feed. When used with sheep, the suction trap was mounted horizontally in the side of a wire-mesh cage containing the animal.

(3) *Lard-can type No. 6 trap:* This is based on the lard-can trap designed by Bellamy & Reeves (1952), but is smaller (Jupp, 1969). Each trap was baited with a pigeon enclosed in a cage of nylon mosquito screen which prevented mosquitoes from feeding. Traps were suspended 1,2–1,8 metres above ground level.

(4) *Net trap:* This trap, which was described by Jupp & McIntosh (1967), was baited either with about 2,5 kg of solid CO_2 inside a cardboard box, or with a sheep, penned in a wire cage within the net.

(5) *Human-baited catches:* These were undertaken at sunset with 2 or more catchers and lasted up to 1 hour. Each catcher operating outdoors alone, collected mosquitoes alighting on his legs with test tubes. Light was provided by an electric torch shaded with a red cloth cover.

The total number of trap-nights recorded with each method is listed below as well as the number of man-hours for the catches off human bait.

Light trap.....	(a) With coarse filter.....	145
	(b) With mosquito filter..	65
	(c) With coarse filter and CO_2 bait.....	8
Suction trap....	(a) Pigeon-baited.....	58
	(b) Sheep-baited.....	5
Lard-can trap...	Pigeon-baited.....	157
Net trap.....	(a) CO_2-baited.....	34
	(b) Sheep-baited.....	14
Human-baited catches.....		16½ man-hours

Mosquito and Culicoides *identification*

The nomenclature used for Anopheline mosquitoes follows that of Gillies & De Meillon (1968), and for culicines that of Knight & Stone (1977), except in the case of the 2 members of the *Culex pipiens* complex occurring in South Africa which are referred to as *Cx.* (*Culex*) *pipiens* L. and *Cx.* (*Cx.*) *quinquefasciatus* Say. The abbreviations used for generic and subgeneric names follow those suggested by Reinert (1975). Certain changes in nomenclature occurred in 2 aedine subgenera during the course of the study, 2 new species being named in the subgenus *Neomelaniconion* (McIntosh, 1971) and 1 in the subgenus *Ochlerotatus* (McIntosh, 1973), which were all found in the study area. It seems likely that the 2 *Neomelaniconion* species, *Ae. luridus* McIntosh and *Ae. unidentatus* McIntosh, were previously confused with *Ae. lineatopennis* Ludlow, and the *Ochlerotatus* species *Ae. juppi* McIntosh with *Ae. caballus*. Hence collections of *Neomelaniconion* and *Ochlerotatus* mosquitoes made prior to the recognition of these new species are referred to in the report as *lineatopennis* group and *caballus* group respectively.

The nomenclature used for *Culicoides* (*Cul.*) follows that of Fiedler (1951) with 3 exceptions as follows: Fiedler's *Cul. pallidipennis* Carter, Ingram & Macfie is referred to as *Cul. imicola* Kieffer and *Cul. babrius* De Meillon as *Cul. tropicalis* Kieffer according to the revision by Kremer (1972); while his *Cul. hirtius* De Meillon & Lavoipierre is referred to as *Cul. brucei* Austen after the revision by Khamala & Kettle (1971). One new *Culicoides* species with clear wings (*Cul.* spec. nov. 1.) and a second with spotted wings (*Cul.* spec. nov. 2.) were discovered during the study and are in the process of being described by Dr M. Cornet of ORSTOM and a co-author of this paper (E.M.N.).

Results

Traps

Mosquitoes were caught in all the traps used except in the light traps fitted with a mosquito filter, while *Culicoides* were collected only in suction and light traps. The pigeon-baited lard-can traps collected far fewer mosquitoes per trap night than suction traps, but they collected a higher proportion of *Cx. theileri*, as was also suggested during previous collections on the Highveld (Jupp, 1973). The net trap, baited with either CO_2 or a sheep, and the light trap, particularly when used with CO_2 bait, were found effective for collecting a wider range of mosquito species including the seasonal *Aedes*.

Species prevalence

A total of 22 798 adult female mosquitoes belonging to 23 species, divided among 4 genera, were caught at Bethulie and Luckhoff (Table 1). *Anopheles*, with 5 species, was represented by 852 insects and composed only 3,7% of the total catch. *Aedes*, with 9 species, totalled 8 931 insects (39,2% of catch). *Culex*, with possibly 8 species, totalled 12 709 insects and composed the largest proportion of the catch (55,7%). *Culiseta* was represented by a single species.

TABLE 1 Numbers of adult female mosquitoes collected at Bethulie and Luckhoff by all methods used over 69 nights during 4 summers (1968/69, 69/70, 71 & 76)

	No.	No. as % of total(c)
Anopheles (*An.*) *coustani*.....	8	
Anopheles (*An.*) *implexus*.....	1	
Anopheles (*Cel.*) *cinereus*.....	2	
Anopheles (*Cel.*) *listeri*.....	142	
Anopheles (*Cel.*) *squamosus*.....	693	3,0
Anopheles spp. undet.....	6	
Aedes (*Ad.*) *dentatus*.....	21	
Aedes (*Ad.*) *durbanensis*.....	1	
Aedes (*Ad.*) *hirsutus*.....	1	
Aedes (*Neo.*) *lineatopennis*.....	689	3,0
Aedes (*Neo.*) *luridus*.....	50	
Aedes (*Neo.*) *unidentatus*.....	4	
Aedes (*Neo.*) *lineatopennis* group(a).....	4 118	18,0
Aedes (*Och.*) *caballus*.....	981	4,3
Aedes (*Och.*) *juppi*.....	962	4,2
Aedes (*Och.*) *caballus* group (b).....	2 092	9,1
Aedes (*Stg.*) *aegypti formosus*.....	1	
Aedes spp. undet.....	11	
Culex (*Cx.*) *annulioris*.....	19	
Culex (*Cx.*) *pipiens*.....	2 379	10,4
Culex (*Cx.*) *quinquefasciatus*.....	54	
Culex (*Cx.*) *theileri*.....	4 108	18,1
Culex (*Cx.*) *tigripes*.....	1	
Culex (*Cx.*) *univ[illegible]tus*.....	5 677	24,7
Culex (*Mat.*) *sali[illegible]ensis*(d).....	58	
Culex spp. undet.....	413	1,8
Culiseta (*All.*) *longiareolata*.....	306	1,3
Total Culicidae.....	22 798	

(a)=*luridus*, *lineatopennis* and *unidentatus*—see results
(b)=*caballus* and *juppi*—see results
(c)=In this and subsequent tables a blank indicates less than 1%
(d)=Examination of larvae showed that both *salisburiensis* and the subspecies *naudeanus* occurred at Bethulie.

Among *Aedes*, the subgenus *Aedimorphus* was poorly represented, as was the subgenus *Stegomyia*, with only a single specimen. Three species of *Neomelaniconion* and 2 of *Ochlerotatus* were collected. Because 3 of these 5 species were discovered only during the study, collections prior to their discovery are referred to as the *lineatopennis* group and *caballus* group respectively. Collectively, these were a dominant part of the mosquito fauna of the region and in numbers collected accounted for 39,0% of the total catch, although active females were only plentiful after heavy rains. From collections in which species identifications were possible among *Neomelaniconion* mosquitoes, *Ae. luridus* was most prevalent at Bethulie and *Ae. lineatopennis* at Luckhoff, although all 3 species in this subgenus were prevalent at Luckhoff. In collections at Luckhoff in 1976 *Ae. caballus* and *Ae. juppi* were present in approximately equal numbers.

TABLE 2 Numbers of male and female *Culicoides* (*Cul.*) collected in 40 light-trap-nights at Bethulie and Luckhoff during 2 summers (1969/70, 1971)

	No.	♂ as % of total
Cul. pycnostictus	2 314	46,6
Cul. similis	843	16,9
Cul. spec. nov. 1.(a)	720	14,5
Cul. schultzei	286	5,7
Cul. nivosus	275	5,5
Cul. onderstepoortensis	226	4,5
Cul. distinctipennis	63	1,2
Cul. neavei	55	1,1
Cul. tropicalis	49	
Cul. imicola	44	
Cul. bedfordi	36	
Cul. magnus	23	
Cul. brucei	15	
Cul. huambensis	2	
Cul. gulbenkiani	1	
Cul. spec. nov. 2.(b)	1	
Cul. spp. undet	11	

(a)=A clear-winged species
(b)=A species with spotted wings
Both species are being described by M. Cornet (ORSTOM) and E. M. Nevill

TABLE 3 Mosquitoes attracted to different animal baits and to man

	Pigeon (215)(a)		Sheep (19)(a)			Man(b)	
	No.	No. as % of total	No.	No. as % of total	Mean % fed(c)	No.	No. as % of total
An. coustani			5		0	3	
An. listeri			13		0		
An. squamosus	6		49	2,7	53	1	
Ae. caballus group	22		147	8,3	39	86	15,8
Ae. dentatus			2		0	11	2,0
Ae. luridus	9		3		0		
Ae. lineatopennis group	19		83	4,7	59	240	44,1
Aedes spp. undet			2		0		
Cx. annulioris	18						
Cx. pipiens	2 081	27,2	13		0		
Cx. quinquefasciatus	41						
Cx. salisburiensis	1						
Cx. theileri	405	5,3	1 381	78,4	75	202	37,2
Cx. univittatus	4 625	60,6	47	2,6	9		
Culex spp. undet	333	4,3	14		0		
Cs. longiareolata	63		2		0		

(a)=No. of trap-nights
(b)=16,5 man-hours collecting over 8 days
(c)=Arithmetic mean of percentage fed in each collection

Culex was dominated by 3 species, *Cx. univittatus*, *Cx. theileri* and *Cx. pipiens*, which between them accounted for 53,4% of the total catch. The presence of both *Cx. salisburiensis* and its subspecies *naudeanus* were established from larval identification. Even during the 1969–70 summer, when there was a severe drought (Fig. 1), appreciable numbers of *Cx. univittatus* (898) and *Cx. theileri* (727) were still collected.

Total catches of *Culicoides* and Simuliidae were not always recorded, partly because of the large numbers of *Culicoides* present. Random samples, consisting of a total of 16 031 *Culicoides* and 2 791 Simuliidae, were counted, and these figures are probably a fair representation of the relative numbers of these 2 groups of insects in the collections. In addition 16 species of *Culicoides*, collected in 40 light trap-nights, were identified and counted (Table 2).

Host preferences

The results of mosquito collections with the use of either pigeon, sheep or human baits are given in Table 3. While a variety of species were caught in pigeon-baited traps, these catches were dominated by *Cx. univittatus* and *Cx. pipiens*, with *Cx. theileri* also present in moderate numbers. Sheep-baited traps yielded largely *Cx. theileri*, but fair numbers of the *caballus* and *lineatopen. is* groups were also caught. A high proportion of these mosquitoes was recorded as having fed on the sheep, as was also the case for *An. squamosus*. With the exception of *An. squamosus* the same species and species groups also predominated in the man-baited collections.

Table 4 gives the numbers of *Culicoides* species collected in pigeon-baited suction traps. *Cul. pycnostictus* and *Cul. nivosus* dominated these collections

at an even higher relative proportion than in light trap collections (Table 2). It therefore seems likely that these 2 species are attracted to and would feed on birds in the Karoo in significant numbers. Conversely, *Cul. similis*, *Cul.* spec. nov. 1., *Cul. schultzei* and *Cul. onderstepoortensis*, which were present in fair numbers in the light trap collections, were either poorly represented (*Cul.* spec. nov. 1.) or absent in the pigeon-baited collections, which indicates that probably none of these species are attracted to birds.

TABLE 4 *Culicoides* (*Cul.*) collected in 27 trap-nights in pigeon-baited suction traps

	No.	No. as % of total
Cul. pycnostictus	809	80,5
Cul. nivosus	149	14,8
Cul. neavei	18	1,7
Cul. spp. undet.	17	1,6
Cul. spec. nov. 1.	10	
Cul. imicola	1	

At Luckhoff in January, 1971, 39 *Cul. pycnostictus* were collected in sheep-baited suction traps during 5 nights, and in March, 1971, a suction trap set inside a sheep-baited net trap during 4 nights collected 11 of the same species. These results suggest that *Cul. pycnostictus* also feeds on sheep in the Karoo.

Collections made in pigeon-baited suction traps at Bethulie in 1971 indicated that some species of Simuliidae are strongly attracted to birds.

Discussion

Mosquitoes

Because of unavoidable variables resulting from individual trap bias, times which collections are made and siting of traps, it is extremely difficult to determine accurately the relative prevalence of species among a mosquito fauna. The numbers of the various mosquito species shown in Table 1 must therefore be assessed with this in mind. None the less, a variety of collecting methods were used during various climatic conditions over several years and the figures are meaningful in a particular context. They are the result of collections made for a specific purpose, namely, to investigate adult female mosquitoes as possible vectors of viruses. To some extent the vector species were known and the collecting methods were designed partly with these species in mind. Since our main interest was those species feeding on birds, domestic ungulates and man in a particularly arid region of the inland plateau in South Africa, a numerical presentation of the collections serves a useful purpose to this end.

The collections showed a considerable numerical predominance of *Cx. univittatus*, *Cx. theileri*, *Cx. pipiens* and certain members of the *Ae. lineatopennis* and *Ae. caballus* groups. All these species have in varying degrees been implicated as virus vectors during other studies (Gear, De Meillon, Le Roux, Kofsky, Rose-Innes, Steyn, Oliff & Schultz, 1955; Jupp & McIntosh, 1967; Jupp, 1976a, b; McIntosh, Jupp, Dos Santos & Meenehan, 1976). By revealing their prevalence in the Karoo, the present collections, indicate that they could act as vectors in this region. *Culex quinquefasciatus* and *Culiseta* (*Ali*) *longiareolata* were collected in only small numbers but, judging from the frequency with which they occurred in larval collections (Jupp, 1978), apparently both are prevalent species.

The difference in life histories among members of the *Aedes* and *Culex* genera has implications for their roles as vectors. *Aedes* pass through adverse climatic periods as drought-resistant eggs on the ground. In this form they remain viable for long periods as, for example, from April 1969 until December 1970, a period of 20 months, when the 1969–70 summer rains failed. Although adults of certain *Aedes* species appear in enormous numbers after sufficient rain has inundated the dormant eggs, the adults are short-lived and their ability to transmit viruses is correspondingly restricted in time. Despite this handicap, they possess great potential as vectors because they produce large populations, albeit for a short period. The collections showed that members of the *Ae. lineatopennis* and *Ae. caballus* groups belong to this category.

The eggs of *Culex* species are not drought resistant; these species survive adverse conditions as adults, pupae or larvae. They utilize permanent or semi-permanent ground pools for breeding and consequently the active adult female is present throughout the summer and autumn and their ability to transmit viruses extends over a longer period of the year. Where ground pools occur, *Culex* species will usually be found, as in the present study. The collections suggested that even during dry summers, virus transmission in the Karoo by *Cx. univittatus* and *Cx. theileri* would be possible. Since humans and domestic animals in an arid environment tend to cluster around permanent water, this habit of *Culex* species has obvious epidemiological implications.

Numbers of various mosquito species caught in the baited collections (Table 3) concurred with host preferences recorded in the Highveld region for those species common to both regions (Jupp & McIntosh, 1967; Jupp, 1973). In both regions *Cx. univittatus* and *Cx. pipiens* were strongly ornithophilic and poorly anthropophilic, while *Cx. theileri* was strongly attracted to ungulates and man besides being moderately ornithophilic. Members of the *Ae. lineatopennis* and *Ae. caballus* groups were shown to feed on sheep and man. Because of apparent differences in prevalence among species of these groups in the Highveld and Karoo, it seems that *Ae. luridus*, *Ae. lineatopennis*, *Ae. caballus* and *Ae. juppi* could be important vectors in the Karoo, whereas in the Highveld only *Ae. unidentatus* and *Ae. juppi* would assume this role.

Culicoides

Assuming the different *Culicoides* species are equally attracted to light traps, the numbers of the various species given in Table 2 are probably a fair representation of their relative abundance. *Cul. pycnostictus* probably dominates all other species in the Karoo and a further 5 species are fairly prevalent.

The predominance of *Cul. pycnostictus* on most of the farms at the 2 localities and the prevalence of bluetongue virus in the 2 areas suggest that this species could be the most likely vector of bluetongue virus in this region, particularly as the proven vector *Cul. imicola* is rare. In this connection it seems that *Cul. pycnostictus* does feed on sheep, judging by its collection in suction traps set near a sheep at Luckhoff. Its preference for birds shown here (Table 4) agrees with bloodmeal identifications for *Cul. pycnostictus* collected at Onderstepoort in the Transvaal

(Nevill & Anderson, 1972). *Cul. nivosus* was rare in the collections previously made at Onderstepoort by Nevill & Anderson (1972), but in the Karoo it was fairly common and apparently ornithophilic.

The densities of *Culicoides* were probably higher than is suggested by the study since trapping was mainly directed towards the collection of mosquitoes. *Culicoides* are probably better adapted to the arid Karoo than mosquitoes because their immature stages are less dependent on rainfall and it is their habit to develop in moist soil or dung rather than water. Thus it is that during droughts they are probably maintained at higher levels than mosquitoes. The collections showed that trapping sites, season and rainfall all influence catches of these midges.

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14.1

A Morphological Study of *Culex* (*Culex*) *univittatus* Theobald and *Culex* (*Culex*) *neavei* Theobald from Various African Countries

by

P. G. Jupp
Arbovirus Research Institute
South African Institute for Medical Research
Johannesburg[1/]

Mosquito samples from various African countries consisting of specimens ascribed to *Culex univittatus* and *Culex neavei* were studied morphologically and compared with these species from South Africa in an attempt to make specific determinations. A few *univittatus* were identified from Niger, Upper Volta and Dahomey in West Africa. However, a similar number of the specimens from Niger and the majority of those from the other 2 countries were identified as *neavei* and an aberrant form close to *neavei*, while these 2 forms only occurred in samples from Nigeria and Mauritania. Small samples from Tanzania, Kenya and Reunion were exclusively *neavei*, while that from Egypt probably contained only the aberrant form. This form, which is recognized from its male genitalia, may represent a distinct species. A single specimen from Ethiopia was *univittatus*.

Culex univittatus Theobald

Culex univittatus Theobald, 1901, Mon. Cul. 2:29; Edwards, 1911, Bull. ent. Res. 2:262; Edwards, 1941, Mosq. Ethiopian Region 3:306; Jupp, 1971, J. ent. Soc. sth. Afr. 34:339. (For complete bibliography, including that of synonyms, see Stone *et al.*, 1959.).

TYPE DATA. Holotype female, 4 paratype females, 5 paratype males and 1 paratype male genitalia, Salisbury, RHODESIA. All types in British Museum.

Note on type locality of *Culex univittatus* Theobald

In the first description Theobald (1901) describes the "habitat" of *univittatus* as Durban (Natal), Salisbury (Rhodesia) and Singapore. Stone *et al.* in their catalogue of mosquitoes (1959) refer to the type localities as Durban and Salisbury. However, it appears that only Salisbury should be considered as the type locality of *C. univittatus*. Edwards (1941) gives this single locality and furthermore, Mattingly (*in litt.*) has informed me that the type material held in the British Museum all originates from Salisbury.

Culex neavei Theobald

Culex neavei Theobald, 1906, Rep. Wellcome Lab. 2:76, and 1907, Mon. Cul. 4:429; Edwards, 1914, Bull. ent. Res. 5:67; Jupp, 1971, J. ent. Soc. sth. Afr. 34:339.

Culex univittatus var neavei Edwards, 1922, Bull. ent. Res. 13:85; Edwards, 1941, Mosq. Ethiopian Region 3:308.

TYPE DATA. Holotype female and 1 paratype female, Lualas, SUDAN, 1 paratype female, Lado, SUDAN. Types in British Museum.

1/ Work financed jointly by the South African Institute for Medical Research and the Poliomyelitis Research Foundation.

INTRODUCTION

A study of the morphology, geographical relationship and genetics of 2 forms of *C. univittatus* present in South Africa was recently reported (Jupp,1971). In this study it was found that one form, restricted to high altitude, was conspecific with type material of *C. univittatus* Theobald, 1901. The other form, of lowland distribution, was conspecific with the types of *C. univittatus var neavei* Edwards, 1922, and was raised to specific status. Morphologically the former was identical to *univittatus* and the latter similar to *neavei* except in regard to markings on terga and sterna. Morphological study of specimens collected in the area of contact between the ranges of the 2 forms indicated that they were largely reproductively isolated and cross-mating between laboratory colonies of the 2 forms failed to occur because of ethological isolation. Subsequently, material considered as belonging to the 2 species from other African countries has been made available to the author. In an attempt at specific determinations this material has been compared with South African material with emphasis on characters found to be reliable in the earlier study for distinguishing the 2 species.

MATERIALS AND METHODS

Mosquitoes

All the pinned specimens studied from countries outside South Africa were borrowed from the collection of the Office de la Recherche Scientifique et Technique Outre-Mer held in Bondy, France, except those from Egypt which were borrowed from Dr. K. L. Knight of North Carolina State University, Raleigh. Samples originated from Upper Volta, Dahomey, Nigeria, Mauritania and Niger in West Africa; from Tanzania, Kenya and Ethiopia in East Africa and from Reunion and Egypt. With the exception of Mauritania and Egypt which are Palaeartic, all these countries are within the Ethiopian region. As far as could be ascertained none of the localities in each country exceeded 500 m in altitude, except probably Ataie in Ethiopia and possibly some localities in Niger.

Identification Procedure

Eight characters were looked for in specimens of both sexes to determine their occurrence and another 2 were studied in the genitalia of males. Table 1 lists all these characters showing the frequency with which each occurs in *univittatus* or *neavei* from South Africa or, in the case of the genitalia, the appearance of the structure concerned. Terminology used to describe structures in the male genitalia is that of Knight and Laffoon (1971). Two structures were examined: the leaflet borne by the subapical lobe of the gonocoxite and the outer division of the lateral aedeagal plate (LAP). The leaflet was inspected to see whether its shape was narrow or broad and this was usually expressed as a leaflet index (R_1) (Jupp, 1971). The spine borne by the outer division of the LAP was classified as short, long or intermediate in length.

The examination of South African material reported previously (Jupp, 1971) and summarized in table 1 showed that *univittatus* and *neavei* may be distinguished in the female by the mid femur and in the male by the leaflet. Female *univittatus* has an anterior white stripe on the mid femur which is missing in *neavei*: male *univittatus* possesses a narrow leaflet on the gonocoxite with an R_1 index of 33-50 while *neavei* possesses a broad leaflet with an R_1 index of 50 or more. The previous study did not include mention of the second character in the male genitalia. Examination of the spine borne by the LAP in South African material

shows that it is always long in *neavei* (fig. 2c) while in *univittatus* it is sometimes long but more often of intermediate length (fig. 2b). Hence, this character is precluded from being suitable for separating the 2 species but it was examined in males in the present study after the discovery that some individuals possessed a short spine (fig. 2a) quite distinct from that of *univittatus* or *neavei*. Thus, in classifying the present material females were identified as *univittatus* or *neavei* on the basis of the mid femur while, depending upon the appearance of the 2 characters in their genitalia, male specimens were identified as follows. Specimens with a narrow leaflet and long or intermediate LAP spine were considered *univittatus*, those with a broad leaflet and long or intermediate LAP spine as *neavei*, while any specimen with a short LAP spine was designated the "short-spined form". The observations made on the remaining 7 characters in both sexes served to indicate any differences from South African *univittatus* or *neavei*.

Technique for male genitalia

The method of Edwards (1941), with modifications, was used for preparing mounts of male genitalia stained with carbol fuchsin. Gonocoxites were mounted separately, the leaflet either being measured while still attached to the subapical lobe or after detachment. Where the size of the sample from a country was large, in a proportion of the specimens the phallosome was mounted intact and the LAP examined in situ. However, in most cases the outer divisions of the LAPs were dissected from the remainder of the phallosome and mounted separately. Preparations were examined under a compound microscope at a magnification of 540. Camera lucida drawings were made of one or more of the leaflets in males from each country. Similar drawings were also made of half or more of the preparations of the LAP from each country before the category of spine borne was determined.

RESULTS

C. univittatus

Only 5 females and 5 males were identified as *univittatus* as shown in table 2. Three of the females had a faint stripe on the mid femur and 2 a clear stripe. The males all possessed leaflets with a narrow shape very similar to that of *univittatus* from South Africa, although in most cases they tended to be shorter and slightly more convex (see figs. 1 a-c). The 2 broadest leaflets belonging to the 2 males from Niger had an R_1 index of 50 which is the bottom of the range determined for *neavei* (Jupp, 1971). However, their shape appeared nearest to that of *univittatus*. For all the males the spine borne by the LAP was of intermediate length as is most usual in *univittatus* from South Africa. The remaining characters assessed in both sexes, where the condition of a specimen permitted a full examination, occurred as in South African material except that one male from Niger lacked pale scales at the base of the costa in the wing.

C. neavei

Most of the female specimens were identified as *neavei*. The frequencies at which 5 of the characters occurred in the samples designated *neavei* are given in table 3. The remaining 3 characters, not included in this table, are a striped mid femur, post spiracular scales and an apical

spot on the hind tibia. The first was absent and the others present in all the specimens. From table 3 it can be seen that most of the samples agreed well with *neavei* in all respects. Important exceptions to this were the absence of dark apical lateral markings on the sterna in some individuals from Upper Volta and Egypt and the absence of basal costal scales in the wings of some specimens from Upper Volta and Dahomey.

After the few *univittatus* specimens had been removed from the male material, of the 66 males remaining, 34 were identified as *neavei* and 32 as the short-spined form. Table 4 includes a list of the samples of males identified as *neavei*, together with their R_1 values. These specimens all possessed bro d leaflets with R_1 index, when measured, ranging from 50-84. Leaflet shape was very similar to that exhibited by *neavei* from South Africa (figs.1.e-k), except in the case of males from Egypt (fig. 1.d). In these it appeared to be intermediate between that of *univittatus* and *neavei* in spite of having an R_1 index within the range of that shown by *neavei*. The spine on the LAP was of intermediate length in all the samples (e.g., figs. 2c, h & k) except in those from Tanzania and Kenya where it was long (figs. 2f & i). Drawings of the outer division of the LAP from these 2 countries were identical to that of *neavei* from South Africa shown in fig. 2c. A faintly striped mid femur occurred on the one side of a single specimen from Upper Volta and Reunion respectively, and on both sides of 1 specimen from Dahomey; a striped mid femur has not been encountered in South African *neavei*. The other characters examined occurred in all the specimens except for 2 which lacked basal costal scales on the wing.

Short-spined form

Table 4 also shows those male specimens identified as the short-spined form. Examples of the outer division of the LAP from such specimens can be seen in figs. 2, a, d, g, j, l. & m. Most individuals belonging to this form had the broad leaflet characteristic of *neavei* with R_1 values of 50-81 except 4 specimens which possessed the narrow leaflet characteristic of *univittatus* with R_1 values of 43-48. None of the specimens exhibited a striped mid femur except 1 from Upper Volta and 1 from Dahomey where a faint stripe occurred. The remaining 7 characters were always present.

DISCUSSION

The distribution of *univittatus*, *neavei* and the aberrant short-spined form in the samples examined from each country are given in table 5. Out of a total of 128 specimens only 10 were identified as *univittatus*, the remainder being identified as *neavei*, or the short-spined form. As this aberrant form is only detectable in the male it is thought that some of the female specimens ascribed to *neavei* must have corresponded to it. This is probably the case for all the females from Egypt as all males from this country belonged to the aberrant form. It would appear, therefore, that *univittatus* is not so widespread in Africa as previously believed and that it has frequently been confused with *neavei* or the short-spined form. In South Africa *univittatus* was found to be restricted to the highlands and *neavei* to the lowlands (Jupp 1971). The results of the present study tend to be in accord with this, as *univittatus* was rare and *neavei* common in samples which nearly all originated from localities of low altitude. Also, the collection localities in Ethiopia and Niger where some of the *univittatus* specimens originated may be high.

In the phallosome of male genitalia only 2 types of LAP were clearly distinguishable - one with a long spine (those classified as intermediate or long) and one with a short spine on the outer division. Males of the short-spined form may represent a sibling species of *neavei*. Further morphological study on larger samples, including reared siblings would be necessary towards an assessment of whether this is so or whether the aberrant form merely represents polymorphism. Crossing experiments would also probably be necessary before the taxonomic status of this form could be finally established. Mattingly (1954) drew attention to 3 forms in Africa based on the LAP character which he ascribed to *univittatus* and suggested might be climatypes. These were a western Mediterranean form (long spine), an eastern Mediterranean form (short-spine) and an intermediate form (spine of intermediate length). According to Mattingly the western Mediterranean form agrees closely with the type form of *univittatus*. Senevet *et al.* (1957) have also reported intermediate and eastern Mediterranean forms in Algeria. The eastern Mediterranean form with the short spine is said to correspond to *Culex perexiguus* Theobald, one of the synonyms of *univittatus*. However, examination of the holotypes of this synonym (Mattingly - *in litt.*) reveals that it differs from typical *univittatus*: the female holotype lacks a striped mid femur as does *neavei*, while the leaflet in the male holotype is similar to that of *univittatus*. It would seem likely therefore, that the eastern Mediterranean form is the same as the short-spined form of the present study. The latter occurred in the collections from all West African countries and from Egypt which is in agreement with the distribution of the eastern Mediterranean form given by Mattingly (1954). As males of the short-spined form and of *neavei* occurred together in 4 of the West African countries, often originating from the same localities, this precludes them from being climatypes.

Since both *univittatus* and the short-spined form are important as viral vectors, the taxonomic status of the form concerned needs to be established. In South Africa *univittatus* is believed to be the maintenance vector of West Nile and Sindbis viruses (McIntosh *et al.*, 1967; Jupp and McIntosh, 1967). In Egypt "*univittatus*" which, in the light of the present study, almost certainly belongs to the short-spined form is an important vector of West Nile and probably also Sindbis viruses (Taylor *et al.*, 1955, 1956). Furthermore, in Israel this same form of mosquito may be involved in the transmission of West Nile virus. It is noteworthy that the Egyptian form has host preferences which differ from those of *univittatus* in South Africa (Hurlbut and Weitz, 1956; Jupp and McIntosh, 1967).

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Table 1. *C. univittatus* and *C. neavei* from South Africa; the frequency with which 8 morphological characters occur in both sexes and the nature of 2 characters in genitalia of males.

	C. univittatus		*C. neavei*	
	Female	Male	Female	Male
Post-spiracular scales	Present	Present	Present	Present
Terga; basal white bands	"	"	Rarely absent	"
Sterna; median dark scales	"	"	Sometimes absent	"
Sterna; dark apical lateral triangles	"	"	Present	"
Hind tibia; apical white spot	"	"	"	"
Hind tibia; anterior white stripe	"	"	Rarely absent	Rarely absent
Mid femur; anterior white stripe	"	Usually present	Absent	Absent
Wing; pale scales at base of costa	"	Present	Present	Often absent
Genitalia				
Subapical lobe of gonocoxite; leaflet shape		Narrow R_1* 33-50		Broad R_1 50 or more
LAP+; length of spine borne by outer division		Intermediate length or long		Long

*Leaflet index. + Lateral aedeagal plate.

Table 2. Number of female (F) and male (M) specimens identified as *C. univittatus*; values for the leaflet index (R_1) are given for males.

		No.	R_1
Upper Volta	F	1	
	M	1	48
Dahomey	F	1	
	M	2	43,47
Niger	F	2	
	M	2	50,50
Ethiopia	F	1	

Table 3. Female specimens identified as C. *neavei*; the occurrence of certain morphological characters - all specimens lacked a striped mid femur.

Country	No.	TERGA white basal bands	STERNA Median dark scales	STERNA Dark apical lateral triangles	HIND TIBIA anterior white stripe	WING pale basal costal scales
WEST AFRICA						
Upper Volta	18	9	3/17*	0/4	14	16
Dahomey	5	2/4	2	3/3	4	4
Nigeria	1	1	1	1	1	1
Mauritania	4	4	3/3	2/2	4	4
EAST AFRICA						
Tanzania	1	1	0	?	1	1
Kenya	2	2	2	?	2	1/1
REUNION	2	2	1/1	?	2	2
EGYPT	19	19	11/18	6/8	19	19

*Where the condition of some specimens precluded the assessment of a character for the whole sample, the numerator is the number of specimens possessing the character and the denominator the number on which it could be examined.

Table 4. Male specimens identified as C. *neavei* and the short-spined form; appearance of leaflet and values for the leaflet index (R_1).

Country	C. *neavei* (all with broad leaflets) No.	C. *neavei* R_1	Short-spined form No.	Short-spined form No. with broad leaflet	Short-spined form No. with narrow leaflet	Short-spined form R_1
WEST AFRICA						
Upper Volta	13	51-71(10)*	12	10	2	50-81(9) 47(2)
Dahomey	8	50-62(7)	6	5	1	50-58(3) 43(1)
Nigeria			3	3		52-56
Mauritania	2	50(1)	2	1	1	48(1)
Niger	2	52,53	2	2		56,56
EAST AFRICA						
Tanzania	4	56-73				
Kenya	2	67,84				
REUNION	3	54-67				
EGYPT			7	7		50-60

* The bracketed figures indicate the number of individuals for which R_1 values were calculated when determinations were not made on the full sample.

Table 5. Numbers of specimens of C. *univittatus*, C. *neavei* and the short-spined form in the collections from different African countries; figures in parenthesis are female specimens and the others are male specimens.

Species	South Africa	Upper Volta	Dahomey	Nigeria	Mauritania	Niger	Tanzania	Kenya	Ethiopia	Reunion	Egypt
C. *univittatus*	Present	1(1)	2(1)			2(2)		(1)	(1)		
C. *neavei*	Present	13(18)	8(5)	(1)	2(4)	2	4(1)	2(2)		3(2)	(19)
Short-spined form		12	6	3	2	2					7

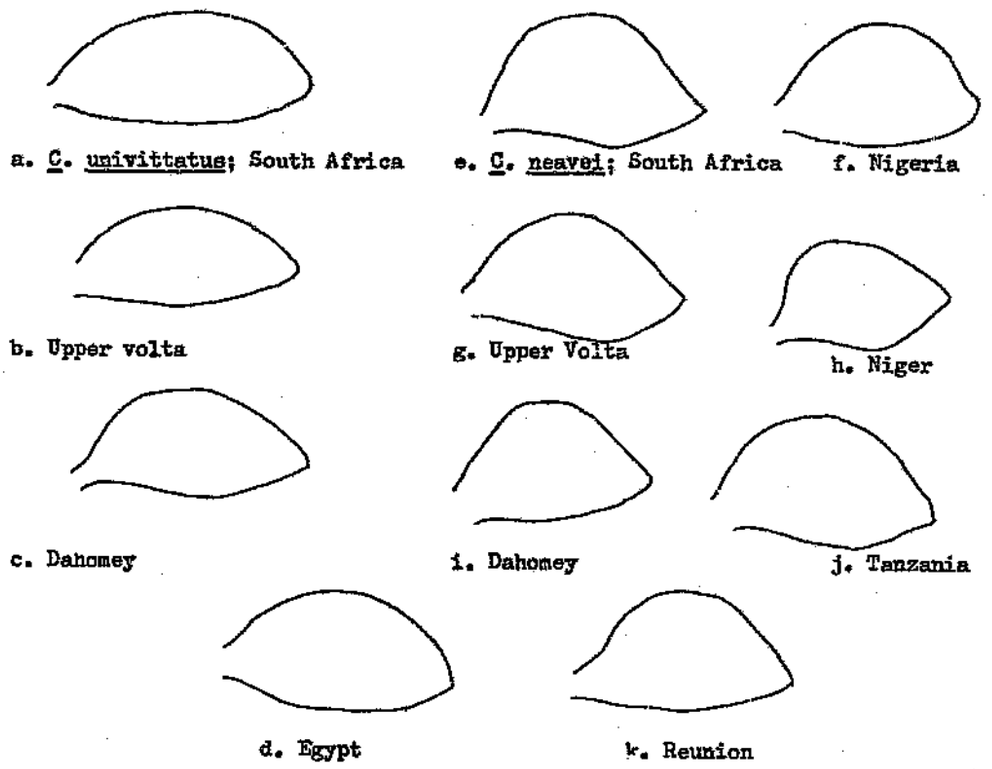

Fig. 1 Drawings of the leaflet from the gonocoxite of various male specimens; b and c are similar to that from C *univittatus* (a), f-k are similar to that from C. *neavei* (e), while d appears intermediate in shape.

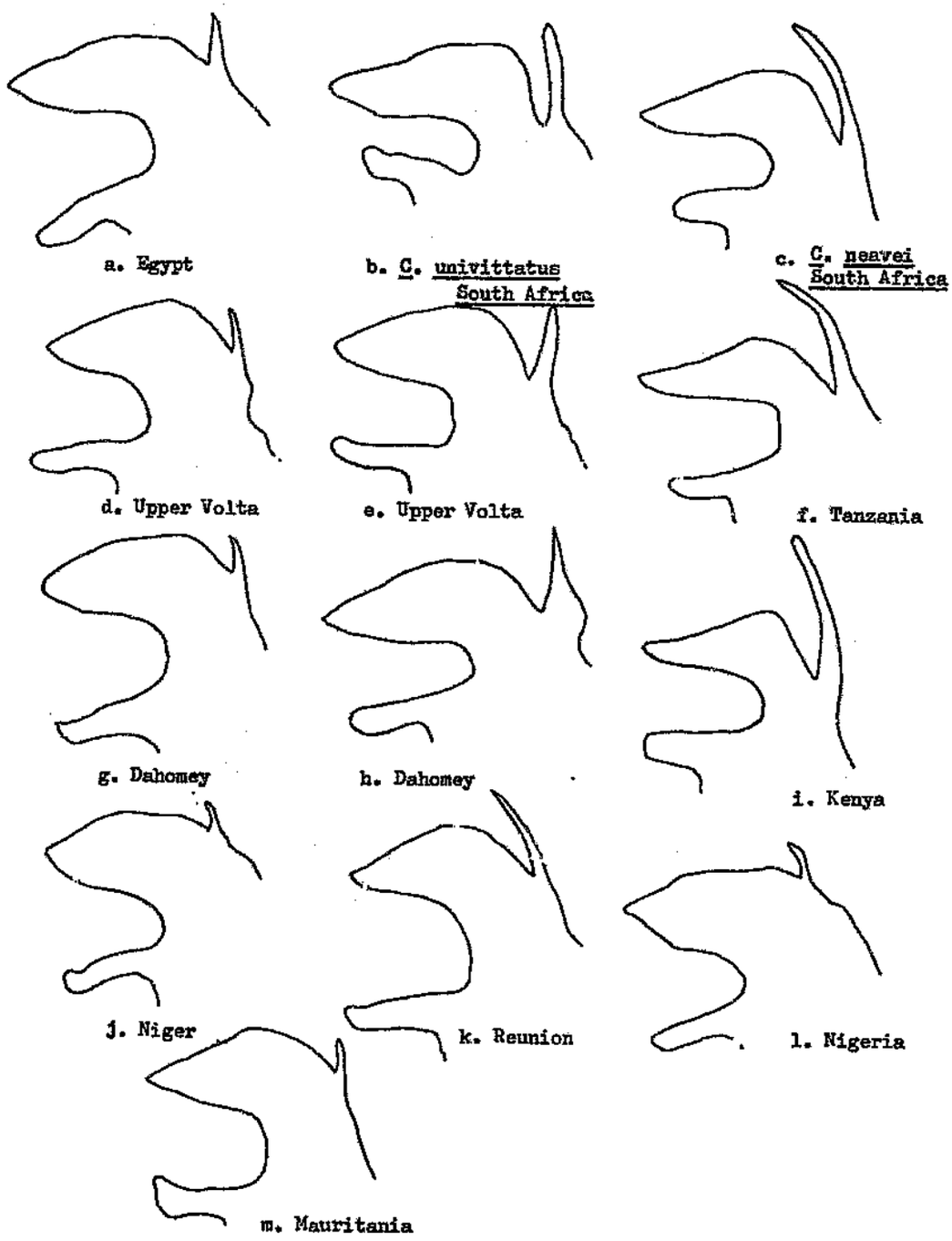

Fig. 2 The outer division of the lateral aedeagal plate; drawings to show different spine lengths.

1.4.2

Arch. Inst. Pasteur Madagascar, 1987,56,1,287-295

PRESENCE DU MOUSTIQUE *CULEX (CULEX) NEAVEI* A MADAGASCAR, IMPORTANCE DANS LA TRANSMISSION DES ARBOVIRUS

D. FONTENILLE (1) & P.G. JUPP (2)

R E S U M E

L'étude de mâles et de femelles du groupe Culex (Culex) univittatus, *de différentes régions de Madagascar, a permis de mettre en évidence, pour la première fois, la présence de* Culex neavei *Theobald, 1906 à Madagascar, en particulier dans la région de Tsiroanomandidy, et de confirmer la présence de* Culex univittatus *Theobald, 1901 dans diverses autres régions. L'examen de 25 mâles et de 43 femelles de ce groupe montre, qu'en raison de nombreuses formes atypiques, la détermination précise ne peut se faire que sur l'observation de l'appareil génital mâle.*

Les conséquences de la présence de Cx. neavei, *dans la transmission des virus West-Nile et Babanki sont discutées.*

Mots-clés : CULICIDAE, CULEX NEAVEI, CULEX UNIVITTATUS, VIRUS WEST-NILE, VIRUS BABANKI, SYSTEMATIQUE, MADAGASCAR.

S U M M A R Y

The presence of Cu x (Culex) neavei in Madagascar and its relevance to arboviruses transmission

A study was made of 25 male and 43 female mosquitoes belonging to the Culex (Culex) univittatus *group, from different localities in Madagascar, Indian Ocean. This showed that* Culex neavei Theobald, *1906 is present on the island, at least in the Tsiroanomandidy region. As demonstrated previously,* Cx. univittatus *Theobald, 1901 is also present. Because of the presence of some atypical forms, firm identifications was made only in males according to the structure of the leaflet of the sub-apical lobe of the gonocoxite. The relevance of the presence of* Cx. neavei *to the epidemiology of West Nile and Babanki viruses in Madagascar is discussed.*

Key-words : CULICIDAE, CULEX NEAVEI, CULEX UNIVITTATUS, WEST-NILE VIRUS, BABANKI VIRUS, TAXONOMY, MADAGASCAR.

(1) Institut Pasteur de Madagascar, B.P. 1274, Antananarivo, Madagascar.

(2) Arbovirus Unit, National Institute for virology, Private Bag X 4, 1231 SANDRINGHAM, République of South Africa.

1955

Dans le cadre du programme d'étude des arbovirus à Madagascar, le Laboratoire d'Entomologie Médicale de l'Institut Pasteur de Madagascar a capturé, depuis 1983, près de 200000 Culicidae en différentes régions de l'île.

Jusqu'à présent, les auteurs ayant travaillé à Madagascar avaient toujours considéré que seul *Culex univittatus* Theobald, 1901 était présent sur l'île.

L'isolement du virus West-Nile d'un lot de femelles du groupe *Cx. univittatus* nous conduit à revoir la position taxonomique de ce groupe à Madagascar, en particulier à la lumière des données obtenues en Afrique du Sud.

Dans cette région, *Culex neavei* Theobald, 1906 et *Cx. univittatus* sont tous deux présents. Jupp (1971, 1972) a donné, pour les mâles et les femelles, les critères de discrimination entre ces deux espèces très proches, mais à capacité vectorielle pour les arbovirus sensiblement différente.

M A T E R I E L S E T M E T H O D E S

Nous avons examiné des adultes mâles et femelles montés sur minuties et numérotés. Les captures ont été effectuées sur homme de nuit, aux pièges lumineux et en faune résiduelle. Les genitalia des mâles ont été disséqués et montés sur lame dans un milieu conservateur (Euparal).

Les adultes ont été observés au stéréomicroscope à des grossissements de X6,4 à X80. Les appareils génitaux mâles ont été observés au microscope à des grossissements de X100 à X640. Les mesures ont été effectuées au micromètre oculaire.

Les spécimens présentés tableau I ont été étudiés.

Pour la détermination, nous avons utilisé les descriptions d'Edwards (1941), reprises par Jupp (1971, 1972), qui a montré que, pour *Cx. neavei*, la description d'Edwards comportait des imprécisions.

Nous n'avons pas pu observer suffisamment de stades larvaires pour déceler d'éventuelles différences spécifiques. Hopkins (1952) et Jupp n'en reconnaissent pas.

A Madagascar, les adultes de *Cx. univittatus* et *Cx. neavei*, se différencient des autres *Culex* du sous-genre *Culex* par les caractères suivants :

- présence d'écailles post-spiraculaires et pré-alaires (caractère par ailleurs commun à *Cx. simpsoni*, *Cx. comorensis* et *Cx. striatipes*).
- Ligne antérieure d'écailles blanches sur le tibia postérieur, et
- présence d'écailles pâles à la base de la costa de l'aile.

Après examen des spécimens présentés tableau I nous avons donc retenu, pour les adultes, les critères suivants (tableau II) inspirés de Jupp (1972) :

- taille de la bande basale d'écailles blanches sur les tergites

- présence d'une ligne antérieure d'écailles blanches sur les fémurs médians, et pour l'appareil génital mâle :

- le rapport largeur (l) sur longueur (L) de l'appendice foliacé de la touffe de soies du lobe subapical du coxite (figure 1)

$$\text{tel que } R_1 = \frac{l}{L} \times 100$$

TABLEAU I

Culicides du groupe Culex univittatus *étudiés selon la localité*

LOCALITE	FEMELLES	MALES	TOTAL
ANTANANARIVO	16	7	23
ANJIRO	2	11	13
PERINET	1	0	1
ANDAPA	1	0	1
MAHASOLO	3	0	3
TSIROANOMANDIDY	16	6	22
MORONDAVA	1	1	2
NOSY BE	1	0	1
BERENTY	2	0	2
	43	25	68

Les autres caractères reconnus par Jupp ne nous ont pas semblé suffisamment discriminants pour Madagascar. Ce sont :

- longueur de la ligne dorsale d'écailles sombres du fémur postérieur
- écailles noires médianes et triangles apicaux latéraux d'écailles noires sur les sternites
- longueur de l'épine portée par le bras latéral de la division interne du phallosome (longue dans tous les cas observés)
- forme de la soie f de la touffe sub-apicale du coxite figure 1),
- et comme nous l'avons déjà vu la présence d'une ligne blanche sur le tibia postérieur, et des écailles claires sur la costa.

TABLEAU II

Différences entre Cx. univittatus *et* Cx. neavei, *à Madagascar*

CARACTERES	*Cx. univittatus*		*Cx. neavei*	
	FEMELLES	MALES	FEMELLES	MALES
* ligne basale d'écailles blanches sur les tergites	présente (parfois étroite)	présente large	étroite ou absente	présente large
* bande antérieure d'écailles blanches sur le fémur médian	présente (parfois seulement quelques écailles peu visibles)	en général absente sinon peu marquée	absente (parfois quelques écailles)	absente
* appendice foliacé du lobe subapical	-	étroite R_1<50	-	large R_1>50

RESULTATS

Après examen des caractères énoncés précédemment, les adultes que nous avons pu étudier se répartissent comme présenté tableau III et carte 1.

Les mâles ont pu être identifiés sans difficulté. La forme de l'appendice foliacé du lobe subapical du coxite est un bon caractère qui apparaît constant dans une population donnée : toujours long chez *Cx. univittatus* et toujours large chez *Cx. neavei*. Pour les mâles et les femelles, la présence d'une ligne d'écailles blanches bien marquée sur le fémur médian est caractéristique de *Cx. univittatus*. Elle manque chez *Cx. neavei* et chez certains *Cx. univittatus*.

La taille de la bande basale d'écailles blanches sur les tergites est un complément d'aide au diagnostic pour les femelles une bande large étant caractéristique de *Cx. univittatus*, alors qu'une bande étroite, voire absente, ne permet pas de conclure.

Cette variabilité de caractères nous a amenés, pour certaines femelles, à ne pas prendre de décision et à les considérer comme "atypiques" (par exemple, femelle avec quelques écailles blanches alignées, ne formant pas une vraie ligne sur le fémur médian, et bandes des tergites très étroites).

En définitive, à Madagascar, comme sans doute ailleurs, seule l'observation de l'appareil génital mâle au microscope permet d'identifier l'espèce de manière certaine.

De nos observations, il ressort que *Cx. univittatus* est présent à Tananarive, à Anjiro, à Périnet, à Mahasolo, et à Morondava.

La présence de *Cx. neavei* n'est certaine qu'à Tsiroanomandidy où tous les mâles observés sont typiquement de cette espèce (R_1 : 50 à 62).

TABLEAU III

Espèces présentes selon les régions étudiées

LOCALITES (altitude en m)	*Cx. univittatus*: MALES (R_1)	Fem-melles	FEMELLES "ATYPIQUES"	*Cx. neavei*: MALES (R_1)	Fem-melles
TANANARIVE (1400)	7(30à40)	6	2	0	7
ANJIRO (950)	11(30à42)	1	1	0	0
PERINET (900)	-	3	1	-	0
ANDAPA (500)	-	0	0	-	1
BERENTY (50)	-	0	0	-	2
NOSY BE (0)	-	0	0	-	1
MAHASOLO (900)	-	3	0	-	0
TSIROANOMANDIDY (900)	0	0	5	6(50à62)	11
MORONDAVA (0)	1 (32)	0	0	0	1

- : absence de mâles

D I S C U S S I O N

Culex univittatus a une large répartition mondiale. Knight et Stone (1977) signalent sa présence dans toute l'Afrique sub-saharienne, dans la région méditerranéenne et le moyen-orient.

Culex neavei a été signalé dans de nombreux pays africains : Mauritanie, Burkina Faso, Benin, Nigeria, Niger, Soudan, Congo, Zaïre, Angola, Ethiopie, Ouganda, Kenya, Afrique du Sud, et autour de Madagascar en Tanzanie et à l'Ile de La Réunion.

En Afrique du Sud, Jupp a montré que la répartition de ces deux espèces était fonction de l'altitude. *Cx. univittatus* est généralement localisé en altitude, jusqu'à plus de 1500 m, et *Cx. neavei* dans les basses terres, avec des zones de sympatrie à altitude intermédiaire.

Nous n'avons pas assez de données à Madagascar pour évaluer si une telle répartition existe, en fonction de l'altitude ou de facteurs climatiques. Notons simplement que des mâles de *Cx. univittatus* ont été capturés en bord de mer vers Morondava et jusqu'à 1400 m dans les environs de Tananarive. A Tsiroanomandidy, à 900 m d'altitude, seuls des mâles de *Cx. neavei* ont été récoltés.

Dans aucune zone, nous n'avons trouvé ensemble des mâles de ces deux espèces.

Lors de toute étude sur les arbovirus, la connaissance précise des espèces en cause dans une zone donnée est de la plus grande importance. *Cx. neavei* et *Cx. univittatus* sont tous deux des vecteurs des virus West-Nile et Sindbis, mais leur capacité vectorielle est sensiblement différente (Jupp et Col. 1986).

Cx. univittatus est un bon vecteur de ces deux virus alors que *Cx. neavei* a une capacité vectorielle réduite, surtout pour le virus West-Nile.

A Madagascar, le virus West-Nile est abondant et les Alphavirus sont représentés par un virus très proche de Sindbis : le virus Babanki (Fontenille 1989).

Il est intéressant de noter que dans la région de Tsiroanomandidy, de nombreuses souches de virus West-Nile et une de virus Babanki ont été isolées de *Cx. decens* et *Cx. antennatus*, mais aucune de Culex du groupe *univittatus* représenté dans cette région par *Cx. neavei* (Fontenille et col. 1988).

En revanche, vers Anjiro, où le groupe est représenté par *Cx. univittatus* s.s., une souche de virus West-Nile a été isolée de cette espèce.

Ces données sont en faveur d'une meilleure capacité vectorielle de *Cx. univittatus* par rapport à *Cx. neavei*, également à Madagascar.

C O N C L U S I O N

L'étude détaillée de 43 femelles et de 25 mâles du groupe *Cx. univittatus* nous a permis de mettre en évidence, pour la première fois, la présence de *Cx. neavei* à Madagascar.

La détermination précise de l'espèce ne peut se faire, sans risque d'erreur, que sur l'observation de l'appareil génital mâle. En effet, de nombreuses femelles observées présentent des caractères atypiques, pouvant être source de confusion.

A partir de nos exemplaires, nous n'avons pu conclure à la présence de *Cx. neavei*, que dans la région de Tsiroanomandidy.

La constitution de séries de mâles de diverses origines géographiques est maintenant nécessaire pour connaître la répartition de cette espèce à Madagascar et pour voir si elle est soumise à des facteurs climatiques comme en Afrique du Sud.

En raison de la capacité vectorielle probablement différente vis-à-vis des virus West-Nile et Sindbis, la connaissance de cette répartition s'avère d'une grande importance, dans l'étude des arbovirus de Madagascar.

R E M E R C I E M E N T S

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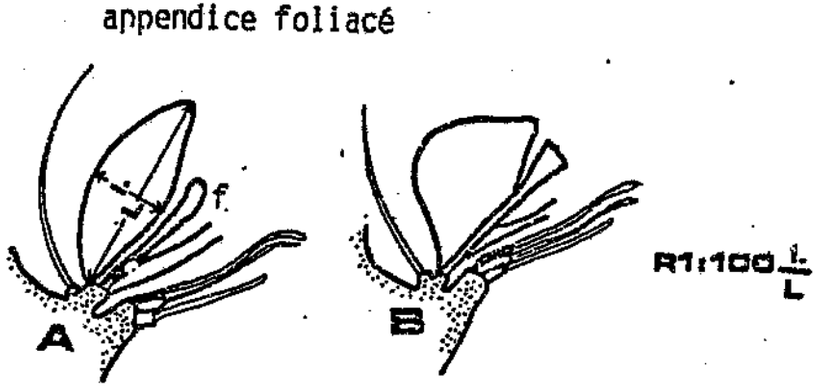
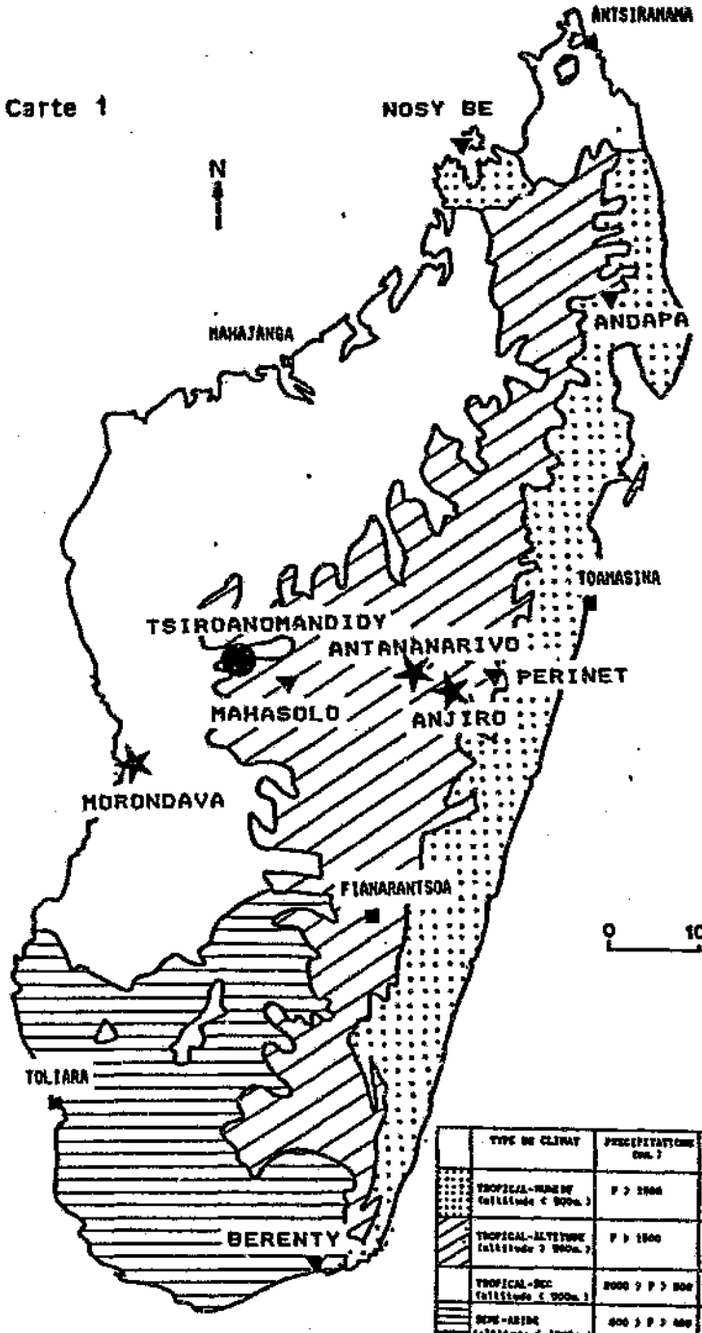


Fig. 1. Lobe sub-apical du gonocoxite
A : Cx. univittatus, B : Cx. neavei
(d'après Jupp. 1971).

Carte 1



	TYPE DE CLIMAT	PRECIPITATIONS (mm.)	TEMP. MOYENNE DU MOIS LE PLUS FROID	N° DE MOIS SECS
	TROPICAL-HUMIDE (altitude < 900m.)	P > 2000	t > 18°	0-2
	TROPICAL-ALTITUDE (altitude > 900m.)	P > 1500	8° < t < 18°	0-4
	TROPICAL-SEC (altitude < 900m.)	2000 > P > 800	t > 20°	0-6
	SEMI-ARIDE (altitude < 1000m.)	600 > P > 400	t > 20°	8-9

D'après : Association des géographes de Madagascar, 1969, Atlas de Madagascar, BDPA et FTM, Tananarive.

● Localités avec des cônes de Cr. pauciseminatus

✱ Localités avec des cônes de Cr. microphyllus

▼ Localités avec des familles uniquement

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CROSSMATING AND MORPHOLOGICAL STUDIES OF *CULEX NEAVEI* AND *CULEX PEREXIGUUS* (DIPTERA: CULICIDAE) TO ELUCIDATE THEIR TAXONOMIC STATUS[1]

PETER G. JUPP[2] AND RALPH E. HARBACH[3]

ABSTRACT. Comparative studies were conducted on *Culex perexiguus* and *Cx. neavei*, two members of the medically important *univittatus* complex, to determine if they represent the same or different species. Specimens of *neavei* from countries south of the Sahara were compared with specimens of *perexiguus* from countries in northern Africa and southwestern Asia. Slight and variable differences were observed in the ornamentation of adults, males differed constantly in a single feature of the genitalia, and several tenuous distinctions were noted in larvae. A high degree of unidirectional incompatibility occurred when *neavei* females from South Africa were mated with *perexiguus* males from Egypt. It is concluded that *perexiguus* and *neavei* represent different, largely allopatric species.

INTRODUCTION

The *univittatus* complex of the subgenus *Culex* includes three nominal forms: *univittatus* Theobald, 1901 (type locality Salisbury, Zimbabwe), *perexiguus* Theobald, 1903 (type locality Sidon, Lebanon), and *neavei* Theobald, 1906 (type locality Lualas, Sudan). The nominotypical form appears to be restricted to the temperate highlands of southern and eastern Africa and the southwestern corner of the Arabian Peninsula. *Culex neavei* seems to occur throughout the subtropical and tropical lowlands south of the Sahara. *Culex perexiguus* inhabits the arid areas of northern and eastern Africa and southwestern Asia eastward into India.

Culex neavei was originally described as a variety of *univittatus*. It was elevated to specific status based on morphological discontinuity and reproductive isolation (Jupp 1971). *Culex perexiguus* was synonymized with *univittatus* by Edwards (1912), treated as a form of *univittatus* by Mattingly (1954), and recognized as a variety of this species by Mattingly and Knight (1956). Jupp (1972) performed a morphological comparison between *neavei* and specimens attributable to *perexiguus* and showed that the two forms were distinguishable by minor characteristics of the male genitalia. Two other differences between *neavei* from South Africa and *perexiguus* (as *univittatus*) from Egypt became evident during laboratory colonization and vector competency tests involving West Nile and Sindbis viruses. While *perexiguus* readily adapts to mating in 30-35 cm^3 cages under normal laboratory conditions (Hurlbut and Weitz 1956, Gad et al. 1987), *neavei* does not and requires a simulation of crepuscular lighting similar to that required by *univittatus* in South Africa (Jupp and Brown 1967, Jupp 1971). *Culex perexiguus* in Egypt is a superior vector of both West Nile and Sindbis viruses (Hurlbut 1956, Taylor et al. 1955) as compared with *neavei* in South Africa (Jupp et al. 1986).

Jupp (1972) suggested that *perexiguus* and *neavei* may represent sibling species, but added that further morphological and crossing experiments were necessary before the taxonomic status of these forms could be settled. White (1975) apparently disregarded this statement when he recognized *perexiguus* as a valid species in his "Notes on a Catalog of Culicidae of the Ethiopian Region." White stated that "*perexiguus* is now reinstated to full species rank, on the evidence marshalled by Jupp (1971, 1972)." But since the evidence presented by Jupp was meager and inconclusive, the status of *perexiguus* and *neavei* remained uncertain. In this paper we describe the results of morphological studies and crossmating experiments which support the contention that these nominal forms represent different species.

MATERIALS AND METHODS

Morphology. Morphological studies were conducted on specimens deposited in the National Museum of Natural History (Smithsonian Institution), the British Museum (Natural History), and the London School of Hygiene and Tropical Medicine. A total of 253 specimens of *neavei* (111 females, 52 males, 44 male genitalia, 14 pupal exuviae, 18 larval exuviae, and 14 fourth-instar larvae) were examined from 15 countries south of the Sahara. Some 2,696 specimens of *perexiguus* (735 females, 474 males, 92 male genitalia, 895 pupal exuviae, 446 larval exuviae, and 54 fourth-instar larvae) were examined from seven countries in southwestern Asia and nine in northern Africa (Harbach 1988). Ethiopia, Sudan, and Upper Volta were the only countries from which specimens of both forms were

[1]The views of the authors do not purport to reflect the position of the Department of the Army or the Department of Defense.

[2]Department of Virology, University of Witwatersrand and the Arbovirus Unit, National Institute for Virology, Private Bag X4, Sandringham, 2131 South Africa.

[3]Walter Reed Biosystematics Unit, Department of Entomology, Walter Reed Army Institute of Research, Washington, DC 20307-5100. Mailing address: Walter Reed Biosystematics Unit, Museum Support Center, Smithsonian Institution, Washington DC 20560.

examined. The morphological terminology and the abbreviations used in the figures follow Harbach and Knight (1980).

Crossing experiments. Virgin mosquitoes drawn from laboratory colonies of *neavei* (F_4) and *perexiguus* (F_{29}) were used for the crosses. The *neavei* colony was started with specimens collected at Mtubatuba, northern coastal Natal, South Africa and the *perexiguus* colony was started with specimens collected at Kom Ombo, Egypt (collection number 055 of Harbach et al. 1988). Reciprocal crosses were done in duplicate in 35 cm^3 cages using adult mosquitoes not more than seven days old. From 221 to 269 mosquitoes of each sex were placed in each cage. The cages were maintained at 25-26° C and 75-80% RH. The daily light-cycle was provided by controlled artificial lighting and consisted of a day period of 12 hours, a night period of 10 hours, and twilight periods of 60 minutes each when the light intensity was either gradually increased (dawn) or decreased (dusk). Hamsters were exposed to the mosquitoes as a source of blood. Gravid mosquitoes were placed individually in tubes for oviposition 5-6 days after feeding.

Hybrid progeny resulting from the crosses were backcrossed with the parental strains. These crosses were performed under the same conditions as the primary crosses. The mosquitoes were 1-13 days old.

The larvae and adult males resulting from all of the crosses were examined for evidence of abnormal morphology. Larvae and male genitalia were mounted on slides and compared with specimens from the parental colonies.

Egg rafts were placed in cedar oil and examined under a stereomicroscope to determine the percentage of hatched, unhatched-infertile (unembryonated), and unhatched-embryonated eggs. For purposes of discussion, rafts containing eggs that hatched are termed "viable rafts" while those containing only unhatched eggs are termed "nonviable rafts." Spermathecae of a sample of females used in the crosses were examined for the presence of sperm.

RESULTS

Morphology. *Culex neavei* and *perexiguus* are remarkably similar in all life stages. Slight and variable differences between the adults of these forms and *univittatus* were listed previously by Harbach (1988, Table 41). Differences observed between *neavei* and *perexiguus* are compared in Table 1. The most reliable distinction between females of these forms is the presence or absence of pale scales at the base of the costa. A short line of pale scales is present in *perexiguus* and apparently absent in *neavei*. Males of these forms appear to differ constantly in a single character of the genitalia, i.e., the length of the ventral arm of the lateral plate. The ventral arm (VA) is always long (projecting beyond distal margin of lateral plate, LP) in *neavei* (Fig. 1) and short (not projecting beyond distal margin of LP) in *perexiguus* (Fig. 3).

Too few larval and pupal specimens of *neavei* were available for detailed study. Most of the available specimens were from South Africa and no significant differences were found to distinguish them from larval and pupal specimens of *perexiguus* from southwestern Asia and northern Africa (primarily Egypt). The pupae of these forms appear to be indistinguishable (*cf.* Figs. 1 and 3) and only vague and variable differences were noted in the larvae (*cf.* Figs. 2 and 4). Equivocal morphological differences between the larvae of these forms include the following: (1) pecten spines (PS) with more denticles in *neavei*; (2) seta 1-S usually in 5.5 or 6.0 pairs in *neavei* (5.0-6.5) and 5.0 pairs in *perexiguus* (4.5-6.0); (3) seta 2-I frequently (frequency of nearly 50%) inserted directly anterior or anterior and slightly lateral to seta 3-I in *perexiguus* (as shown in Fig. 4) and anterior and normally well mesad of 3-I in *neavei*; (4) seta 5-IV double or triple in both forms but more often triple in *neavei* and double in *perexiguus*; and (5) seta 2-X often double and frequently triple in *neavei* (rarely with 4 branches) but almost always double in *perexiguus* (rarely single or triple).

Crossing experiments. Table 2 records the viability of egg rafts deposited by females used in the reciprocal crosses. The viabilities of egg rafts resulting from crossing *perexiguus* females with *neavei* males were 90.2 and 96.7%. These percentages were on the same order of magnitude as the viabilities for the parental colonies, i.e., 95.5% for *neavei* and 85.1% for *perexiguus*. Indices for egg rafts resulting from the reciprocal cross were only 5.7 and 12.9%, thus there was a measure of unidirectional incompatibility between the two forms.

The results of spermathecal examinations are given in Table 3. All of the *neavei* females crossed with *perexiguus* males which deposited viable rafts were inseminated, while both inseminated and uninseminated females deposited nonviable rafts. In contrast, judging from the samples examined, all nonviable rafts resulting from the cross between *perexiguus* females and *neavei* males were deposited by uninseminated mosquitoes. This further indicates that there was some incompatibility in the cross between *neavei* females and *perexiguus* males.

The classification of eggs comprising viable rafts obtained from the primary crosses and the parental colonies is compared in Table 4. This table contrasts the mean percentage of hatched, unhatched-embryonated, and infertile (unembryonated) eggs contained in all of the rafts examined in each case. Of interest is that *neavei* females crossed with *perexiguus* males produced rafts which contained few eggs that hatched and that a large proportion of embryonated eggs failed to hatch.

The mean percentages of unhatched-embryonated and infertile (unembryonated) eggs comprising nonviable rafts that resulted from the primary crosses are compared in Table 5. Nonviable rafts from *neavei* females crossed with *perexiguus* males contained a mixture of embryonated and

Table 1. Morphological differences observed between *Culex perexiguus* and *Cx. neavei*.

Character	*perexiguus*	*neavei*
1) Ventral surface of proboscis	pale except at base, weakly pale on distal 0.25	inconspicuously pale in middle
2) Postspiracular area	tendency for scales to cover more than dorsal 0.5	tendency for scales to occur in small patch near spiracle
3) Forefemur	usually with indistinct anterior pale stripe	no anterior pale stripe
4) Hindtibia	with distinct anterior and posterior pale stripes on proximal 0.8, partly separated on proximal 0.5 or less by weak ventral dark stripe; with distinct apical pale spot	with rather indistinct anterior and posterior pale stripes ending before base; with rather indistinct apical pale spot
5) Wing - costa	with short line of pale scales at base	without pale scales at base
6) Abdomen - pale bands of terga	normal	reduced or absent
7) Male genitalia - ventral arm of lateral plate	short	long

Table 2. Viability of egg rafts from primary crosses and parental stocks.

Cross/colony	Cage	Proportion of viable rafts	Percent viable
neavei ♀ x *perexiguus* ♂	1	7/122	5.7
	2	11/85	12.9
perexiguus ♀ x *neavei* ♂	1	111/123	90.2
	2	116/120	96.7
neavei	-	42/44	95.5
perexiguus	-	40/47	85.1

Table 3. Proportion of inseminated females which deposited viable and nonviable rafts.

Cross	Cage	Inseminated ♀s /viable rafts	Inseminated ♀s /nonviable rafts
neavei ♀ x *perexiguus* ♂	1	7/7	9/20
	2	5/5	15/20
perexiguus ♀ x *neavei* ♂	1	ND	0/11
	2	ND	0/2

ND = Not determined.

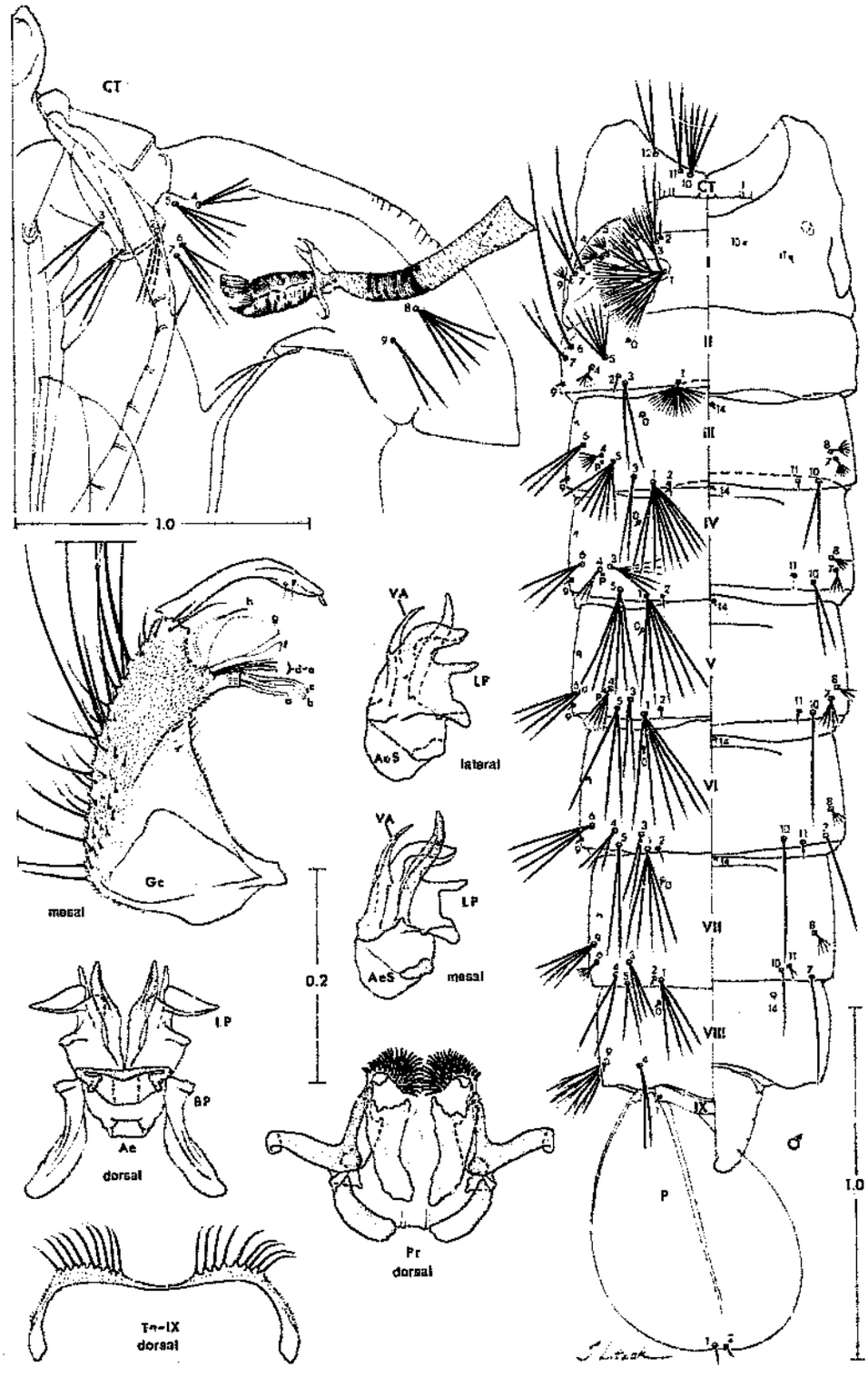

Fig. 1. *Culex (Culex) neavei.* Male genitalia and pupa, South Africa.

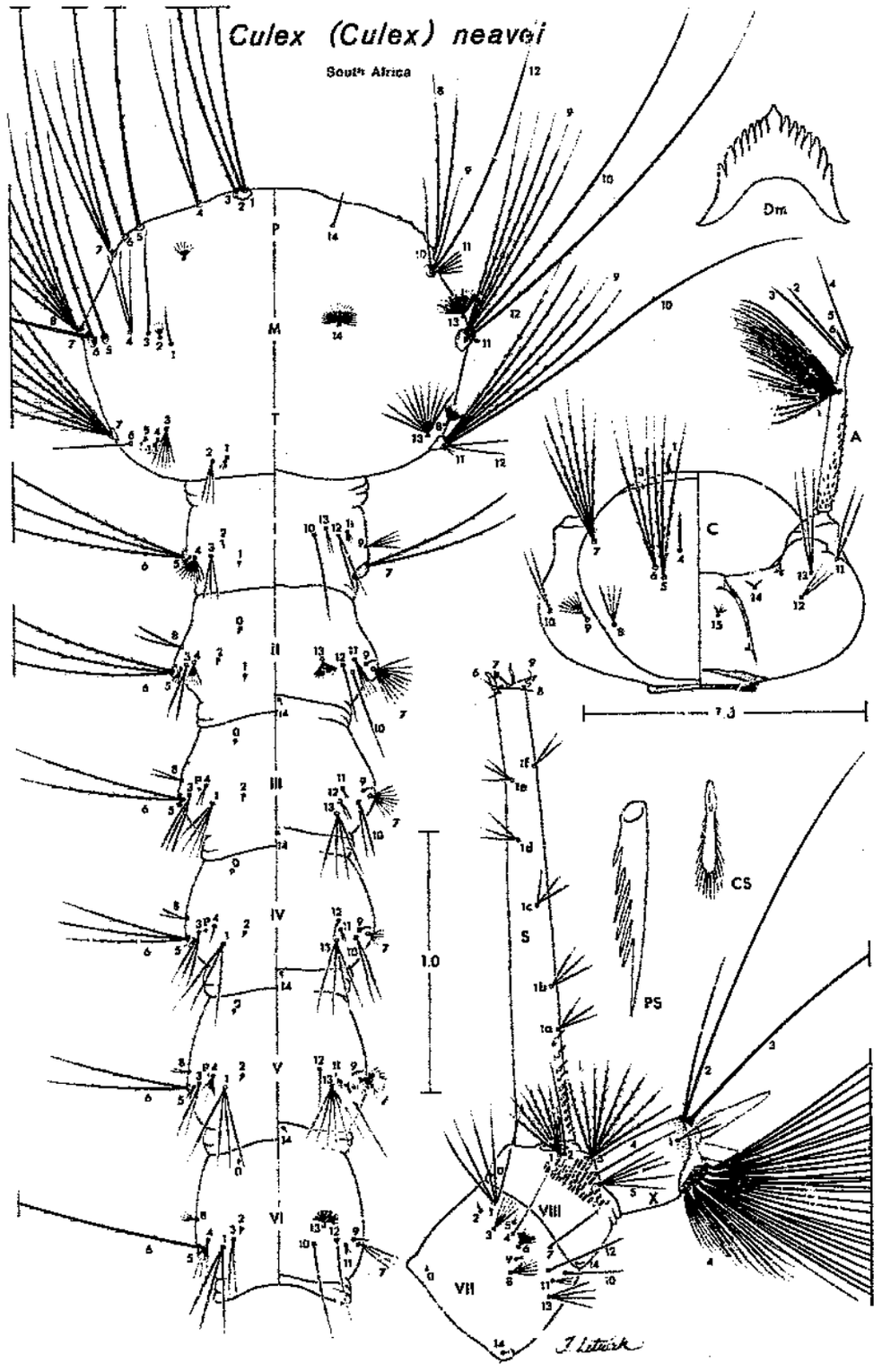

Fig. 2. *Culex (Culex) neavei.* Larva, South Africa.

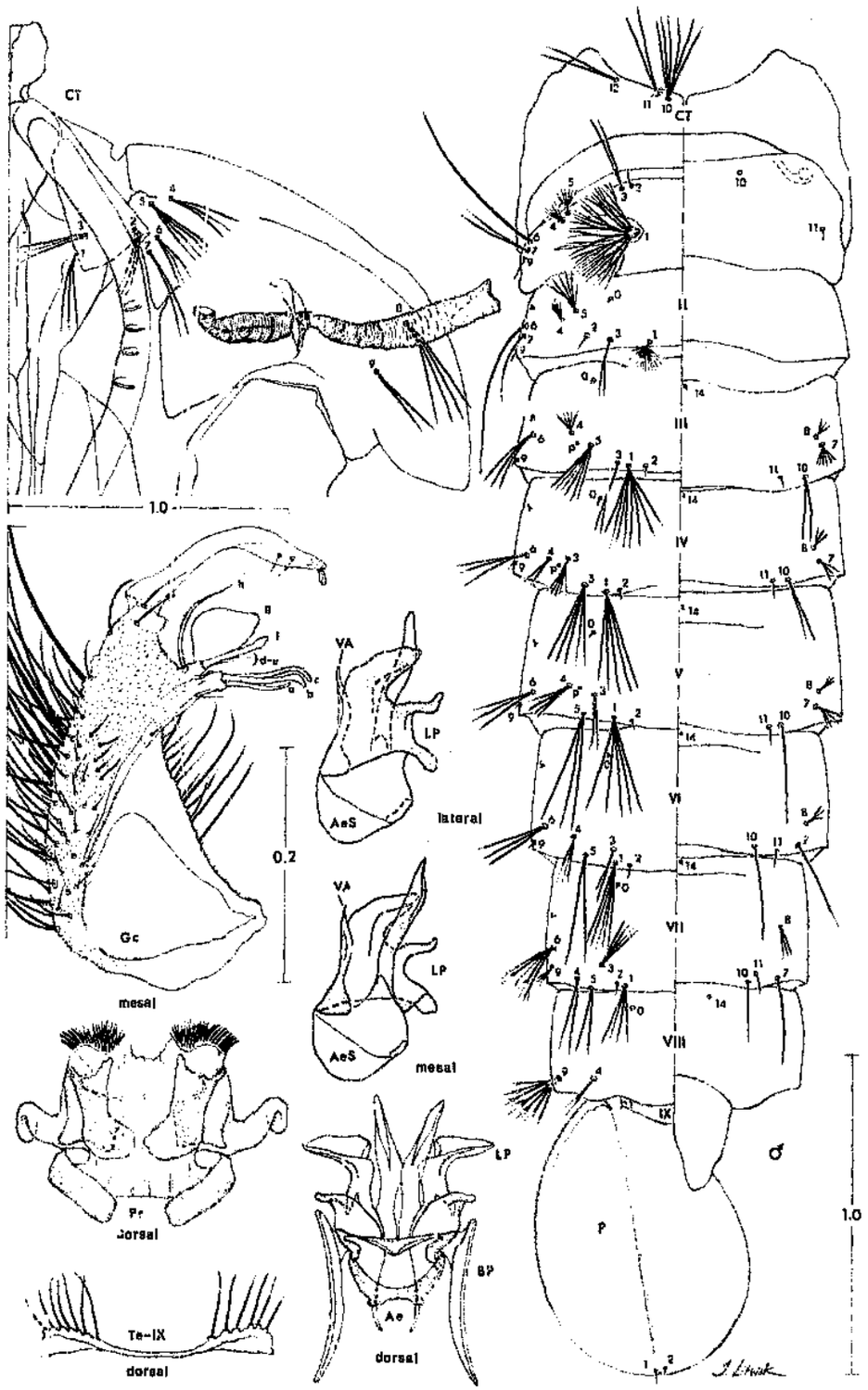

Fig. 3. *Culex (Culex) perexiguus.* Male genitalia and pupa, Egypt.

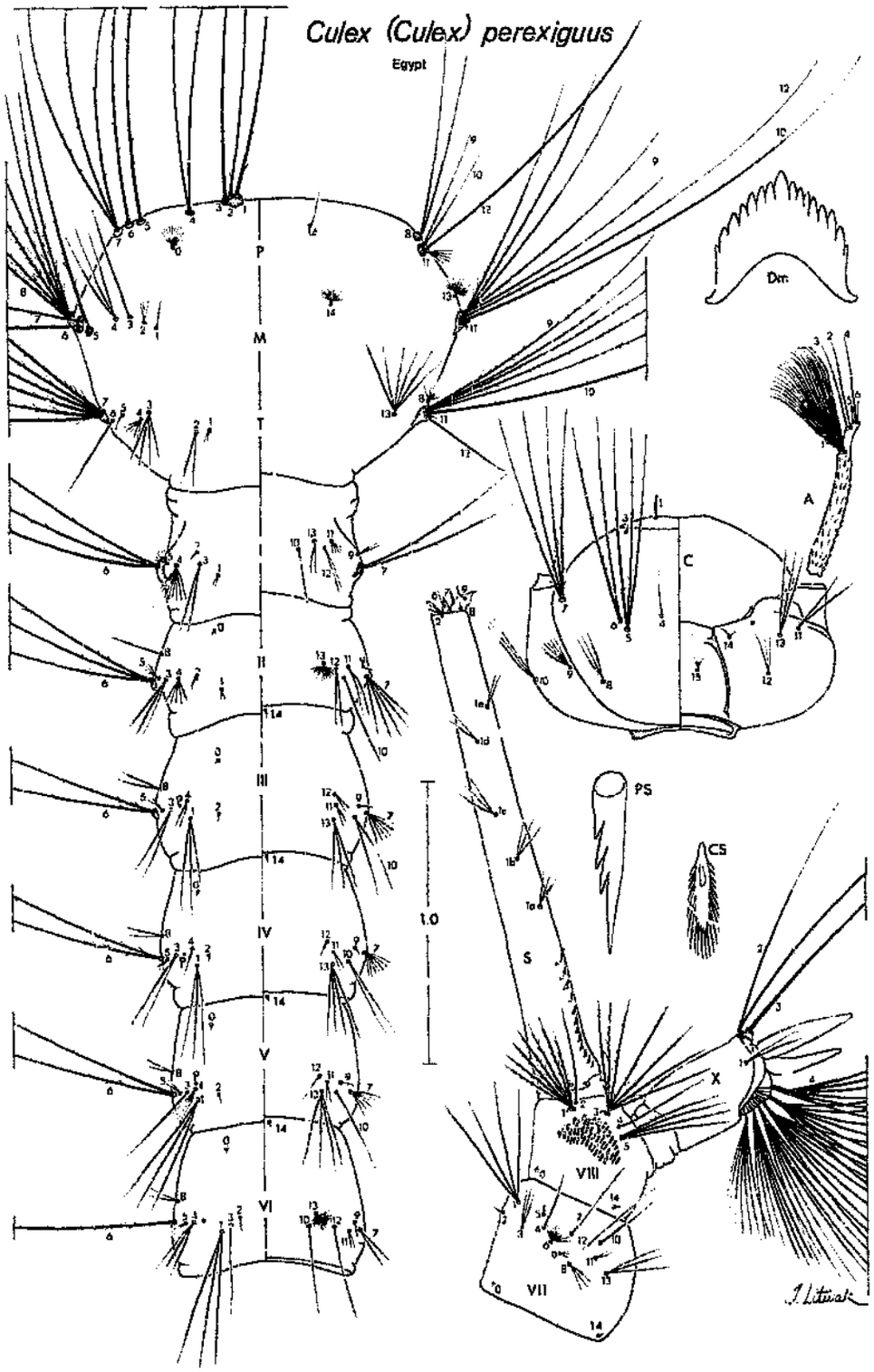

Fig. 4. *Culex (Culex) perexiguus.* Larva, Egypt.

Table 4. Classification of eggs in viable rafts from primary crosses and parental stocks.

Cross/colony	Cage	No. rafts examined	Mean % of eggs		
			Hatched	Unhatched-embryonated	Infertile (unembryonated)
neavei ♀ x *perexiguus* ♂	1	7	2	96	2
	2	11	3	95	2
perexiguus ♀ x *neavei* ♂	1	16	72	2	26
	2	22	89	1	10
neavei		23	95	3	2
perexiguus		21	93	2	5

Table 5. Classification of eggs in nonviable rafts from primary crosses.

Cross	Cage	No. rafts	Mean % of eggs	
			Embryonated	Infertile (unembryonated)
neavei ♀ x *perexiguus* ♂	1	48	44	56
	2	49	50	50
perexiguus ♀ x *neavei* ♂	1	12	0	100
	2	4	4	96

infertile (unembryonated) eggs while unhatched eggs from the reciprocal cross were virtually all infertile (unembryonated).

The hybrids that resulted from the crosses had normal sex ratios and developed normally, without morphological abnormalities, to produce healthy, vigorous adults. However, only a limited number of F_1 adults resulted from the cross between *neavei* females and *perexiguus* males because of the limited number of viable rafts produced, each with a low egg hatch. In comparison, a large number of F_1 adults resulted from the reciprocal cross. These hybrids were backcrossed with the parental stocks to obtain the results shown in Tables 6 and 7. Table 6 shows that viabilities were high for the egg rafts resulting from each of the backcrosses. The ensuing progeny developed at a normal rate, no morphological abnormalities were observed, and healthy adults were produced which exhibited a normal sex ratio.

The classification of eggs comprising viable rafts deposited by females used in the backcrosses is shown in Table 7. The overall viability of the eggs that resulted from backcrossing the hybrids was not always as high as it was for eggs in rafts obtained from the parental colonies. However, a high percentage of eggs hatched in some of the rafts that resulted from each of the backcrosses.

DISCUSSION

The results of the crossing experiments show that there is a high degree of unidirectional incompatibility when *neavei* females are mated with *perexiguus* males. The high proportion of females which were inseminated in this cross (*viz.* 36/52, 69.2%), and which deposited eggs that developed embryos, indicates that there was little or no premating isolation between the laboratory colonies of these forms, presuming, of course, that normal premating isolating mechanisms were not disrupted in the laboratory. The effect of crossing *neavei* females with *perexiguus* males is certainly indicative of a measure of postmating isolation, but also could be due to the phenomenon of cytoplasmic incompatibility caused by a rickettsia-like symbiont similar to that found in different geographical populations of *Cx.*

Table 6. Viability of egg rafts from backcrosses between F_1 hybrids and parental forms.

Cross	Proportion of viable rafts
hybrid ♀ x *neavei* ♂	89/98 (90.8%)
hybrid ♀ x *perexiguus* ♂	107/111 (96.4%)
neavei ♀ x hybrid ♂	74/76 (97.4%)
perexiguus ♀ x hybrid ♂	62/92 (67.4%)

Table 7. Classification of eggs in viable rafts from backcrosses and parental stocks.

Cross/colony	No. rafts examined	Mean % of eggs		
		Hatched	Unhatched-embryonated	Infertile (unembryonated)
F_1 hybrid ♀ x *neavei* ♂	31	80	8	12
F_1 hybrid ♀ x *perexiguus* ♂	30	68	21	11
neavei ♀ x F_1 hybrid ♂	30	73	20	7
perexiguus ♀ x F_1 hybrid ♂	30	86	8	6
neavei	23	95	3	2
perexiguus	21	93	2	5

pipiens Linnaeus (Yen and Barr 1973, Irving-Bell 1974, Jupp 1987). However, if the evidence obtained from the crossing experiments is considered in combination with the morphological differences that exist between the two forms, then the conclusion is reached that *neavei* and *perexiguus* are separate, largely allopatric species.

ACKNOWLEDGMENTS

Appreciation is expressed to the following individuals: Bruce A. Harrison, F.L. Peyton, Ronald A. Ward and Richard C. Wilkerson for commenting on the manuscript; Taina Litwak for preparing the illustrations; and James Pecor for typing the manuscript. Figures 3 and 4 were published previously by the American Entomological Institute in Harbach (1988).

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Culex (Culex) pipiens pipiens Linnaeus and *Culex (Culex) pipiens quinquefasciatus* Say in South Africa: Morphological and Reproductive Evidence in Favour of Their Status as Two Species

by

P. G. Jupp
National Institute for Virology
Department of Health
Private Bag X4
Sandringham 2131
South Africa

ABSTRACT. The study showed that both larvae and adult males of *C. pipiens pipiens* and *C. pipiens quinquefasciatus* from Johannesburg, Transvaal, can be distinguished morphologically. Males were separable either by the structure of their genitalia or according to the proportion of the shaft of the maxillary palp which bears hairs. Ninety six percent of adult females were distinguishable by a quantitative character in the wing venation. Laboratory colonization showed that *quinquefasciatus* was stenogamous and *pipiens* eurygamous which suggests that reproductive isolation is due to an ethological premating mechanism. In 9 crossing experiments there was no cross-insemination between the 2 taxa except in one experiment where 2 *quinquefasciatus* females were inseminated by male *pipiens*. The $F_1 + F_2$ hybrids subsequently reared were vigorous and fertile indicating the absence of postmating isolating mechanisms. After study of these laboratory hybrids a search was made for natural hybrids among mixed field collections from Johannesburg and from Bethulie, Orange Free State. Not one such hybrid was found. From this evidence that *pipiens* and *quinquefasciatus* are not interbreeding and because of their considerable morphological difference it is concluded that the 2 taxa in South Africa should be classified as separate species.

INTRODUCTION

No definite agreement has yet been reached concerning the taxonomic status of the various members of the *Culex pipiens* complex. In the latest reviews written on the systematics of the complex (Mattingly, 1967; Laven, 1967) it was concluded that the complex should be treated as a single polytypic species embracing six named taxa; these were *C. pipiens pipiens* Linnaeus, *C. pipiens quinquefasciatus* Say*, *C. pipiens australicus* Dobrotworksy and Drummond, *C. pipiens var. molestus* Forskal, *C. pipiens var pallens* Coquillet, *C. pipiens var comitatus* Dyar and Knab. *C. australicus* was raised to specific rank by Belkin (1962) and Knight and Stone (1977) upheld this view in their recently revised "Catalogue of mosquitoes of the world". However, some of these supposedly infraspecific

*The name *quinquefasciatus* Say 1823 is regarded here as a senior synonym to *fatigans* Wiedemann, 1828 (see White (1975), Belkin (1977), Knight and Stone (1977), Sirivanakarn and White (1978).).

taxa have certain natural populations displaying different morphological, ecological, behavioural and physiological characters, which raises the possibility that all or some might exist as distinct species (Rozeboom, 1951; Paterson and James, 1973). The difficulty is to show that 2 sympatric forms are not interbreeding in nature - the critical evidence for distinguishing 2 species. In a recent study of sympatric populations of members of the complex in Australia, Miles (1976) tackled this problem by using isoenzymes as genetic markers and showed that not only *C. globocoxitus* but *C. p. australicus* and *C. p. quinquefasciatus* were coexisting at Perth without interbreeding.

In South Africa there are 2 members of the complex, *C. p. pipiens* and *C. p. quinquefasciatus*, which differ in morphology and other ways suggesting that these taxa are also specifically distinct. *C. quinquefasciatus* is an important nuisance mosquito which occurs throughout the country, particularly in urban environments, although it has occasionally been collected in rural areas in the vicinity of farmsteads in the Orange Free State province (Jupp *et al* - in preparation). *C. pipiens*, on the other hand, occurs more frequently in rural than in urban and peri-urban habitats, although both taxa often occur sympatrically. Both are ornithophilic, particularly *pipiens*, but while *quinquefasciatus* also enters houses and bites man readily, *pipiens* is poorly anthropophilic (Jupp 1973). The larvae of *quinquefasciatus* are more commonly encountered in artificial containers whereas larvae of *pipiens*, although also occurring in this habitat, prefer natural ground pools, vleis, river margins and irrigation furrows (Jupp, 1967, and unpublished observations). Laboratory colonies of the 2 taxa also have certain differences.

This paper describes morphological and reproductive studies undertaken on *pipiens* and *quinquefasciatus* in South Africa in an attempt to establish whether they are specifically distinct. The extent of morphological difference observed between them constitutes evidence for the existence of 2 taxa and the question of whether these were biological species was approached in 2 ways. Firstly evidence for reproductive isolation was sought through a study of laboratory mating behaviour and by carrying out cross-mating experiments. Secondly, after making a study of laboratory hybrids and gaining a picture of hybrid morphology it was possible to search for naturally occurring hybrids.

MATERIALS AND METHODS

Mosquitoes studied morphologically

Field specimens originated from in or near Johannesburg, Transvaal province, except where indicated otherwise. All the *pipiens* and some of the *quinquefasciatus* examined were the progeny reared from wild-caught gravid females. Part of the egg raft deposited by each such wild-caught female was reared out and a larva, pupa, adult male and adult female studied in each case. The remaining *quinquefasciatus* specimens collected were larvae which were identified and reared in isolation to obtain a series of associated immature and imagine specimens. Four F_1 larvae arising from a cross-mating experiment were preserved from each of 3 egg rafts. The remaining larvae were then pooled for rearing F_1 adults, a sample of which was killed for examination. Eighteen F_2 rafts

were obtained, each of which was reared in isolation, so that a larva, adult male and adult female could be kept for examination. Genitalia were also examined from males reared from larvae of the 2 taxa collected together in the same water at Johannesburg and at Bethulie in the southern part of the Orange Free State province.

Morphological characters

Two larval and 3 adult characters were studied as shown below. Terminology used is that recommended by Knight and Laffoon (1970, 1971 a,b).

1. Larva; siphonal Index. The siphonal index was calculated, that is the length of the siphon (excluding the valves and acus) divided by its greatest width at the basal ring.

2. Larva; siphonal setae. The number of branches borne by the siphonal setae 1a-S and 1b-S was counted. These subventral tufts of setae are the first 2 proximal pairs on the siphon and both in each pair were examined.

3. Adult; cross vein index in wing. The length of each cross vein (c & d) and their distance apart (a) were measured in the wings of male and female mosquitoes, as shown in fig 1, so that a "cross vein index" could be calculated. This was the ratio of the combined lengths of the cross veins to their distance apart.

4. Maxillary p ir index in adult male. In the male maxillary palp the length of the shaft (segments 2 + 3) was measured and the proportion of its length bearing hairs (figure 2). A "hair index" was calculated which was the ratio of total shaft length to length with hairs.

5. DV/D index in male genitalia. The DV/D index (Sundararaman, 1949) was calculated from measurements made on the lateral plate of the aedeagus (figure 3).

Siphonal setae on the larva were studied by floating each specimen in a small dish containing a 70% ethanol solution with glycerol added to 5% of volume, while to measure the siphon the larva was transferred, in the same solution, to a hollow ground microscope slide. Examinations were made under a stereomicroscope at a magnification of 50 x (siphonal index) or 100 x (setae). Measurement of the maxillary palp was made after removing the mosquito's antennae. Each such male mosquito was examined on a hollow ground slide in ethanol-glycerol under a stereomicroscope at a magnification of 50 x. For measuring the cross veins, adult wings were mounted permanently on slides in polyvinyl lactophenol; and permanent euparal mounts were made of male abdomens with aedeagus still intact for measurements on the aedeagus. Slides of both wings and genitalia were examined under a compound microscope at a magnification of 80 x (wings) or 500 x (genitalia).

Mating behaviour in the laboratory.

Various attempts to establish laboratory colonies of *quinquefasciatus* and *pipiens* were carried out, starting with adults reared from gravid females collected in or near Johannesburg. This work produced information on the laboratory mating behaviour of each taxon.

Crossing experiments.

As a laboratory colony of *pipiens* was unavailable at the time the crossing experiments were performed, individuals of *pipiens* used were the progeny reared from gravid females collected near Johannesburg. Pupae harvested from among such *pipiens* progeny and from *quinquefasciatus* colonies were reared individually and on emergence the adult mosquitoes were separated according to sex and placed in cages. Exceptions to this were some of the male *quinquefasciatus*, which were taken directly from the colonies.

Nine experiments were carried out in which the 2 taxa were mixed together in 35 cm^3 cages. This was done because any cross-matings would reveal whether postmating isolating mechanisms existed between the 2 taxa. In 6 experiments *pipiens* females w re mixed with *quinquefasciatus* males and in 3 experiments the reciprocal cross was attempted. From 50 to 100 mosquitoes of each sex, usually 1-20 days old, were used. Temperature was 25 - 26° C and relative humidity 75 - 80%. The daily light cycle, provided by controlled artificial lighting, consisted of a day period of 12 hours, a night period of 10 hours and twilight and dawn periods of 70 minutes each when the light intensity gradually decreased or increased respectively. An interval of 1 week or more elapsed before mosquitoes were fed on pigeons or young chickens. Five days or more after feeding, gravid mosquitoes were confined individually in "laying tubes" (Jupp 1967) for oviposition. Rafts deposited were regarded as infertile if they had not hatched after 3 days.

RESULTS

1. MORPHOLOGICAL DISTINCTION BETWEEN *C. PIPIENS* AND *C. QUINQUEFASCIATUS* FROM JOHANNESBURG.

Examination of eggs and pupae under the light microscope revealed no differences between the 2 taxa. Morphological differences found in larvae and adults are shown in table 1.

Siphonal index of larva. The larvae of *pipiens* usually had a longer, more slender siphon than those of *quinquefasciatus*. Out of a total sample of 100 (50 *pipiens* and 50 *quinquefasciatus*) specimens only 2 had siphonal indices in the area of overlapping distribution of values.

Siphonal setae of larva. Setae 1a-S and 1b-S both had 1 - 3 branches in the specimens of *pipiens*, while seta 1a-S had 4 - 11 and seta 1b-S 4 - 10 branches in the specimens of *quinquefasciatus*.

Cross vein index in wing. Five different ratio s were calculated from preliminary measurements made on the wings of adult females as follows: -

$$\frac{x}{y}, \frac{a}{b}, \frac{x}{a}, \frac{a}{c}, \quad \frac{a+b}{c}$$

The meaning for all these symbols are shown in fig. 1. The values for all these ratios determined on samples of *pipiens* and *quinquefasciatus* overlapped

considerably. However, when values for the cross vein index $\frac{(c + d)}{a}$ were determined there was overlap in only 5 out of 98 (4%) of female specimens as shown in table 1, although in males the proportion was bigger with 9 out of 91 (10%) overlapping.

Male maxillary palp hair index. It was found that in the case of *pipiens* hairs extend about halfway down the shaft of the palp from its distal end (index of 2.3 - 3.1) while in *quinquefasciatus* they only occur at the tip of the shaft (index 3.2 - 8.7). Edwards (1941) described this difference although he did not express it quantitatively.

Male DV/D index. Fig 3 shows drawings of the inner division of the lateral aedeagal plates. In *pipiens* the ventral arm never overlapped the dorsal arm so that the DV/D index remained zero, while in *quinquefasciatus* there was always overlap so that the values of the index were 0.6 - 0.9. The tip of the dorsal arm also differed in the 2 forms of mosquito, being blunt-ended in *pipiens* but pointed in *quinquefasciatus*.

2. REPRODUCTIVE ISOLATION IN THE LABORATORY

Mating Behaviour.

C. quinquefasciatus mated readily in cages as small as 16 cm^3 which showed that the species was stenogamous. Three colonies were thus established easily in the laboratory. No special lighting regime was necessary and 2 of these colonies provided *quinquefasciatus* for the crossing experiments.

Colonization of *pipiens* was much more difficult. Previously the author had attempted to colonize this species in 35 cm^3 cages exposed to lighting which represented a 12 hour day - 12 hour night and subsequently in a larger cage 75 x 50 cm in cross-section and 150 cm high, exposed to illumination simulating a 12 hr day, 1 hr dusk of constant intensity and 11 hr night. However, the mosquitoes did not mate because of the eurygamous nature of this species until the method described for *C. univittatus* (Jupp and Brown, 1967) was used with a walk-in cage measuring 3.0 metres high, 2.9 m long and 1.8 m wide and with lighting incorporating a gradual increase in the morning and decrease in the evening. With this environment a high raft viability index of about 80% was attained by the 8th generation which indicated a similarly high frequency of matings.

At the 11th generation this colony was transferred to small 35 cm^3 cages kept in identical conditions. The percentage viability indices for F_{12} - F_{22} rafts were 3, 2, 9, 22, 9, 8, 8, 30, 14, 33 and 49 but none of 228 F_{23} rafts hatched so that the colony was then lost. The sudden cessation of mating by the F_{22} adults was probably due to these adults being moved out of the main insectory room with its special illumination for a period while this room was being repainted.

Crossing experiments

Altogether 136 egg rafts were deposited in the 9 crossing experiments. These all failed to hatch except in one experiment where 100 *pipiens* males had

been mixed with 45 *quinquefasciatus* females. In this experiment 2 rafts hatched out of 13 and one of the females which had deposited one of these viable rafts was re-fed and a second raft was laid. Hence the F_1 hybrid generation originated from 3 viable rafts in each of which the proportion of eggs which hatched was normal and the numbers of each sex equal. Vigorous F_1 adults were reared, given a blood-meal and they subsequently deposited 30 F_2 rafts, 25 of which hatched. These in turn lead to vigorous F_2 adults. No attempt was made to rear a third generation although it is unlikely that any difficulty would have been encountered in doing this.

Morphology of hybrids

Table 1 also shows the results of a study of the morphology of the F_1 and F_2 hybrids and how they compare with the morphology of *pipiens* and *quinquefasciatus*. It can be seen that in both the F_1 and F_2 larvae the range of values for both the siphonal index and the number of branches on setae 1a-S and 1b-S overlaps the ranges of both *pipiens* and *quinquefasciatus*. Similarly the range of values for the female wing cross vein index and the male maxillary palp hair index determined on adult F_1 and F_2 hybrids showed the same kind of overlap. The DV/D indices determined on the genitalia of 45 F_1 hybrids were all intermediate between the zero index of *pipiens* and the range of indices obtained for *quinquefasciatus*. This was also the case in 11 out of 12 of the F_2 hybrids, the twelfth mosquito having an index of 0.6 which just falls in the *quinquefasciatus* range. Drawings of the lateral aedeagal plates in the genitalia of *pipiens*, *quinquefasciatus* and hybrid males are shown in fig. 4 from which it is clear that those of *pipiens* and *quinquefasciatus* are readily distinguishable. Furthermore, most hybrid genitalia would probably be recognizable without measurement.

3. SEARCH FOR HYBRIDS AMONG FIELD SPECIMENS

It was thought that the newly gained knowledge of the morphology of laboratory hybrids described above should prove useful for recognition of hybrids if they existed in nature. Accordingly certain slides of male genitalia prepared from mosquitoes previously collected were re-examined. These mosquitoes had been reared from collections of larvae which had comprised both *pipiens* and *quinquefasciatus* co-existing in the same water.

Table 2 shows the DV/D indices determined on samples originating from Bethulie and Johannesburg respectively. The indices determined for the F_1 and F_2 laboratory hybrids are included for comparative purposes. As can be seen none of the resulting indices corresponded to one determined on a laboratory hybrid if the solitary F_2 hybrid with a ratio of 0.6 is discounted. Rather the indications are that the field samples consisted of distinct *pipiens* and *quinquefasciatus* only, as indicated in table 2. In addition to the specimens shown in table 2 for which the DV/D index was determined, a further 86 males from Bethulie and 112 males from Johannesburg were identified as *pipiens* or *quinquefasciatus* from their male genitalia without any measurement.

DISCUSSION

It is evident from the morphological study that it is possible to distinguish Johannesburg populations of *pipiens* and *quinquefasciatus* by examination of the larva and adult male. The number of branches in the first 2 pairs of siphonal setae is diagnostic for differentiating larvae while the value for the siphonal index is almost diagnostic. Adult males can either be distinguished by examination of the lateral aedeagal plate in their genitalia or according to the proportion of the shaft of the maxillary palp which bears hairs. The second character is easier to use than the genitalia as slide mounts are unnecessary. The adult females cannot be differentiated with complete certainty, although the majority of specimens (96%) can be identified by means of the cross vein index in the wing. There are other characters which are helpful in recognising females of the 2 taxa. *C. quinquefasciatus* usually has a buff-coloured scutum and well marked white tergal bands as compared to *pipiens*, which tends to have a darker rust brown scutum and narrower tergal bands. The considerable morphological difference between *pipiens* and *quinquefasciatus* suggests that they are 2 separate species, as they were formerly regarded by Edwards (1941).

Previously the author attempted to cross the 2 taxa by the artificial mating technique; this was unsuccessful, which prevented the study of a large sample of cross-inseminated females. However, the insemination of 2 *quinquefasciatus* females by *pipiens* males in one of the present crosses, with deposition of viable egg rafts and subsequent production of vigorous F_1 and F_2 mosquitoes, shows that a postmating isolating mechanism does not exist.[1] It is likely that the absence of interbreeding between *pipiens* and *quinquefasciatus* in South Africa is due to an ethological premating isolating mechanism and that this explains why there were only 2 successful insemi ions in the crossing experiment.

Laboratory colonization of the 2 taxa showed very clearly that *quinquefasciatus* was stenogamous and *pipiens* eurygamous although by selection over a number of generations the latter became stenogamous. This difference indicates that *quinquefasciatus* probably has a quite different requirement for swarm formation in nature than *pipiens* and that this is probably the basis for the ethological isolation. In Australia isolation between 2 members of the *C. pipiens* complex, *C. globocowitus* and *C. australicus* seems to have a similar basis (Paterson and James, 1973).

The search for naturally occurring hybrids among male mosquitoes reared from mixed larval collections did not reveal a single hybrid. The samples of such males on which the DV/D index was measured were small, but the genitalia of many more potential hybrid males were examined from the field without detecting any intermediates. This is thought to indicate that hybridization either does not occur between *pipiens* and *quinquefasciatus* at Johannesburg and Bethulie and probably throughout South Africa, or that it occurs only very rarely.

The evidence that the 2 taxa are not interbreeding, together with their considerable morphological difference means that their present taxonomic status needs to be re-evaluated. There are now grounds for referring to the 2 taxa in South Africa as separate species.

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TABLE 1. Morphological comparison between *C. pipiens*, *C. quinquefasciatus* and laboratory-reared hybrids.

	Character	*C. pipiens*	*C. quinquefasciatus*	F_1 hybrids	F_2 hybrids
Larva	Siphonal index	4.8-6.1 (48) 4.3-4.4 (2)	3.5-4.7 (50)	4.1-4.9 (12)	3.9-5.0 (18)
	No. branches on seta 1a-S; 1b-S	1.3; 1-3 (50)	4-11; 4-10 (50)	3-6; 2-5 (12)	2-6; 2-5 (18)
Adult female	Wing; cross-vein index	1.4-5.0 (47) 1.2 (1)	0.7-1.3 (51) 1.4-1.7 (4)	1.0-1.9 (50)	0.9-1.5 (18)
Adult male	Maxillary palp; hair index.	2.3-3.1 (50)	3.2-8.7 (50)	2.4-4.6 (50)	2.6-4.9 (18)
	Genitalia; lateral aedeagal plate - DV/D index	0.0 (32)	0.6-0.9 (31)	0.2-0.4 (45)	0.2-0.6 (12)

Numbers in parenthesis are the numbers of specimens

TABLE 2. DV/D indices for adult males reared from collections of larvae which each contained both *C. pipiens* (C.P.) and *C. quinquefasciatus* (C.Q.) as compared to indices for laboratory hybrid males.

Locality	No. specimens	DV/D index	Identity
Bethulie	19	0.0	C.P.
(Orange Free State)	13	0.6-1.1	C.Q.
Johannesburg	16	0.0	C.P.
(Transvaal)	14	0.6-1.7	C.Q.
Laboratory specimens	45	0.2-0.4	F_1 hybrids
	11	0.2-0.4	F_2 "
	1	0.6	F_2 "

Fig. 1. Diagram of portion of wing to show various measurements made on the venation including those used for the cross-vein index $\frac{c + d}{a}$.

Fig. 2. Maxillary palp of male mosquito to show the two measurements made for calculation of the palp hair index.

Fig. 3. Diagram of the inner division of the lateral aedeagal plate to show ventral arm (VA), dorsal arm (DOA) and measurements made for the DV/D ratio (after Sundararaman 1949).

Fig. 4. Camera lucida drawings of the lateral aedeagal plates in *C. pipiens*(a), *C. quinquefasciatus* (b), F_1 laboratory hybrid (c) and F_2 hybrid (d).

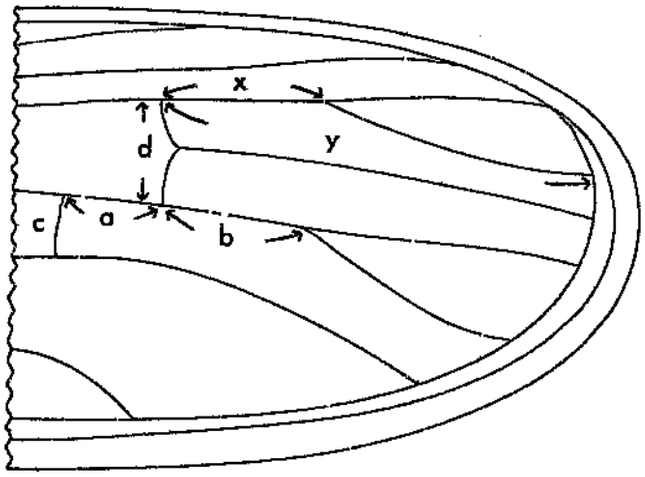

Fig.1

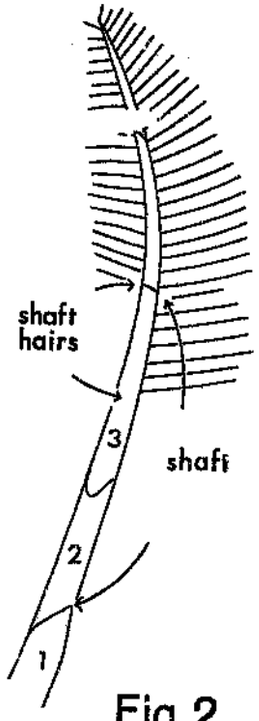

Fig.2

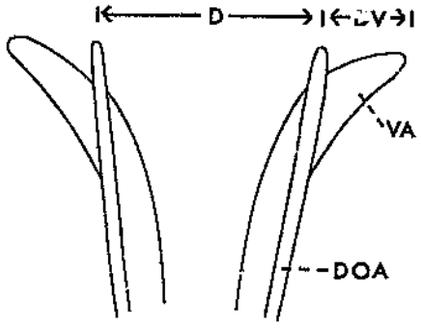

Fig.3

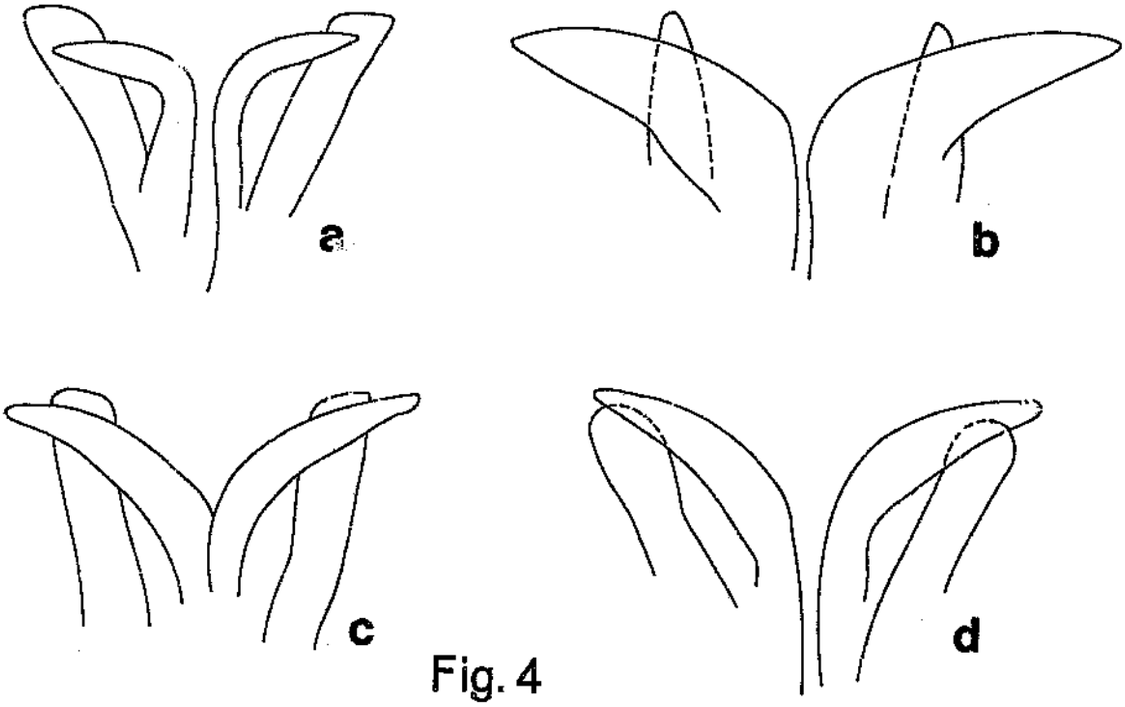

Fig. 4

Culex (Culex) pipiens and *Culex (Cx.) torrentium* (Diptera: Culicidae) in England: Notes on their Taxonomy and Biology

P. G. Jupp
Arbovirus Research Unit
National Institute for Virology
Private Bag X4
Sandringham 2131
South Africa

ABSTRACT. The sympatric sibling species *Culex torrentium* Martini and *Cx. pipiens* L were collected in Surrey and *Cx. pipiens* in Huntingdonshire, southern England. The 2 species were studied taxonomically mainly to find a diagnostic character for separating the females and some biological observations were also made.

Males could only be separated by previously described genitalia differences, while in the female study of the pre-alar scales showed that a diagnosis could be made according to the number of these scales.

At Silwood Park in Surrey *Cx. torrentium* comprised a high proportion (62.1%) of the total collection of both species, while *Cx. pipiens* occurred alone in a collection of hibernating mosquitoes from Huntingdonshire.

Hibernating *Cx. pipiens* taken by air to South Africa were reactivated by an increase in temperature and day length (provided by artificial illumination) at 17 days and at 5 months after collection, so that they took bloodmeals and oviposited.

INTRODUCTION

In the 1977 summer specimens of *Culex pipiens* L. were collected in England as part of a broader taxonomic study on members of the *Culex pipiens* complex from several countries. Large numbers of *Culex torrentium* Martini occurred in these collections which made it necessary to be able to separate the 2 species.

Apart from Mattingly's (1951) brief report on the discovery of *Cx. torrentium* in England, Service (1968) is the only worker who has investigated *Cx. torrentium* and its relationship to *Cx. pipiens*. He did studies on both taxonomy and biology and his findings on the taxonomic separation of the 2 species, with terminology after Knight (1971) and Knight & Laffoon (1971), were as follows:

(1) Eggs and larvae cannot be distinguished.

(2) Pupae can be separated to some extent: when seta 4 on abdomen segment VIII is single on one or both sides the pupa can be confidently ascribed to *Cx. torrentium* but specimens which have 2 branches on both sides cannot be identified with certainty.

(3) The genitalia are completely diagnostic for adult males owing to differences in the structure of the phallosome (see also Mattingly, 1951). In *Cx. torrentium* the basal lateral projection of the paraprocts is very well developed whereas in *Cx. pipiens* it is vestigial. In addition the dorsal arm of the inner division and the outer division of the lateral aedeagal plate have differently shaped tips.

(4) The possession of pre-alar scales is not diagnostic for males of *Cx. torrentium* as 10% of a sample of this species was found to lack these scales on both sides of the thorax, making them indistinguishable from *Cx. pipiens*. No pre-alar scales were found in males of *Cx. pipiens*. Owing to these findings in males Service cautioned against relying on pre-alar scales for identification of females.

The present paper reports taxonomic and some biological studies carried out on the 2 sympatric sibling species. A search was made for morphological characters by which larvae, adult females and adult males of the 2 taxa could be separated. A means of identifying the female mosquito was the principal objective.

MATERIALS AND METHODS

The collections were all made in the grounds of the Field Station of the Imperial College of Science and Technology at Silwood Park near Ascot in Surrey, southern England, except for one which was made at Monks Wood in Huntingdonshire. Egg rafts, larvae and pupae were collected at Silwood Park. At Monks Wood a sample of adult *Cx. pipiens* females was collected by mechanical aspirator from the walls of an underground room where this species had just entered into hibernation.

Each field-collected larva or pupa was reared in isolation so that each emergent adult could be preserved along with its associated pelt or pelts for later taxonomic study. Each field-collected egg raft was allowed to hatch and a sample of the resulting larvae reared so that associated larvae, larval pelts, pupal pelts and male and female adults all originating from the same individual egg raft could be preserved. This procedure was carried out for a number of egg rafts so that different batches of associated immature-adult specimens could be obtained i.e. different sibling groups. Thus a sibling group was the progeny reared from one egg raft deposited by one female mosquito. A number of such different sibling groups were also reared from rafts subsequently deposited by the mosquitoes collected at Monks Wood.

For the taxonomic studies, with the exception of certain instances stated in the 'Results' section, one larva, one adult male and one adult female were used from each of the sibling groups which had been reared. The male was identified from the structure of its genitalia, so that the identity of the associated larva and female mosquito was known. Using specimens identified in this way an evaluation was made of 4 morphological characters for separating the 2 species. These were as follows:

(1) The number of branches borne by the siphonal setae 1a-s and 1b-s in the larva.

(2) The value for the cross-vein index in the wing of the adult female, that is the ratio of the combined lengths of the 2 cross-veins to their distance apart.

(3) The value for the maxillary palp hair index in the adult male, that is the ratio of the length of the shaft of the palp bearing hairs to its total length (segments 2 + 3).

(4) The presence and number of pre-alar scales in the female mosquito.

Characters (1) and (3) had previously proved diagnostic and (2) almost diagnostic for distinguishing *Cx. pipiens* and *Cx. quinquefasciatus* Say in South Africa (Jupp, 1978).

For clarity the methods used for handling the hibernating mosquitoes are included in the 'Results' section.

RESULTS

Taxonomy

The first 3 morphological characters evaluated proved valueless for separating the 2 species as their examination on small samples showed an overlap between the 2 taxa. However, study of the fourth character, the occurrence of pre-alar scales in females, showed that in 15 *Cx. torrentium* belonging to different sibling groups 1 or more pre-alar scales were present on both sides of the thorax. In a further 12 *Cx. torrentium*, which were siblings of 9 of the above mosquitoes, there were 2 specimens that had scales on only one side of the thorax - 3 scales and 1 scale respectively. However of 35 *Cx. pipiens* belonging to different sibling groups, 3 mosquitoes possessed a single pre-alar scale on one or both sides of the thorax. In view of this, all the available female siblings of these 3 mosquitoes were examined which revealed that the occurrence of pre-alar scales varied within each of the sibling gruops as follows:

Number of siblings in sibling groups examined	Number individuals with: 1 scale on each side	1 scale on one side	No scales
2	1	0	1
6	1	3	2
6	0	2	4

These findings show that the presence or absence of pre-alar scales is not absolutely diagnostic for separating females of the 2 species, although the presence of 2 or more scales on one or both sides of the thorax would identify a specimen as *Cx. torrentium*. This criterion was used in identifying the emergent females reared from immatures collected in the field and in those cases where only 1 scale occurred, or where a specimen was damaged, the associated pupal pelt was mounted and examined. For each of these pelts a single-branched seta 4 on one or both sides of abdominal segment VIII confirmed that they were all *Cx. torrentium*.

Biology

At Silwood Park, larvae, pupae and egg rafts were collected from artificial containers of water, including metal water tanks, wooden barrels and concrete animal drinking troughs, and in one instance from the stagnant margins of a stream. A total of 206 emergent mosquitoes were identified. This figure was made up of 95 males and 86 females which had been reared individually from larvae or pupae collected in the field and of 25 sibling groups of mosquitoes successfully reared from egg rafts. Out of this total of 206 mosquitoes, 128 individuals or different sibling groups were identified as *Cx. torrentium* i.e. 62.1%. All of the 11 larval collections made contained *Cx. torrentium* and 7 of them also contained *Cx. pipiens*.

Seventy one individuals of a sample of about 250 *Culex* mosquitoes collected hibernating at Monks Wood on 30th August, were all shown to be *Cx. pipiens* from examination of males reared from egg rafts they subsequently deposited. Hence it is likely that the complete sample was only *Cx. pipiens*. The sample was placed in a 20 cm^3 cage of cotton netting on the top of which was placed a thick wad of cotton wool soaked in 4% sucrose solution. Thereafter this wad was kept moist and renewed when necessary. The cage was enclosed in a card board box, just big enough for it, so that the relative humidity was 80-84% and the light excluded. The temperature was not controlled but the box was kept in a cool garden shed until flown to Johannesburg 14 days after the mosquitoes had been collected. On arrival in Johannesburg the box was stored in a cool garage and after 3 days the mosquitoes were divided into 2 groups of about 100 insects each in 35 cm^3 cages. The mosquitoes in the first cage were released from hibernation immediately by placing this cage in an environment which, although humid, (RH 68-70%) had a higher temperature of 26-27°C and artificial lighting to simulate a day length of 16 1/2 hours (an English summer's day) and a night of 7 1/2 hours. Seven days after placing this cage under these conditions 6 of the insects engorged on a pigeon offered to the mosquitoes. However, many others died from uncertain causes, possibly because of insecticide on the feathers of

the pigeon used for feeding. Over the next 3 weeks opportunity to feed on pigeons was provided 4 more times. This resulted in 25 insects taking bloodmeals. The last 4 surviving mosquitoes, which remained unfed, were dissected and found to contain large (1 mosquito), medium-sized (1 mosquito) or small (2 mosquitoes) fat bodies.

The second cage was kept humid (RH 68-70%) and in the dark at 16°C, in conditions similar to those which prevailed in their natural hibernating site at Monks Wood. When this cage was finally placed under the conditions to terminate hibernation, it was found that only one mosquito had died after 5 months of hibernation in an artificial environment. Three days later a large proportion of the mosquitoes engorged on a pigeon and 40 insects subsequently deposited rafts, all of which hatched. The higher feeding rate by this group of mosquitoes was thought to be due to the fat bodies having been depleted in a higher proportion of individuals before the pigeon was offered to them.

DISCUSSION

Service (1968) showed that *Cx. torrentium* could not be distinguished in the larval stage from *Cx. pipiens* and that males of *Cx. torrentium* could not be distinguished by the pre-alar scale character but only according to the structure of the genitalia. The present findings do not alter this conclusion since the siphonal setae proved to be of no value for diagnosis in the larva and the maxillary palp hair index was likewise valueless in males. However, examination of female specimens in the present study indicated that although the 2 species could not be separated according to their wing venation, they could be differentiated by the pre-alar scale character. In the rather small samples studied it was shown that all females of *Cx. torrentium* except one specimen could be identified by the presence of 2 or more pre-alar scales on 1 or both sides of the thorax. Since siblings of this single exception could also be identified it is thought that the specimen in question may have lost 1 or more scales due to being rubbed. *Cx. pipiens*, although usually having no scales, occasionally had only a single scale on 1 or both sides. This method of diagnosis needs to be tested out on larger samples of female mosquitoes of known identity belonging to both species.

Cx. torrentium was more prevalent than *Cx. pipiens* at Silwood Park accounting for 62.1% of the total collections of both species. This is a much higher proportion than Service (1968) found in collections made at Poole, Dorset, from 1964-1966 (37.2%), Monks Wood (15.9%) and from a large area over the breadth of southern England (32.3%). The collections at Silwood Park suggest that a future survey in Britain might reveal that *Cx. torrentium* has displaced or partly displaced *Cx. pipiens* in certain areas.

No *Cx. torrentium* occurred among the 71 mosquitoes identified from the sample which was collected hibernating in an underground room at Monks Wood. Service (1968) also failed to find adults of this species hibernating in man-made shelters. Hibernating *Cx. pipiens* transferred to the laboratory in South Africa were reactivated at 2 different times after collection - after 17 days and after 5 months. Increased temperature and day-length,

the latter provided by artificial illumination, stimulated the mosquitoes to feed and develop their ovaries. This shows that *Cx. pipiens* can be transferred from one country to another while in a state of hibernation and subsequently reactivated. The same could well apply to other species of *Culex* from temperate countries.

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Comparative studies on morphology and laboratory biology of *Culex (Culex) pipiens* Linnaeus (Diptera: Culicidae) from South Africa and England

by

P. G. JUPP

Arbovirus Unit, National Institute for Virology and Department of Virology, University of the Witwatersrand. Private Bag X4, Sandringham, 2131

Culex pipiens Linnaeus from South Africa and England and *Culex pipiens* var. *molestus* Forskal from England were compared in respect of five quantitative morphological characters to assess their taxonomic status. Ranges of values for each of the characters in the 3 mosquito forms overlapped. A laboratory colony was established with *Cx. pipiens* from England and compared to *Cx. pipiens* from South Africa. During simulated twilight they exhibited a different flight behaviour. It was concluded that *molestus* should continue to be regarded as a variety and that on the available evidence *pipiens* from South Africa and England should continue to be regarded as conspecific, although these two populations probably represent distinct species. Evidence suggesting this is their different overwintering mechanisms and laboratory behaviour as well as the absence of the gonadal symbiont *Wolbachia* in South African *pipiens* which is present in *pipiens* from England.

INTRODUCTION

The two most important members of the *Culex pipiens* complex, *pipiens* Linnaeus and *quinquefasciatus* Say, have usually been regarded along with the other members as belonging to a single polytypic species (Mattingly 1967; Laven 1967; Barr 1981). However, Miles (1976) obtained evidence that *quinquefasciatus* in Australia is a separate species. In South Africa, Jupp (1978) argued on the basis of morphological and reproductive evidence that the two forms occurring there should be regarded as distinct species, i.e. *Culex pipiens* and *Culex quinquefasciatus*. The question still left unanswered is whether *pipiens* from South Africa is conspecific with *pipiens* from the type locality. The latter, given by Linnaeus is very broad, i.e. Europe, Lapponia and America (Knight and Stone 1977), so it was decided to compare South African *pipiens* with *pipiens* from England since an opportunity arose to collect live mosquitoes there. Since this work was completed Harbach *et al.* have recently (1985) designated a neotype for *pipiens* from Vererŏd, Scania, Sweden.

Culex pipiens occurs throughout the temperate areas of the world, except in extremely cold areas. It is spread throughout South Africa although it occurs more frequently in rural than in urban and peri-urban areas. In Europe the nominate subspecies occurs together with its much rarer variety, *molestus* Forskal, both of which were

studied in detail by earlier workers. In England these studies showed that there was considerable morphological and biological variation among populations of *pipiens* (Jobling 1938; Marshall and Staley 1937; Tate and Vincent 1936; Christophers 1945). Differences noted between *pipiens* and *molestus* led the earlier workers to regard these forms as distinct species (Marshall and Staley 1937). Subsequently, however, when the characters of populations from a variety of localities were taken into account it appeared that these differences were not consistent and the status of *molestus* was reduced to that of a variety (Mattingly, 1951). This view is still upheld (Harbach, *et al.* 1984).

The present study included a comparison of selected anatomical features for South African *pipiens* (SAPIP), English *pipiens* (GBPIP) and English *molestus* as well as some observations on the laboratory behaviour of SAPIP and GBPIP.

MATERIAL and METHODS

Morphology

The SAPIP originated from near Johannesburg, Transvaal, while GBPIP originated from Monks Wood, Huntingdonshire in the south of England. Specimens examined were the progeny of wild-caught females. Part of the egg raft deposited by each such wild-caught female was reared out and a single larva, adult male and adult female studied from each. Some male siblings were also studied. The larvae and adults of *molestus* came from a long established colony maintained at the London School of Hygiene and Tropical Medicine.

Morphological characters studied were as follows:

1. The number of branches of siphonal setae 1a-S and 1b-S in the larva.
2. The cross vein index for the wing of the adult female, i.e. the ratio of the combined lengths of the two cross veins to their distance apart.
3. The number of setae on the lateral areas of the ninth tergite in the female.
4. The maxillary palp hair index in the adult male, i.e. the ratio of the length of the shaft of the palp bearing long hairs to its total length (segments 2 & 3).
5. DV/D in male genitalia (Sundararaman 1949), i.e. the ratio of the distance between the apices of the ventral arm of the inner division of the lateral plate and the dorsal arm of the outer division on one side (DV) to the distance between the apices of the dorsal arms of the outer divisions (D), measured from the dorsal aspect (pre-rotation sense) in undissected specimens with the genitalia in the normal resting position.
6. Other structures of the male genitalia including the setae of the subapical lobe of the gonocoxite, the length of the basal lateral arm borne by the paraproct and the components of the lateral plate of the phallosome.

Characters (1), (4) and (5) previously proved diagnostic, and (2) almost diagnostic, for distinguising *pipiens* and *quinquefasciatus* in Johannesburg (Jupp 1978). Marshall and Staley (1937) found the mean value for (3) useful for separating GBPIP from most English populations of *molestus*, although it was not diagnostic.

Laboratory Behaviour

A laboratory colony of GBPIP was established from adults reared from females collected at Silwood Park near Ascot, Berkshire, in the south of England. The females were collected in the autumn on the 3rd September, at the start of their hiber-

nation, and the following day were brought to Johannesburg where they were maintained in a state of diapause by keeping them humid (RH 68–70%) and in the dark at 16 °C for 4 months. Diapause was broken by placing them in an environment with the same humidity, a higher temperature (26–27 °C) and artificial lighting simulating an English summer's day (day length of 16,5 hours and night of 7,5 hours). These methods were essentially the same as those used previously (Jupp 1979).

The adult progeny reared from the eggs obtained from the wild-caught females were placed in three 350 × 350 × 350 mm cages which were designated as the 'P generation' and the following generation the F_1. Temperature was maintained at 25–27 °C and relative humidity at 75–80%. The daily light cycle, provided by controlled artificial lighting, consisted of a day period of 12 hours, a night period of 10 hours and dusk and dawn periods of 60 minutes when the light intensity gradually decreased or increased respectively. Beginning with the third generation, the day and night periods were changed to 14 hours and 8 hours respectively in order to increase the mosquitoes' activity and hence the number of mosquitoes which fed on pigeons exposed to them overnight.

RESULTS

Morphology

The results of the examination of morphological characters (1) to (5) expressed quantitatively are shown in Table I. Ranges of values for the three mosquito forms overlapped in the case of each of the characters. In addition to the male genitalia on which DV/D ratios were determined (Table I), 17 male genitalia of GBPIP and 24 of SAPIP were examined for structural differences, with negative results. Finally, the phallosome was removed and closely examined in respect of 20 siblings of each form, but this failed to reveal any consistent differences between the forms.

TABLE 1. Morphological comparison between *Cx. pipiens* (South Africa), *Cx. pipiens* (England) and *Cx. pipiens* var *molestus* (England).

	Character	*pipiens* South Africa	*pipiens* England	var *molestus* England
Larva	No branches on seta 1a–S: 1b–S	1–3; 1–3 (50)*	2–4; 2–4 (20)	3–4; 2–4 (5)
Adult female	Wing, cross-vein index	1,2–5,0 (48)*	1,3–3,0 (29)	2,6–4,8 (24)
	No. setae on 9th tergite	9–19 (14)	10–16 (9)	14–21 (14)
Adult male	Maxillary palp; hair index	2,3–3,1 (50)*	2,2–3,2 (8)	2,6–2,9 (10)
	Genitalia; lateral plate of phallosome – DV/D index	0,0 (32)*	0,0 (24)	0,0 (9)

Numbers in parenthesis are the numbers of specimens studied.
*These results were reported previously (Jupp 1978).

Laboratory Behaviour

Only 1/91 (1%) of the GBPIP egg rafts proved viable and the single fertile mosquito which had deposited this raft was refed four times and four further rafts obtained. Thus the F_1 Generation was composed entirely of siblings. Percentage viability indices thereafter were 30% (F_2), 43% (F_3) and 18% (a mixed F_2/F_3 cage).

A cage of F_1 GBPIP and a cage of progeny reared from SAPIP were observed during the twilight period. This period began at 1800 hours, the light growing gradually dimmer until darkness was reached at 1900 hours. By 1820 hours both sexes of mosquito in both cages had increased their flying activity. At 1848 hours the SAPIP hovered close to the sides and top of the cage, while the GBPIP swarmed with larger flight movements which took them 10–15 cm from the sides and top of the cage. By 1845 hours when the light intensity was very low, a few matings on the wing appeared to occur among the GBPIP, but not one was seen among the SAPIP.

DISCUSSION

The failure to detect any significant morphological difference in *molestus* when it was compared with GBPIP and SAPIP is in agreement with the conclusions of Mattingly (1951) and Harbach *et al.* (1984) that *molestus* should be regarded as a variety. It is, however, possible that the long history of laboratory colonization of the *molestus* mosquitoes examined may have altered their morphology. This could not be ascertained since it was not possible to collect *molestus* from the field on account of its present rarity in England.

The morphological study showed considerable similarity between GBPIP and SAPIP and it was clearly not possible to regard the two forms as distinct species on the basis of the characters and techniques used. The slight variation in values for indices and setal counts in the two forms could be regarded as being within normal morphological variation among populations of the same species. It is possible, however, that a study with the scanning electron microscope might reveal some consistent difference in structure.

The biology of GBPIP and SAPIP has both similarities and differences: both feed mainly on birds, to a lesser extent on domestic mammals, and rarely on man (Jupp 1973; Anderson 1967; Tate and Vincent 1936; Service 1968) and both are eurygamous. However, SAPIP is more strongly eurygamous than GBPIP and differs in overwintering mechanism although the latter could be accounted for as resulting from adaptation by allopatric conspecific populations to markedly different local climatic conditions.

When SAPIP was previously colonized (Jupp 1978) it proved more difficult than the colonization of GBPIP in the present study because of its greater eurygamy. Mating only occurred readily in a large cage measuring 3000 × 2900 × 1800 mm provided with a special lighting regime entirely of artificial light. This consisted of a 12 hour day, 10 hour night and 60 minute dusk and dawn periods during which there was a gradual decrease and increase in intensity respectively. No matings occurred in cages 350 × 350 × 350 mm or in one 750 × 500 × 1500 mm. However, it is possible that the absence of properly controlled dawn-dusk lighting in the case of this second cage may have contributed to this failure of the mosquitoes to mate. In contrast, workers in England have been able to colonize mosquitoes from that country in relatively small cages, smaller than that necessary to establish a colony from South African populations. Tate

and Vincent (1936) showed that mosquitoes, presumably originating from Cambridge, would mate in cages down to a minimum of 600 × 600 × 600 mm. Using cages of this size up to ones 750 × 750 × 750 mm they showed a viability of 15–54% (mean 33%) in the egg rafts deposited over 11 generations. Service (pers. comm.) established a colony from mosquitoes collected at Brownsea Island on the south coast of England in a 900 × 900 × 900 mm cage which he kept for eight months through several generations. In both these colonies the lighting was important but did not appear so critical as that for the South African form. Both workers utilized a combination of sunlight through windows and artificial illumination throughout the night (Tate and Vincent 1936) or for 15 hours per day (Service, pers. comm.). It appears that the artificial light was necessary to prevent the mosquitoes hibernating rather than for stimulating mating activity. The near absence of mating among the progeny of mosquitoes from Silwood Park in 350 × 350 × 350 mm cages observed in the present study concurs with the above observations – more matings would have probably occurred if a larger cage had been used.

English workers have known for a long time that *pipiens* in their country overwinters by means of hibernating (diapausing) females (Tate and Vincent 1936; Clements 1963). However, on the Transvaal highveld this does not appear to be the case. Instead, observations indicated that the winter is bridged by quiescent adults, larvae and pupae with emergence and bloodfeeding occurring during mild periods when temperatures increase (Jupp 1969, 1975). This difference is probably related to the climatic differences between the two countries, the shorter day length and lower minimum temperatures in the English autumn and winter triggering and maintaining hibernation.

In conclusion it may be said that it is doubtful that populations of *pipiens* Linnaeus in South Africa and England can be distinguished morphologically. However, biologically they differ slightly, the main difference being in their overwintering mechanisms. South African *pipiens* also differs from *pipiens* in England in not possessing the gonadal symbiont *Wolbachia* (Barr, pers. comm. and Irving Bell, 1974), which strongly suggests they differ taxonomically. It would therefore be worthwhile cross mating the two forms to ascertain whether they are reproductively isolated. In order to do this it would first be necessary to colonize each form in a 'walk-in cage', i.e. one large enough for the researcher to enter and stand up in. Such a cage provides sufficient space for male mosquitoes to swarm and for females to enter the swarm and mate on the wing.

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Mosquitoes feeding at two horizontal levels in gallery forest in Natal, South Africa, with reference to possible vectors of chikungunya virus.*

by

B. M. McINTOSH, P. G. JUPP and J. DE SOUSA

Arbovirus Research Unit, South African Institute for Medical Research, Johannesburg

The relative biting prevalence of mosquito species in the canopy and at ground level was studied in a gallery forest where monkeys had probably been infected with chikungunya virus in the canopy. Several species, some of which fed readily on monkeys, were observed to feed in significant numbers in the canopy. Considering prevalence, host preferences, arboreal tendencies, and vector capability in species where this is known, it is concluded that *Aedes* (*Dicrorompia*) *furcifer* (Edwards) and/or *Aedes* (*Dicrorompia*) *taylori* Edwards and *Mansonia* (*Mansoniodes*) *africana* (Theobald) seem most likely to have been involved as vectors.

Following the disclosure in 1964 that an immune rate of 54% to chikungunya (CHIK) virus existed in a vervet monkey population in the Ndumu Game Reserve in the coastal plain of northern Natal (McIntosh, 1970) studies were started in the area in an attempt to identify a possible mosquito vector. The indications were that the monkeys had been infected during the night in the canopy in gallery forest along the Usutu River near its confluence with the Pongola River.

Collections of mosquitoes were made simultaneously in the canopy and at ground level in this forest during 8 periodic visits, each of about 8 days, from November, 1964 to April 1967.

Over this period surveillance was also maintained on possible activity by CHIK virus in the Reserve. All mosquitoes collected were tested for virus and monkeys were trapped, bled, marked and released in a survey of their antibody status. CHIK virus was not isolated from the mosquitoes (McIntosh, Jupp & de Sousa, in preparation) nor was there evidence of a recrudescence of infection by this virus among the monkeys (McIntosh, 1970). While the study on the feeding habits of mosquitoes was in progress therefore it seems unlikely that any active transmission by this virus occurred, and in retrospect it now seems most likely that the monkeys had been infected during a single epizootic, possibly of limited duration, which had occurred shortly before the presence of antibody had been discovered. Therefore, since there is no reason to believe that the monkeys are perpetuating CHIK virus in this particular forest an incidental vector with a relatively short existence during the adult stage could have been involved just

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as well as spec. , which are present more or less continuously as adults. If so, those *Aedes* species which survive the long dry season at Ndumu in the egg stage came into consideration, other factors being equal, with the same degree of suspicion as *Culex* and *Mansonia* species which for most, if not all, of the time have a population of adults in the area.

Apart from CHIK virus the study is relevant to other mosquito-borne viruses which occur at Ndumu. Some of these have been associated with certain mosquito species and a determination of the preferred feeding level of these species would help towards an understanding of the ecology of the viruses concerned.

METHODS

Mosquitoes were collected using either man, monkey or fowl as bait. In respect of class of bait, collecting site, time and duration of collecting all collections were so arranged that observations at one horizontal level would be comparable with those at the other level. For the majority of collections three sites were used, evenly spaced over about 1,5 kilometres of gallery forest. Man-baited collections were always at the same site where an open platform had been erected, 12 metres up, in a giant fig tree, *Ficus sycamorus*, a dominant species in the gallery. At the other two sites fowl and monkey baits were alternated on successive nights, the same class of bait always being used at both levels at particular sites.

Man-baited collections

On the tree platform and on the ground at the foot of the tree two persons collected mosquitoes in test-tubes as they landed on their exposed legs. Torches with the light dimmed by a cover of red cloth provided light. Catches usually started ½ hour before sunset and ended 1½ to 2½ hours later.

Fowl- and monkey-baited collections

In each fowl-baited trap one fowl, restrained within a nylon stocking, was used in a black, circular trap of the "lard-can" type designed by Bellamy and Reeves (1952). The trap had an inverted mosquito gauze cone at each end and mosquitoes entered through a hole 2,5 cm in diameter in the centre of the cone. The trap was 66 cm long and 33 cm in diameter. Monkey-baited traps were similar in design but larger (76 ×

TABLE 1. Number of collections for each class of bait during various months. 1964–1967.

Month	Number collections*			Total
	man	monkey	fowl	
January	8	0	0	8
February	9	9	9	27
March	3	7	5	15
April	16	9	8	33
November . . .	11	12	11	34
December . . .	15	11	14	40
Totals	62	48	47	

* Each collection refers to a pair, one on the ground and the other in the canopy.

43 cm) and the monkey was restrained within a wire cage (20 × 20 × 25 cm) with mesh of 12 × 25 mm. Traps were set in the late afternoon and collected shortly after sunrise. They were set at 1 metre and at 12 metres above ground, a rope on a pulley used to the branch of a tree being used to raise traps to the higher level.

Duration of collecting

Table 1 shows the total number of paired collections for each class of bait during the six wet months of the year during which observations were made. The

TABLE 2. Numbers, M_w's and persistence of mosquito species collected off man during 62 paired collections

Species	Persistence index	Number		M_w	
		ground	canopy	ground	canopy
Anopheles coustani	11	15	1	0,1	
,, tenebrosus	40	106	8	0,7	0,1
,, ziemanni	5	9	1	0,1	
,, funestus group	35	293	3	0,9	
,, gambiae complex	24	89	2	0,4	
,, pharoensis	29	40	12	0,3	
,, squamosus	13	28	23	0,1	0,1
Coquillettidia chrysosoma	2	1			
,, fuscopennata	3		2		
Mansonia africana	100	10107	942	96,1	6,5
,, uniformis	92	697	155	5,9	1,2
Eretmapodites silvestris	2	1			
Aedes (Mucidus) lucianus	24		80		0,5
,, ,, sudanensis	18	6	99		0,4
,, (Aedimorphus) albocephalus	3	2			
,, ,, cummunsii	39	292		1,3	
,, ,, durbanensis	10	6	1		
,, ,, fowleri	2	1	1		
,, ,, kennethi	2	1			
,, ,, marshalii	10	34		1,7	
,, (Neomelaniconion) circumluteolus	90	5034	25	32,6	0,2
,, ,, lineatopennis	2	1			
,, (Diceromyia) furcifer/taylori	68	6	284		2,0
Culex (Neoculex) insignis	2	2			
,, (Culex) annulioris	31	73	2	0,6	
,, ,, antennatus	29	76	13	0,4	0,1
,, ,, aurantapex	2	1			
,, ,, bitaeniorhynchus	2	1			
,, ,, neavei*	87	419	236	2,8	2,1
,, ,, poicilipes	71	2	219		1,9
,, ,, thalassius	2	1			
,, ,, zombaensis	85	461	43	3,7	0,4
Totals		17 805	2 152		

* Referred to previously by us as *univittatus*.

number of collections between different baits varied as a result of unavoidable discarding of some collections and human bait was used more extensively than the others.

Analysis of the results

The results are given as the total number of each species collected at each level and, in cases where numbers were large enough to justify it, also as Williams' modification of the geometric mean (M_w) to offset bias resulting from exceptionally large individual catches (Haddow, 1960). Greater significance has been given to the M_w than to the arithmetical total.

A "persistence index" is given for each mosquito species with each class of bait. This is the proportion, expressed as a percentage, of the days on which the species was collected to the total number of collecting days, and is a measure of the relative time each species was encountered as a blood-seeking female.

The number of engorged females of each species collected in the fowl and monkey-baited traps was recorded and the proportion engorged to the total number collected is given as a percentage.

TABLE 3. Numbers, M_w's, persistence and percent "blooded" of mosquito species collected in monkey-baited traps during 48 paired collections

Species	Persistence index	% blooded	Number		M_w	
			ground	canopy	ground	canopy
Coquillettidia aurea/wahlbergi	8	0		7		0,1
,, chrysosoma	13	8	2	11		0,1
,, fuscopennata	6	7		14		0,1
,, metallica	4	0	1	1		
,, microannulata	8	0		7		0,1
Mansonia africana	100	14	3595	97	47,1	1,2
,, uniformis	58	21	115		1,6	
Aedes (Mucidus) lucianus	8	37		30		0,2
,, ,, sudanensis	17	55	4	139		0,4
,, (Neomelaniconion) circumluteolus	38	15	109	2	0,8	
Culex (Neoculex) insignis	6	0	2	3		
,, (Culex) annulioris	6	20	4	1		
,, ,, antennatus	10	0	13	4	0,1	
,, ,, aurantapex	2	0	1			
,, ,, bitaeniorhynchus	2	0	2			
,, ,, guiarti	2	0		1		
,, ,, neavei	92	4	658	1271	6,1	10,6
,, ,, pipiens	4	0	5	3		
,, ,, policilipes	40	9	5	71		0,6
,, ,, zombaensis	90	3	529	28	5,5	0,4
Totals			5045	1690		

THE GALLERY HABITAT

The gallery forest in the collecting zone varies from about 25 to 75 metres in width with trees of about 15 to 25 metres high. The canopy is discontinuous and in the open parts a dense cover of shrubs, creepers and grasses cover the ground. While this cover extends to some extent into the more closed parts of the forest, in parts where the canopy is thickest vegetation is largely absent, ground cover in these parts being confined to fallen leaves and the remains of fallen trees. Parts of the ground are frequently flooded from the Usutu River and for most of the summer conditions are shady, humid and warm. There are a large number of treeholes suitable for mosquito breeding. Close by in the adjoining savanna there are several large swamps and permanent pans.

Monkeys seen at night in the gallery were occupying the upper half of the understorey, slightly above the higher level at which mosquito collections were made. At times dense populations of mosquitoes rested, during daylight, on the ground in the shady parts of the forest amongst low vegetation or fallen leaves. They showed little inclination to feed on man at this time.

TABLE 4. Numbers, M_w's, persistence and percent "blooded" of mosquito species collected in fowl-baited traps during 47 paired collections

Species	Persistence index	% blooded	Number		M_w	
			ground	canopy	ground	canopy
Anopheles coustani		0		1		
Coquillettidia aurea/wahlbergi	13	78	2	7		0,1
,, chrysosoma	9	80	1	4		
,, fuscopennata	6	100	1	2		
,, metallica	11	50	5	1		
,, microannulata	2	0	1			
Mansonia africana	100	90	2566	28	29,7	0,3
,, uniformis	46	99	120	1	1,0	
Eretmapodites silvestris	2	0	1	1		
Aedes (Mucidus) lucianus	13	92		12		0,1
,, ,, sudanensis	17	83		18		0,2
,, (Neomelaniconion) circumluteolus	26	73	36	2	0,3	
,, (Diceromyia) furcifer-taylori	2	100		2		
Culex (Lutzia) tigripes	2	0		1		
,, (Neoculex) insignis	4	100	1	2		
,, (Culex) annulioris	19	0	9		0,1	
,, ,, antennatus	8	85	11	2	0,1	
,, ,, bitaeniorhynchus	4	100	1	1		
,, ,, neavei	100	80	1115	1731	11,8	18,07
,, ,, pipiens	8	42	28	8	0,1	
,, ,, poicilipes	51	64	7	50	0,2	0,7
,, ,, thalassius	2	100	1			
,, ,, zombaensis	74	68	539	36	2,8	0,3
Totals			4245	1910		

RESULTS AND DISCUSSION

Previous experience had shown that the lardcan traps fail to collect *Aedes* and *Anopheles* species adequately but it was hoped that the man-baited series would to some extent overcome this deficiency. Several species which failed to enter the lardcans but were attracted to man in these collections were shown to feed readily on monkeys in the laboratory and there is perhaps some justification for the belief that most species attracted to man would also feed on monkeys.

On the other hand it is clear that the present collections fell short of a complete coverage of the existing mosquito fauna although many of the species not collected would be very rare and consequently of no probable importance as vectors. Worth & Paterson (1961) listed 75 culicine species as occurring at Ndumu most of which probably feed in the gallery so the total of 25 culicine and 7 anopheline species collected in the present study is only about a third of the total fauna. Attention should be drawn, in particular, to *Aedes aegypti* which was not collected and which might well have been involved in the epizootic. It is noteworthy that while this species is extremely common in the immature stages in treeholes in the forest it has rarely been collected as the adult during previous studies in the Reserve, either during the day or night, and little is known of its feeding preferences. Man is apparently not a preferred host.

It soon became apparent that the method used of restraining the monkey bait within a wire mesh cage decreased mosquito feeding rates in comparison to rates on fowls enclosed in a stocking in which greater immobility and accessibility of bait were achieved and in assessment of feeding rates on monkeys allowance should be made for this fact.

At times of very high mosquito densities the landing rate of mosquitoes in the man-baited collections on the ground was so high that it became impossible to collect all mosquitoes. Hence numbers collected at this time are an inadequate reflection of the real densities which were higher than those recorded.

The total numbers and M_w of each species collected are given separately for man, monkey and fowl baits in tables 2, 3 and 4 respectively. Human bait was most productive of variety of species as well as of numbers of each species collected. The results will now be discussed in sequence under the respective genera or subgenera.

Anopheles Meigen

Only one specimen of a single species entered the lardcan trap and in the man-baited catches all species, except *squamosus*, which was collected in equal numbers at both levels, were ground-haunting.

Coquillettidia Dyar

None of the five species collected was sufficiently numerous for reliable conclusions to be drawn although all species were attracted to both animal baits and high feeding rates were recorded with fowls. With the exception of *metallica* they showed arboreal tendencies and it seems likely that these species would feed at least to a limited extent on monkeys in the canopy. Observations in Uganda have shown that species of this genus feed readily in the canopy and that birds are the preferred hosts (Haddow, 1961; Williams, 1963).

Mansonia Blanchard

M. africana was the numerically dominant species on the ground with all three classes of bait and formed 60% of the total ground-catch. In the man-baited

series large numbers were not collected until it had become quite dark, usually about an hour after sunset. It had the highest persistence of any mosquito, being recorded daily with all classes of bait, fed avidly on fowls and reasonably well on monkeys. It was also collected quite frequently in the canopy, was the most numerous species in the man-baited series at that level and ranked second in the monkey-baited series. Hence this species is clearly a frequent feeder on monkeys in the gallery. Although able to transmit CHIK virus it is probably an inefficient vector (McIntosh *et al.*, 1965) and the extent to which it is capable of initiating and maintaining an epizootic among monkeys is not clear, but it appears very likely that this species was implicated to some extent in the epizootic which occurred. Because of its habit of vertical movement between ground level and canopy and its host preferences this species might well transfer CHIK virus from simian to human cycles to man.

In the man-baited series *uniformis* was the third most prevalent species at ground level and it also showed high persistence. It also fed readily on both animal baits at ground level and was also collected off man in fair numbers in the canopy. It is reasonable to believe that it would feed on monkeys in the canopy and during the epizootic it may have played a similar but minor vectorship role to its more prevalent congener.

Eretmapodites Theobald

Because the three species of this genus in Southern Africa are known to feed readily on man the small numbers collected in the present man-baited series are an indication of their rarity in the gallery at Ndumu and their importance there is probably slight.

Aedes (*Mucidus*) Theobald

Of the two species of this subgenus collected, *sudanensis* (previously referred to as *scutaphagoides*) was collected alone during the early stages of the study while *lucianus* was collected alone at a later stage. In retrospect it seems possible that some misidentifications may have occurred among those recorded as *sudanensis*. In any event both species occur in the gallery and both species as recorded showed strong arboreal tendencies, were attracted to all baits and fed avidly on monkeys and fowls. Although their adults are seasonal in appearance these species must clearly be considered as possible vectors of CHIK virus over a short period. Haddow (1961) reported *Mucidus* species as being highly arboreal in East Africa.

Aedes (*Aedimorphus*) Theobald

These species failed to enter the lardcan traps and off human bait only *cumminsii* and *marshallii* were collected in moderate numbers. Both species were ground-haunting which appears to be the favoured level for members of this subgenus (Haddow, 1960). It is noteworthy that although *kennethi* is one of the most common species breeding in treeholes in the gallery only a single specimen was collected.

Aedes (*Neomelaniconion*) Newstead

Off human bait *circumluteolus* ranked third in persistence and second in numbers collected at ground level although relatively few were collected in the canopy. Even on the ground however, only small numbers entered the monkey- and fowl-baited traps probably because *circumluteolus*, like other aedine species, is to some extent deterred from entering these traps although the presence of engorged specimens in these traps

clearly indicates that they would feed on these animals if circumstances were suitable. It is nevertheless doubtful, in fact, whether the monkeys are fed upon by *circumluteolus* to any significant extent. The scarcity of blood-seeking *circumluteolus* in the canopy at night as well as the reluctance of this mosquito to feed during daylight when monkeys are on the ground would seem to ensure minimal feeding on monkeys. This is borne out by the low immune rate in monkeys at Ndumu to such viruses as Pongola, Wesselsbron and Spondweni which have been repeatedly isolated from *circumluteolus* and which it has been shown capable of transmitting (Muspratt et al. 1957; Worth et al. 1961; McIntosh, 1970). *Ae. circumluteolus* is also an apparent non-vector of CHIK virus (McIntosh & Jupp, 1970) which is a further reason for eliminating this species from involvement in the epizootic. Because of its high feeding rate on man it seems that *circumluteolus* would readily infect him with the viruses it does transmit provided he entered its bush haunts at night. In the present series only small numbers were collected off man in the half hour before sunset; large numbers being first encountered only about half-an-hour after sunset.

Aedes (*Diceromyia*) Theobald

Both *furcifer* and *taylori*, which are inseparable in the female, occur at Ndumu although the former is probably more common and most specimens referred to this group were probably *furcifer*. Members of this group failed to enter the lardcans in large numbers but were readily attracted to man and were predominantly arboreal. *Ae. furcifer*, at least, is a proven vector of CHIK virus and has fed readily on monkeys in the laboratory (Paterson & McIntosh, 1964). Members of this group were suspected as vector during an epidemic of chikungunya in Rhodesia in 1962 (McIntosh et al. 1964) and it seems that they should also be considered as one of the more likely vectors during the epizootic at Ndumu. Their disappearance as adults during the dry season at Ndumu would however preclude them from maintaining transmission at this time.

Culex (*Lutzia*) Theobald

Only a single specimen of *tigripes* was collected. This was in a fowl-baited trap in the canopy.

Culex (*Neoculex*) Dyar

Although *insignis* was collected only in very small numbers in the present catches experience at Ndumu over several years has suggested that it is more common than these collections indicate. It was collected at both levels off monkeys and fowls. Judgment on this species as well as on two other members of this subgenus, *rima* and *horridus*, which were not collected but which do occur at Ndumu should prudently be withheld until more information on them can be obtained.

Culex (*Culex*) Linnaeus

C. annulioris, *antennatus* and *zombaensis* were collected in sufficient numbers to show that they are predominantly ground-haunting although small numbers of *antennatus* and *zombaensis* would also feed in the canopy. They fed on all three classes of bait and *zombaensis* particularly fed on monkeys in the canopy in small numbers. Its high persistence level might even ensure that as many *zombaensis* would feed on monkeys as the predominantly arboreal but seasonal *Mucidus* species. *C. neavei* and *poicilipes* were also attracted to all three classes of bait. The lardcan collections showed *neavei* to possess strong arboreal tendencies although large numbers were also collected on the

ground and in the man-baited series a larger number were in fact collected at ground level. *C. neavei* is now recognised as a sibling species of *Culex univittatus* Theobald which does not occur at Ndumu and which rarely feeds on man. It was, in fact, the relatively higher feeding rates of *neavei* on man at Ndumu which partly led to the recognition of *neavei* as specifically distinct (Jupp, 1971). In the present collections *neavei* had a high persistence level and was by far the most numerous species collected in monkey-baited traps in the canopy. It is, however, a non-vector of CHIK virus (McIntosh & Jupp, 1970) and it seems should be discounted as vector during the epizootic. *C. poicilipes* was definitely arboreal and the fairly large number collected off man is suggestive that the lardcans deterred this species to some extent and its real monkey-biting attributes might be higher than the collections indicate. De Meillon et al. (1957) also reported on the prevalence and arboreal tendencies of *poicilipes* on the Pongola River and it seems that this species is an important component of the arboreal mosquito fauna in gallery forest in northern Natal. Experimentally, however, it also is a non-vector of CHIK virus (McIntosh & Jupp, 1970), and should therefore be absolved from involvement in the epizootic.

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Rural Epidemic of Chikungunya in South Africa with Involvement of *Aedes* (*Diceromyia*) *furcifer* (Edwards) and Baboons

B. M. McIntosh, P. G. Jupp and I. dos Santos

Arbovirus Research Unit, National Institute for Virology, Department of Health, Private Bag X4, Sandringham 2131, South Africa.

The epidemiological features of a small rural epidemic of chikungunya in the wooded savanna of South Africa are described. Sixteen isolations of chikungunya virus were made from 538 females of the Aedes (Diceromyia) furcifer/taylori *group in a locality where human infection was high. All 92 baboons (*Papio ursinus*) sampled at this locality after the epidemic were immune. It is concluded, since identification of male mosquitoes of the group indicated that* Aedes furcifer *was the numerically dominant species, that this species was the main vector in both baboon and human infection. Baboons are regarded as the primary vertebrate host and extensive feedback of virus from them to the vector was the probable cause of most human infection.* Ae. furcifer *was shown to be strongly attracted to baboons and monkeys, greater numbers were attracted to these animals in the understorey compared to the ground, but some were also attracted to man on the ground, and it is concluded that this species could therefore readily transfer virus from baboons to a rural human population. Floral and topographical features believed to favour chikungunya virus transmission in the affected area are described.*

Die epidemiologiese kenmerke van 'n klein plattelandse epidemie van chikungunya in die Transvaalse bosveld van Suid-Afrika word beskryf. Sestien kwekings van chikungunyavirus is verkry vanaf 538 wyfies van die groep Aedes (Diceromyia) furcifer/taylori *op 'n plek waar merkbare menslike infeksie plaasgevind het. Al 92 bobbejane (*Papio ursinus*) afkomstig van dieselfde gebied wat na die epidemie getoets is, was immuun. Identifisering van manlike muskiete het aangedui dat* Aedes furcifer *die getalsterkste lid van die groep was, en die gevolgtrekking word gemaak dat hierdie muskietsoort grotendeels verantwoordelik is vir infeksie van beide mense en bobbejane. Bobbejane word beskou as die primêre gewerwelde virusgasheer en uitgebreide terugvoering van virus tussen hulle en die virusdraende muskiet was vermoedelik die oorsaak van die meeste gevalle van menslike infeksie.* Ae. furcifer *word sterk aangelok deur ape en bobbejane veral as hulle in bome is, maar hulle is ook gedeeltelik aangelok deur mense op die grond. Daar is tot die gevolgtrekking gekom dat virus maklik van bobbejane na 'n plattelandse bevolking oorgedra kan word. Plantkundige en topografiese kenmerke wat vermoedelik die verspreiding van chikungunyavirus in die aangetaste gebied begunstig, word beskryf.*

Active infection by chikungunya (CHIK) virus has been infrequently recognized in Southern Africa over the past two decades since the presence of the virus was established. It has consequently been difficult to obtain reliable evidence implicating either vectors or wild vertebrate hosts, although antibody surveys[1] and susceptibility studies[2] had indicated that the baboon, *Papio ursinus* (Kerr), and vervet monkey, *Cercopithecus aethiops* (Cuvier), were important natural hosts. However, in 1976 an epidemic occurred in South Africa, during which further evidence was obtained on the role of baboons and for the first time virus was isolated from mosquitoes.

The mosquito species found infected in this outbreak were members of the *Aedes furcifer/taylori* group, of which *Ae. furcifer* was the species actually implicated. Suspicions against these species had arisen before in studies following an epidemic in Rhodesia in 1962[3], and later in South Africa in field studies on possible vectors which had infected vervet monkeys.[4] Furthermore, in laboratory transmission tests with wild-caught females of the group, which were probably mainly *Ae. furcifer*[5], and with a laboratory colony of *Ae. furcifer*[6], a reasonably efficient level of vector capability was recorded.

The recent outbreak, which occurred during March and April, 1976, was in the wooded savanna region of the eastern Transvaal, the so-called lowveld, where previous human infection had been recognized in 1956 and 1975. During the outbreak field studies were concentrated on two adjoining farms, Hope and Lillie, where human infection had been intense, and which are located some 40 km west of the town of Phalaborwa. On these farms is a cluster of granite outcrops, rising some 50–150 m above the surrounding plain, which provided a safe overnight refuge for baboons which were using the summits as dormitories. Cattle ranching was the main agricultural activity and consequently the woodland areas were still largely in their natural state. Large trees were plentiful and the habitat was typical of the kind known to favour the tree-hole breeding *Ae. furcifer/taylori* group.

Mosquitoes were collected on Hope and Lillie for attempts at virus isolation, and acute and convalescent-phase blood specimens were obtained from residents on these farms and at surrounding localities for virus isolation and serological studies. Blood specimens were again collected from humans in May, 1976, and from baboons in August, 1976, to determine post-epidemic immune rates. During March, 1977, mosquito collections were made on Hope to determine the extent that the *Ae. furcifer/taylori* group fed on monkeys and baboons; the results are included in this paper.

Methods

Mosquitoes were collected off volunteer human bait on Hope and Lillie from 7–14 April, 1976. Collections started at sunset and continued for 2–3 hours. Collecting was in woodland, on the ground, or in the understorey of trees, and on occasions on or near the summits of the granite hills. Mosquitoes were identified

the following day, pooled according to species, and stored in liquid nitrogen. After processing in the laboratory, mosquito suspensions were inoculated intracerebrally into 1–2-day-old suckling mice. Sera collected from patients during the acute phase of the illness were also inoculated into suckling mice in an attempt to isolate virus. All strains of virus isolated were identified in micro-complement fixation tests, and a few also in neutralization tests conducted in suckling mice inoculated intracerebrally.

Antibody determinations on sera were made in micro-haemagglutination inhibition (HI) tests, using 2-fold dilutions (1/10–1/2560) of serum against 8 units of antigen. Antigens of all arboviruses known to infect man in Southern Africa were used and among the Alphaviruses they included the H817 strain (isolated in Rhodesia in 1962) of CHIK virus, the Angmom strain of o'nyong-nyong virus, the prototype strain of Semliki Forest virus, and the AR86 strain of Sindbis virus. All sera were also tested in neutralization tests in suckling mice against H817 strain.

In the mosquito collections in 1977, intended to determine feeding rates of *Ae. furcifer/taylori* on monkeys and baboons, four methods were used:

1) Mosquitoes were collected on the ground off human bait in test-tubes as they alighted on the bait;
2) as in (1) by a single operator as they alighted on an anaesthetized baboon lying on the ground;
3) in battery-operated suction traps (portable light-trap minus light) placed 9 cm below a 5-cm wire mesh platform suspended 1 m above ground-level and on which was secured an anaesthetized baboon or vervet monkey;
4) as in (3) except that the platform was suspended at a height of 10 m in the understorey of a tree.

In the (3) and (4) series, collections at the two levels were identical in respect of bait species and setting-time at any one site. All these collections took place at one of the localities on Hope where infected *Ae. furcifer/taylori* had been collected in 1976, and occurred between 18h00 and 20h00, sunset being at about 18h00.

Results

Human infection

Strains of virus, all subsequently identified as CHIK, were isolated from the serum of 8 patients, who had been bled between 10 March and 15 April, 1976. As far as is known all of them had been in rural areas in the vicinity of the towns of Phalaborwa, Hoedspruit and Gravelotte, two resided on Hope, and two on Lillie. In two donors a convalescent-phase blood specimen was obtained and in both the isolation was confirmed by demonstration of sero-conversion. A serological diagnosis was made on a further 19 humans, all from the same area, who had recently been ill. While o'nyong-nyong infection could not be excluded serologically in these cases, HI titres being high and usually the same against this virus and CHIK, all isolates, even as wild type virus from man and mosquito, were typically CHIK being highly lethal to suckling mice. All those tested were strongly neutralized by H817 antiserum.

Mosquito infection

The numbers of the various species collected on Hope and Lillie during the epidemic and tested for virus are shown in Table 1. The collections consisted largely of the *Ae. furcifer/taylori* group, which group yielded all 16 isolations of CHIK obtained. Infection rates in the group were high: 1 infected mosquito per 34 tested. Infected mosquitoes were present on both farms. As often occurs with the *Ae. furcifer/taylori* group, a considerable proportion of males were also collected off the baits. All were identified, 47 being *Ae. furcifer* and 3 *Ae. taylori*. A somewhat similar proportion was maintained in all subsequent collections from which it seems justifiable to conclude that *Ae. furcifer* comprised the majority of the females collected and it is probable that this was the species largely involved although *Ae. taylori* cannot be excluded from a minor subsidiary role.

Table 1. Numbers of various mosquito species collected on Hope and Lillie during April, 1976, with the number of isolations of CHIK virus

Species	No. mosq.	No. pools	CHIK isolat.
Ae. (Dic.) furcifer/taylori	538	55	16
Ae. (Dic.) adersi	1	1	
Ae. (Stg.) aegypti	22	1	
Ae. (Stg.) vittatus	4	1	
Ae. (Stg.) metallicus	4	1	
Ae. (Adm.) cumminsii	1	1	
Cx. (Cux.) poicilipes	1	1	
Cx. (Cux.) nebulosus	1	1	
Cx. (Cux.) pipiens	1	1	
An. (Ano.) coustani	3	1	
An. (Cel.) rufipes	1	1	
An. (Cel.) pretoriensis	3	1	
An. (Cel.) gambiae complex	6	1	
An. (Cel.) funestus group	5	1	
An. (Cel.) squamosus	2	1	

Ae. = Aedes, Cx = Culex, An. = Anopheles, Dic = Diceromyia, Stg = Stegomyia, Adm. = Aedimorphus, Cux. = Culex, Ano. = Anopheles, Cel. = Cellia.

Post-epidemic immunity in man and baboons

Table 2 shows the post-epidemic immune rates among humans and baboons on Lillie and Hope as well as other farms in the area. Immune humans, including children, with high HI antibody titres indicative of recent infection, were present on all farms, the rates on Hope and Lillie being higher than the other farms. Among the baboons, while the sample of 15 baboons from one farm was negative, animals with high HI antibody titres were present on each of the remaining farms. In view of the high human immune rate on Hope and Lillie, it is noteworthy that all 92 baboons sampled on these two farms were immune.

Habits of Ae. furcifer/taylori

The results of the collections of the *Ae. furcifer/taylori* group with various kinds of bait are presented in Table 3. Significant numbers of the group were attracted to all three species of bait, and far exceeded all other mosquito species combined. A total of 1451 females of the group was collected in these and a few other baited collections made on Hope compared to only 351 of 16 other mosquito species. It would seem, therefore, that, at least at

Table 2. CHIK immune rates in man and baboons at various localities in epidemic area

Locality	Immune rates (%)		
	Man		Baboon.
	Juvenile	All ages	All ages
Grietjie		40 (5)[a]	
Môrelag			0 (15)
Lillie	100 (9)	100 (19)	100 (21)
Hope	56 (9)	87 (46)	100 (71)
Marble Bath	25 (4)	19 (21)	
Oxford	33 (12)	38 (34)	
Cambridge			18(16)
	54 (34)	57 (125)	70 (123)

[a] Figure in parenthesis is the total number tested.

Table 3. Numbers of females of the *Ae. furcifer/taylori* group and males of both species attracted to man, monkey and baboon at two horizontal levels

	Number of mosquitoes by collecting method						
Method	Hand	Hand	Suction	Suction	Suction	Suction	Suction
Bait	Man	Baboon	Monkey	Monkey	Baboon	Baboon	None
Level	G	G	G	U	G	U	G
Ae. furcifer/taylori ♀	212	159	27	206	38	410	2
Ae. furcifer ♂	12		42	443	37	740	9
Ae. taylori ♂				11		28	
Total trap/hours	93.5	5.5	8.0	8.0	8.0	8.0	14.3
Number females per trap/hour	2.3	28.9	3.4	25.8	4.8	51.0	<0.1

G = ground level. U = 10 m above ground in understorey of tree.

certain periods of the year, a large majority of the mosquitoes biting man and wild primates in this area during the two hours after sunset would consist of members of this group.

As shown in Table 3, the males of the group collected were predominently *Ae. furcifer*. That such large numbers of males were collected suggests that mating in these species is related temporally and spatially to blood-feeding. The collections by hand off human and baboon bait on the ground indicated that the latter is much the preferred host, but nevertheless significant numbers are attracted to man. Their attraction for man is more evident from collections not shown in Table 3. For example, during the epidemic, hourly alighting rates per man varied from 2 to 17 mosquitoes, and in February, 1977, on Hope, on two nights when climatic conditions were favourable and mosquito numbers high, rates of 55 and 120 were recorded.

In the suction-trap series with wild primate bait there was a somewhat greater preference evident for baboon over monkey and, with regard to activity at two horizontal levels, an 8 to 10-fold greater number was collected in the understorey. In the series in which the group was collected by hand off baboons, it was observed that mosquitoes of the group showed a strong preference for alighting on the hairless or relatively hairless parts of the body, such as the muzzle, lips, feet, around the eyes and anal regions. It was also noticed during collections made at the entrance to caves and holes on the granite outcrops that relatively large numbers of the group were collected, which suggests that they used these places as resting sites.

Discussion

From the high infection rate demonstrated among the *Ae. furcifer/taylori* group on Hope and Lillie during the outbreak, its prevalence and feeding habits, it is reasonably certain that the main epidemic vector was a species of this group. From the large numbers of males of *Ae. furcifer* collected and the known vector capability of this species, the indications are that *Ae. furcifer* was the species involved in this particular outbreak. The observations on relative attraction to various baits showed that this species is strongly attracted to baboons and, although feeding mainly at high horizontal levels, sufficient feed on the ground to infect man readily should he be present in the sylvatic environment where *Ae. furcifer* occurs. These conditions were fulfilled on Hope and Lillie and offer an explanation for the high human infection rate on these farms.

Previous collections at various localities in South Africa and Rhodesia have indicated that *Ae. furcifer* and *Ae. taylori* co-exist over large areas of the wooded savanna of Southern Africa, the habitat to which CHIK virus seems best adapted, and it seems likely that both species, should *Ae. taylori* also possess the required vector capability, could be the main vectors in rural epidemics over this entire region.

From the observations reported here and the fact that they develop high levels of viraemia, it seems probable that baboons were the primary vertebrate host during the epidemic and human infection was largely the outcome of massive feedback of virus from viraemic baboons to *Ae. furcifer*. In this regard it is significant that human infection was high on Hope and Lillie, where baboon immunity was also high, and that most human infection was apparently contracted in a rural environment. While there is no evidence to show that baboons or monkeys are essential for the maintenance of the virus, there is little doubt that these species and perhaps other wild primates are a dominant factor in the ecology of the virus and in the epidemiology of the disease in Africa.

Our observations indicate that certain habitats favour CHIK virus transmission through their influence on the prevalence of wild primates and the *Ae. furcifer/taylori* group. The wooded savanna would seem to be an essential feature of tree-hole breeding vectors and within this macrohabitat certain microhabitats may be discerned. The large tree, particularly that which dominates the flora of the narrow strip of riverine bush, is one such microhabitat, since it provides suitable primate dormitory sites on an abundant scale and is a relatively prolific source of larval habitats. Another, particularly evident on Hope and Lillie, is the high granite outcrop. This provides a safe overnight refuge for baboons and, within its numerous caves and smaller holes, a relatively humid resting site for adult mosquitoes, thereby enhancing their longevity and increasing the chances of successful transmission. The riverine habitat is also particularly attractive to man, for purposes of camping and recreation.

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2.3

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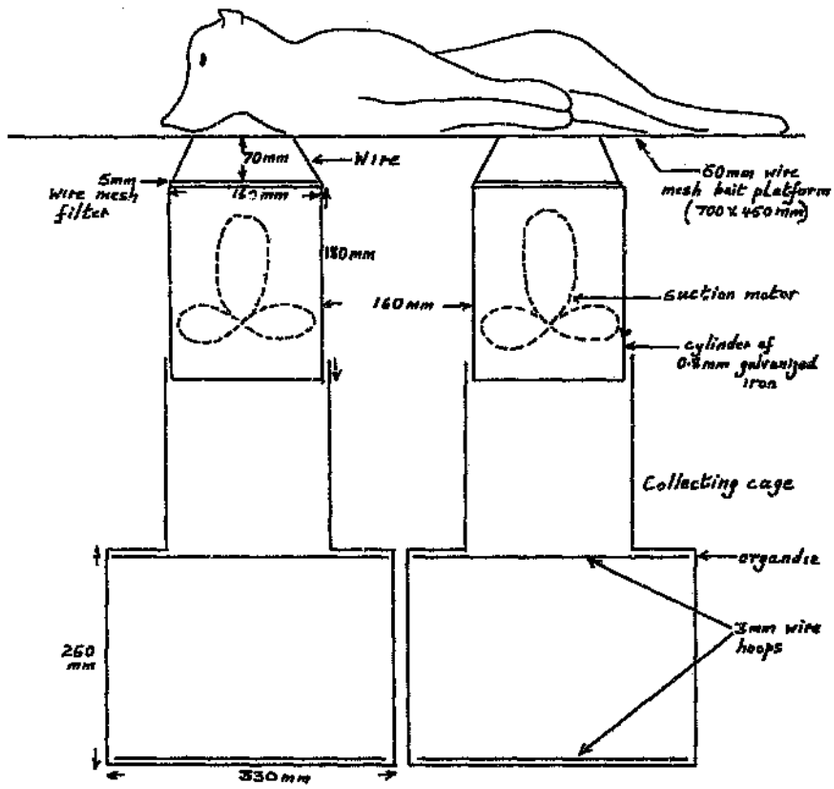
A TRAP TO COLLECT MOSQUITOES ATTRACTED TO MONKEYS AND BABOONS

P. G. JUPP

Arbovirus Research Unit,
National Institute for Virology,
Private Bag X4,
Sandringham,
Johannesburg, South Africa 2131

Previously diffi- · has been experienced in South Africa i uately sampling those mosquito species ing on vervet monkeys at localities where the epidemiology of Chikungunya (CHIK) virus has been investigated. During studies in gallery forest along the Usuzu river near Ndumu in the coastal plane of Northern Natal (McIntosh et al. 1972) some mosquito species failed to enter monkey-baited "lard-can" type traps in large numbers, notably the *Aedes (Diceromyia) furciferitaylori* group of which *Ae. furcifer* is an important vector of CHIK virus. This group of mosquitoes was, however, readily attracted to man on a tree platform 12 m above the ground. For this reason when a recent epidemic of CHIK virus (McIntosh et al. 1977) made another study of the primate-feeding habits of the *Ae. furcifer/taylori* group necessary, a different type of trap was employed baited with either a baboon or a monkey. This new trap proved most efficient for sampling this group of mosquitoes at a farm near Mica in the epidemic area which lies in the wooded savanna region of the north-eastern Transvaal. A description of the trap is given below.

Fig. 1 shows how the trap is constructed and



set up. A young baboon (*Papio ursinus* (Kerr) or vervet monkey (*Cercopithecus aethiops* (Cuvier)) is anaesthetized by intramuscular injection with "Sernylan" (phencyclidine hydrochloride) and roped to the bait platform which consists of a piece of 50 mm wire mesh. This platform can either be supported by poles of electric conduit tubing 1 m above ground level or is suspended on a rope at a height of 10 m or more in the tree understorey. Two rubber-bladed suction fans housed in galvanized iron cylinders, painted black outside, are suspended from the bait platform as shown so that the openings of the cylinders through their 5 mm wire mesh filters are about 70 mm away. Eight-watt "autofan" motors operated by a 12 volt car battery are used in the suction units. Insects are sucked downwards into 2 organdy collecting cages as they come near the bait to feed.

In the eastern Transvaal this collecting method was employed during the 2 hr after sunset, the bait animals usually remaining asleep over this period, although occasionally a 2nd injection of anaesthetic became necessary. Large numbers of female and male mosquitoes of the *Ae. furciferltaylori* group were collected at 2 levels, particularly by traps in the understorey. Identification of the males from their genitalia showed that they were mainly *Ae. furcifer*. Unbaited control traps set 1 m from the ground gave almost negative catches. Fair numbers of Phlebotomines and *Culicoides* species were also sampled in the traps baited with vervet monkeys.

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ATTEMPTS TO TRANSMIT CHIKUNGUNYA VIRUS WITH SIX SPECIES OF MOSQUITO[1]

By B. M. McIntosh and P. G. Jupp[2]

Abstract: Females of *Culex univittatus, Culex poicilipes, Aedes circumluteolus, Aedes calceatus, Aedes simpsoni* and *Aedes aegypti*, originating from populations in southern Africa, were tested for infection rates and ability to transmit chikungunya virus between vervet monkeys, *Cercopithecus aethiops*. The first 5 species failed to transmit the virus and, with the exception of *Ae. simpsoni*, infection rates were low. With *Ae. aegypti* 3.0 logs of virus failed to infect whereas at 5.3 logs 74–92% were infected. After 21 days the transmission rate with this species was 78% and after 49 days 12%. Despite the lower transmission rate there was no definite evidence that the level of virus in the mosquito had decreased at the latter time.

In attempts to identify potential vectors of chikungunya (CHIK) virus in the tropical and subtropical savannah of southern Africa various mosquito species have been tested by us in transmission experiments. Species from the fauna of this region were selected on the basis of their implication as likely vectors on epidemiological grounds, largely on account of their distribution, prevalence and host preference. Some of the species dealt with in the present paper have been reported on in a previous publication dealing with the transmission of CHIK virus (McIntosh et al. 1963). Further attempts to transmit this virus by these species were made as additional and more precise information on their vectorial capacity seemed necessary.

In the methods used we have attempted to duplicate as nearly as possible conditions likely to exist in the particular region of Africa concerned. With this in mind the vervet monkey, *Cercopithecus aethiops*, which is frequently infected in southern Africa, has been used as the source of the infective feed as well as the recipient host in attempted transmissions. For the same reason the mosquitoes used have been collected at localities in southern Africa, and colonies with a lengthy history of laboratory maintenance have been avoided. In all species, except *Ae. aegypti*, first-generation females reared from feral eggs or blooded females were used.

Mosquitoes

Culex univittatus Theobald: Reared from blooded females collected in a fowl-baited trap set at Ndumu in the coastal plain of northern Natal, South Africa. It has been shown by one of us (P.G.J., unpubl.) that *C. univittatus* in southern Africa consists of 2 different allopatric forms both deserving specific rank. The mosquitoes we used are known to us as the "coastal" form to distinguish them from the "highveld" or type form occurring in the inland plateau region where CHIK virus does not occur.

Culex poicilipes (Theobald): Reared from females collected off human bait near Salisbury, Rhodesia.

Aedes circumluteolus (Theobald): Reared from blooded females collected off vegetation at Ndumu.

Aedes calceatus Edwards: Collected as eggs laid in bamboo pots hung in trees at Bushbuckridge, eastern Transvaal, South Africa.

Aedes simpsoni (Theobald): Collected in the same manner and locality as the previous species.

Aedes aegypti (L.): Females from a laboratory colony in its ± 25th generation originating from Ndumu and at nearby Maputa in 1966. Although they are of the dark "formosus" form which is the common form present in southern Africa there are pale scales on the first tergite. The colony originated from eggs collected in bamboo pots and were presumably from a feral population.

METHODS

To provide infective meals vervet monkeys were inoculated intramuscularly with the H817 strain of CHIK virus in its 2nd intracerebral mouse passage. When viraemic, 48 hr later, the monkeys were anaesthetized and exposed to mosquitoes held in a small canister with gauze end strapped to the monkey's flank. Feeding was allowed to take place in a darkened insectary for 1 to 2 hr. Immediately before exposure a blood sample was collected to determine the concentration of virus present. Titrations of virus were done in infant mice inoculated intracerebrally and titers given refer to 0.02 ml of mouse inoculum.

Mosquitoes were encouraged to feed by denial of sugar water for 10 or 24 hr prior to the infective feed. Blooded mosquitoes from each individual monkey were kept separate and held at 25–26°C and 80% relative humidity.

Transmissions were usually attempted on the 13th

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[2]Arbovirus Research Unit, South African Institute for Medical Research, Johannesburg.

to 21st days after the infective feed by feeding "infected" mosquitoes on known susceptible vervet monkeys, exposing single monkeys to many mosquitoes. In one experiment with *Ae. aegypti*, 2 series of transmissions were attempted (on the 21st and 49th days) and in both series single monkeys were exposed to individual mosquitoes. In these attempts a transmission rate, indicating the proportion or percentage of infected mosquitoes which successfully transmitted, is given. Successful transmission was determined by testing monkeys for viraemia 48 hr after mosquitoes fed and/or for antibody against CHIK virus 2 weeks later.

Infection rates in mosquitoes were determined by testing mosquitoes individually for virus by inoculation of a group of 12 infant mice. For this purpose mosquitoes were usually killed 48 hr after attempted transmission and stored on solid carbon dioxide until tested for virus. In experiments in which the number of mosquitoes was small, infectivity tests were also made on some of those which had died before the transmission attempt was made. In these cases mosquitoes were not tested before the 8th day after the infective meal. In groups of mice in which there was any suspicion that mortality might not have been due to CHIK virus, brains of sick or dead mice were tested for the presence of this virus with the complement-fixation test.

RESULTS

In TABLE 1 the results are shown of 2 experiments with each of 5 species of mosquito. Firstly, it can be seen that none of the species successfully transmitted the virus despite the ingestion of reasonably high concentrations of virus in all experiments. *C. univittatus* appeared as the least susceptible species since none of 26 mosquitoes tested for virus was infected. *C. poicilipes*, *Ae. circumluteolus* and *Ae. calceatus* appeared to have somewhat the same degree of susceptibility since all showed low infection rates of between 4 to 10%. A single infected *Ae. circumluteolus* and 2 *Ae. calceatus* engorged during the transmission attempt. *Ae. simpsoni* proved highly susceptible to infection with a rate of 72% with a viral dose of 3.5 logs, the lowest dose recorded of all experiments shown in TABLE 1. In the transmission attempts with this species, in which 4 monkeys were used, a total of 12 infected mosquitoes engorged.

TABLE 2 shows the results of 4 experiments with *Ae. aegypti*. In the first, 3.0 logs of virus failed to infect the 25 mosquitoes that were tested for virus. In the unsuccessful transmission attempt 47 mosquitoes engorged which included the 25 known to be uninfected and another 22 which were not tested for virus. In experiment 2 and 3 a viral dose of 5.3 logs infected 74 and 92% of mosquitoes respectively. In both experiments each group of 14 and 22 infected mosquitoes engorging successfully transmitted the virus.

In experiment 4 infection tests and transmission attempts were made twice, at approximately 3 and 7 weeks after the infective meal. Mosquitoes tested for virus on the 23rd and between the 48th–52nd days gave infection rates of 79 and 69% respectively. These differences are not statistically significant ($P>.05$) and titration of individual mosquitoes at these 2 times still revealed high concentrations of virus on the 48th–52nd days, although one mosquito at this time did have a virus titer of only 2.8 logs, much lower than any other mosquito tested.

In the transmission attempts on the 21st day, 7 out of 9 infected mosquitoes feeding singly on

TABLE 1. Vectorial capacity of 5 species of mosquito with CHIK virus.

Species	Expt.	Infect. titer	Mosquito infection			Transmission attempts			
			Day tested	Rate	%	Day tested	Number mosq. feeding: Uninfected	Number mosq. feeding: Infected	Results
C. poicilipes	1	5.0*	15–20	0/14		18	2	0	Neg.
	2	5.6	8–20	1/10	10	18	2	0	Neg.
C. univittatus	1	4.5	21	0/13		20	5	0	Neg.
	2	4.9	21	0/13		20	5	0	Neg.
Ae. circumluteolus	1	5.0	20–21	0/13		19	9	0	Neg.
	2	5.6	11–21	1/12	8	19	3	1	Neg.
Ae. calceatus	1	4.0	22–23	2/24	8	21	19	2	Neg.
	2	5.4	21–23	1/25	4	21	7	0	Neg.
Ae. simpsoni	1	3.5	18–20	18/25	72	18	3	3	Neg.
						18	0	3	Neg.
	2	5.5	19–21	24/25	96	21	0	2	Neg.
						21	0	4	Neg.

*Expressed as $\log_{10}$ per 0.02 ml of mouse inoculum.

TABLE 2. Vectorial capacity of *Ae. aegypti* with CHIK virus.

		Mosquito infection				Transmission attempt						
							Number mosquitoes feeding					
Expt.	Infect. titer	Day tested	Rate	%	Titer*	Day tested	Total	Uninfected	Infected	Result	Rate	%
1	3.0	21	0/25			19	47	25	?	Neg.		
2	5.3	21	14/19	74		19	19	5	14	Pos.		
3	5.3	21	22/24	92		[illegible]	24	2	22	Pos.		
4	6.5	23	30/38	79	5.5, 5.8, 6.1	[illegible]	13	4	9	Pos.	7/9	78
		48–52	20/29	69	2.8, 5.0, 5.1, 5.2, 5.5, 5.7	49	12	4	8	Pos.	1/8	12

*Of selected individual mosquitoes.

individual monkeys transmitted the virus. On the 49th day only one out of 8 infected mosquitoes successfully transmitted. These figures reveal a statistically lower ($P<.05$) transmission rate on the 49th day as compared to the 21st day.

DISCUSSION

Because of low infection rates despite reasonably high viral infecting doses, it would seem that *C. univittatus*, *C. poicilipes*, *Ae. circumluteolus* and *Ae. calceatus* could not be vectors of epidemiological importance of CHIK virus. Although *Ae. simpsoni* was readily infected it would seem that it is a non-vector because of its apparent inability to transmit the virus. Failure to transmit also occurred in earlier attempts with this mosquito (McIntosh et al. 1963). All 5 species were strongly under suspicion as vectors on epidemiological grounds and one species, *Ae. calceatus*, had previously transmitted the virus (McIntosh et al. 1963), but it seems that all should be exonerated from any major role as vectors of CHIK virus.

However, caution is indicated because of our ignorance of the natural transmission cycles, and it is not known to what extent poor vectorial capacity can be compensated for by high vector populations. There may be unknown wild vertebrate hosts which circulate the virus at higher concentrations than vervet monkeys. Nevertheless, in so far as this monkey is concerned, it hardly seems likely that these 5 species could be involved on any significant scale.

Since *Ae. aegypti* has been an important vector during epidemics the result of laboratory transmission experiments are of interest from one aspect in that they serve to provide some standard by which the requirements and attributes of an efficient vector can be assessed. Compared to the other 5 species it is apparent that *Ae. aegypti* readily transmits the virus although, rather surprisingly, a fairly high level of viraemia seems necessary to infect. *Ae. simpsoni*, an apparent non-vector, seems in fact to become infected more readily than *Ae. aegypti*. Our failure to infect *Ae. aegypti* with 3.0 logs/0.02 ml agrees well with the figure of slightly less than 4.3 logs/1.0 ml obtained by Paul & Singh (1968). It seems that the "formosus" form in southern Africa has a vectorial capacity with CHIK virus apparently equivalent to populations of this species in other parts of the world (Gilotra & Shah 1967).

A question that arises in experiments on vectorial capacity is how long after the infective meal should transmission be attempted. It is often not practical, particularly with non-colonized species, to make attempts repeatedly at various periods and it seems that to make valid comparisons between different species there is some justification for using a uniform period. In our experiments transmissions were attempted between the 18th and 21st days, but it could be argued that mosquitoes which failed to transmit at these times might have done so earlier as there is evidence that vectorial capacity decreases after long periods (Chamberlain et al. 1954, Chamberlain & Sudia 1955) and in our own experiments with *Ae. aegypti* diminished transmitting ability was evident on the 49th day.

However, Gilotra & Shah (1967) showed a constant transmission rate of CHIK virus for at least 14 days among several species of mosquito and Chamberlain et al. (1954) showed that transmission of western equine encephalitis virus by *Ae. aegypti* remained at peak rates for 27 days before a decline occurred. In our experiments also there was a high transmission rate by *Ae. aegypti* on the 21st day so that there is little to indicate that the period used has penalized the species tested.

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Scientific Note

The laboratory colonization of *Culex (Culex) theileri* Theobald and *Aedes (Diceromyia) furcifer* (Edwards) (Diptera: Culicidae) *

by

P. G. JUPP

Arbovirus Research Unit, South African Institute for Medical Research, Johannesburg

The methods are briefly described, with notes on the early history of the colonies. *Culex theileri* mated only when illumination simulating the diurnal cycle was provided.

Culex theileri Theobald

Previous attempts to colonize this mosquito were unsuccessful owing to the absence of mating. However, mating occurred when a special artificial lighting arrangement giving simulated dawn, day and dusk periods was used (Jupp & Brown, 1967).

The methods used to establish and maintain the colony were similar to those previously described for *Culex univittatus* (Jupp & Brown, 1967), except that the initial use of a "walk-in" cage was unnecessary. Starting material was a collection of gravid females from Lake Chrissie in the eastern Transvaal highveld from which egg rafts were obtained. The mosquitoes reared from these were housed in cages 35 cm square which were illuminated as just described. Viability indices for the F_1–F_4 rafts were 26, 60, 36 and 38% respectively. Although these indices are rather low for mosquito colonies, all stages of the mosquito were robust, with very little mortality, and it is expected that mating will take place more readily after further conditioning and selection in the laboratory.

The F_3 adults were observed during the dusk period. The males did not swarm. Hovering near the side of the cage, mainly by males, increased as the light dimmed. Males copulated with females that were resting on the sides of the cage; sometimes a male disengaged while the female was still stationary, while at other times the pair flew *in copulo* to the bottom of the cage before disengaging. The frequency of mating increased as the light intensity decreased.

A variety of animals were used as the source of the blood-meal, including infant and adult mice, chickens and sheep. The mosquitoes were starved for 24 hours

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before a blood-meal, which was offered over part or all of the night period. When fed on a sheep the mosquitoes were transferred to smaller cages which were strapped to the animal's shaven side.

The laying tubes described for *C. univittatus* (Jupp & Brown, 1967) were used for oviposition with two modifications: an infusion was substituted for tap water and sugar-water pledgets were not provided. The infusion, prepared by soaking dried green kikuyu grass (*Pennisetum clandestinum*) in water for 5 days and filtering this mixture through organdie, stimulated oviposition. Oviposition, which occurred in the dark, was inhibited if the mosquitoes imbibed sugar solution, and also if beakers of infusion were placed in the cages instead of confining the mosquitoes in laying tubes. Upon hatching, which took place 1–2 days after oviposition, approximately 200 newly hatched larvae were placed in each of a series of white enamel trays with internal measurements 86 × 24 × 5 cm. The larval cultures were treated in the manner already reported for *C. univittatus*, except that dried green kikuyu grass was added to the tap water. The duration of the larval stages, at a water temperature of 24–25°C, varied from 7 to 23 days and averaged 14 days. The pupal period usually lasted 2 days.

Aedes furcifer (Edwards)

This colony was started with gravid females collected near Newington in the eastern Transvaal lowveld. Eggs were obtained by confining these mosquitoes individually in laying tubes containing a layer of moist cotton wool about 1 cm thick at the bottom covered with a disc of filter paper. The usual strip of filter paper was provided down the side of the tube. These eggs were stored in an insectary at a temperature of 25–26°C and a relative humidity of 75–80% for 5 months. They were then placed in white enamel trays containing water to which some dried kikuyu grass was subsequently added. Treated in this manner the eggs hatched from the 2nd to the 8th day after submersion. Larvae were reared as just described for *C. theileri*. Adults were housed in 35 cm square cages which were exposed to a 12-h day period of artificial light and a 12-h night period, the dawn and twilight periods being omitted. The identity of all the male mosquitoes was confirmed by examination of their terminalia to ensure that the original collection of *A. furcifer* had been homogeneous, and not mixed with *Aedes taylori*.

It was learned that the mosquito was stenogamous. Mating readily occurred on the sides of the cage during the day period. Females fed readily, without starvation, when a human hand was placed inside the cage during the light period. Monkeys and guinea pigs also proved suitable hosts. Gravid mosquitoes failed to oviposit on moist cotton wool provided in petri dish lids placed within the cage, but most did so within 24 h when confined individually in laying tubes.

The F_1 eggs were stored for 1 month before the laying tubes were flooded with an infusion. The latter was made by soaking dry poplar leaves for a week in tap water and then filtering this mixture through organdie. This proved a successful hatching medium, for a large proportion of the eggs hatched within 2 h of flooding. A smaller number of additional eggs hatched over the following 3 days. Egg batches containing viable eggs were produced by 78% of the mosquitoes. After 3 days the eggs were permitted to dry in their tubes and were then stored in the insectary for a second but shorter period (17–23 days) before they were re-flooded. This resulted in the hatching of further eggs in the batches from 71% of the mosquitoes, showing that in most cases not all the eggs laid by a mosquito hatch after the first flooding; in the case of the egg batches

derived from 4 mosquitoes which had completely failed to hatch after the first flooding, some eggs proved viable when flooded a second time.

The newly hatched F_1 larvae were reared in trays containing tap water added to dried poplar leaves; pieces of dead branch were also added in an attempt to simulate the larval environment found in the tree holes utilized by *A. furcifer* in nature. Farex-yeast food powder was also added daily, and the cultures were aerated when this became necessary. In dishes which contained up to 200 larvae the duration of the larval stages at a water temperature of 24–25°C varied from 11 to 30 days and averaged 16 days. The pupal period lasted from 3 to 5 days.

So far the colony has reached the stage where F_4 eggs have been obtained. While mortality of larvae and pupae has been negligible, adult mortality was high after oviposition in the laying tubes.

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Laboratory vector studies on six mosquito and one tick species with chikungunya virus

P. G. Jupp, B. M. McIntosh, I. dos Santos

Arbovirus Unit, National Institute for Virology, Private Bag X4, Sandringham 2131, South Africa

and

P. de Moor

Marianhill Hospital, Pinetown 2260, South Africa

Summary

The tick *Ornithodoros savignyi* and the mosquitoes *Culex horridus, Culex quinquefasciatus, Aedes fulgens, Ae. furcifer* and *Mansonia africana* were tested for infection rates and ability to transmit chikungunya virus. *O. savignyi* and *Cx quinquefasciatus* did not become infected and *Cx quinquefasciatus* failed to transmit the virus between vervet monkeys, *Cercopithecus aethiops*. Only one of 17 *Cx horridus* feeding on a blood-virus mixture became infected which included infection of the salivary glands. *Ae. fulgens* had a high infection rate and transmitted the virus between *Mystromys albicaudatus* rodents. *Ae. furcifer* and *Ma. africana* both transmitted virus between vervet monkeys: the 50% infection threshold and the transmission rate were $< 4{\cdot}5$ logs and 25% respectively for *Ae. furcifer* and *c.* 5·5 logs and 29% for *Ma. africana*. In a further test, *Ae. furcifer* transmitted virus from a monkey to hamsters at a transmission rate of 32%. Attempts to demonstrate transovarial transmission of the virus in *Ae. aegypti* and *Ae. furcifer* were unsuccessful.

It is concluded that *Ae. furcifer* is fitted for its suspected role as epidemic vector and that *Ma. africana* could also act as an important epidemic vector in southern Africa.

Introduction

Our Unit has an ongoing programme of transmission experiments to investigate the vector capability of selected mosquito species and other arthropods with chikungunya (CHIK) virus, which is endemic in the tropical and subtropical wooded savanna of southern Africa. The present paper deals with one argassid tick species and six species of mosquito, four of which have been reported on previously in studies concerning the transmission of CHIK virus (McIntosh *et al.*, 1963; Paterson & McIntosh, 1964; McIntosh & Jupp, 1970).

Four of the mosquito species studied, *Aedes (Diceromyia) furcifer* (Edwards), *Ae. (Finlaya) fulgens* (Edwards), *Culex (Eumelanomyia) horridus* Edwards and *Ae. (Stegomyia) aegypti formosus* (Walker) are prevalent on the farm "Hope" in the wooded savanna region of the north-eastern Transvaal near Phalaborwa (unpublished studies) where an epidemic of chikungunya occurred in 1976 (McIntosh *et al.*, 1977) and which led to their choice in the present experiments. *Ae. furcifer* was believed to be the main vector in this epidemic and was probably likewise involved in an epidemic in Rhodesia in 1962 (McIntosh *et al.*, 1964).

Mansonia (Mansonioides) africana (Theobald) was considered as a candidate for vector because it was the most consistently prevalent species feeding on monkeys in the canopy of riverine forest at Nd nu, Natal, where vervet monkeys had become infected with CHIK virus (McIntosh *et al.*, 1972; McIntosh, 1970), and because it was the most common species at Mopeia in Mozambique where high immune rates to CHIK virus were found in human and non-human primates (unpublished studies).

Cx (Culex) quinquefasciatus Say from Wankie, Rhodesia, was investigated since it was the most abundant mosquito during an outbreak of suspected chikungunya there in 1973. This species had also been suspected as a vector in an earlier outbreak in Zambia (Rodger, 1961).

Ornithodoros savignyi Audouin, the sand tampan, was studied to determine whether it, or possibly other argassids, might act as a viral maintenance vector. This seemed necessary since Taufflieb *et al.* (1968) isolated the virus from *O. erraticus sonrai* Sautet & Witkowski collected in a rodent burrow in Senegal.

Materials and Methods

Arthropods

Details of the geographical origin and laboratory history of the mosquito species and the tick species used are included in the tables. While the age of the wild-caught females of *Ma. africana* was unknown, that of the other species varied from one to 19 days. All the experiments with mosquitoes were undertaken in an insectary where these insects were maintained at 75 to 80% relative humidity and 24 to 26°C. The ticks were kept in an unhumidified room at 25 to 26°C but the sand in their rearing bowls was moistened weekly.

Virus

The virus used was the H817 strain of CHIK virus at the second or third mouse-passage level.

Infective feeds

Attempts to infect mosquitoes were made by feeding them on either the vervet monkey (*Cerco-*

pithecus aethiops) or the white-footed rat (*Mystromys albicaudatus*) while these animals were viraemic after inoculation of virus 48 hours (monkeys) or 71 hours (rodents) previously. Monkeys were anaesthetized and exposed to mosquitoes held in 10 cm diameter canisters with mesh-covered ends strapped to the monkey's chest and abdomen, while anaesthetized rodents were placed inside cages of mosquitoes. For feeding ticks, each anaesthetized rodent's abdomen was shaved and two open 2·5 cm diameter Perspex rings were glued to its skin. The ticks were then placed within these rings until they had engorged. Immediately before feeding started a blood sample was collected from each animal to determine its virus content. Titrations of virus were done in infant mice inoculated intracerebrally and titres given refer to 0·02 ml of mouse inoculum.

For one mosquito, *Cx horridus*, it was necessary to use an artificial feeding method because this species would not feed on monkeys or rodents. The mosquitoes were placed singly in test tubes which were then plugged with cotton wool soaked in blood-virus suspension and inverted. The suspension was prepared by mixing virus with defibrinated rabbit's blood and, as a phagostimulant, adding adenosine triphosphate to a 0·001 M concentration.

Infection rates

The infection rate, i.e. the proportion of arthropods feeding which became infected, was determined for each group of mosquitoes or ticks. For this purpose arthropods were tested individually for virus by inoculation of a suspension of the arthropod into infant mice. In cases where mosquitoes had been used in transmission attempts these determinations were done one day after feeding. Hence the "infection status" of all individuals used in transmission attempts was known.

In presenting the infection rates in Tables I and II the data for different groups of mosquitoes were usually pooled if their infecting titres did not differ more than 0·5 logs.

Transmission

Infected mosquitoes were held for 10 to 25 days before transmissions were attempted; the actual time in each case is given in Tables I and II. Ticks were not fed a second time in an attempt to transmit virus. Transmission attempts were undertaken either by feeding single "infected" mosquitoes on each monkey or Syrian hamster or by feeding several mosquitoes on the same vervet monkey or *Mystromys*. The former method permitted a transmission rate to be determined, i.e. the proportion of infected mosquitoes feeding which transmitted virus.

Transmission of virus was determined by testing for haemagglutination-inhibition antibodies in the serum 21 days after the transmission was attempted or, if the infection proved fatal as in *Mystromys*, by testing the livers of dead animals for virus by mouse inoculation. With *Cx horridus* the salivary glands were removed and tested for virus separately from the remainder of the mosquito, because the mosquito failed to feed when transmission to hamsters was attempted.

Transovarial transmission

Adult male and female progeny of female *Ae. aegypti* and *Ae. furcifer* which had fed on viraemic monkeys or rodents were tested for virus by two methods to determine whether virus had been transmitted transovarially. Suspensions of pools of up to 100 adult females or males were (i) inoculated directly into infant mice or (ii) first inoculated intrathoracically into *Ae. aegypti* adult females which were then, after 14 days, inoculated into infant mice. The technique of ROSEN & GUBLER (1974) was used for mosquito inoculation. From each group of 15 to 25 *Ae. aegypti* inoculated a pool of 10 mosquitoes was inoculated into mice.

Results

The results for the infection and transmission tests with *Ae. furcifer* are shown in Table I. Mosquitoes used were either the progeny reared from wild-caught females or they belonged to the early generations of two laboratory colonies as indicated in the table. Six groups of insects which fed on viraemic monkeys circulating 4·5-6·8 logs of virus had high infection rates when tested 8 to 18 days later, the 50% infection threshold being much less than 4·5 logs. From the 10th to the 12th day after the infective feed three attempts were made to transmit the virus, one with mosquitoes from one laboratory colony to hamsters and two with mosquitoes from a second colony to monkeys. In the latter, one of the two tests also included the progeny of wild-caught mosquitoes. The transmission rate to hamsters was 6 out of 19 (32%) and the combined results of the two attempts to transmit to monkeys gave a rate of two out of eight (25%). In the two insect groups which fed on viraemic *Mystromys* the infection rates were only 48 and 59%, indicating a 50% infection threshold of about 6·9 logs—much higher than that obtained using viraemic monkeys.

Table II shows the results of tests done with four further mosquito species and the tick species. *Ae. fulgens* was highly susceptible to infection and a successful transmission occurred when three infected mosquitoes fed on a single *Mystromys*. Only one individual *Cx horridus* became infected and this had infected salivary glands. None of the *Cx quinquefasciatus* became infected and this mosquito failed to transmit to three monkeys. *Ma. africana* was moderately susceptible to infection (50% infection threshold > 5·2 logs) and two out of seven (29%) mosquitoes infected at 5·2 logs transmitted the virus to monkeys. The nymphs of the tick *Ornithodoros savignyi* failed to become infected after feeding on a viraemic *Mystromys* and most of the nymphs failed to moult and died. Although it seems possible that this may have been associated with the blood meal no explanation can be offered.

As shown in Table III, the adult progeny of three groups each of colony mosquitoes of *Ae. furcifer* and *Ae. aegypti* were tested for virus in an attempt to determine if transovarial transmission of the virus had occurred. Each group of parental mosquitoes had fed on monkeys or rodents with high viraemias and the resulting infection rates in

Table I—Results of infection and transmission tests with *Ae. furcifer*

Mosquito colony No., generation and origin	Infective feed: Viraemic animal	Infective feed: Titre[1]	Mosquito infection: No. days after infective feed mosquito tested	Mosquito infection: Rate[2]	Transmission: No. days after infective feed	Transmission: Rate[3]
A.27; $F_1 + F_2$, Othawa, Transvaal	*Cercopithecus*	6·8	9-14	35/43 (81%)	10	1/5 (20%); monkeys
A.27; progeny[4] + F_1	*Cercopithecus*	6·3	8-13	11/15 (73%)		ND[5]
						2/8 (25%)
A.27; progeny[4] + F_1	*Cercopithecus*	5·8	8-13	22/31 (71%)	10	1/3(33%); monkeys
A.27; F_3	*Cercopithecus*	4·5	9-14	11/15 (73%)		ND
A.47; $F_1 + F_2$ Phalaborwa, Transvaal	*Cercopithecus*	6·4	9-18	34/42 (81%)		ND
A.47; $F_1 - F_4$	*Cercopithecus*	5·7	9-18	47/62 (76%)	11-12	6/19(32%); hamsters
A.47; F_1	*Mystromys*	7·3	21-29	10/17 (59%)		ND
A.47; F_1	*Mystromys*	6·9	21-29	22/46 (48%)		ND

[1] expressed as $\log_{50}$ per 0·02 ml of mouse inoculum
[2] numerator = No. mosquitoes infected, denominator = No. mosquitoes tested
[3] numerator = No. mosquitoes which transmitted virus, denominator = No. infected mosquitoes feeding
[4] progeny reared from wild-caught females
[5] ND = not done

Table II—Results of infection and transmission tests with four species of mosquito and a tick

Species	Origin of arthropods used	Infective feed: Viraemic animal	Infective feed: Titre	Arthropod infection: No. days after infective feed arthropods tested	Arthropod infection: Rate	Transmission: No. days after infective feed	Transmission: Result
Ae. fulgens	Reared from eggs ex Phalaborwa, Transvaal	*Mystromys*	5·7	9-12	29/33 (88%)	10	3 mosquitoes transmitted to one *Mystromys*
Cx horridus	Reared from larvae ex Phalaborwa	Cotton pledgets[1]	5·4	25	0/ 2	25	Salivary glands negative for virus in all 17 except the one infected mosquito
			4·6	14	1/15 } 1/17 (6%)	14	
Cx quinquefasciatus	Progeny of adult females ex Wankie, Rhodesia Zimbabwe	*Cercopithecus*	5·3	20-22	0/19	22	3 groups of 5-9 mosquitoes failed to transmit to 3 monkeys
Ma. africana	Wild-caught females ex Mopeia, Mocambique	*Cercopithecus*	5·2	8-15	16/40 (40%)	14	2 of 7 mosquitoes infected at 5·2 logs transmitted to monkeys
			4·7	8-15	7/27 (26%)	ND	ND
Ornithodoros savignyi	Wild-caught 1st and 2nd nymphal instars ex Bray, Cape Province	*Mystromys*	6·6	50-61	0/11[2]	ND	ND

[1] See "Methods"
[2] A single pool of 18 ticks was also uninfected

Table III—Showing number of F_1 *Ae. furcifer* and *Ae. aegypti*, reared from groups of infected parents, found negative for virus

Species, colony No. and origin	Parental female mosquitoes: Colony generation	Titre of infective feed	Infection rate[1] (%)	No. F_1 progeny tested: No. males	No. females
Ae. furcifer, A.47, Phalaborwa, Transvaal	F_1	6·7—7·3	42—59	76	84
	$F_1 + F_2$	5·7; 6·4	79—81	72	123
	$F_3 + F_4$	5·7	76	452	588
			Total	600	795
Ae. aegypti, A. 49, Glenmore Beach, Natal	Progeny	7·8	100	215	194
	$F_2 + F_3$	7·2	89	568	850
	$F_1 + F_2$	5·9—6·8	88	574	757
			Total	1357	1801

[1] 14 days or more after infective feed

the samples tested varied from 42 to 81% in *Ae. furcifer* and from *88* to *100*% in *Ae. aegypti*. Despite these high infection rates none of the progeny tested, totalling 1395 *Ae. furcifer* and 3158 *Ae. aegypti*, was found to be infected. 52% of the total of 4,547 progeny mosquitoes tested for virus were inoculated into *Ae. aegypti*.

Discussion

The tests indicate that *Ae. furcifer* is a moderately efficient vector of CHIK virus and consequently uphold the earlier findings, based on epidemiological evidence (McIntosh *et al.*, 1964; 1972; 1977) that one or both members of the *Ae. furcifer-taylori* group are the main vectors in human and wild primate infections in southern Africa. These experiments, using two colonies of *Ae. furcifer* (one augmented with the progeny of wild-type mosquitoes) which originated from insects collected at two localities in the eastern Transvaal are in agreement with previous experiments (Patterson & McIntosh, 1964). In this former study, 82% of wild-caught *Ae. furcifer-taylori* (probably mainly *Ae. furcifer*) collected in Rhodesia became infected after feeding on monkeys circulating 6·7-7·2 logs of virus and transmitted virus to monkeys as well as accidentally from monkey to man.

In the present experiments the 50% infection threshold in *Ae. furcifer* was not accurately established. Inexplicably, this appeared to differ depending upon the species of viraemic animal used to infect the mosquitoes but, when monkeys were used, this threshold seemed well below 4·5 logs of virus, a concentration often exceeded in monkeys and baboons which are the suspected wild vertebrate hosts in southern Africa. In the transmission of virus to susceptible hosts *Ae. furcifer* showed a rather low capability but, nevertheless, the rates obtained seem quite adequate for this mosquito to act as a fairly efficient vector. With the one laboratory colony there were two transmissions among eight monkeys and with the other there were six transmissions among 19 hamsters.

The virus failed to infect the tick *O. savignyi* and the mosquitoes *Cx quinquefasciatus* and, with the exception of one individual, *Cx horridus*. Since viral infecting doses were reasonably high it would seem that these mosquitoes and the tick can be excluded as epidemiologically important vectors of CHIK virus. Our failure to infect *O. savignyi* concurs with the observations of Camicas *et al.* (1978) who concluded from an experiment with *O. erraticus sonrai* that this species could not act as a vector. Failure to infect the mosquitoes and transmit the virus also occurred in a previous experiment with *Cx quinquefasciatus* (McIntosh *et al.*, 1963).

From the infection rates obtained in the experiments with *Ma. africana*, a 50% infection threshold of about 5·5 logs seems likely which is higher than for *Ae. furcifer*, although transmission rates in the two species seem the same. However, since monkeys can circulate the virus at levels of up to 6·8 logs (Table I) and baboons up to 6·4 logs (McIntosh *et al.*, 1963), significant numbers of *Ma. africana* could become infected. Furthermore, the frequent prevalence of *Ma. africana*, its occurrence in forest canopy where it may feed on monkeys (McIntosh *et al.*, 1972) and its high feeding rate on man suggest that this species could transmit virus from wild primate to man.

A study of the tree-hole breeding *Aedes* mosquitoes at "Hope" near Phalaborwa indicated that *Ae. fulgens* was a common species in the epidemic area. The single experiment carried out on this species suggests that it is an efficient vector and further tests are needed.

Our failure to demonstrate transovarial transmission in both *Ae. aegypti* and *Ae. furcifer* suggests that such transmission of virus does not occur in these two species.

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2.7

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*[1] 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

[1] 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 8 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_2 was released through 3-mm bore polythene tubing close to the opening (Jupp et al. 1980). The source of the CO_2 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 $\log_{10}LD_{50}$/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[a]			Monkey-baited suction traps 10 m above ground in trees[b]			Light traps with CO_2 at ground level[c]		
	No.	No. as % of total	No./ man-hour	No.	No. as % of total	No./trap hour	No.	No. as % of total	No./trap night
Tree hole *Aedes* species									
Ae. (Dic.) furcifer/cordellieri females[d]	1,968	83.9	40.2	3,995	43.6	16.6[e]	174	4.8	2.8
Ae. (Dic.) furcifer males				4,825	52.6	20.1	265	7.4	4.2
Ae. (Dic.) cordellieri males				49	0.5	0.2			
Ae. (Dic.) adersi							2		
Ae. (Dic.) fascipalpis							11	0.3	0.2
Ae. (Adm.) vittatus	2						31	0.9	0.5
Ae. (Stg.) aegypti	2			2			13	0.4	0.2
Ae. (Stg.) ledgeri							2		
Ae. (Stg.) metallicus	8	0.3	0.2	2			43	1.2	0.7
Ae. (Stg.) unilinectus							2		
Other species									
Cx. (Cux.) poicilipes	28	1.2	0.6	271	3.0	1.1	334	9.3	5.3
Aedes (Adm.) 6 spp.	1						20	0.6	0.3
Aedes (Neom.) 2 spp.							25	0.7	0.4
Anopheles 11 spp.	334	14.2	6.8				2,498	69.6	39.7
Culex 11 spp.	1			12	0.1		149	4.2	2.4
Mansonia 2 spp.	1						9	0.3	0.1
Uranotaenia mashonaensis							12	0.3	0.2
Total	2,345			9,174			3,590		

[a] Mosquitoes collected in 49 man hours over 12 days during 2 summers (March 1977 and February 1978).
[b] Collected in 240.5 trap hours over 36 days in March 1977, February 1978 and January–June 1980.
[c] Collected in light traps baited with CO_2 over 26 nights (63 trap nights) in February–April 1977 and February–June 1980.
[d] Proportion of *Ae. cordellieri* 1.0–3.5% based on males attracted to females in traps.
[e] No./trap hour varied from 0 to 77.5, depending on the month.

significant numbers were *Culex poicilipes* (Theobold) 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* Theobold (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 $\log_{10}LD_{50}$/ml. (*Ae. aegypti*) and < 6.7 $\log_{10}LD_{50}$/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 $\log_{10}LD_{50}$/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)[a]	1977–78 (30)	1980 (49)	1981 (29)	Mean (155)	1980 (134)[b]
Ae. (Adm.) cumminsi	2	0	0	0	1	0
Ae. (Alb.) haworthi	6	10	18	31	16	0
Ae. (Alb.) marshalli	2	10	4	0	4	0
Ae. (Adm.) vexans	6	0	0	0	2	0
Ae. (Adm.) vittatus	15	17	33	45	27	1
Ae. (Dic.) furcifer/cordellieri	4	3	14	48	16	1
Ae. (Fin.) fulgens	64	37	33	62	48	1
Ae. (Fin.) nyasae	0	3	6	0	3	0
Ae. (Stg.) aegypti	98	93	100	90	96	78
Ae. (Stg.) ledgeri	89	97	100	100	96	87
Ae. (Stg.) metallicus	96	67	71	62	75	46
Ae. (Stg.) simpsoni	2	0	0	0	1	0
Ae. (Stg.) subargenteus	4	0	0	0	3	0
Ae. (Stg.) unilineatus	2	0	6	7	4	2
Cx. (Cui.) cinereus	27	ND	3	ND	17	1
Cx. (Cui.) nebulosus	57	ND	14	ND	39	1
Cx. (Eum.) horridus	21	ND	67	ND	40	23
Cx. (Lut.) tigripes	0	ND	2	ND	1	0
Tx. (Tox.) brevipalpis	0	ND	3	ND	4	1

ND = larval collections of these species not done.
[a] No. of pots exposed.
[b] No. of pairs of wooden paddles removed from bottles and larval samples.

Table 3. Number of mosquitoes[a] tested for virus: wild caught as adults and reared from bamboo pots.

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

[a] All mosquitoes were females except where indicated otherwise.
[b] Includes catch in Feb–March after first rains.
[c] Number of collecting days.
[d] 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: Titer of infective feed ($log_{10}LD_{50}$/ml)	Mosquito infection: Rate[a]	Mosquito infection: 50% infection threshold[b] ($log_{10}LD_{50}$/ml)	Transmission to hamsters: Days after infective meal	Transmission to hamsters: No. hamsters exposed	Transmission to hamsters: No. mosquitoes feeding	Transmission to hamsters: Results
Ae. aegypti	7.2	10/16 (63%)	6.7	14	1	16	+
	6.7	6/12 (50%)		14	1	12	+
Ae. ledgeri	7.2	1/27 (4%)	>7.2	14	2[c]	79	+;–
	6.7	1/17 (6%)		14	2[c]	10	+;–
Ae. metallicus	7.2	12/34 (35%)	>7.2	14	2[c]	37	+;–
	6.7	4/22 (18%)		14	2[c]	23	–;–
Ae. vittatus	6.7	10/10	<6.7	13	1	12	+
Ae. fulgens	6.7	4/6 (67%)	<6.7[d]	13	1	4	+

[a] Numerator = no. mosquitoes infected; denominator = no. mosquitoes tested 15 days after their infective meal.

[b] The titer of virus needed to infect 50% of the mosquitoes.

[c] 2 hamsters exposed simultaneously in the same cage of mosquitoes.

[d] 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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Chapter 20

2.8

CHIKUNGUNYA VIRUS DISEASE

P. G. Jupp and B. M. McIntosh

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I. HISTORICAL BACKGROUND

A. Discovery of Agent and Vectors

The word "chikungunya" was first used by the indigenous people of Southern Province, Tanganyika Territory (Tanzania), in reference to a disease which afflicted them in epidemic form in 1952—1953.[1-3] The disease was characterized mainly by a sudden onset, fever, rash, and joint pains. The latter were often severe and sometimes persisted as a recurrent arthralgia for some time. The word is Swahili meaning "that which bends up" and refers to the stooping posture adopted by patients because of the severity of the joint pains. The incidence of the disease was highest on the Makonde Plateau, but it also occurred to a lesser extent in the adjoining lowlands of the Ruvuma River valley. From there it was believed at the time that the disease had been introduced into the highly susceptible plateau population by movement of infected humans and subsequently spread throughout the plateau by the same means. Man-biting *Aedes aegypti*, the suspected vector, was abundant in the villages as were their larvae in water storage jars in the huts. During the outbreak, a previously unknown virus was isolated from humans and mosquitoes, including *Ae. aegypti*,[3,4] and its etiological role confirmed serologically by the demonstration of specific antibodies in recovered patients.[3] In 1956, experimental transmission of the virus by *Ae. aegypti* was reported,[5] and its identification as a group A arbovirus (*Alphavirus*), with a close antigenic relationship to Mayaro and Semliki Forest viruses, was reported in 1957 and 1959.[6,7] The Makonde Plateau is virtually devoid of woodland and, in retrospect, it seems certain that the virus did in fact originate in the lowland woodland and was transmitted on the plateau by a highly efficient domiciliary vector which would explain the high incidence of the disease in the plateau population which was only infrequently exposed to infection, and consequently had only a low immunity.

Studies in Africa have uncovered a sylvan transmission cycle between wild primates and aedine mosquitoes of the *Stegomyia* and *Diceromyia* subgenera in the tree canopy of moist forest and semiarid savannah-woodland. First indications of this were reported from Uganda in 1958 when chikungunya (CHIK) virus was isolated from the primatophilic *Ae. africanus* collected in the forest canopy and from a mosquito catcher who may have been infected in the canopy.[8] Later studies during the 1960s in Uganda further implicated *Ae. africanus* in forest redtail monkey transmission cycles, but also suggested that the virus was not being maintained in the isolated forests around Entebbe with their small monkey populations.[9] In West Africa, the same mosquito, as well as *Ae. luteocephalus* and the *Ae. furcifer/Ae. taylori* species pair, were implicated in both feral and human transmission in the 1960s and 1970s.[10-13] In southern Africa, where the *Ae. furcifer-taylori* pair appears to be the only important rural vector, it was implicated in human and wild primate transmission in Zimbabwe in 1963,[14] and in South Africa in studies during 1964 to 1967[15] and again in 1976.[16] In West Africa, *Ae. aegypti* was implicated as an urban vector during an outbreak in 1969 in Ibadan, Nigeria,[17] and in 1970—1971 in Luanda, Angola.[18] Since 1954, the virus has been identified as the cause of epidemics in the Philippines,[19,20] Thailand,[19,21] Kampuchea,[22]

Table 1
LOCATION AND YEAR OF HUMAN OUTBREAKS OF CHIK DIAGNOSED ON CLINICAL AND/OR LABORATORY BASES

Tanzania (Makonde)	1952—1953[12]
Philippines (Manila, Amlan)	1954, 1956,[19,20] 1968[42]
South Africa (Transvaal Province)	1956,[43] 1975, 1976,[16] 1977[44,45]
Thailand (Bangkok)	1958,[19] 1962, 1963, 1964[21]
Zaire	1958[30]
Zambia	1959[46]
Senegal	1960,[47] 1966,[10] 1982[48]
Kampuchea	1961, 1962[22]
Uganda (Entebbe)	1961—1962,[9] 1968[9]
Zimbabwe	1962,[49] 1971[50]
India (various localities)	1963,[23] 1964,[51] 1965,[25] 1973[52]
Burma	1963,[53] 1970—1972[29]
Nigeria (Ibadan)	1964,[17] 1969,[17] 1974[54]
Vietnam	1964[55]
Sri Lanka	1965[27]
Angola (Luanda)	1970—1971[18]
Central African Republic	1978—1979[13]

India,[23-26] Sri Lanka,[27] Vietnam,[28] and Burma.[29] In at least some of these outbreaks, *Ae. egypti* was implicated as the main vector.

Evidence of infection in wild primates in Africa concerned both the presence of antibodies as well as the isolation of virus from several primate species. Reports on the presence of antibodies emanated from Zaire (1960),[30] Zimbabwe (1964),[14] Nigeria (1968),[31] South Africa (1970, 1977),[16,32] and Uganda (1971).[9] Virus isolations were reported from Senegal in 1969, 1979, and 1984.[12,33,34]

Previous recent reviews on CHIK were published in 1975,[35] 1981,[36-38] and 1984.[39]

B. History of Epidemics

Carey,[40] after study of the historical literature, advanced the thesis that there has been confusion between dengue (DEN) and CHIK, but since the isolation of the causal viruses, it is now possible to differentiate these illnesses on clinical grounds alone. DEN is typically characterized by a diphasic fever lasting about 1 week, headache, retroorbital pain, backache with generalized body pains, and rash, the acute illness being followed by residual asthenia. CHIK differs in that the pains are predominantly located in the joints rather than the muscles; the febrile period is shorter and usually not diphasic; there is an absence of mortality; and some patients experience persistent arthralgia following the acute episode, but no asthenia. Using these clinical differences as a basis, Carey suggested that epidemics of CHIK can be said to have occurred in 1779 (Batavia-Jakarta; Cairo), 1823 (Zanzibar), 1824—1825 (India), 1827—1828 (West Indies; New Orleans; Charleston, S.C.), 1870 (Zanzibar), 1871—1872 (India), 1901—1902 (Hong Kong; Burma; Madras), and 1923 (Calcutta). It was in Cuba in 1828 that the term "dengue" was first used, which is possibly derived from the word "dinga" used in Zanzibar in 1923. Since Mayaro virus is now known to cause an illness very similar to CHIK virus, including residual arthralgia,[41] it seems that the outbreaks in the Western Hemisphere might have been caused by that virus.

Table 1 lists the epidemics due to CHIK which have occurred since the isolation of the prototype CHIK virus in 1952—1953 until the present. CHIK appears to be enzootic throughout much of tropical Africa from where it has apparently spread to other parts of the world; there is historic evidence for this. Such evidence includes the chronology of the outbreaks, their infrequency in Asia and the very high morbidity prevailing there when they did occur,[40] and the failure so far to find evidence of a feral transmission cycle outside of Africa.

II. THE VIRUS

A. Antigenic Relationships

The initial antigenic classification of CHIK virus was based on reactions obtained with hemagglutination-inhibition (HI) and complement-fixation (CF) tests as well as neutralization (N) tests in mice, which demonstrated its relationship to certain group A viruses available at the time, particularly to Mayaro and Semliki Forest viruses.[6,7] These studies were confirmed and extended by others using variations of the N test in cell cultures,[56-60] which gave better definition of antigenic complexes within the genus *Alphavirus*. At present, 6 complexes are recognized among the 25 members of the genus.[60] CHIK virus is one of the four species of the Semliki Forest complex, the others being Semliki Forest, Getah, and Mayaro. CHIK and o'nyong-nyong (ONN) viruses are regarded as subtypes of the CHIK virus species. In the N test, there is a partial one-way relationship between CHIK and ONN with CHIK antisera neutralizing both viruses equally and ONN antisera neutralizing the homologous virus to a significantly greater degree.[41,57]

B. Vertebrate Host Range

In the laboratory, 1- to 4-day-old mice die 2 to 3 days after intracerebral (i.c.) or intraperitoneal (i.p.) inoculation, while 3- to 4-week-old mice usually survive and develop antibodies,[61] although a variant strain of the virus has been developed which is lethal to adult mice by the i.c. route.[61] Guinea pigs, hamsters, and rabbits do not develop viremia after inoculation, but survive with development of antibodies.[61] African rodents of the genera *Mastomys, Arvicanthis,* and *Aethomys* develop a low viremia, while *Mystromys* does so with high-level viremia, and they all develop antibodies.[62] Viremia followed by antibodies develops in rhesus monkeys, vervet monkeys, and baboons (*Macaca radiata, Cercopithecus aethiops,* and *Papio ursinus*) and day-old chickens.[61,63,64] Adult fowls,[65] domestic sparrows, and pigeons[66] are refractory to infection by the virus. One of nine Indian fruit-eating bats, *Rousettus leschenaulti*, inoculated with virus, showed a trace of viremia and eight developed low titer hemagglutinating and/or neutralizing antibodies.[66] Furthermore, two African bats of the genera *Tadarida* and *Pipistrellus* circulated virus after inoculation.[67] Adult cats are refractory to infection, although inoculation of newborn kittens may lead to viremia.[65]

C. Strain Variation

African and Asian strains of CHIK virus differ biologically, viz. differences in plaque size and heat stability, although these differences may possibly be due to the relatively greater number of mouse passages of the African strains.[68] Strains from Calcutta have been shown by the kinetic HI test to be more closely related to a strain from Thailand than to an African strain, but differences are slight and probably of little importance in vaccines.[23,68] The KLA-16 strain of CHIK virus which was recovered from a child with hemorrhagic fever in Thailand[69] differs from other strains by its effects on infant mice, rats, and hamsters. These animals develop a hemorrhagic syndrome, mainly associated with the intestine, and also characterized by thrombocytopenia, prolonged bleeding and clotting times, and a decrease in the time for formation of prothrombin.[69,70] Strains isolated from Calcutta also occasionally produced intestinal hemorrhage in the course of their isolation in mice.[71]

Small and large plaque (SP and LP) variants of CHIK virus have been produced experimentally.[72] These have been compared serologically with ONN virus to elucidate the one-way antigenic relationship between ONN and CHIK viruses.[59] The results suggest that similar antigens are present on both ONN and CHIK variants, but in the latter, the antigens are differently distributed which prevents them from participating in the neutralization of the virus by ONN antibodies. ONN virus could not be distinguished from CHIK SP on the basis of plaque morphology, although it is clearly distinct because of its inability to multiply in

Ae. aegypti cells. CHIK SP virus differs in its greater ability to replicate in *Ae. aegypti* mosquitoes than either CHIK prototype virus of CHIK LP virus. All three viruses had the ability to multiply in *An. gambiae* cells, although ONN was the only virus to infect *An. gambiae* mosquitoes. The similarities between ONN and CHIK viruses might be due to convergent evolution.[59]

D. Methods for Assay

For primary isolation and cultivation of laboratory strains of CHIK virus, both suckling mice and a wide range of vertebrate and mosquito cell lines are available. Cell lines which have been used successfully include VERO,[73] BHK-21,[74] and the C6-36 *Ae. albopictus*[75] and *Toxorhynchites amboiensis*[76] cell lines. Mosquito cells do have a disadvantage because of the absence or irregular production of cytopathology,[77] although this is irrelevant where immunofluorescence is used for the detection of viral antigens.[78] The enzyme-linked immunosorbent assay (ELISA) has not been used yet for detection of CHIK antigen, but it should be applicable for the assay of antigen in sera or in individual mosquitoes as has been done in the case of Rift Valley fever virus.[79] It does seem that cell lines from *Aedes* mosquitoes are more sensitive to CHIK than those from *Culex*.[77] For primary isolation, suckling mice still have certain advantages over cell cultures because of their high susceptibility, the ease by which CF and hemagglutinating antigens can be obtained from their brains, the lower level of technique required in their usage, and the higher tolerance they possess to the toxic effects of inoculating pools consisting of large numbers of mosquitoes. Also, because of their high susceptibility to a wide spectrum of arboviruses, mice are certainly better in infectivity surveys in which as many arboviruses as possible are being investigated. The sensitivity of mice can be increased in primary isolation if the inocula are first inoculated into male *Ae. aegypti* or species of *Toxorhynchites* mosquitoes.[80]

III. DISEASE ASSOCIATIONS

A. Humans

CHIK is a feverish illness characterized by sudden onset, chills, flushed face, nausea, vomiting, backache, headache, photophobia, lymphadenopathy, arthralgia, and rash.[44,55,81-83] The acute illness lasts 3 to 5 days, with recovery in 5 to 7 days. The incubation period is usually about 2 to 4 days and the single most significant symptom is the arthralgia, presenting in 70% of cases. It may be severe, affecting one joint or several. Reddening and swelling of the joint may occur. The arthritis may persist in a small proportion of cases for months or years and mimic rheumatoid arthritis.

Patients with persistent joint pain and stiffness have shown high viral antibody titers. The rash, appearing most commonly on the trunk, is macular or maculopapular, and rarely petechiae may be present. It may be pruritic and occur in short-lived episodes.[43,44,49,55,82] Biopsy specimens from the skin lesions of CHIK patients show a perivascular lymphocytic infiltrate in the upper half of the dermis and red blood cell extravasation is seen around the superficial capillaries.[45] In Asia, comparison of the symptomatology of DEN and CHIK infections indicated that hemorrhagic manifestations rarely occur in cases of CHIK and that CHIK should not be listed as a hemorrhagic fever.[36] Severe hemorrhagic symptoms have not been reported in CHIK cases in Africa. Viruses associated with severe hemorrhagic fever in south and Southeast Asia from 1956 to 1963 were either DEN alone or DEN and CHIK.[84] In Africa, ONN fever has similar symptoms to CHIK, but ONN may be distinguished by the presence of lymphadenitis which is absent in CHIK patients.[85]

B. Domestic Animals and Wildlife

There are no records of clinical disease in domestic animals or wildlife due to CHIK virus.

C. Applicable Diagnostic Procedures

CHIK fever can be confused clinically with ONN, DEN, Sindbis, and West Nile infections, so diagnosis should be confirmed by virus isolation as well as serologically. Virus is most readily isolated from the blood within 48 hr of the onset of illness, but has been isolated as late as 6 days afterwards using infant mice.[71] In Africa, it may be necessary to differentiate CHIK from ONN virus which is difficult to do serologically (see Section II.A). However, the two can be separated because CHIK has a greater pathogenicity for infant mice.[86] Apart from ONN, the HI test will readily differentiate CHIK from other *Alphavirus* infections. In India, the virus was readily isolated and identified in 72 hr using mice and CF or HI tests with antigens prepared from the mice.[24] Diagnosis is thus based on either virus isolation or a fourfold or greater rise in antibody titer following the illness. Usually a serum sample collected within 5 days of onset of fever will be free of HI, CF, and N antibodies.[21,26] A sample collected 2 weeks or more after onset should have HI and low-level N antibodies; CF antibodies develop more slowly.[21,26] N antibodies can be measured by the virus dilution method in infant mice, by the serum dilution method in cell cultures by CPE, or by plaque assay methods. Radial hemolysis has been shown to correlate well with the HI test.[87] Methods for virus isolation are by inoculating acute-phase serum or other tissues i.c. into infant mice[3] or into cell cultures. CHIK virus produces cytopathogy in a variety of vertebrate cell lines, but VERO or BHK-21 cells have been commonly used. For the rapid identification of virus in sera, these may be inoculated into cell cultures and a diagnosis made after 9 hr by the indirect immunofluorescence test. Alternatively, after 24 hr the infected cells are negatively stained and examined with the electron microscope.[78] Indirect immunofluorescence has also been used to distinguish CHIK from the closely related Mayaro and Semliki Forest viruses.[88]

IV. EPIDEMIOLOGY

A. Geographic Distribution

Table 1 lists the countries that have experienced CHIK outbreaks from 1952 onwards. The virus seems to be enzootic throughout tropical Africa. Outbreaks, sometimes major epidemics, have been documented for East Africa (Tanzania and Uganda), southern Africa (northeastern Transvaal and Zimbabwe), West Africa (Senegal, Nigeria, and Angola), and Central Africa (Central African Republic, Zambia, and Zaire). According to antibody surveys, human infection has also occurred in Mozambique, northern Botswana, and northeastern Namibia.[89,90]

CHIK appears to have spread to other parts of the world from Africa to cause pandemics in both the American and Asian tropics.[40] India has had a history of epidemics from 1824 until 1965 when the virus spread to Sri Lanka. CHIK became established endemically in Southeast Asia during the late 1950s to early 1960s and was continuously transmitted in the towns and cities in Thailand, Kampuchea, and Vietnam, probably largely by *Ae. aegypti*. Outbreaks have been recorded in Burma in 1963, 1970, and 1973 and in Manila in 1954, 1956, and 1968. Serological surveys alone have recorded the presence of the virus in South Vietnam (Laos),[91] Vietnam (Saigon),[28] the Malaysian Peninsular,[92] Pakistan,[93] and the Pacific Islands.[94]

B. Incidence and Serologic Epidemiology

In the various outbreaks studied, human infection rates have varied depending upon factors relating to the immune status of the population, the size and density of the population, whether infection was urban or rural, the extent to which human infection was dependent upon the feral transmission cycles, and the efficiency of the particular vector system. In Asia, where most recognized outbreaks have been in large urban populations with trans-

mission effected by *Ae. aegypti*, outbreaks have been on a large scale. In contrast, in Africa they have been smaller, have tended mainly to involve rural populations, transmission has often been by feral vectors and evidently less efficient, and outbreaks have often been conditional upon viral activity in the wild primate transmission cycles.

In India, where infection rates were obviously very high, it was estimated that during the outbreak in Madras in 1964, nearly 400,000 cases occurred.[25] In the town of Barsi in Central India in 1973, the overall morbidity was 37.5%.[52] In Thailand during 1962 to 1964, CHIK was common in Bangkok. During the 1962 outbreak alone it was estimated that between 44,000 and 70,000 cases were seen in outpatient children, and the infection rate, based on 1887 persons bled before and after the 1962 rainy season, was 31%.[95] Immune rates by age in 1962 were 10 to 20% in 1 to 2 year olds, 70% in 15 to 19 year olds, and 70 to 85% in 20 to 70 year olds.[95] Antibody surveys among adults from different regions outside of Bangkok indicated that immune rates were the same as in Bangkok, i.e., 80 to 100%, while conversion rates of 30 to 50% were obtained in 337 school children; it was evident from both studies that infection was widespread in rural Thailand.[96] After the 1964 outbreak in Vietnam, immune rates among 472 children were 33.3% in those less than 2 years old and 81.6% in the 10- to 15-year age group.[28] However, recent evidence indicates that the virus had virtually disappeared from Bangkok by the early 1980s despite the continued presence of DEN and abundance of *Ae. aegypti*. The reason for this decline was not evident.[97]

The 1952 outbreak in Tanzania involved a population of 150,000 living in small villages scattered over the Makonde Plateau, with many villages more than 8 km from water, thus giving rise to a need to store domestic water which led to large numbers of *Ae. aegypti*.[2] Once virus had been introduced, the outbreak spread rapidly to affect eventually most of the villages, The overall morbidity on the plateau was 47.6% among adults and 50.8% among children. *Ae. aegypti* was also the main vector in Ibadan, Nigeria, in 1969[17] and in Luanda, Angola, in 1970.[98] In Luanda, an antibody survey after the outbreak showed that 65% of sera reacted only with CHIK antigen; 33.7% with both CHIK and ONN, but usually to a greater degree with CHIK; and 1.3% with ONN alone. In the Central African Republic in 1978—1979, CHIK virus was active in several areas.[13] An antibody survey at this time revealed an immune rate of 17% suggestive of a poor vector system. Prior to this, virus had not been active since 1975 despite continuous surveillance. Studies in Uganda over several years revealed a very low level of human infection, also apparently the result of an inefficient vector system.[9] A sylvan vector *Ae. africanus* was involved and human infection resulted from incidental leakage of virus from transmission cycles involving monkeys in small isolated forests. It seemed that there was a 5- to 7-year — perhaps even a 10-year — periodicity in monkey epizooticity because of the need to replace immune monkeys by susceptibles. In southern Africa, where human infection has been caused by the sylvan vectors *Ae. furcifer-taylori*, a somewhat similar periodicity in human infection has been evident, although human infection has sometimes been quite intense among small localized groups.[16,49] During an outbreak in South Africa in 1976, postepidemic immune rates in a locality with a large baboon population were 77.7% among 18 children and 55.7% among 106 adults, while all of the 92 baboons tested were immune.[16] Antibody surveys in Mozambique in 1957 showed immune rates in adult humans of 37.5% and in children of 4%,[89] in Angola in 1960 of 32.5% in adults and 3.3% in children;[99] in Botswana in 1959 of 25.3% in adults and 13.4% in children;[90] and in northern Namibia in 1966 of 1%.[100]

C. Seasonal Distribution

Outbreaks of CHIK depend upon sufficient rainfall filling the tree-holes or artificial containers preferred for oviposition by the aedine mosquito vectors, resulting in high densities of the vector species. Sufficient nonimmune humans must be present. Rural outbreaks in Africa are also related to the presence of adequate nonimmune populations of wild primates which in turn will also depend to a degree on the rainfall.

In Africa, the 1962 epidemic in Zimbabwe and the 1976 epidemic in South Africa, both rural outbreaks, occurred following unusually heavy rains in the wooded savannah which caused high densities of *Ae. furcifer-taylori* mosquitoes. Furthermore, the original epidemic in 1952—1953 in Tanzania was associated with the wettest year (1952) for a decade.[2] This heavy rainfall probably increased the numbers of *Ae. furcifer-taylori* in the woodland habitat in the lowlands of the Ruvuma River from where infection was brought into the villages on the Makonde Plateau. In 1975, a rural epizootic of CHIK occurred in western Senegal which peaked in November at the end of the rainy season with the highest number of virus isolations obtained from *Ae. furcifer-taylori* and *Ae. luteocephalus* during that month[12] when mosquito populations were actually declining. The explanation for this unexpected peaking of virus transmission so long after the heavy rainfall in July is unclear.

In urban outbreaks where *Ae. aegypti* is the vector, possibly supplemented by *Ae. albopictus* in Asia, the relationship with rainfall pattern has been recorded for several countries. In the 1969 Ibadan, Nigeria, outbreak, the frequency of infections increased and decreased parallel with the rainfall pattern, but the virus did not subsequently reappear during the late rains in October, probably because of high immunity in the human population by that time.[17] A similar pattern occurred in the 1964 epidemic in Vellore, India.[26] The highest densities of *Ae. aegypti* have occurred in Thailand just after the first rains in May and the epidemic peak has followed 2 months later. CHIK virus is only active in the rainy season in Kampuchea,[22] while in Sri Lanka, maximum infection occurred in June and July 1 to 2 months after the heavy rains in May.[27] In other areas of south and Southeast Asia where rainfall is not markedly seasonal, cases of CHIK may occur throughout the year.[84]

D. Risk Factors

Risk factors are related mainly to the degree of exposure of humans to the mosquito vectors. Age, sex, and occupation are irrelevant except insofar as they may increase this exposure. On the other hand, place of residence or temporary visits to high-risk areas have been of primary importance. Thus, risk factors are largely dictated by ecological factors determining the geographical distribution and abundance of the vectors. Where the vector has been the domestic man-biting *Ae. aegypti*, risk to man has been highest among urban populations, especially those of the lower socioeconomic class, where the container habitat for mosquito larvae is usually most abundant and where, in some rural villages, the need to store water is great. Furthermore, the houses occupied by this class of the population are less likely to be mosquito-proof. This probably accounts for the threefold greater infection rate in the lower compared to the higher socioeconomic class in Mandalay, Burma, shown by the 1973—1974 serological survey.[101] Similarly, in the 1965 outbreak in Colombo, Sri Lanka, the lowest income group had the highest attack rate.[27] In Africa, where proximity to the feral transmission cycles has been of importance, high-risk areas are either forest or riverine and savannah-woodland where the habitats of the sylvan vectors and the wild primate hosts occur. In southern Africa, this effect of the wild transmission cycles has been clearly evident as recognized human infection there has been restricted exclusively to the putative distribution of *Ae. furcifer-taylori*,[14,16] and, consequently, human outbreaks have been confined to the tropical region of southern Africa. This region embraces the extreme north of Namibia and Botswana (Ovamboland and the Okavango), the lowlands of Zimbabwe, the northeastern Transvaal and northern Natal in South Africa, and the Mozambique lowlands.[32] Within this tropical region, human infection has been most intense in habitats chosen by wild primates for their dormitories, i.e., riverine woodland and high granite outcrops.

Table 2
CHIK VIRUS ISOLATIONS FROM *AEDES* IN AFRICA

Country	Year	Species (no. isolations >1)
Tanzania	1953	*aegypti* (2)[2,61]
Uganda	1956	*africanus*[8]
	1961	*africanus* (5)[103]
	1968	*africanus* (9)[9]
Nigeria	1963	*aegypti* + *taylori*[a][31]
	1969	*aegypti* (13)[17]
Senegal	1966—1967	*luteocephalus* (2), *aegypti*, *irritans*[33]
	1975	*furcifer-taylori* (28), *luteocephalus* (8), *dalzieli*[12]
	1983	*furcifer* (29), *luteocephalus* (2), *africanus*, *neoafricanus*, *vittatus*[104]
South Africa	1976	*furcifer-taylori* (16)[16]
Angola	1971	*aegypti*[18]
Central African Republic	1978—1979	*africanus* (33)[13]

[a] This pool also contained 3 *Eretmapodites grahami*.

V. TRANSMISSION CYCLES

A. Evidence from Field Studies

1. Vectors

Field evidence implicating vectors largely involves that obtained by isolating the virus from wild mosquito populations complemented by studies on the biology of the suspected vector species. This section deals with virus isolation studies; those concerning vector biology are described in Section VI.

Virus isolation studies have indicated that *Aedes* species are the main CHIK vectors in both wild primate and human transmission cycles. More specifically, the evidence is strongest for species of the *Diceromyia* and *Stegomyia* subgenera, although odd isolations have come from *Aedimorphus* species. Based on isolation frequency in Africa, it is clear that the main vectors in the Afrotropical Region are in probable order of importance the *Ae. (Dic.) furcifer-taylori* pair, *Ae. (Stg.) africanus*, *Ae. (Stg.) luteocephalus*, and *Ae. (Stg.) aegypti*. The evidence in Africa concerning the last species relates solely to domestic populations; in fact, the indications are that the feral form is not involved. Table 2 shows the numbers of virus isolations from African aedine species according to country since the isolation of the prototype strain in 1953. Of the 156 total isolations, 74% came from *Ae. furcifer-taylori*. Since it became possible to separate the females of these species in Senegal, isolations of CHIK virus have been made only from *Ae. furcifer*. Evidence from studies in southern Africa suggests that this species is also the main vector there. There have been a few isolations from *Mansonia* species, (Nigeria, Uganda) and, since *Mansonia africana* is a proven experimental vector,[102] it is possible that this species, at least, could at times be a covector.

In India, virus isolations have been obtained only from *Ae. aegypti*, and it is reliably certain that this species has been responsible for the Asian urban epidemics. There were seven isolations in Thailand in 1962 and nine in 1964.[95] In India, there were 2 isolations in Calcutta in 1964[71] and 111 isolations in South India in 1964.[24] There were no isolations from *Cx. quinquefasciatus* or from the *Cx. vishnui* complex. The highest minimum field infection rate in mosquitoes was obtained over 1 week in Vellore, when it was 18/1000.

Occasional isolations have also been made from *Culex* species in Asia and Africa, but as experimental transmission studies indicate that *Culex* species are not biological vectors, any role these species might have in transmission would evidently be limited to mechanical transmission.

2. Vertebrate Hosts

There have been single isolations of CHIK virus from various vertebrates including a golden sparrow (*Auripasser luteus*) in Nigeria,[17] a ground squirrel (*Xerus erythropus*),[33] and a bat (*Scotophilus* sp.) in Senegal,[34] but the only group of vertebrates for which there is a strong body of field evidence implicating them as primary hosts in CHIK transmission cycles are the wild primates. The evidence comes mainly from studies undertaken in several countries in Africa. The first recorded indirect evidence of the involvement of wild primates in sylvatic infection of CHIK was the isolation of virus from primatophilic *Ae. africanus* in 1956 in Uganda.[8] The first recorded direct evidence was published by Osterrieth and coworkers in 1960[30] who demonstrated immunity in chimpanzees in Zaire. Studies in southern Africa have demonstrated antibodies in vervet monkeys (*Cercopithecus aethiops*) and Chacma baboons (*Papio ursinus*). A year after the 1962 epidemic in Zimbabwe, all the baboons and vervets in small samples tested were seropositive.[14] Similarly, after the 1976 epidemic in the northeastern Transvaal, 70% of the sera from 92 baboons contained antibodies.[16] Immune rates of 25% were found in vervets in northern Natal, South Africa, and of 16% in baboons in the northeastern Transvaal and Zimbabwe sampled in regions which had not experienced epidemics.[32] In Senegal, isolations have been made from vervet monkeys (two), the baboon *P. papio* (one), and the bushbaby *Galago senegalensis* (one).[12,33] Serological studies in Nigeria have shown immune rates in three monkey species: *Erythrocebus patus* (10%), *C. aethiops tantalus* (7%), and *C. mona* (20%).[31] Immunity has been shown in monkeys sampled at various localities in Uganda,[29] and in the Zika forest near Entebbe observations indicated the occurrence of an intense sylvan epizootic of CHIK in a cycle between red-tailed monkeys. (*C. ascanius schmidti*) and *Ae. africanus*. These long-term studies at Zika have provided evidence that suggests a 5- to 7-year periodicity in CHIK activity in the forest related to the reproductive replacement of immune monkeys by nonimmune monkeys.[9]

Serological tests done on a variety of wild primates imported into the U.S. have shown significant immune rates in gorillas, chimpanzees, baboons, vervets from Africa, and orangutans and macaque monkeys (*Macaca mulatta*) from Asia.[105] Other evidence for infection in Asian monkeys was obtained in a serological survey from 1962 to 1970 in Malaysia which showed a low immunity rate.[106]

In Uganda, no antibodies were found in 224 *Tadarida* bats and 78 other bats of 7 other species, nor in samples of birds, rodents, and cattle.[9,107] In Bankok, however, antibodies were reported in cattle, water buffalo, horses, pigs, dogs, rabbits, and bats, but not in cats or rodents.[91] Titers of virus recorded in patients during the Calcutta epidemic of 1963—1964 ranged from 1 to 7.0 log/mℓ.[23,71] The higher titers would allow significant numbers of *Ae. aegypti* and other vectors to become infected and subsequently transmit virus.[108]

B. Evidence from Experimental Infection Studies

1. Vectors

The first vector competence test with CHIK virus was done by Ross[5] with *Ae. aegypti* from Uganda: 87% of the mosquitoes became infected after feeding on a virus suspension through a bat wing membrane, and 25% of the infected mosquitoes transmitted the virus into blood suspensions through other membranes.[5] Since this experiment, reported in 1956, there have been several tests with *Ae. aegypti*, frequently attempting to transmit the virus to infant mice. Whether the population of *Ae. aegypti* is the feral form (subspecies *formosus*) or the domestic form has not been stated in most reports. The 50% infection thresholds determined range from 6.7 to 9.2 log/mℓ, and transmission rates were 13, 78, 27, and 90 to 100% in mosquitoes previously fed on infective meals with titers 7.3, 8.2, 8.9, and 9.2 log/mℓ, respectively.[64,108-112] Since this virus probably does not circulate much above 7.0 log in humans, these figures suggest that some populations of *Ae. aegypti* would be rather inefficient vectors during man-to-man transmission. Mechanical transmission between mice

Table 3
RESULTS OF VECTOR COMPETENCE TESTS WITH VARIOUS MOSQUITO SPECIES AND CHIK VIRUS

Species	Country/continent	50% infection threshold ($log_{10}/m\ell$)	Transmission (host animal)	Ref.
Ae. furcifer	South Africa	<6.2	+, 25%, 32% (monkeys, hamsters)	102
Ae. vittatus	South Africa	<6.7	+ (hamsters)	112
Ae fulgens	South Africa	ca. 7.0	+ (hamsters)	102
Ma. africana	South Africa	ca. 7.0	+, 29% (monkeys)	102
Ae. metallicus	South Africa	>7.2	± (hamsters)	112
Ae. ledgeri	South Africa	>7.2	± (hamsters)	112
Ae. simpsoni	South Africa	<5.2	− (monkeys)	110
Ae. circumluteolus	South Africa	>7.3	− (monkey)	110
Ae. apicoargenteus	Uganda	+	+ (monkey)	116
Ae. togoi	Asia	+	+	111
Er. chrysogaster	Tropical Africa	+	+	109, 111

Note: + = infection or transmission; − = no transmission; % = transmission rate (proportion of infected mosquitoes transmitting virus).

by *Ae. aegypti* has been shown to occur up to 8 hr after the infective feed and the most efficient transmission occurred within the first hour.[113] All *Culex* species so far tested — *Cx. quinquefasciatus*,[14,102] *Cx. molestus*,[109] *Cx. horridus*,[102] *Cx. poicilipes*, and *Cx. univittatus*[110] — have been found refractory to infection with CHIK virus. Tests have shown *Anopheles* species to be barely susceptible to infection (*An. gambiae*[63,115] and *An. stephensi*[114]) or susceptible but unable to transmit (*An. albimanus*[109]).

Tests on other aedine mosquitoes that have been incriminated as vectors in field studies have been done in the case of *Ae. furcifer* in South Africa[102,115] and *Ae. africanus* in Uganda.[116] *Ae. furcifer* has a 50% infection threshold of <6.2 log/mℓ and a transmission rate of 25 to 32% which would enable it to participate in transmission cycles involving vervet monkeys and baboons which circulate virus at levels up to about 7.0 and 8.0 log/mℓ, respectively. Furthermore, two laboratory personnel were accidentally infected by bite by *Ae. furcifer* which had previously fed on viremic monkeys.[115] *Ae. africanus* has also experimentally transmitted the virus between rhesus monkeys.[116]

The results of the experiments done on *Ae. furcifer* as well as those done on other species are shown in Table 3. The 50% infection threshold for *Ae. vittatus*, *Ae. fulgens*, and *Mansonia africana* would permit them to become infected in nature by feeding on viremic vervets or baboons so that the measure of their susceptibility and ability to transmit the virus makes them potential vectors. *Ae. metallicus*, *Ae. ledgeri*, and *Ae. circumluteolus* were poorly susceptible and largely failed to transmit; *Ae. simpsoni*, although susceptible, did not transmit the virus. Some populations of *Ae. albopictus*, the Asian species, have been shown as better vectors than populations of *Ae. aegypti*,[64,108,111] while other populations appear to be less efficient vectors.[114] Hence, *Ae. albopictus* can be regarded as a potential feral vector in India and Thailand.

2. Vertebrate Hosts

In one study, cattle, sheep, goats, horses, and various species of birds showed no viremia after inoculation of CHIK virus. There also was no antibody response except for low-titer hemagglutinating antibodies in some of the goats and sheep.[63] In another study, a significant number of horses and cattle developed hemagglutinating antibodies after inoculation, but

the positive reactions in horses may have resulted from cross reactions with antibodies to Getah virus.[117]

In Uganda, inoculation of the fruit-eating bat *Eidolon helvum* failed to elicit either viremia or an antibody response.[118] However, inoculation of the insectivorous bats *Pipestrellus nanus* and *Tadarida aegyptiaca* in South Africa caused viremias lasting at least 3 days ranging from 4.9 to 6.7 and 3.0 to 5.3 log/mℓ, respectively, and the bushbaby or night ape (*Galago senegalensis*) circulated virus for at least 3 days at concentrations of from 4.1 to 7.2 log/mℓ.[67] Inoculation of virus into South African rodents showed that only one species, *Mystromys albicaudatus*, the white-tailed rat, circulated virus at high levels of from 4.2 to 8.1 log/mℓ.[62] The distribution of this rodent does not, however, coincide with the distribution of CHIK virus.

Studies with Indian rhesus monkeys (*Macaca radiata*) and South African vervet monkeys (*Cercopithecus aethiops*) and baboons (*Papio ursinus*) demonstrated that all circulated the virus at high concentrations and developed hemagglutinating and N antibodies. Viremia in rhesus monkeys lasted up to 6 days and titers were 3.3 to 7.5 log/mℓ by mouse titration.[64] Similar tests done with African and Asian strains of the virus using the less sensitive monkey kidney cells for titration of virus showed viremia of up to 5 days duration and titers of 3.5 to 5.0 and 4.5 to 5.5 log/mℓ, respectively.[119] Titers in vervet monkeys and baboons ranged from 3.5 to 7.0 and 4.6 to 8.2 log/mℓ, respectively. and the viremia lasted up to 4 days.[63]

C. Maintenance/Overwintering Mechanisms

From observations in a small, isolated, moist equatorial forest at Zika near Entebbe, Uganda, it was postulated in 1971 that CHIK virus, together with Zika virus, were not being maintained in a monkey *Ae. africanus* cycle within this forest.[9] Instead, it seemed that viral activity in the monkeys followed the introduction of virus, and the subsequent monkey epizootics were in time terminated because of rising monkey immunity and could not recur until adequate replacement of immunes had taken place. This resulted in a 5- to 7-year periodicity in their activity and, during the interepizootic years, virus was absent as became apparent by the failure to isolate the viruses from *Ae. africanus*. It was concluded that CHIK virus could only be maintained in a very large forested area by a series of epizootic foci moving continually because of the changing pattern of wild primate immunity. Movement of virus within forests could be accounted for by long-range dispersal of *Ae. africanus* and extra-forest *Ae. africanus* could transfer virus between forests.

Observations on yellow fever virus in West Africa by workers in Senegal, the Ivory Coast, Upper Volta, and the Central African Republic led them to postulate that this virus is maintained at low levels in an enzootic cycle between *Ae. africanus* and monkeys in moist equatorial forests.[13,39,120-122] Furthermore, the evidence indicated that after heavy rains, yellow fever virus spills over from this enzootic forest cycle into the surrounding savannah-woodland so that high rates of transmission occur involving monkeys, man, and other sylvan *Aedes* species. This region surrounding the forests was thus named the zone of emergence.[122] The general belief now is that CHIK virus has a similar ecology.[39] After heavy rains, CHIK virus also appears to spill over from the enzootic forest cycle to the savannah-woodland where an epizootic cycle is initiated between *Ae. furcifer taylori* or *Ae. luteocephalus* and monkeys.[10-12] This extension of infection to the savannah-woodland region is thought to be affected by either viremic monkeys or infected *Ae. furcifer-taylori*.

In southern Africa where there are no extensive evergreen forests with *Ae. africanus* or an equivalent species as vector, it does not seem likely that the virus could be maintained by a similar enzootic forest cycle. The occurrence of adult females of *Ae. furcifer-taylori* in the wooded savannah areas is relatively short lived and dependent upon adequate summer rains with sometimes long intervals of up to several years during drought periods when adults are either absent or present in only small numbers. This suggests that the only

mechanism that would permit the virus to persist is survival in the dormant egg stage of *Ae. furcifer-taylori* mosquitoes followed by vertical (transovarial and transstadial) transmission to the next generation. Studies in southern Africa have so far failed to provide evidence of vertical transmission in *Ae. furcifer-taylori* either experimentally in the laboratory,[102] or in the field by testing newly emerged adults which were the progeny of naturally infected females of the previous summer, or by exposing in the field eggs laid by experimentally infected females and testing the adults reared out the following spring.[112] There is, in fact, only the smallest of evidence for the natural occurrence of transovarial transmission in the case of any *Alphavirus*, and the only evidence in support of aedine transovarial transmission is the laboratory transovarial transmission of Ross River virus in *Aedes vigilax*.[139] There is, however, evidence that *Ae. furcifer-taylori* may be involved in the vertical transmission of yellow fever as isolations of this virus have been made from males of this mosquito in nature.[123]

In both Africa and Asia, the virus can doubtless survive for considerable periods in the epidemic man-*Ae. aegypti* cycle, moving from locality to locality according to the availability of sufficient numbers of susceptible human hosts.[22] Whether this is the only way by which it has survived in Asia or whether there is a feral maintenance cycle, similar to the African ones, which occurs there remains to be ascertained. The apparent disappearance of CHIK virus from Bangkok in the presence of *Ae. aegypti*[97] does not support transovarial transmission in this species.

VI. ECOLOGICAL DYNAMICS

A. Climate and Environment

CHIK virus is strictly tropical in distribution which is clear from its geographical distribution pattern in southern Africa where the virus is absent from the temperate areas. The location of past human outbreaks and antibody surveys in humans and wild primates in South Africa suggest that low temperature, and not low rainfall, is the main factor excluding virus from the temperate areas. This is particularly evident in Natal where the southern limits of the virus apparently correspond with the 18°C midwinter isotherm.[32] It seems that temperature exerts its effect through the ecological requirements of the vector which, in southern Africa, is *Ae. furcifer-taylori* rather than the wild primate hosts. For the same reasons, it is essentially a virus of lowlands and woodland, the latter being necessary because the sylvan vectors breed exclusively in tree-holes. Within the tropics of Africa, the virus occurs in a variety of woodland. In Central and West Africa, it is apparently enzootic in large rainforests from where it probably emerges periodically during the rainy season into the adjoining semihumid savannah-woodland and subsequently even beyond that into semiarid savannah-woodland; the geographical limits of emergence are dependent upon the degree of precipitation and availability of susceptible wild primates and humans. In regions where forest is limited, e.g., southern Africa, the identity of the enzootic environment remains obscure, although wild primate epizooticity and human outbreaks have been linked unquestionably to the occurrence of abnormally wet summers in mixed savannah-woodland. In tropical Asia, where outbreaks have been temporally associated with heavy rainfall, the original source of the virus has not been determined.

B. Virus Transmission

A computer-simulated model of a CHIK transmission cycle indicated that provided there was a reasonably large number of vertebrate hosts present, the most critical factor in the transmission cycle is the survival probability of the vector.[124] It seems that vector longevity could decisively influence transmission in both human and wild primate cycles transmitted by *Ae. furcifer-taylori* in the savannah-woodlands of Africa, since these species are only

active for a relatively short period coinciding with the warm rainy season. During the outbreak in Zimbabwe in 1962, which started in January, the outbreak ended dramatically with the onset of cold weather in early May.[14,49] In retrospect, it also seems likely that short-lived *Ae. furcifer-taylori* terminated a monkey epizootic in gallery forest in South Africa.[15,32] However, it would [illegible] that CHIK transmission cycles are influenced more frequently by rising immune rat[illegible]he vertebrate hosts. This could well be the limiting factor in *Ae. aegypti*-transmitted urban outbreaks where very high postepidemic immune rates have been recorded in Asia and where outbreaks have ended without any vector control measures being applied. Rising immune rates are also believed to be the limiting factor in localized *Ae. africanus*-transmitted monkey epizootics in rain forest,[9] and probably also frequently end *Ae. furcifer-taylori*-transmitted wild primate epizootics in savannah-woodland.[16]

C. Vector Species

1. Aedes africanus

This vector is the most consistently numerically dominant arboreal mosquito species in the rainforests of West and Central Africa.[120,125] Studies in Uganda showed that this species had a maximum density at 12 to 18 m in the forest canopy where its single sharp peak of biting activity occurred from 1800 to 2200 hr at the time when the monkeys had settled for the night in the canopy of their dormitory trees.[125,126] In Uganda, this species can also survive as an aestivating adult during periods of marked drought. In both West Africa and Uganda it was observed that during the day the mosquitoes moved from the canopy to the ground and that at this time feeding on humans was greater on the ground than at night.[125,127] Mosquitoes oviposited at about the same frequency in tree-holes as in the fruits of *Saba senegalensis* hollowed out by monkeys and dropped on the forest floor. In the semihumid savannah-woodland of the Central African Republic, biting females with a remarkably constant parous rate were collected throughout the year, although their numbers dropped to very low levels by the end of the dry season.[128] Observations showed that the gonotrophic cycle lasts 4 to 8 days and daily adult female survival rates vary from 0.925 in June in the first half of the [illegible]y season to 0.95 in October at the end of the rainy season. Hence, longevity increases in the second half of the rainy season.[129]

2. Aedes luteocephalus

In the savannah of eastern Senegal, *Ae. luteocephalus* is the predominant species in mangrove gallery forest, where humidity is high and temperature is fairly constant, and it is still abundant in the adjoining drier forest zone but is rare in more distant arid zones. In the mangroves, *Ae. luteocephalus* is active for 5 months of the year compared to only 3 months in drier forest. This mangrove forest was thus regarded as the "zone of intensification" of virus transmission.[1] It seems that this species has a major role in amplification of CHIK virus in the early part of the rainy season, but that *Ae. furcifer* assumes this role later in the season.[130]

3. Aedes furcifer-taylori

The ecology of *Ae. furcifer-taylori* has been studied in the savannah-woodland of eastern Senegal[47] where it is the numerically dominant mosquito. These mosquitoes prefer to oviposit in tree holes with small openings and have a low preference for bamboo pots.[131] The eggs hatch in installments after successive immersion by successive rains. There is possibly only one generation a season with long intervals between the hatching of different groups of eggs. More recent work in eastern Senegal, done since it became possible to separate the females of *Ae. furcifer* and *Ae. taylori*, has shown that *Ae. taylori* is more active in the canopy, biting little at ground level in villages,[104,130] and suggests that this species is more exclusively simiophilic than *Ae. furcifer*. It seems to persist longer than *Ae. furcifer* after the end of the

rains probably because of a greater longevity. *Ae. furcifer*, in spite of a marked canopy activity, is also quite common at ground level, particularly in the nearby villages. Biting is mainly nocturnal with a peak between 1800 and 2100 hr — the first 3 hr after sunset. Observations suggest that *Ae. furcifer-taylori* takes two blood meals in one gonotrophic cycle, which would enhance transmission of virus. Mosquitoes probably use resting sites in the canopy rather than on the ground. The proportions of *Ae. taylori* to *Ae. furcifer* in Senegal was 1:3.[104]

The observations described above agree largely with those made in the wooded savannah of southern Africa where it has not as yet been possible to separate females of the two species.[16] Studies there indicated that *Ae. furcifer* and *Ae. taylori* usually coexist and that *Ae. furcifer* is probably more abundant than *Ae. taylori*. For example, in several series of collections based on the collection of males coming to human and wild primate bait, the *furcifer* to *taylori* ratio was 32:1. *Ae. furcifer-taylori* was by far the most prevalent species feeding on wild primates in the canopy and was 10 times more prevalent in the canopy than on the ground for 2 hr after sunset, although at times large numbers were collected on the ground on human bait.[16] In western Zimbabwe, the only occasion when *Ae. taylori* was evidently the numerically dominant species, it was not collected on the ground at a time when large numbers were collected in the canopy, suggesting that *Ae. taylori* may play a relatively smaller role in human infection.

4. Aedes aegypti

The man-biting habits of domestic *Ae. aegypti* appear to vary in different countries where CHIK epidemics have occurred. On the Makonde Plateau in Tanzania in 1952—1953 it fed on man mainly inside the huts but also outside on the verandahs,[2] while in Bangkok in 1962 biting was largely indoors. In Bangkok, the epidemic dissemination of CHIK virus accompanied the annual monsoon and varied directly with the density of *Ae. aegypti*.[132] Since about 1963, populations of *Ae. aegypti* in Calcutta appear to a large extent to have competitively displaced those of *Ae. albopictus* in the urban areas.[133]

If *Ae. aegypti* were to be eradicated from these areas, *Ae. albopictus* might well come in from the adjoining rural areas to occupy the vacated urban habitat. A feature of the 1964 epidemic in Madras and Vellore was the rapid build-up of human cases so that within 12 weeks nearly 40% of the population had become infected. It was suggested that this rapid spread of virus might have been due to the daytime biting habit of the local *Ae. aegypti*. During the day the human hosts are more likely to disturb the mosquitoes, thus interrupting their feeds, so that a mosquito moves to another host to finish feeding thus transmitting the virus mechanically.[113]

The present population growth in Africa with its associated urbanization could lead to large-scale future epidemics on the scale already experienced in Asia, with the possible maintenance of the virus in an *Ae. aegypti*-man cycle.

D. Movements and Migrations of Vectors and Hosts

Monkeys in the savannah-woodland of eastern Senegal sometimes move from woodland to the nearby villages in search of food.[11] In this way, they probably take infection from the feral to the domestic transmission cycles. An alternative way for virus to reach the villages is probably by *Ae. furcifer* flying from woodland, as it has been shown to fly distances of up to 3 km.[104] This strong flying ability, together with its habit of feeding both in the canopy and on the ground in woodland and on the ground in the villages[130] would enable it to fulfill this role. In evergreen forests in West Africa and Uganda,[127,134] the propensity of *Ae. africanus* to move from the canopy to the ground in the daytime and to migrate above the forest canopy would enable this species to disseminate virus between forests and from sylvan transmission cycles to man.

VII. PREVENTION AND CONTROL

Although several experimental inactivated vaccines have been produced,[135-138] vaccines have not yet been used to control outbreaks of CHIK. However, with the possible increase in human infection in the future, there may well be a need for a live virus vaccine. Control measures would therefore center around the avoidance of mosquito bites and the reduction in the density of vectors. In rural areas of Africa, the feral vectors are most active for only a few hours after sunset which facilitates the avoidance of these mosquitoes. In urban areas infested by *Ae. aegypti* but free of disease, quarantine measures could be applied to prevent the introduction of virus. Mosquito control may be needed in urban epidemics. The breeding sites of *Ae. aegypti* should be eliminated by reducing the number of water containers in- and outside homes and supplying these with piped water. Insecticidal control of adult mosquitoes and perhaps larvae may be necessary. Surveillance of *Ae. aegypti* densities by regular collection of larvae should form the background of any such control program.

VIII. FUTURE RESEARCH

Aspects of CHIK epidemiology requiring investigation include the possible role of bats in viral dissemination and maintenance; the identity of the wild primate species in the forests of Africa primarily concerned with viral maintenance or amplification; the possible existence of a feral transmission cycle in Asia; the mechanism of viral survival in nature, whether it is in fact through continuous transmission in moving foci of wild primate infection in large rain forests or whether it is primarily dependent upon vertical transmission in the mosquito host; the biology and taxonomy of the four important vectors; and the development of a live virus vaccine.

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The Aedes (Diceromyia) furcifer group (Diptera: Culicidae) in southern Africa; identification and occurrence of Ae. furcifer (Edwards) and Ae. cordellieri Huang

by

P.G. Jupp, B.M. McIntosh and A. Kemp

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

In southern Africa, the Aedes subgenus Diceromyia Theobald comprises Ae. fascipalpis (Edwards), Ae. adersi (Edwards) and the Ae. furcifer group of mosquitoes. The latter is important medically as it contains vectors of yellow fever, dengue and chikungunya viruses. The Ae. furcifer-taylori group was implicated as a vector of yellow fever in the Sudan in 1940 by Lewis (1942; 1943) and has been deeply incriminated as such a vector in West Africa (Cornet et al. 1979a; Port & Wilkes 1979). This mosquito group is also a vector of dengue-2 virus in the Ivory Coast and Burkina Faso (Upper Volta) (Cordellier et al. 1983; Roche et al. 1983) while Ae. furcifer (Edwards) has been incriminated as a vector of chikungunya virus in Senegal (Cornet et al. 1979b; Anonymous 1984).

The Ae. furcifer-taylori group is a vector only of chikungunya virus in southern Africa. During early work on mosquito vectors in relation to activity of

chikungunya virus, mosquitoes collected at Chipinda Pools in south-eastern Zimbabwe (McIntosh et al. 1964), at Ndumu in northern Natal-KwaZulu (McIntosh et al. 1972), and at Mica in the north-eastern Transvaal (McIntosh et al. 1977) were identified as belonging to the Ae. furcifer-taylori group. More recently, however, Huang (1986) revised the taxonomy of the Ae. furcifer group and described a third species, Ae. cordellieri Huang. In the light of her work, male mosquitoes from Mica previously identified as Ae. taylori were reexamined by Jupp and McIntosh (1990) and found to be Ae. cordellieri. Owing to this development we reexamined all the male specimens of the Ae. furcifer group in our collection to determine the distribution in southern Africa of Ae. furcifer and Ae. cordellieri and whether Ae. taylori does in fact occur.

From an examination of specimens from the Ivory Coast, Huang (1986) distinguished between females of Ae. furcifer and Ae. cordellieri on the basis of the ornamentation of the abdominal tergites. Her conclusion was that Ae. cordellieri could be distinguished from Ae. furcifer by the absence of pale scales on the dorsomedian areas of tergites II to VII and apparently by the absence of basomedian white bands on tergites II to VII. Ae. furcifer had prominent basomedian white bands and scattered dorsomedian pale scales. We examined female mosquitoes belonging to these two species in South Africa to see whether they were separable.

Except during periods of heavy rainfall in wooded savanna in the tropical region of South Africa, the furcifer group is seldom taken in human-baited catches. Because of this, we sampled the group by exposure of bamboo pots for oviposition in riverine forest at Ndumu. Aedine eggs deposited in the pots were reared to

adulthood and Ae. furcifer group mosquitoes thus obtained were pooled together in cages measuring 20 x 20 x 20 cm. They were allowed to feed on hamsters and separate families were subsequently reared from the gravid females. Slides of genitalia were prepared from 1-7 males in each of 12 families to make a firm identification and to confirm the absence of any possible hybridisation which might possibly have occurred between Ae. cordellieri and Ae. furcifer while they were still mixed together in the rearing cage. Tergite ornamentation was examined on all females and some males representing each family.

Ae. cordellieri oviposited poorly and eggs proved difficult to rear so that only a small number of females were obtained for study. From this parental generation, three families of Ae. cordellieri were reared in which there were five, one, and one females respectively. Yellow and white dorsomedian scales were either present or absent on tergites II to VII in these seven mosquitoes (and, notably, in the five siblings), indicating that this character is polymorphic in Ndumu populations of Ae. cordellieri. Furthermore, white basomedian bands were nearly always present on all tergites indicating a further difference between Ae. cordellieri from South Africa and the Ivory Coast. Hence it wasn't possible to distinguish between females of the two species from Ndumu. Males of the two species can not be separated by tergite morphology either so that examination of male genitalia is essential for diagnosis. This is straightforward as genitalia can be readily distinguished without their removal from the mosquito: Ae. furcifer possesses a tuft of long, curled, orange setae on the apicomesal area of the gonocoxite which is entirely absent in Ae. cordellieri. The

available records for distribution of the two species in southern Africa are listed below. These are all based on identification from male genitalia.

Ae. furcifer and Ae. cordellieri.

Robin's Camp, Hwange (Wankie) Reserve, Zimbabwe (McIntosh unpublished);

Chipinda Pools, S.E. Zimbabwe (McIntosh et al. 1964; McIntosh unpublished);

Mica, N.E. Transvaal (McIntosh et al. 1977; Jupp & McIntosh 1990);

Skukuza, E. Transvaal (Kemp & Jupp 1991);

Ndumu, N. Natal-KwaZulu (McIntosh et al. 1972; Kemp & Jupp 1991).

Ae. furcifer.

Newington, E. Transvaal (McIntosh unpublished);

Othawa, E. Transvaal (McIntosh unpublished);

Rolle Siding, E. Transvaal (Edwards 1941, p.216);

Tzaneen, N.E. Transvaal (Ingram & De Meillon 1929, p.139);

Potgietersrust, N. Transvaal (Muspratt 1955);

Malelane, E. Transvaal (Ibid.);

Durban, Natal (Kemp & Jupp 1991).

Ae. cordellieri.

Gwalaweni & Dukuduku forest, N. Natal-KwaZulu (McIntosh unpublished).

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RIFT VALLEY FEVER

2. ATTEMPTS TO TRANSMIT VIRUS WITH SEVEN SPECIES OF MOSQUITO*

B. M. McINTOSH**, P. G. JUPP**, D. ANDERSON** AND D. B. DICKINSON**

SUMMARY

Seven species of mosquito among the fauna of Southern Africa were tested for their ability to transmit Rift Valley fever virus. *Anopheles coustani, Aedes lineatopennis* and *Aedes circumluteolus* were readily infected by high doses of virus but attempts at transmission of virus failed. These were regarded as inconclusive because only a small number of mosquitoes was fed. *Culex theileri, Culex zombaensis, Culex neavei* and *Eretmapodites quinquevittatus* all successfully transmitted virus at varying degrees of efficiency. *Culex theileri* was the most efficient vector and 92 to 100 per cent of females of this species were infected by 6,5—8,5 log LD_{50} of virus and 11 out of 20 mosquitoes transmitted virus. Seventy-five per cent of *Culex zombaensis* were infected by 5,8 log LD_{50} of virus and at least 2 out of 5 mosquitoes transmitted virus. Between 31 and 69 per cent of *Culex neavei* were infected by 6,6—8,5 log LD_{50} of virus and about 3 out of 22 mosquitoes transmitted virus. The poorest vector was *Eretmapodites quinquevittatus* which was readily infected but only about one out of 22 mosquitoes transmitted virus. Attempts to demonstrate transovarian transmission of virus with this species failed.

INTRODUCTION

When Rift Valley fever (RVF) appeared in domestic animals in Southern Africa in 1969, only *Aedes aegypti* [1] and *Aedes caballus* [2] among the mosquito fauna of this region had been shown capable of transmitting the virus. *A. aegypti* has not been implicated in the transmission of this virus during epizootics and seems unlikely to become so in the future. Gear *et al.* [3] had collected *A. caballus* during an outbreak in sheep near Luckhoff, Orange Free State, exposed three lots of these mosquitoes to laboratory mice, and successfully transmitted virus with one lot. Subsequent attempts, however, to transmit virus in the laboratory with *A. caballus* collected near Johannesburg had failed (Arbovirus Research Unit, unpublished work). It has now become apparent that what is at present known as *A. caballus* in South Africa probably consists of two species, as yet unnamed, and it will probably never be possible to establish which of these were used in the aforementioned experiments, because both species probably occur at Luckhoff and Johannesburg, the localities whence the mosquitoes used in the tests originated. These experiments, therefore, can no longer be interpreted satisfactorily in terms of transmission in the field. Information on species capable of transmitting RVF was therefore minimal during the recent epizootics, making it difficult to identify the important vectors, particularly when transmission was observed to occur in the absence of species of the *A. caballus* group.

In an attempt to remedy this deficiency, transmission tests were conducted with seven species of mosquito. These had come under suspicion as vectors because of their feeding habits, distribution and prevalence, and, in some species, also because of the isolation of RVF virus from wild-caught specimens.

METHODS

With the exception of *Culex theileri*, the females of all species used in the experiments were first generation progeny reared from wild-caught females. Those of *C. theileri* were from a laboratory-maintained colony in its 12th generation, established from material collected at Lake Chrissie, Transvaal. The *Anopheles coustani, Aedes lineatopennis* and *Culex zombaensis* came from near Salisbury, Rhodesia, the *Aedes circumluteolus* and *Culex neavei* from Ndumu, Natal, and the *Eretmapodites quinquevittatus* from Port Shepstone, Natal.

Attempts to infect mosquitoes were made by feeding them on either calves or hamsters while these animals were viraemic after inoculation of wild-type RVF virus. Shortly before feeding started, a blood sample was collected from the viraemic animal to deter-

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**Arbovirus Research Unit, South African Institute for Medical Research, Johannesburg.

mine its virus content. This was done by titration intracerebrally in infant mice and virus concentrations are expressed as the logarithm of the LD_{50} per 0,02 ml of mouse inoculum.

Infection of individual mosquitoes was determined by inoculation of a suspension of the mosquito into infant mice. In cases where mosquitoes had been used in transmission attempts, these determinations were done on all mosquitoes two days after feeding. Hence, the 'infection status' of all mosquitoes used in attempted transmission was known.

'Infected' mosquitoes, i.e., all those which had engorged on the viraemic animal, were held at 80% relative humidity and 26°C for periods of from 9 to 30 days before transmissions were attempted; the actual time in each experiment is given in the table.

Transmission attempts were made by feeding 'infected' mosquitoes on either a calf, lamb, hamsters or 6 days old mice, as indicated in the table, exposing each animal to single or several mosquitoes. These attempts were considered valid only when one or more of the mosquitoes feeding were shown later to contain virus.

With the calves and lamb a positive or negative transmission was established by antibody tests on serum collected two weeks after mosquito feeding, with hamsters and mice by death of the animal and confirmation of the cause of death by histological examination of the liver and usually also by isolation of virus from the dead animal. In addition, surviving hamsters were bled after three weeks for an antibody test on the serum to exclude the possibility of a non-fatal infection.

RESULTS

Anopheles coustani

From the table it can be seen that three attempts were made to infect this species, two from a viraemic calf and one from a hamster. At a virus concentration of 7,5 log LD_{50}, 100 per cent of mosquitoes were infected whereas at the lowest concentration of 4,3 log LD_{50}, 45 per cent became infected.

Table: RESULTS OF ATTEMPTED INFECTION AND TRANSMISSION OF RVF VIRUS WITH SEVEN MOSQUITO SPECIES

Species	Viraemic Animal	Virus concentration in blood	Mosquito infection		Transmission				
			Day	Rate %	Day	Host	Result	No. infective mosquitoes feeding	Rate
An. coustani	Hamster	6,0	8—20	71(8)§			NT		
	Calf	>7,5	14—18	100(10)	16	Lamb	Neg.	1	
	Calf	4,3	8—16	45(11)			NT		
A. lineatopennis	Calf	6,4	6—14	43(7)	10	Mouse	Neg.	2	
A. circumluteolus	Calf	6,4		100(4)			NT		
	Calf	4,8	14—38	24(59)	14	Calf	Neg.	2	
					19	Mouse	Neg.	1	
					23	Mouse	Neg.	1	
C. zombaensis	Hamster	5,8	8—32	75(24)	11	Hamster	Pos.	? 1*	2/5
					15	Hamster	Pos.	2	
					30	Hamster	Neg.	2	
C. neavei	Hamster	6,6	18—20	31(26)	17	Mouse	Pos.	? 1*	3/22
					17	Hamster	Neg.	5	
	Hamster	7,5	19—20	69(16)	17	Hamster	Pos.	4	
					18	Hamster	Neg.	2	
	Hamster	8,5	19—20	68(19)	18	Mouse	Neg.	1	
					18	Hamster	Pos.	7	
					18	Hamster	Neg.	2	
C. theileri	Hamster	6,5	20—22	100(25)	19	Mice†	Pos.	2	1/2
	Hamster	8,0	19—22	95(44)	18	Mice	Pos.	11	5/11
	Hamster	8,5	21—22	92(24)	20	Mice	Pos	7	5/7
E. quinquevittatus	Hamster	5,5	17—69	82(22)	22	Hamster	Neg.	10	1/22
	Hamster	6,0	21—77	57(28)	22	Hamster	Neg.	5	
					24	Hamster	Neg.	2	
	Hamster	6,6	8—35	71(7)	19	Mouse	Pos.	2	
	Hamster	7,3	9—29	80(20)	9	Mouse	Neg.	3	

§Figure in parenthesis indicates number of mosquitoes on which percentage is calculated.
*Successful transmission resulting from an unknown number of mosquitoes probing but not imbibing blood.
†In all transmission attempts with C. theileri each mouse was exposed to one mosquito.
NT = no test.

Unfortunately, during the attempted transmisison of virus, only one infected mosquito was induced to feed. This occurred on a lamb on the 16th day and no transmission resulted. Hence, apart from the observation that this species is easily infected at the dosages used, no conclusion on its vector potential can be arrived at, because inadequate numbers of mosquitoes fed during the attempted transmission.

Aedes lineatopennis

Considerable difficulty was experienced in getting adequate numbers of this species to feed even at their first feeding. At a virus concentration of 6,4 log LD_{50}, 43 per cent (based on only seven mosquitoes) were infected. On the 10th day two mosquitoes failed to transmit to mouse. Here again it is believed that numbers of mosquitoes were insufficient for a reliable conclusion to be made.

Aedes circumluteolus

In one of the two experiments with this species only 24 per cent of mosquitoes were infected at 4,8 log LD_{50} and in the other all four mosquitoes imbibing 6,4 log LD_{50} of virus became infected. In the former experiment attempts at transmission were made on the 14th, 19th and 23rd days and quite large numbers of mosquitoes fed on the calf and mice but because of the low infection rate only four were infective. These failed to transmit the virus. While this species should not be classified as a non-vector on the basis of only four mosquitoes, it would seem that, at best, this species would be a vector of only moderate capability because of low susceptibility to infection. Nevertheless, this species occurs in prodigious numbers on the coastal plains of northern Natal and Moçambique: it is quite posible that high populations may well compensate for its poor vectorship to make this species an important vector.

Culex zombaensis

Seventy-five per cent of this species were infected by 5,8 log LD_{50} of virus. There were successful transmissions to hamsters exposed to groups of mosquitoes on the 11th and 15th days. On the former occasion none of the mosquitoes engorged, the transmission in this instance resulting from probing without the mosquito imbibing any blood. An attempted transmisison on the 30th day with two mosquitoes failed. If it is assumed that only a single mosquito transmitted virus on the 11th and 15th days, an over-all transmission rate of 40 per cent is arrived at. Although RVF virus has never been isolated from *C. zombaensis*, the results show this species to be another potential vector in the lowlands of Natal and Moçambique, where it is prevalent and a frequent feeder on the larger domestic animals.

Culex neavei

In three experiments this species was shown to possess a moderate degree of susceptibility to infection and mosquitoes successfully transmitted the virus on three occasions to either mice or hamsters. There was a considerable number of failures, however, and with a possible over-all transmission rate of only 14 per cent, this species seems to possess only a fair degree of vector capability.

Culex theileri

This species was easily infected (92—100 per cent) at the three rather high concentrations of virus used. In the transmission tests, in all of which individual mice were exposed to single mosquitoes, a total of 11 out of 20 mosquitoes engorged and transmitted virus, giving an over-all transmission rate of 55 per cent. The actual transmitting ability of this species is probably even higher as there were a further six successful transmisisons by mosquitoes probing without engorging. These were excluded from consideration in calculaing the transmission rate, because it could not be determined how many mosquitoes had probed without transmitting. These results indicate that this species is an efficient vector of RVF virus.

Eretmapodites quinquevittatus

In the four experiments with this species the mosquitoes were easily infected, although infection rates were not fully consistent in relation to virus concentration in that the lowest concentration yielded the highest rate. It seems that the rate of 82 per cent at 5,5 log LD_{50} should be considered as abnormally high, since the remaining values were in proportion to virus concentration. There were five attempted transmissions, involving altogether 22 mosquitoes. Only one, in which two mosquitoes engorged on a mouse on the 19th day, was successful. With a transmisison rate of only 5 per cent it may be concluded that this species is a poor vector.

While the experiments with *E. quinquevittatus* were in progress, it was noticed that this species was mating successfully in the

cages, with the result that numbers of eggs, pupae, larvae and adults, mostly the progeny of infected females, became available. These were used to test the possibility that transovarian infection occurs with RVF virus. With this in mind, groups of eggs, larvae, pupae and adults, collected randomly, were ground up, suspensions prepared and these were inoculated separately into infant mice to determine whether they contained virus. In all, 289 eggs, 50 larvae, 50 pupae, 178 females and 295 males were tested in 17 lots. None contained virus and because of the high infection rate among the parental females it seems unlikely that transovarian transmission could be of significance in nature.

DISCUSSION

In all the experiments high infecting doses of virus were used intentionally. While this procedure favours the species under test, the purpose was to determine vector capability at virus concentrations occurring in sheep and cattle, the investigation of epizootic vectors being the main objective. From a practical viewpoint, also, it is an advantage in the initial screening of species of unknown susceptibility to have many infected specimens available when the second feeding is attempted. Nevertheless, although high viraemia levels do occur in cattle and sheep, it should be borne in mind that at lower infecting doses than those used in the tests fewer mosquitoes would become infected resulting in a lower vector efficiency.

The tests showed that four species of the local mosquito fauna are capable of transmitting RVF virus and on epizootological grounds all of these are likely to play some part in nature. It is unfortunate that because of insufficient numbers the tests with *Anopheles coustani, Aedes lineatopennis and Aedes circumluteolus*, all potentially important vectors, were inconclusive.

Of all the species tested, *Culex theileri* emerged as the most efficient vector and from the degree of vector capability shown it is believed that this species is probably the most important vector on the South African highveld. As discussed elsewhere, there is strong epizootiological evidence in support of this opinion [8].

The isolation of RVF virus in *Eretmapodites* species both in South Africa [3] and Uganda [4] under circumstances unassociated with epizootics in domestic animals is suggestive that these species might be involved in a maintenance transmission cycle. *E. quinquevittatus* would appear to possess some potential as maintenance vector of this virus in Natal. It is quite common along the forested coastal zone and is exceptionally long-lived as an adult, in which stage it probably overwinters. The poor level of vector capability shown, however, is against this possibility. It would seem that if this species had been a vector over a long period of time, which would be the case if it were a maintenance vector, selection would probably have resulted in a strain of virus more invasive for the salivary gland of this mosquito with a more efficient level of transmission.

Unlike most mosquitoes, *E. quinquevittatus* is able to produce viable eggs without a blood meal. Because of this, the possibility that virus might reside in the mosquito population for long periods of time without the need for frequent transmission between vertebrate animals seemed worth investigating, although it has never yet been demonstrated conclusively to occur regularly with any mosquito-borne virus. The failure to demonstrate transovarian passage of virus, however, seems to rule out the possibility that this species could act as a virus reservoir in this manner.

3.2

. . Vector Studies on Rift Valley Fever Virus in South Africa

B. M. McINTOSH, P. G. JUPP, I. DOS SANTOS, B. J. H. BARNARD

SUMMARY

Attempts to isolate Rift Valley fever virus from wild-caught haematophagous flies were made over an 8-year period in the coastal lowlands of Natal, where the virus causes enzootic infection of cattle, and on infected farms in the inland plateau region of South Africa during epizootics in 1974/1975. In Natal the virus was not isolated from 71 581 mosquitoes, 10 514 *Culicoides* spp., or from fewer numbers of simulliid, phlebotomine and tabanid flies. On the plateau there were 12 isolations from *Culex theileri* and 6 from 3 other mosquito species. There were no isolations from 12 368 *Culicoides* spp., and smaller numbers of simulliids, tabanids and *Stomoxys* flies. In laboratory transmission tests the virus was transmitted by 7 mosquito species: *Cx. theileri, Cx. univittatus, Cx. quinquefasciatus, Aede. juppi, Ae. lineatopennis, Ae. aegypti* and *Eretmapodites quinquevittatus*. Two mosquito species, *Ae. cabellus* and *Cx. rubinotus*, became infected but failed to transmit by bite. *Cx. theileri* was shown to be the most efficient vector. The tick, *Ornithodoros savignyi*, failed to become infected or to transmit virus by bite. It is concluded that *Cx. theileri* is the main epizootic vector among sheep and cattle on the plateau, where it probably causes some human infection. In the same region *Ae. juppi* and *Ae. lineatopennis* are believed to act as vectors of lesser importance. Failure to isolate the virus from mosquitoes in enzootic foci in Natal is in accordance with previously observed low isolation rates from mosquitos in such localities. It is concluded that there is no definitive evidence to indicate that mosquitoes are concerned in viral maintenance.

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Although Rift Valley fever (RVF) virus was first isolated in South Africa in 1951, uncertainty still exists regarding the identity of the vectors. In part this has been the result of the long quiescent periods shown by the virus in nature and the low frequency with which it has been isolated from wild populations of mosquitoes, the generally suspected vectors. The situation in South Africa is further complicated because the virus is present in two ecologically distinct regions, where vectors almost certainly differ, viz. the temperate inland plateau west of the Drakensberg escarpment, and the subtropical coastal lowlands of Natal. Viral activity on the plateau has been most evident as an epizootic infection of sheep and cattle, involving man secondarily, and usually only appearing during abnormally wet years. During years of normal rainfall localized enzootic infection in domestic animals on the plateau seems likely and this type of infection has occasionally been recognized.[1,2] In the lowlands of Natal, viral activity has been observed as a sporadic infection of cattle, probably occurring annually, with minimal infection of man.

In previous attempts to isolate the virus from mosquitoes in South Africa four species were found to be infected. During epizootics on the plateau in 1953 Gear *et al.*[3] isolated the virus 6 times from *Aedes caballus* and 3 times from *Culex theileri*, and wild-caught specimens of the former mosquito also transmitted the virus to mice by bite. In later field studies on the plateau, 2 isolations were made from *Cx. theileri* in 1956[4] and 5 from the same species in 1970.[5] In the coastal region of Natal the virus was isolated twice from *Ae. circumluteolus* in 1955[4] and once from *Eretmapodites quinquevittatus* in 1971.[5]

In previous transmission studies with South African mosquitoes successful transmissions were obtained with *Cx. theileri, Cx. zombaensis, Cx. neavei* and *Er. quinquevittatus*, whereas *Anopheles coustani, Ae. lineatopennis* and *Ae. circumluteolus* failed to transmit.[6]

We report here further attempts to isolate the virus from mosquitoes and other haematophagous flies in the field as well as attempts to transmit the virus in the laboratory with nine mosquito and one tick species. The wild-caught flies sampled for virus included collections made in the coastal region of Natal over several years and on infected farms in the plateau during epizootics in 1974 and 1975. Further transmission tests were regarded as necessary to determine vector competence more precisely in certain species previously tested as well as to include hitherto untested species.

MATERIALS AND METHODS

Virus Isolation

Tests for virus in insects from Natal formed part of an ongoing surveillance for arboviruses in that region. Collections of insects were made periodically during summer and autumn from 1972 to 1979 at several collecting sites near the town of Empangeni on the north coast and Port Shepstone on the south coast. A variety of collecting methods was used in diverse rural microhabitats to ensure collection of as many species as possible. These methods included CO_2 or goat-baited net traps, CO_2-baited light traps, or capture by hand in test tubes off volunteer

National Institute for Virology, Sandringham, Johannesburg
B. M. McINTOSH, D.V.SC.
P. G. JUPP, M.SC., PH.D.
I. DOS SANTOS, LIC. MED. VET.
Veterinary Research Institute, Onderstepoort, Tvl
B. J. H. BARNARD, B.V.SC., M.MED.VET.

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TABLE I. DETAILS OF ARTHROPODS USED FOR TRANSMISSION EXPERIMENTS

Species	Individuals used	Locality of origin
Ae. aegypti	Colony, F_{23}*	Maputa and Ndumu, Natal
Ae. caballus	Wild-caught	Luckhoff and Jacobsdal, south-western OFS
Ae. juppi	Wild-caught	Luckhoff and Jacobsdal, south-western OFS
Ae. lineatopennis	Wild-caught	Delareyville, Tvl
Cx. quinquefasciatus	Colony, F_{18}	Johannesburg
Cx. rubinotus	Wild-caught	Mtubatuba, Natal
Cx. theileri	Colony, F_3 + F_{11} and wild-caught	Delareyville, Tvl
Cx. univittatus	Colony, F_{28} and progeny of wild-caught females	Johannesburg
Er. quinquevittatus	Progeny of wild-caught females	Port Shepstone, Natal
Ornithodoros savignyi	d-caught 1st nymphal instars	Bray, CP

* Laboratory-maintained colony in its 23rd generation.

human bait. The same methods were used in the plateau to collect insects on ten farms at varying periods after the disease had been diagnosed in domestic animals. Insects were identified on the day of collection and stored in liquid nitrogen. Mosquitoes containing ingested blood were pooled and tested separately from unengorged specimens. In the laboratory insects were stored at −70°C until tested for virus. For this purpose suspensions of the insect pools were inoculated intracerebrally into 2-day-old suckling mice. Viruses isolated were identified in complement fixation tests, and in some cases, identification was confirmed in neutralization tests.

Virus Transmission

The nature and geographical origin of the mosquito and tick species used are shown in Table I. Infected mosquitoes were held at 25° - 26°C and 75 - 80% relative humidity. The ticks were held at 26°C in an unhumidified room, but the sand in the rearing bowls was moistened weekly. Attempts to infect mosquitoes and ticks were made by feeding them on hamsters which were viraemic after inoculation of the AN 1830 strain of RVF virus in its 2nd or 3rd intracerebral mouse passage level. The concentration of virus in the blood of the hamsters was determined by titration in adult mice of a sample collected shortly before feeding. Mosquitoes feeding on hamsters with approximately similar viraemias were pooled in some cases into a single lot for subsequent experimentation, and the mean viraemia level was taken as the concentration of virus in the feed. Virus concentrations are expressed as the log LD_{50} per 0,02 ml. Mosquitoes and ticks were held for varying periods before they were fed singly or in groups on 8-day-old mice, hamsters or sheep in attempts to transmit virus. The number of mosquitoes or ticks infected was determined by testing them individually for virus by mouse inoculation, usually on the day after their second feed. A 50% infection threshold, the concentration of virus infecting half of the mosquitoes, was calculated by the probit method,[4] where the data was sufficient to allow for this; otherwise it was estimated. First nymphal instars of the tick were given their infective feed, then moulted into second instars which were fed for the transmission attempts on the 66th and 67th days. With the exception of 2 species noted below, transmission attempts with mosquitoes were made by exposing each animal to a single mosquito. Only visibly engorged and infected mosquitoes were considered in estimating transmitting efficiency. This is expressed as the percentage or proportion of infected mosquitoes transmitting. Transmission of virus was established by isolation of virus from the livers of dead animals by examining the livers histologically, or by an antibody test on the serum of surviving animals. In transmission attempts with *Cx. quinquefasciatus* and *Er. quinquevittatus* (Table VI) small groups of mosquitoes were exposed repeatedly to hamsters, a different hamster being used each time. Since more than one mosquito sometimes fed on the same animal, transmission rates could not be determined.

Transovarial and Trans-stadial Transmission

In 17 of the 9 mosquito species, first-generation progeny of females from infected lots were tested for virus to determine whether virus was transmitted through the egg to the following generation. Suspensions of pools of up to 100 adult males, females, larvae or eggs were inoculated intracerebrally into suckling mice to test for the presence of virus.

RESULTS

Virus Isolation

Table II shows the number of insects from the coastal region of Natal which were tested for arboviruses from 1972 to 1979. Included among the mosquitoes, which predominated numerically, were 71 species and all the known epidemiologically important genera in the region. There were 4 264 *Er. quinquevittatus* and 1 687 *Ae. circumluteolus*, the 2 species from which RVF virus had been isolated previously in the coastal region. The subgenus *Eumelanomyia* was largely made up of 21 613 *Cx. rubinotus*, a species feeding mainly on wild rodents. *Cx. theileri* and *Ae. lineatopennis*, both rare species in the region but prevalent on the plateau, were represented by only 13 and 25 specimens respectively.

RVF virus was not isolated from either mosquitoes or other flies although, as listed in a footnote to Table II, there were 279 isolations of 8 other arboviruses from the mosquitoes.

Table III shows the results of virus isolation attempts on insects collected on infected farms on the plateau. RVF virus was isolated from unfed specimens of 4 mos-

TABLE II. BLOOD-FEEDING FLIES FROM COASTAL REGION OF NATAL TESTED FOR ARBOVIRUSES, 1972 - 1979

Family/subfamily	Genus	Subgenus	No. species	No. pools	No. insects
Culicidae	*Anopheles*		12	218	8 296
	Uranotaenia		6	52	1 479
	Coquillettidia	*Coquillettidia*	6	287	8 311
	Mansonia	*Mansonioides*	2	149	6 964
	Eretmapodites		3	201	4 714
	Aedes	*Stegomyia*	7	115	2 376
	Aedes	*Aedimorphus*	13	151	4 550
	Aedes	*Neomelaniconion*	3	86	2 526
	Culex	*Eumelanomyia*	3	1 156	21 816
	Culex	*Culiciomyia*	2	14	290
	Culex	*Culex*	14	306	10 237
Total Culicidae			71	2 714	71 561
Simuliidae				20	1 092
Ceratopogonidae	*Culicoides*			97	10 514
Phlebotominae				46	391
Tabanidae				19	77
Muscidae	*Stomoxys*			26	252

Viruses isolated from mosquitoes: Ilanzi (115 isolations), Germiston (99), Witwatersrand (73), Sempamwara (2), Middelburg (1), Sandfly fever (1), Pongola (1), Shakwe (1).

TABLE III. BLOOD-FEEDING FLIES TESTED FOR RVF VIRUS ON INFECTED FARMS ON THE INLAND PLATEAU, 1974/1975, SHOWING NUMBER OF VIRUS ISOLATIONS

Species/family	No. pools	No. insects	No. RVF isolations
Cx. theileri	257	12 738	12
Ae. juppi	93	2 945	3
Ae. lineatopennis	38	1 315	2
An. cinereus	30	881	1
Other Culicidae — 16 species	118	3 843	
Total Culicidae	536	21 722	18
Simuliidae	16	1 131	
Ceratopogonidae (*Culicoides*)	80	12 368	
Tabanidae	4	22	
Muscidae (*Stomoxys*)	18	182	

quito species; *Cx. theileri* yielded 12 of the total of 18 isolations. Infection in *Cx. theileri* was irregularly distributed among the ten farms (Table IV), as 11 of the isolations came from one farm. On eight farms no isolations were made from *Cx. theileri* even though on some farms, e.g. Skatryk and Goedendacht, fair numbers were tested after only a short period had elapsed since the last identified RVF case in domestic animals. Tests for virus among mosquitoes on infected farms indicated therefore that *Cx. theileri* was most frequently infected, but even in this species infection rates were usually low. The overall infection rate was about one infected *Cx. theileri* per 1 000 of the population, but it is evident that on some farms it was even lower. *Cx. theileri* was present on all infected farms and often in large numbers, whereas the other infected species were fewer and often absent.

The collections also revealed the persistence of *Cx. theileri* well into winter as the collections on Goodhope and Acacias which were made in July during cold weather showed this species to be present and still active.

Virus Transmission

Thirty specimens of the tick, *Ornithodoros savignyi*, failed to transmit virus when they fed on 12 hamsters on the 66th and 67th days after their infective feed. Twenty-six of these ticks, when tested for virus on the day following their second feed, were negative. It was concluded that the virus did not multiply in the ticks and this accounted for their failure to transmit.

In contrast to the tick the virus infected all 9 species of mosquito, although to a varying degree (Table V). *Cx. theileri* was the most susceptible species with a 50% infection threshold of about 4,0 log. *Ae. aegypti* and *Cx. univittatus* and possibly *Er. quinquevittatus* appeared nearly as susceptible while the remaining species were more resistant to infection, *Cx. rubinotus* being most resistant.

The virus was transmitted by *Ae. aegypti*, *Ae. juppi*, *Ae. lineatopennis*, *Cx. theileri*, *Cx. univittatus*, *Cx. quinquefasciatus* and *Er. quinquevittatus*, the results obtained with the two last-named species being shown in Table VI.

In the transmission attempts with *Ae. aegypti* and in some tests with *Cx. theileri* and *Cx. univittatus*, the mosquitoes fed on mice. For reasons not clearly understood but possibly related to the excessive probing indulged in by mosquitoes when feeding on 8-day-old mice, apparently because of difficulty in obtaining blood, the use of mice enhances vector efficiency. This is evident in the tests with *Cx. theileri* infected at 5,3 log in which both mice and hamsters were used. With mice a transmission rate of 70% was obtained, whereas with hamsters the rate was only 16%. A further example is the result with *Cx. univittatus* in which transmissions were obtained with

mice but not with hamsters. This is a phenomenon we have also observed with chikungunya virus, and we believe that positive transmissions obtained using mice should be interpreted cautiously in evaluating vector potential in the field. [illegible] group of *Cx. theileri* infected at 8,0 log and fed on sheep the transmission rate was 26%, a result closer to that obtained with hamsters than mice, and suggesting that hamsters give a more realistic epidemiological assessment of vector efficiency.

Excluding those results obtained using mice, the lest

TABLE IV. ISOLATIONS [illegible] VF VIRUS FROM 4 MOSQUITO SPECIES ON 10 INFECTED FARMS ON THE PLATEAU, 1974/1975

Farm	Elapsed time*	No. tested with isolations			
		Cx. theileri	*Ae. juppi*	*Ae. lineatopennis*	*An. cinereus*
Goodhope	3 mo.	892			
Potfontein	1 mo.	411			801
Fairfield	1 wk	3 597 (11)†		2	80 (1)
Prospect	1 mo.	815	2 461 (3)	367	
Skotryk	2 wks	1 012	179	330	
Goedendacht	1 d	1 485	127	387 (2)	
Bospan	1 d	1 826 (1)	162	185	
Vlakpan	1 d	250	16	44	
Vogelfontein	3 wks	2 417			
Acacias	1 mo.	33			
		12 738 (12)	2 945 (3)	1 315 (2)	881 (1)

* Months, weeks or days since last RVF case in domestic animals.
† Figures in parentheses indicate number of isolations of RVF virus.

TABLE V. RESULTS OF TRANSMISSION TESTS WITH RVF VIRUS AND 9 SPECIES OF MOSQUITO

Species	Titre of infective feed in logs	Mosquito infection: Period tested*	Mosquito infection: Rate†	Mosquito infection: 50% infection threshold	Transmission: Days tested	Transmission: Test animal	Transmission: Rate‡
Ae. aegypti	5,8	15 - 29	53/76 (70%)	<5,5	14; 28	Mice	3/44 (7%)
Ae. caballus	7,8	10 - 22	18/30 (60%)	6,3	12; 13	Hamster	0/12
	5,6		12/30 (40%)				0/3
	5,0		33/116 (28%)				0/11 } 0/27
	3,6		16/87 (19%)				0/1
Ae. juppi	>8,0	8 - 21	20/30 (67%)	7,0	17	Hamster	0/6
	7,8		25/52 (48%)				1/4
	4,9		25/61 (41%)				0/4 } 1/18 (6%)
	3,6		5/23 (22%)				0/2
Ae. lineatopennis	7,0	12 - 18	11/15 (73%)	6,4	10; 17	Hamster	0/3
	6,2		10/33 (30%)				0/2 } 1/6 (17%)
	5,5		12/37 (32%)				1/1
Cx. quinquefasciatus	4,0	10 - 28	13/71 (25%)	>5,0		See Table VI	
Cx. rubinotus	6,9	9 - 11	11/34 (32%)	>7,0	9; 10	Hamster	0/3
Cx. theileri	8,0	12 - 34	77/78 (99%)	c. 4,0	11; 13	Sheep	6/23 (26%)
	6,4		53/70 (76%)		19	Hamster	2/15 (13%)
	5,3		30/44 (68%)		20	Mice	7/10 (70%)
					21	Hamster	3/19 (16%)
	4,0		16/30 (53%)				
Cx. univittatus	6,5	20 - 21	26/30 (87%)	5,7	19	Mice	2/6 (33%)
	6,0		18/30 (60%)			Hamster	0/8 } 0/11
	5,1		6/30 (20%)			Hamster	0/3
Er. quinquevittatus	8,2	5 - 81	23/31 (74%)	<7,0		See Table VI	
	7,2	11 - 81	31/38 (82%)				

* Days after infective feed that arthropods were tested for infection.
† Numerator = No. mosquitoes infected; denominator = No. tested.
‡ Numerator = No. mosquitoes transmitting; denominator = No. infected feeding.

TABLE VI. RESULTS OF TRANSMISSION ATTEMPTS WITH RVF VIRUS AND 2 MOSQUITO SPECIES IN WHICH MOSQUITOES WERE FED IN GROUPS

*Cx. quinquefasciatus**			*Er. quinquevittatus**		
No. of days after infective feed	Total No. mosquitoes feeding	Proportion of transmission attempts successful†	No. of days after infective feed	Total No. mosquitoes feeding	Proportion of transmission attempts successful†
16	c. 95	1/3	7	8	0/1
24	c. 101	2/3	11	11	0/1
30	c. 62	1/3	19	2	0/2
			33	9	1/3
			39	12	1/3
			47	8	1/3
			54	12	2/4
			57	5	1/2
			61	9	0/4
			68	9	0/4
			75	6	0/4
			82	c. 6	0/4

* Only 25% of Cx. quinquefasciatus but 74 - 82% of Er. quinquevittatus were infected (see Table I).
† In each transmission attempt a different group of mosquitoes fed on a different hamster.

TABLE VII. NUMBER OF PROGENY REARED FROM LOTS OF INFECTED MOSQUITOES FOUND NEGATIVE FOR RVF VIRUS

Species	Titre of infective feed of parental mosquitoes	Infection rates of parental females (%)	No. found negative for virus			
			Eggs	Larvae	Males	Females
Ae. aegypti	5,4 - 6,0	70	c. 500	192	978	1 141
Ae. caballus	4,8 - 7,8	28 - 60		124	6	44
Ae. juppi	3,6 - 7,2	22 - 50			16	65
Ae. kneatopennis	6,0 - 7,0	30 - 73			172	229
Cx. theileri	6,5	76			394	428
Cx. univittatus	6,5	87	15 rafts	203		
Er. quinquevittatus	7,2	82		464	455	366

indicated that *Cx. theileri*, with the lowest infection threshold and transmission rates of from 13 to 26%, is the most efficient vector.

Although *Cx. quinquefasciatus* and *Er. quinquevittatus* transmitted virus on several occasions (Table VI), the mosquitoes were fed in groups and those feeding were not tested individually for infection. A reliable estimate of transmitting efficiency was therefore not possible, but the trends indicated that both species could be vectors of importance. Their infection thresholds appeared fairly low and *Cx. quinquefasciatus* transmitted to 4 out of 9 hamsters after only a moderately high infective feed of 4,6 log. *Er. quinquevittatus* transmitted to 6 out of 15 hamsters from the 33rd to 57th days, although infection doses were high. There were no transmissions before or after this period, indicating an unusually long extrinsic incubation period and a possible loss of transmitting ability with passage of time.

Transovarial and Trans-stadial Transmission

Table VII shows the numbers of specimens of various life stages, reared from infected lots of 7 mosquito species, and tested for virus. Virus was not isolated from any stage. As a high proportion of the parental females probably contained virus the results indicate that RVF virus does not pass transovarially or trans-stadially in mosquitoes.

DISCUSSION

As we had obtained evidence that RVF virus was enzootic in the coastal region of Natal, it was hoped that a long-term survey of biting insects in likely enzootic foci would result in virus isolations and possible identification of a maintenance vector. The virus had been isolated from *Er. quinquevittatus* in forest near Port Shepstone in 1971.[1] Domestic animals were apparently absent from the mosquito-collecting site, suggesting that a wild vertebrate was the source of this infection and thereby indicating the possible existence of a forest enzootic focus. Enzootic infection also seemed likely from surveillance of cattle for immunity at Port Shepstone and Empangeni, which had revealed their sporadic

annual infection.[1] In the initial surveys immune cattle were found in 13 out of the 15 herds sampled, with an overall immune rate of 18% among the total of 708 cattle. Surveillance among marked animals over the next 2 years revealed 8 seroconversions among 58 cattle in 1972 and 11 seroconversions among 244 cattle in 1973. In 1974 RVF virus was also isolated from a cow that died on one farm. During 4 consecutive years, 1971 - 1974, evidence of infection by RVF virus had therefore been obtained in the same areas from which insects were collected.

Included among the mosquitoes tested for infection in Natal were moderate numbers of *Er. quinquevittatus, Mansonia africana, Ma. uniformis* and *Ae. circumluteolus*, mosquito species from which RVF virus had been isolated previously.[1-3] The subgenus *Eumelanomyia*, of which very large numbers were tested for virus, consisted almost exclusively of *Cx. rubinotus*, which had been suspected as a possible maintenance vector because it feeds mainly on wild rodents which have been suggested as natural hosts, and because it is present in both coastal and plateau regions.

We consider the failure to isolate the virus from large numbers of mosquitoes collected in an enzootic area over several years significant in an evaluation of mosquitoes as maintenance vectors. It is also in agreement with observations over many years in Uganda in which isolations from mosquitoes were infrequent and no one species could be identified as a possible maintenance vector.[4] We conclude, therefore, that after many years of observations no definitive evidence has been found to indicate that mosquitoes are involved in the maintenance of RVF virus. Nevertheless, there is substantial evidence that mosquitoes transmit the virus in nature and are epidemiologically important vectors. Isolations of the virus from possible vectors have been made almost exclusively from mosquitoes. Several mosquito species, including some species found infected in nature, have transmitted the virus experimentally, and there is supportive evidence from feeding habits, seasonal prevalence and geographical distribution to incriminate particular mosquito species as vectors among domestic animals in the plateau of South Africa.

Attempts in the present study to isolate the virus on the inland plateau during epizootics demonstrated infection in mosquitoes only, and most isolations came from *Cx. theileri* which had been implicated in earlier studies on the plateau. While this species failed to yield virus on some recently infected farms, this could be explained by low infection rates because of dilution of infected individuals among the large populations of *Cx. theileri* usually present and the relatively small size of the sample tested.

The importance of mosquitoes, and of *Cx. theileri* in particular, was supported by the experimental transmission tests. The virus failed to infect the tick, whereas it infected all 9 mosquito species tested and 7 of these species transmitted the virus by bite. The previous[5] and present series of transmission experiments showed that 4 mosquito species from the plateau fauna, *Cx. theileri, Cx. univittatus, Ae. juppi* and *Ae. lineatopennis*, 4 from the Natal coast, *Ae. aegypti, Er. quinquevittatus, Cx. zombaensis* and *Cx. neavei*, and one from both regions, *Cx. quinquefasciatus*, can transmit the virus, although some of these species are inefficient vectors.

In so far as the present transmission tests can be interpreted quantitatively, which is justified in the case of some species, e.g. *Cx. theileri, Cx. univittatus, Ae. caballus, Ae. juppi* and *Ae. lineatopennis*, *Cx. theileri* emerged as the most efficient vector. Of practical significance was the demonstration that this species transmitted virus to sheep, the epidemiologically important vertebrate host on the plateau. Sheep develop a high viraemia[5] and would therefore infect large numbers of *Cx. theileri*, of which many would be potential vectors. Transmission between sheep of virus mediated by *Cx. theileri* is therefore possible. Since cattle also become highly viraemic,[5] *Cx. theileri* could also transmit virus among these animals. Apart from showing the highest natural infection and experimental transmission rate there are other factors present implicating *Cx. theileri* as the most important vector on the plateau. It occurs in enormous numbers throughout the epizootic area, adult populations persist from early summer to midwinter, and it feeds mainly on sheep and cattle, which have apparently superseded the probable original antelope hosts. As it also feeds readily on man and commonly enters houses, mosquitoes of this species, which acquired virus from sheep or cattle, must also be suspect as a cause of at least some human infection.

RVF virus was isolated from *Ae. juppi* and *Ae. lineatopennis*, and both were also shown experimentally to be vectors. At times these species are extremely prevalent, they feed readily on sheep and cattle, occur widely on the plateau and we believe they are responsible for some transmission. Their lower vector competence and greater dependence on heavy rainfall to produce large numbers, however, indicate a lesser vector role compared with *Cx. theileri*.

In our tests *Ae. caballus* failed to transmit and its implication in earlier studies is now in question because *Ae. juppi*, a newly described species,[10] could have been confused with *Ae. caballus*, since both species coexist over large areas of the plateau including the south-western OFS where the earlier studies were carried out.

The tick *Ornithodoros savignyi* was tested for vector competence as it seemed a possible viral maintenance host because of its habit of living in animal burrows. The failure of the virus to multiply in this tick would exclude such a possibility.

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Epidemiological Aspects of Rift Valley Fever in South Africa with Reference to Vectors

B.M. McIntosh and P.G. Jupp

National Institute for Virology, Private Bag X4, Sandringham, South Africa

On the temperate, inland plateau in South Africa, Rift Valley fever (RVF) has been most evident as an epizootic infection of sheep and cattle, with secondary involvement of man. The epizootic area is extensive, coinciding with the sheep-farming regions; *Culex theileri*, the suspected epizootic vector, is also highly prevalent. It includes the Karoo, the Cape Province north of the Orange River, the southern half of South-West Africa, the Orange Free State Province, and the southern half of the Transvaal Province, excluding the eastern Lowveld region of that province (fig. 1).

The western half of the epizootic area is arid, with an annual precipitation of less than 500 mm, and includes the Panveld of the Orange Free State and northern Cape Province, the Kaap Plateau and the very arid Sandveld in the far west. The habitat in the arid zone consists of a vast plain with scanty, scrub vegetation and little woodland. The soil in Panveld possesses good water-retaining capacity and when heavy rains occur the pans contain water for long periods. Ground burrows which provide shelter for many vertebrate and arthropod species are a prominent feature of the entire arid region. The Panveld and Kaap Plateau experienced the severest outbreaks during widespread epizootics which occurred in 1974 and 1975. The reason for this is not clear, but the prevalence of sheep and *Cx. theileri*, together with the heavy rains in that area, were most likely largely responsible.

The eastern half of the epizootic area is a moist, grassland plain with scattered woodland, known as Highveld. The altitude is relatively high, between 500 to 2,000 m above sea level, and mean temperatures during summer tend to be fairly low. Crop-farming is extensively practiced and sheep and cattle are consequently of relatively less importance.

Antibody surveys among cattle herds in the subtropical, coastal lowlands of Natal, together with the use of sentinel cattle over several years, have indicated that cattle are infected enzootically by RVF virus in that region, and the presence of the virus there has been established by the occasional isolation of the virus from cattle and mosquitoes. Sheep are absent, human infection is minimal, and RVF is of minor economic significance. The habitat in coastal Natal is wooded with patches of evergreen forest and contains many rivers with coastal lagoons fringed with dense vegetation. It has been suggested that outbreaks on the plateau originate from introduction of the virus from the enzootic foci in the coastal region but there is no evidence to support this contention.

Rainfall in the summer rainfall region of South Africa, which includes the epizootic area, has shown a regular quasi 20-year oscillatory pattern since 1910, when regular recording started. This pattern has consisted of a

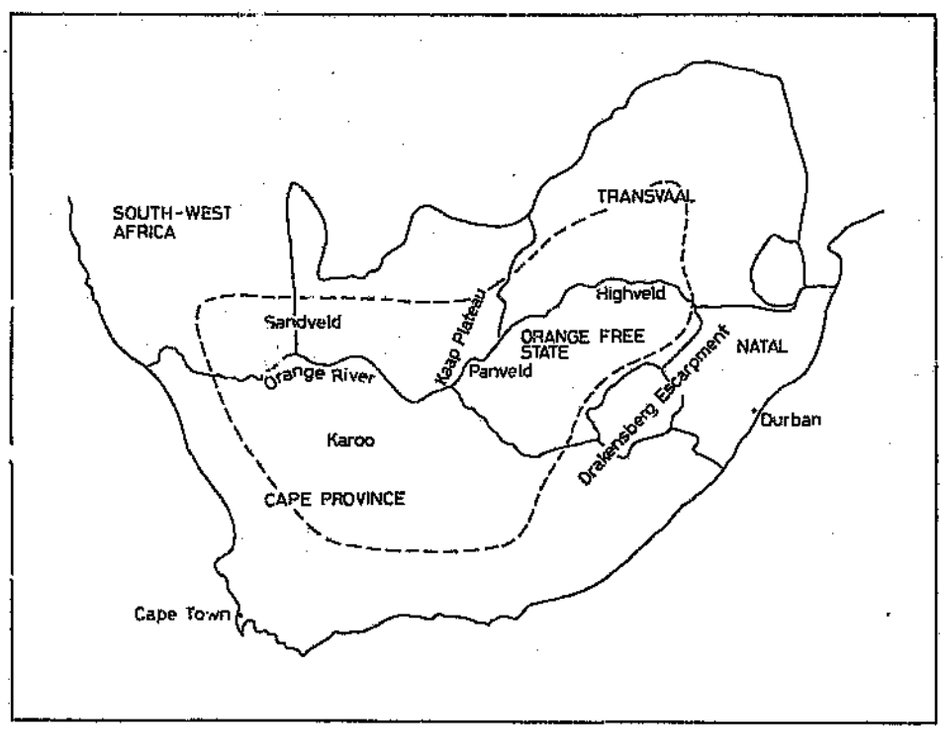

Fig. 1. RVF epizootic area (within broken line) on temperate, plateau region of South Africa.

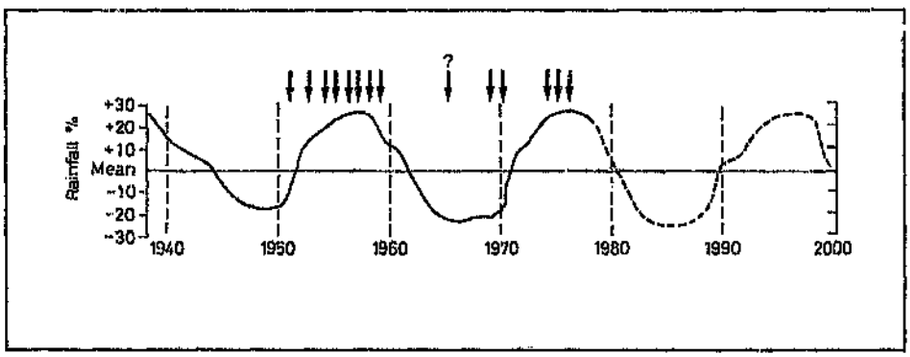

Fig. 2. Rainfall oscillations and outbreaks of RVF (arrows) in South Africa.

decade of above average rainfall, followed by a decade of below average rainfall (7). In figure 2 the occurrence of outbreaks of RVF is shown in relation to the pattern of rainfall since 1938. It can be seen that there is a reasonably good correlation between the moist periods and the occurrence of outbreaks. Mathematical predictions of future rainfall to the year 2000, based on the pattern since 1910 and that shown in figure 2, indicate a high probability of severe outbreaks in the 1990s.

Since 1955, extensive testing of wild populations of mosquitoes for infection by arboviruses has been undertaken in both the epizootic and enzootic areas of South Africa. More recently, these surveys have included other biting Diptera, which have included large numbers of *Culicoides* spp., fair numbers of Simuliidae and small numbers of Phlebotominae. RVF virus has only been isolated from mosquitoes.

In the coastal region of Natal the virus was isolated twice from *Aedes circumluteolus* in 1955 and once from *Eretmapodites quinquevittatus* in 1971 (table I). Between 1971 and 1979 biting flies were tested for viruses in the coast of Natal in localities where cattle were being infected enzootically by RVF virus. Despite tests on 73,543 mosquitoes, 10,514 *Culicoides*, 1,092 Simuliidae and 391 Phlebotominae, no further isolation of RVF virus was made, although there were 279 isolations of eight other arboviruses from the mosquitoes. Although it is difficult to decide in such surveys at which point failure to isolate a virus becomes significant, this low isolation rate is in keeping with similar surveys in Uganda (2, 8) and in Kenya (6) in which isolations of RVF virus were either infrequent from miscellaneous species of mosquitoes or were absent.

Table I. Isolations of RVF virus from mosquitoes in South Africa, 1953 to 1975

Region	Year	Species	No. of insects	No. of isolations	Reference no.
Coastal lowland	1955	*Ae. circumluteolus*	?	2	–[1]
Coastal lowland	1971	*Er. quinquevittatus*	423	1	(3)
Inland plateau	1953	*Ae. caballus*	?	6	(1)
Inland plateau	1953	*Cx. theileri*	?	3	(1)
Inland plateau	1956	*Cx. theileri*	?	2	–[1]
Inland plateau	1970	*Cx. theileri*	1,398	4	(3)
Inland plateau	1970	*Cx. theileri*	144	1	(3)
Inland plateau	1974/75	*Cx. theileri*	12,738	12	(5)
Inland plateau	1974/75	*Ae. juppi*	2,945	3	(5)
Inland plateau	1974/75	*Ae. lineatopennis*	1,315	2	(5)
Inland plateau	1974/75	*An. cinereus*	88	1	(5)
Total isolations				37	

[1] *Smithburn, K.C.; Kokernot, R.H.* (unpublished data).

Attempts to isolate RVF virus from mosquitoes on the South African plateau have been more successful but this was achieved only during outbreaks in domestic ruminants (table I). In 1953, *Gear* et al. (1) isolated the virus six times from *Aedes caballus* and three times from *Cx. theileri.* During the same investigations, wild-caught specimens of *Ae. caballus* transmitted the virus to mice by bite. *Smithburn* and *Kokernot* (unpublished data) made two isolations from *Cx. theileri* in 1956, and *McIntosh* (3) made five from the same species in 1970.

Further isolations were made from unfed *Cx. theileri* and other mosquitoes during the widespread outbreaks in 1974 and 1975 (tables I and II). On infected farms, *Cx. theileri* yielded most isolations although it was found infected on only 4 of the 12 farms, and there were 11 isolations on a single farm. The overall infection rate in this species was 1 infected mosquito per 840 of the population. *Cx. theileri* was present on all infected farms, unlike the two *Aedes* species also found infected on some farms, and it was usually very prevalent.

Attempts to transmit RVF virus in the laboratory were made with the argassid tick, *Ornithodorus savignyi* and 12 mosquito species (4, 5). They were infected by feeding on viremic animals circulating determined quanti-

Table II. Isolations of RVF virus from unfed specimens of 3 mosquito species on 12 infected farms on the plateau, South Africa, 1970–1975

Farm	Elapsed time[1]	Number tested with isolations		
		Cx. theileri	*Ae. juppi*	*Ae. lineatopennis*
Berlin	2 weeks	1,398 (4)[2]		
Olifantsvlei	3 weeks	144 (1)		
Good Hope	3 months	892		
Putfontein	1 month	411		
Fairfield	1 week	3,597 (11)		2
Prospect	1 month	815	2,461 (3)	367
Skatryk	2 weeks	1,012	179	330
Goedendacht	1 day	1,485	127	387 (2)
Bospan	1 day	1,826 (1)	162	185
Vlakpan	1 day	250	16	44
Vogelfontein	5 weeks	2,417		
Acacias	1 month	33		
Total		14,280 (17)	2,945 (3)	1,315 (2)

[1] Months, weeks or days since last RVF case in domestic animals.
[2] Figures in parentheses indicate number of isolations.

ties of virus, held at 26 °C for 2–3 weeks (sometimes longer), then allowed to feed on mice, hamsters or sheep, usually a single mosquito to each animal. The virus failed to infect the tick, nor was this species able to transmit virus by bite to hamsters on the 66th day after attempted infection.

Table III gives a summary of the results of infection and transmission rates obtained in several experiments with 11 mosquito species. All species were infected, some quite readily. Three species did not transmit virus while the remainder transmitted at rather low rates. Of the 11 species, *Cx. zombaensis*, from the coastal zone, appeared the most efficient vector, although the test animals in the transmission attempts were infant mice which apparently increase transmitting efficiency (see table IV). In the tests with *Cx. quinquefasciatus* (fatigans), several mosquitoes fed on each hamster so transmission rates could not be determined, although 4 of the 9 animals bitten became infected.

Table IV shows the results of tests with *Cx. theileri* with the infection and transmission rates obtained in mosquitoes infected at various doses of

Table III. Infection and transmission rates of various South African mosquito species with RVF virus

Species	Titer of infective meal	Infection rate[1]		50% infection threshold	Transmission rate[2]	
Ae. aegypti	5.8[3]	53/76	(70)[4]	< 5.8[3]	3/44	(7)[4]
Ae. caballus	3.6–7.8	79/245	(32)	6.3	0/27	(0)
Ae. circumluteolus	4.8–6.4	18/63	(29)	± 5.5	0/4	(0)
Ae. juppi	3.6–8.0	75/166	(45)	7.0	1/18	(6)
Ae. lineatopennis	5.5–7.0	33/85	(39)	6.4	1/6	(17)
Er. quinquevittatus	5.5–8.2	109/146	(75)	5.5	1/22	(5)
Cx. rubinotus	6.9	11/34	(32)	> 7.0	0/3	(0)
Cx. zombaensis	5.8	18/24	(75)	< 5.8	2/5	(40)
Cx. neavei	6.6–8.5	41/61	(67)	± 7.0	3/22	(14)
Cx. univittatus	5.1–6.5	50/90	(56)	5.7	2/17	(12)
Cx. quinquefasciatus	4.6	18/71	(25)	> 5.0	4/9[5]	

[1] Number of mosquitoes with virus/number of mosquitoes tested.
[2] Number of mosquitoes that transmitted/number of infected mosquitoes that fed.
[3] Titers expressed as $\log_{10}$ per 0.02 ml of mouse inoculum.
[4] Figures in parentheses indicate percentage.
[5] Several *Cx. quinquefasciatus* fed on each animal so transmission rate could not be determined.

Table IV. Infection and transmission rates of *Cx. theileri* with RVF virus

Titer of infective meal	Infection rate[1]	50% infection threshold	Test animal	Transmission rate[2]
4.0	16/30 (53)	± 4.0	–	–
5.3	30/44 (68)		mice	7/10 (70)
5.3	30/44 (68)		hamster	3/19 (16)
6.4	53/70 (76)		hamster	2/15 (13)
8.0	77/78 (99)		sheep	6/23 (26)
8.0–8.5	64/68 (94)		mice	10/18 (55)

[1] Number of mosquitoes with virus/number of mosquitoes tested.
[2] Number of mosquitoes that transmitted/number of mosquitoes that fed.

virus. This species was readily infected even at 4.0 $\log_{10}$ of virus per 0.02 ml and each lot of mosquitoes tested transmitted virus, at high rates to mice and low rates to hamsters and sheep. Of all mosquitoes tested, *Cx. theileri* seemed to be the most efficient vector.

Progeny of the parental females of *Ae. aegypti, Ae. caballus, Ae. juppi, Ae. lineatopennis, Cx. theileri, Cx. univittatus* and *Er. quinquevittatus* used in the above experiments were tested for virus with negative results, suggesting that transovarial passage of RVF virus does not occur in these mosquitoes. Mechanical transmission of virus did not occur when 8 *Cx. theileri* were removed while feeding on a viremic hamster circulating 8.0 $\log_{10}$ of RVF virus and immediately allowed, singly, to complete their meal on other hamsters.

It is apparent therefore that studies in South Africa, conducted in the field and the laboratory, have implicated mosquitoes as biological vectors during outbreaks in domestic ruminants. The studies suggest that *Cx. theileri* is the main epizootic vector; *Ae. juppi, Ae. lineatopennis* and possibly other mosquito species are vectors in a minor capacity. Apart from having shown the highest natural infection rate and experimental transmission rate there are other factors implicating *Cx. theileri.* It is by far the most prevalent and widespread mosquito in the epizootic area, adult populations persist from early summer to midwinter, it is relatively resistant to aridity, and it feeds mainly on sheep and cattle, which have apparently superceded the probable original antelope hosts. As it also feeds readily on man, and commonly enters houses, it must also be suspect as a vector of some human infection.

Although little reliable evidence was obtained in the field to implicate mosquitoes as vectors in the enzootic infection of cattle in the coastal region of Natal, *Ae. circumluteolus, Cx. zombaensis* and *Er. quinquevittatus* seem likely incidental vectors. No evidence was obtained to suggest that mosquitoes are involved in the maintenance of the virus.

Although attempts to transmit the virus mechanically with *Cx. theileri* failed, this form of transmission may well be important in biting flies having other types of mouth parts.

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B.M. McIntosh, DVSc, National Institute for Virology, Private Bag X4, Sandringham 2131 (South Africa)

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Field and Laboratory Evidence Implicating *Culex zombaensis* and *Aedes circumluteolus* as Vectors of Rift Valley Fever Virus in Coastal South Africa

B. M. McIntosh, P. G. Jupp, I. dos Santos and A. C. Rowe

National Institute for Virology, Private Bag X4, Sandringham 2131, South Africa.

During an outbreak of Rift Valley fever in the coastal region of Natal province, 7 isolations of Rift Valley fever virus were obtained from 6621 Culex zombaensis *and one isolation from 1953* Culex neavei. *The outbreak lasted three months, infected about 40% of a herd of 300 cattle, causing 11 abortions and four deaths among adult cattle, and one human infection. At the peak of the outbreak,* Culex zombaensis *was the most prevalent species, comprising 49% of the mosquito catch. Fifteen out of 37 bloodmeals of this species were from cattle. In laboratory tests,* Cx. zombaensis *and* Aedes circumluteolus *were readily infected after feeding on viraemic hamsters circulating high concentrations of virus. At low concentrations* Cx. zombaensis *was more susceptible to infection than the other species. Both species transmitted the virus to hamsters on numerous occasions after ingesting high doses of virus, although an incubation period of more than 14 days was necessary to achieve optimum transmission by* Cx. zombaensis. *It is concluded that* Cx. zombaensis *was the main vector during the outbreak and* Ae. circumluteolus *was possibly a vector during the earlier stages.*

Tydens 'n uitbreking van slenkdalkoors in die kusstreke van Natal is 7 afsonderings van slenkdalkoorsvirus van 6621 Culex zombaensis *en een van 1953* Culex neavei *verkry. Die uitbreking het 3 maande geduur en het omtrent 40% van 'n kudde van 300 beeste besmet wat 11 aborsies en 4 vrektes onder uitgegroeide beeste en een besmetting van 'n mens veroorsaak het.* Culex zombaensis *was die mees verteenwoordigende spesie op die hoogtepunt van die uitbreking, nl. 49% van die muskietvangs. Vyftien van die 37 bloedvoedings van hierdie spesie was van beeste afkomstig. In laboratoriumtoetse is* Culex zombaensis *en* Aedes circumluteolus *maklik besmet na voeding op viremiese hamsters met 'n hoë sirkulasie van viruskonsentrasies. By lae konsentrasies was* Culex zombaensis *vatbaarder vir infeksie as die ander spesie. Albei spesies het die virus verskeie kere oorgedra na die opname van groot dosisse virusse, hoewel 'n inkubasieperiode van minstens 14 dae nodig was om optimale oordrag by* Culex zombaensis *te verkry. Die gevolgtrekking is dat* Culex zombaensis *die belangrikste draer gedurende die uitbreking was en dat* Aedes circumluteolus *moontlik gedurende die vroeër stadium 'n draer was.*

Introduction

Infection of cattle with Rift Valley fever (RVF) virus in the subtropical, coastal region of Natal province in South Africa, is widespread, enzootic, and periodically epizootic. The bovine disease in this region has not been a significant veterinary problem, much of it is clinically unrecognized, while those few outbreaks identified have apparently been localized to individual herds. Furthermore, as could be expected from the low incidence of bovine disease, the disease in humans has rarely been diagnosed. This situation is in marked contrast to the severe, widespread epizootics in sheep and cattle, accompanied by significant secondary involvement of humans, which have intermittently occurred on the temperate, inland plateau over the past 30 years.[1,2]

Field vector studies during the epizootic of 1974 – 75, together with viral transmission experiments in the laboratory, indicated that *Culex theileri* was the main epizootic vector on the plateau.[1] However, this is a scarce species on the Natal coast, and on this account, could not be involved to a significant degree in this region.

Since 1955, as part of a long-term programme of arbovirus surveillance, periodical surveys for infection by these viruses in wild mosquito populations have been carried out on the Natal coast.[1-5] Despite tests on over 400 000 mosquitoes of many species only three isolations of RVF virus have been obtained. These were two isolations from *Aedes circumluteolus* in 195. at Simbu Pan in northern Natal, and a single isolation from *Eretmapodites quinquevittatus* in

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Table 1. RVF immunity survey of cattle and human beings, on the infected farm, April 1982.

		Number positive at indicated HI titres[a]										
	No. tested	Neg.	20	40	80	160	320	640	1280	2560	Deaths	Infection rate
Cattle	246	100	10	18	25	41 (3)[b]	28 (5)	14 (1)	9 (2)	1	4	97/250 (39%)
Humans	33	28		1	3			1				1/33 (3%)

[a]Recently infected animals assumed to have titres of > 80.
[b]Figures in parentheses indicate number of aborting cows with the relevant titres.

1971 at Port Shepstone in southern Natal. Other isolations, both from cattle, of which we have knowledge, include an isolation from an aborted foetus at Empangeni in northern Natal in 1969 and another from a fatal infection in an adult animal in 1974 at Mposa, some 20 km north of Empangeni. Further evidence of RVF virus activity was obtained in 1972 when RVF was diagnosed serologically in a human being who became ill after an autopsy on a cow at Eshowe.

Antibody surveys have also identified human and domestic animal infection in coastal Natal. Smithburn *et al.*[7] found 8 of 138 children (5.8%) and 21 of 145 adults (14.5%) from localities in riverine and pan habitats on the northern coastal plain (Tongaland), positive for neutralizing antibodies. Kokernot *et al.* found 24 of 235 cattle, sheep and goats (10.3%) in the same area to have neutralizing antibodies. Included among the positive reactors was a high proportion of young animals, indicating recent and possible enzootic infection. In the same survey only one of 144 animals on the adjacent Lebombo hills was positive. In 1971 an antibody survey among 708 cattle from 15 herds in the districts of Empangeni and Port Shepstone showed 18% to have antibodies. Positive reactors were present in 13 herds spread over both districts. Surveillance over the following two years of some of these herds, detected 8 seroconversions among 47 cattle in 1972 and 11 seroconversions among 244 cattle in 1973.[1]

Despite this recurring activity by RVF virus, the paucity of isolations from wild-caught mosquitoes has made any firm conclusions regarding the identity of vectors on the coast virtually impossible. On grounds of virus isolation, abundance and host preference, certain species seemed possible vectors and were further evaluated in laboratory transmission experiments.[1,3] In these tests, *Er. quinquevittatus*, *Cx. zombaensis* and *Culex neavei* were readily infected and transmission demonstrated. *Ae. circumluteolus* became infected but transmission was not achieved in a test considered inconclusive, since only four mosquitoes fed during attempted transmission. *Culex rubinotus*, primarily a rodent-feeder, proved relatively refractory to infection and, moreover, failed to yield a single isolation of RVF virus from over 20 000 specimens collected over several years on the Natal coast. Despite this not inconsiderable effort, there was insufficient field evidence to identify vectors: to identify particular species with greater assurance it seemed necessary to obtain multiple isolations from such species during recognized bovine infection.

This evidence was secured recently when several isolations were obtained in November 1981, from *Cx. zombaensis* during an outbreak of RVF in a dairy herd near Mtubatuba, about 50 km north of Empangeni. After these isolations had been made the farm was visited in January and May 1982, after transmission had ceased, to collect adult females of *Cx. zombaensis* and *Ae. circumluteolus* to carry out laboratory transmission tests to determine more precisely the vector capability of these species.

Methods and materials

The area in which the farm lies has a subtropical climate, an altitude of about 100 metres, a mean daily maximum temperature of about 26°C and an average annual rainfall of 1055 mm, of which 67% falls from October to March. In 1981 unseasonally high falls totalling 209 mm occurred in September and October, which were very likely crucial to the occurrence of the outbreak. The natural vegetation on the farm and its environs, like much of coastal Natal, is severely depleted through cultivation of sugar cane and artificial pastures but remnants of indigenous woodland remain along a ravine and as a narrow strip along the Msunduzi river. Ground pools, with associated marshland vegetation, occur along this river, which evidently provide the main aquatic habitats of the predominent species collected.

Mosquitoes were collected overnight from 13 to 20 November in portable light and net traps, both baited with solid carbon dioxide. Light traps and net traps were set for a total of 48 and 22 trap-nights respectively. Mosquitoes were stored at −70°C until identified to species, pooled, ground up, made into suspensions and inoculated intracerebrally into 2–3-day-old mice for attempted virus isolation. Unfed mosquitoes were pooled separately from those containing recognizable ingested blood. Virus isolates were identified by complement fixation; their antigens were prepared by acetone extraction of infected suckling mouse brain. Ascitic fluids obtained from mice hyperimmunized with selected arboviruses were used as a source of viral antibodies.[10] Haemagglutination-inhibition (HI) tests were done by microtitre techniques using acetone-extracted sera and antigens prepared in the same manner as for complement fixation.[10]

Mosquitoes used in transmission experiments were wild-caught females, or their immediate progeny. They were held in the laboratory at 25–26°C and 75–80% relative humidity. They were infected by feeding on viraemic hamsters after inoculation with the AN1830 strain of RVF virus in its fifth intracerebral mouse passage. Viraemic levels were determined by titration in Vero cells or in mice inoculated intracerebrally. Blood was collected immediately before the hamsters were exposed to the mosquitoes. Feeding was usually complete within a half to one hour. The mosquitoes were held for 14 to 36 days, allowed to oviposit, and refed on susceptible hamsters to attempt transmission. With the exception of one experiment, mosquitoes were refed individually on hamsters so that transmission rates could be determined. These are expressed as the ratio or percentage of infected mosquitoes that transmitted. Infection rates were determined by inoculation of mosquitoes individually into suckling mice and this was usually done two days after transmission attempts.

Results

Description of outbreak

About 300 cattle were on the farm during the outbreak, which probably started in September, as the first abortion occurred on 20 September and the cow had an HI antibody titre of 1/640 when tested in April 1982. There were 10 further abortions in which the cows all subsequently showed similar high titre antibodies (Table 1); three in October, six in November and the last on 12 December. RVF virus was isolated from a foetus aborted on 14 November. Four cows died, three during November and the last in mid-December. In three of these fatalities, RVF infection was diagnosed

Table 2. RVF virus isolations, with number of mosquitoes tested for virus during outbreak in cattle, Mtubatuba, 13–20 November 1981.

Genus (subgenus) species	No. pools	No. mosq.	No. isolations
Anopheles (Anopheles) tenebrosus	8	248	
Anopheles (Cellia) pharoensis	3	60	
Anopheles spp. (mostly *tenebrosus*)	20	1 471	
Coquillettidia spp.	3	11	
Mansonia (Mansonioides) uniformis	17	769	
Mansonia (Mansonioides) africana	4	96	
Aedes (Neomelaniconion) circumluteolus	8	267	
Aedes (Neomelaniconion) luteolateralis	6	52	
Aedes (Neomelaniconion) spp.	2	148	
Aedes (Aedimorphus) spp.	17	424	
Aedes spp.	3	10	
Culex (Culex) zombaensis	114	6 621	7
Culex (Culex) neavei	27	1 953	1
Culex (Culex) antennatus	22	1 192	
Culex (Culex) pipiens	4	63	
Culex (Culex) theileri	2	5	
Culex (Culex) spp.	5	64	
Culex (Eumelanomyia) rubinotus	5	78	
	270	13 532	8

histologically from the liver by Dr J. A. W. Coetzer of the Veterinary Research Institute, Onderstepoort. It seems therefore that the outbreak lasted about three months, probably reaching a peak in November, the same month that mosquitoes were collected.

Between 20 and 30 November, the cattle received a single inoculation of inactivated RVF vaccine. In April 1982, 246 cattle and all 33 human beings resident on the farm were bled and their sera tested for RVF HI antibodies. Table 1 shows that 100 cattle were negative for antibodies, 53 positive at titres of 1/20 to 1/80 and 93 positive at titres of 1/160 to 1/2560. The 11 cows that aborted were in the latter category and it is likely that the remaining infected animals were also included in the same group. It seems therefore that the infection rate in the herd was about 40%, certainly not much higher, although it may have been rather lower if a significant number of non-infected vaccinees were included in the group of animals with titres of 1/160 or greater, which seems unlikely. Only one of the humans reacted at high titre and this would appear to be the only infection during the outbreak.

Virus isolations

Collections of mosquitoes on the farm in November 1981, showed that mosquitoes were very abundant and about half of the catch consisted of *Cx. zombaensis*. About two-thirds of the total catch was tested for virus and Table 2 lists the numbers of the various species tested, the number of pools inoculated and the isolations of RVF virus. This shows that of the 13 532 mosquitoes tested in 270 pools half consisted of 6621 specimens of *Cx. zombaensis*, which yielded seven isolations, giving a minimum field infection rate of about 1 per 1000. The only other isolation came from 1953 *Cx. neavei*. All the infected mosquito pools consisted of unfed specimens. Among the *Aedes* tested were two *Neomelaniconion* species, *circumluteolus* and *luteolateralis*, both possible vectors of which only small numbers were obtained, suggesting that both species were scarce at the time collections were made.

Host preferences of Cx. zombaensis

The blood-meals of 37 *Cx. zombaensis*, obtained during the above collections, were tested by Mr D. L. Theron, National Institute for Tropical Diseases, in a precipitin test against bovine and human antisera to identify the host species. Fifteen meals reacted against the bovine and none against the human antiserum. Collections made in goat-baited net traps set on the farm during May 1982, when numbers of *Cx. zombaensis* were much lower than during the outbreak, trapped 72 specimens in 14 trap-nights. Of these, 21 were blood-engorged, 8 gravid and 43 unfed. This gives a feeding rate on the goat of 33%, if the gravid specimens are disregarded.

Laboratory transmission

The results of the tests on the vector capability of *Cx. zombaensis* and *Ae. circumluteolus*, given in Table 3, show that both species

Table 3. Experimental infection of *Cx. zombaensis* and *Ae. circumluteolus* with RVF virus and their transmission of the virus between hamsters.

Species	Virus dose (log_{10}/ml)	Infection rate		Transmission rate	
		Day	Ratio[a] (%)	Day	Ratio[b] (%)
Cx. zombaensis	8.5 – 9.6	16	40/42 (95)	14	1/40 (3)
Cx. zombaensis	7.3 – 9.9	24	37/42 (88)	22	15/37 (41)
Cx. zombaensis	7.3 – 9.9	31	12/22 (55)	29	5/12 (42)
Cx. zombaensis	8.0	28	34/37 (92)		Not done
Cx. zombaensis	5.2	28	17/49 (35)	26	3/17 (18)
Ae. circumluteolus	9.3 – 10.2	16 – 17	27/38 (71)	16	8/23 (35)
Ae. circumluteolus	9.3 – 10.2	27	22/23 (96)	25	7/22 (32)
Ae. circumluteolus	9.3 – 10.2	33	8/8	31	3/8 (38)
Ae. circumluteolus	6.0	30 – 38	0/34	21 – 36	0/13[c]

[a]Number infected/total tested. [b]Number transmitting/number infected feeding. [c]In these transmission experiments hamsters were exposed to groups of mosquitoes: in all, 13 mosquitoes which were not tested for infectivity bit 7 hamsters.

were readily infected at the higher virus doses, but showed either reduced susceptibility (*zombaensis*) or non-susceptibility (*circumluteolus*) at the low dose. Both species transmitted the virus at a moderately high rate after infection at the higher virus doses, although optimum transmission by *Cx. zombaensis* took longer to appear, indicating a rather lengthy extrinsic incubation period. When this had been reached, however, its transmitting ability was marginally superior to that of the other species at the high virus doses and definitely superior at the lower dose. Because of its greater susceptibility to infection and its ability to transmit after ingesting relatively lower virus doses, *Cx. zombaensis* would evidently be a more efficient vector in the field. The usually longer persistence of adult *Culex* populations compared to *Aedes* would also enhance the vector efficiency of this species.

Discussion

The studies clearly indicated that *Cx. zombaensis* was the main vector during the outbreak. The mosquito was the only one to yield multiple isolations of RVF at the height of the outbreak, it was the most prevalent species at that time, it fed on cattle, and transmitted the virus experimentally at an adequate level of efficiency. Although high infecting doses were used in the transmission tests, they were in keeping with viraemic levels often reached in bovine infection and were therefore relevant to transmission in the field. It seems likely therefore that *Cx. zombaensis* was deeply involved in this particular outbreak. Thus this finding identifies a third *Culex* species of the subgenus *Culex* in the role of epizootic vector of RVF virus: the others being *Cx. theileri* on the South African plateau and a member of the *Cx. pipens* complex in Egypt.[1,11]

Some comment is indicated on the possible role of *Cx. zombaensis* in bovine infection generally in coastal Natal, where enzootic bovine infection is evidently the norm, and its possible function in viral maintenance. Since 1955 and prior to the present studies, 5 888 *Cx. zombaensis* (1.4% of the total mosquito catch) from coastal Natal and Mozambique were tested for virus with no isolation of RVF virus. This would tend to absolve this species as a maintenance vector, as the collections were made over a very long period and in several localities, but it would not preclude it as a vector of enzootic bovine infection and occasional epizootic infection in these animals. These roles would be difficult to detect during the intermittent surveys conducted because of the occult nature of enzootic infection and the infrequency of the other in Natal. In the prevailing absence of more field evidence implicating other species, it is reasonable to believe that *Cx. zombaensis* is an important vector of bovine infection in Natal and perhaps elsewhere in tropical Africa. There is supportive ethological and distributional evidence in this regard.

There is good evidence that *Cx. zombaensis* feeds readily on domestic ruminants. In addition to that already mentioned, Paterson *et al.*[12] reported similar findings in this regard in northern Natal. Of 130 blood-meals tested by them, two were from chickens, 19 from human beings, 12 from cattle, 33 from sheep, 17 from bushbuck, and 47 from dogs.

Cx. zombaensis is primarily a tropical species, being absent from the South African temperate plateau. We have found it more prevalent in northern Natal, where it averaged 1.6% of the total catch, than in southern Natal where temperatures are significantly lower, although never before in our studies has it predominated so strongly as in the Mtubatuba collections. Its aquatic habitat is widely distributed in coastal Natal, and it must be regarded as a moderately prevalent and widely distributed species in this region. Its occurrence has been reported from numerous countries in tropical Africa south of the Sahara.

The fact that about 60% of the Mtubatuba herd remained free of infection during an outbreak of three months' duration, probably for most of the time in the presence of high populations of *Cx. zombaensis*, suggests the existence of some factor retarding transmission. One possible factor is the rather long extrinsic incubation period apparently required before *Cx. zombaensis* reaches its optimum transmitting efficiency. Could this be a reason why RVF has failed to develop into the extensive epizootics noted on the South African plateau and in Egypt?

The two isolations of RVF virus from *Ae. circumluteolus* in Natal in 1955, mentioned above, taken together with its ability to transmit the virus as demonstrated in the present transmission tests, indicate that this species, also, must be regarded as a potential vector in Natal. While its numbers were low in November at the peak of the outbreak it is likely that they were high soon after the September rains, as could be expected of an aedine species, in which case it might have had a more positive role in the early stages of the outbreak. It feeds readily on cattle,[12] is locally extremely prevalent in northern Natal,[4] and it seems could have some role in bovine infection in this region.

The low isolation rate of RVF virus from mosquitoes in the absence of infection in domestic ruminants in South Africa and elsewhere in Africa is perplexing in any attempt to explain the persistence of this virus on the basis of mosquito-mediated survival. With the possibility in mind that other insects may be involved in viral maintenance we have attempted to isolate RVF virus from other possible vectors which have included *Culicoides*, Simuliidae and Phlebotominae. Phlebotominae seemed particularly apt in providing an answer to the evident persistence of RVF virus in arid areas in South Africa, and the recent demonstration of serological affinities between RVF and certain phlebotomine-transmitted viruses gave added relevance to phlebotomines as possible maintenance vectors.[13] Efforts in this direction, including tests on over 8000 phlebotomines, have, however, failed to isolate RVF virus.

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Table 5. Amino acid concentrations (mM) in plasma from 3 bile duct ligated pigs compared with plasma from 3 sham-operated pigs.

	Sham-operated	BDL	*P*
Alanine	177 ± 30	250 ± 45	n.s.
Aspartic acid	14 ± 3	69 ± 6	*
Glutamic acid	88 ± 14	296 ± 41	*
Glycine	172 ± 28	317 ± 24	*
Leucine	109 ± 37	165 ± 31	n.s.
Serine	57 ± 8	100 ± 20	*
Taurine	43 ± 9	179 ± 20	*

*$P < 0.05$

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Isolation of Rift Valley Fever Virus from *Aedes* (*Neomelaniconion*) *circumluteolus* and/or *luteolateralis* Collected during an Outbreak in Cattle in the Coastal Region of Natal, South Africa

In a recent issue of this journal[1] we reported 7 isolations of Rift Valley fever (RVF) virus from *Culex zombaensis* and one isolation from *Culex neavei*. These mosquitoes were collected during an outbreak of RVF in November, 1981, on a dairy farm in the coastal region of Natal. *Cx. zombaensis*, which accounted for about half the mosquitoes collected, was shown to be feeding on cattle and to be a moderately efficient laboratory vector of the virus. We thus concluded that this species was the main vector during the outbreak.

Although no virus was isolated from 267 *Ae. circumluteolus*, nor from 148 damaged specimens which consisted mostly of this species collected during the outbreak, laboratory transmission experiments with *Aedes circumluteolus* showed that this species was also a moderately efficient vector and hence a potential epizootic vector.[1] Because of this finding and because of two isolations of RVF virus from this mosquito in Natal in 1955,[2] the balance of the untested *Neomelaniconion* mosquitoes collected during the outbreak were tested for virus, using the same methods as before, except that no attempt was made to separate *Ae. circumluteolus* from *Ae. luteolateralis* because of the high proportion of damaged and specifically unidentifiable specimens. However, it was evident that the mosquito pools consisted mainly of *Ae. circumluteolus*. Out of 695 *Neomelaniconion* mosquitoes tested in 13 pools, there were one isolation of RVF virus and two isolations of Pongola virus. As the pools consisted largely of *Ae. circumluteolus*, it is highly likely that these isolations came from this species.

In our previous article we suggested that *Ae. circumluteolus* might have played a more positive role in the early stages of the outbreak shortly after the September rains, when its population density was probably much higher than during November when the mosquitoes were collected. The isolation of RVF virus from the balance of the *Neomelaniconion* catch lends strong support to this viewpoint.

P. G. JUPP
B. M. McINTOSH
D. L. THOMPSON

Arbovirus Research Unit,
National Institute for Virology, Private Bag X4,
Sandringham, 2131,
South Africa

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14. Dum[illegible]
15. Jacol[illegible]
16. Wadl[illegible] centr[illegible]

of mosquitoes up to 25 minutes after the infective feed but not after 5.5 hours. After shorter intervals, single mosquitoes were also shown to transmit on four occasions. In further experiments, not shown in the table, two *Culex quinquefasciatus* successfully transmitted two minutes after an infective feed on a hamster with a viral titre of 7.3 logs, whereas 8 *Culex theileri* which [illegible] n a hamster [illegible]

3.6

Mech Fever

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TABLE 2. Total number of *B. fusca* and *C. partellus* larvae found with different planting date treatments.

(8 Time intervals × 2 Seasons × 96 = 1536 plants sampled per planting date treatment)

Species	Approximate planting date								Total
	Mid Sept	Beg Oct	Mid Oct	Beg Nov	Mid Nov	Beg Dec	Mid Dec	Beg Jan	
B. fusca	1366	2048	1641	819	152	673	2053	3562	12 314
C. partellus	646	594	135	60	103	89	138	409	2 174
Total	2012	2642	1776	879	255	762	2191	3971	14 488

Mosquito species collected at Richards Bay, Natal. – Over the past decade the development of Richards Bay in northern Natal into an important harbour has led to its increasing urbanization. The high densities of anthropophilic mosquitoes, mainly associated with the reed swamps prevalent in the area, have thus become a concern to the local health authorities. This led to the authors' involvement in the evaluation of suitable mosquito control methods, during which 46 mosquito species were collected. These species, belonging to eight genera and 16 subgenera, are reported on here.

Mosquitoes were collected at Bezani Park, Bay Hall, Meerensee and also at the airport. The methods used were man-baited net-traps and emergence traps. One to three net-traps, each baited with two men, were used for the first three hours after sunset for a total of 81 man-hours from 1st October 1984 to 23rd January 1985. The mosquitoes which had entered each net were collected with aspirators, but because of the extremely large numbers, only the catches made during the first 15 minutes of each hour were identified in full. The emergence traps were the same floating traps previously used at Richards Bay (Appleton, C. C. & Sharp, B. L., 1965. *Journal of the Entomological Society of Southern Africa* 43: 179–184). Seventy traps were set in three reed swamps which were emptied about 5000 times from 6th November 1984 to 22nd May 1985. While only adult female mosquitoes were sampled in the net-traps, both sexes were collected in the emergence traps. The numbers of the 46 species of adult mosquitoes collected, together with percentages of the total catch, are listed in Table 1. The abbreviations used for generic and subgeneric names are those customarily employed. (Reinert, J. F. 1975. *Mosquito Systematics* 7: 105–110.) The 12 commonest species in order of prevalence were *Mansonia uniformis, Ma. africana, Culex neavei, Cx. zombaensis, Anopheles tenebrosus, Coquillettidia microannulata, Mimomyia hispida, Coq chrysosoma, Coq metallica, Cx. theileri, Uranotaenia bilineata* var. *fraseri* and *Cx. thalassius* which are all swamp breeding mosquitoes. The species most frequently entering the net-traps, suggesting they were the most prevalent anthropophilic mosquitoes present, were *Ma. uniformis, Ma. africana, Cx. zombaensis, An. tenebrosus* and *Cx. neavei* in that order.

Previously 10 species of mosquito were collected in man-baited net-traps during a dry period between October 1981 and June 1982 at Richards Bay (Sharp, B. L., Appleton, C. C., Thompson, D. L. & Meenehan, G., *Transactions of the Royal Society of Tropical Medicine and Hygiene* in press), including *Cx. thalassius* which had been recorded in 1975 (McIntosh, B. M., 1975. Mosquitoes as vectors of viruses in Southern Africa.

3.6

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Previously 19 species of mosquito were collected in man-baited net-traps during a dry period between October 1981 and June 1982 at Richards Bay (Sharp, B. L., Appleton, C. C., Thompson, D. L. & Meenehan, G., *Transactions of the Royal Society of Tropical Medicine and Hygiene* in press), including *Cx. thalassius* which had been recorded in 1975 (McIntosh, B. M., 1975. Mosquitoes as vectors of viruses in Southern Africa.

Entomology Memoir No. **43**, Government Printer, Pretoria). Thus the present survey contains 36 species unsampled by Sharp *et al.* which are therefore new records for the locality.

Ma. uniformis and *Ma. africana* appear to be the two commonest anthropophilic species in the area and constitute the most important nuisance mosquitoes. Sharp and co-workers also found *Ma. uniformis* the most prevalent species and Appleton and Sharp (1985) confirmed that *Ma. uniformis* breeds in the swamps. *Cx. thalassius* is widespread in the coastal lakes and river estuaries of Natal, being able to breed in water with a high salinity (Arbovirus Unit, National Institute for Virology, unpublished). In 1966 it reached high densities at Richards Bay after sea water had been pumped into an adjoining swamp during dredging operations (McIntosh, 1975). Although not very prevalent in the present survey it might recur as a pest mosquito in the future if similar dredging operations are resumed.

Since *Aedes (Stegomyia)* species breed in tree-holes or small containers it is unlikely that the two specimens taken in the emergence traps reflect their emergence from the swamp. It is probable that these mosquitoes were resting in the swamp vegetation when they were enclosed by the trap as it was placed on the water. *Stegomyia* mosquitoes do not readily enter net-traps (Jupp, personal communication) and as these species are largely diurnal biters this subgenus was probably not adequately sampled in the catches. For example, *Aedes demeilloni* has previously been collected at Richards Bay (Eckard, unpublished). The presence of man-biting *Ae. aegypti* is noteworthy as this species is a potential vector of dengue virus on the Natal coast and might become infected if this virus enters South Africa. Recently dengue type 1 virus was isolated from a resident returning to Durban from a visit to Saudi Arabia and Pakistan. Recent infections were also strongly suggested on serological grounds in 2 other travellers from Asia (Blackburn, N. K. & Rawal, R. *South African Medical Journal*, in press).

Eretmopodites quinquevittatus has been collected on the Natal South coast where it breeds in natural and artificial containers and bites in the shade during the day (Jupp, personal observation). These habits would preclude the collection of large numbers by the 2 sampling methods used.

Previous collections made at Richards Bay have shown the presence of only *Anopheles merus* among members of the *Anopheles gambiae* complex. Seven additional individuals collected were confirmed electrophoretically as *An. merus* while other individuals possessed 4-banded palps suggesting *An. merus*. This mosquito is regarded as a localized vector of malaria in parts of East Africa although its importance is limited (Gillies & de Meillon, The Anophelines of Africa South of the Sahara, SAIMR, Johannesburg, 1968). In South Africa *An. merus* might be a subsidiary vector in the northern Natal lowlands although high densities would be required to compensate for its low vectorial capacity.

Individuals of the *Anopheles funestus* group collected were probably not *An. funestus* sensu stricto, the important malaria vector which appears to have been absent from Natal and Kwazulu over the last 14 years. This is thought to be due to the insecticidal pressure which has been exerted on a highly anthropophilic mosquito through continual hut spraying with residual insecticides (Eckard, unpublished).

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National Institute for Tropical Diseases, Private Bag X508, Eshowe 3815, and P. G. Jupp, National Institute for Virology, Private Bag X4, Sandringham 2131.

TABLE 1. Numbers of female mosquitoes collected in net-traps (NT) and males and females in emergence traps (ET) at Richards Bay, Natal.

Genera, subgenera and species	NT	ET	Total No.	No. as % of total catch
Aedes Meigen				
1. *(Adm) albocephalus* (Theo.)	8	0	8	
2. *(Adm) argenteopunctatus* (Theo.)		0	1	
3. *(Adm) durbanensis* (Theo.)	19	6	25	
4. *(Adm) vexans* (Meigen)	1	0	1	
5. *(Dic) adersi* (Edwards)	1	0	1	
6. *(Muc) lucianus* Muspratt	b	0	b	
7. *(Neo) circumluteolus* (Theo.)	2	1	3	
8. *(Neo) luteolateralis* (Theo.)	1	0	1	
9. *(Stg) aegypti* (Linnaeus)	7	1	8	
10. *(Stg) subargenteus* Edwards	4	1	5	
Anopheles Meigen				
11. *(Ano) tenebrosus* Dönitz	126	15	141	2.19
12. *(Ano) ziemanni* Grünberg	29	0	29	
13. *(Cel) funestus* group	b	0	b	
14. *(Cel) gambiae* group	1	0	1	
15. *(Cel) marshalli* group	2	0	2	
16. *(Cel) pharoensis* Theo.	b	0	b	
Coquillettidia (Dyar)				
17. *(Coq) aurea/wahlbergi* (Edwards)	12	3	15	
18. *(Coq) chrysosoma* (Edwards)	8	98	106	1.64
19. *(Coq) maculipennis* (Theo.)	0	8	8	
20. *(Coq) metallica* (Theo.)	7	90	97	1.51
21. *(Coq) microannulata* (Theo.)	3	137	140	2.17
22. *Eretmapodites quinquevittatus* Theo.	1	0	1	
Culex Linnaeus				
23. *(Cux) annulioris* Theo.	24	3	27	
24. *(Cux) aurantapex* Edwards	0	1	1	
25. *(Cux) bitaeniorhynchus* Giles	0	1	1	
26. *(Cux) neavei* Theo.	96	286	382	5.93
27. *(Cux) poicilipes* (Theo.)	0	31	31	
28. *(Cux) thalassius* Theo.	39	10	49	
29. *(Cux) theileri* Theo.	54	0	54	
30. *(Cux) zombaensis* Theo.	312	34	346	5.37
31. *(Eum) rima* Theo./*insignis* (Carter)	0	15	15	
32. *(Eum) rubinotus* Theo.	1	3	4	
33. *(Lut) tigripes* DeGrandpré and De Charmoy	0	7	7	

Genera, subgenera and species	NT	ET	Total No.	No. as % of total catch
Mansonia (Blanchard)				
34. *(Mnd) africana* (Theo.)	552	279	831	12,9
35. *(Mnd) uniformis* (Theo.)	3394	497	3891	60,38
Mimomyia Theobald				
36. *(Mim) hispida* (Theo.)	0	119	119	1,85
37. *(Mim) plumosa* (Theo.)	0	14	14	
38. *(Eto) mediolineata* (Theo.)	1	8	9	
Uranotaenia Lynch Arribalzaga				
39. *(Ura) alba* Theo.	b	0	b	
40. *(Ura) alboabdominalis* Theo.	0	6	6	
41. *(Ura) balfouri* Theo.	0	1	1	
42. *(Ura) bilineata* var. *fraseri* Edwards	0	51	51	
43. *(Ura) pallidocephala* Theo.	0	1	1	
44. *(Ura) palmeirimi* De Meillon & Rebelo	0	7	7	
45. *(Pfc) fusca* Theo.	0	1	1	
46. *(Pfc) montana* Ingram & De Meillon	1	2	3	
Total Mosquitoes:			6444	

a = a blank indicates less than 1%
b = present in net but not during the first 15 minutes of each hour.

A comparison of the known life histories of the bethylids *Parasierola* sp. and *Goniozus natalensis* Gordh collected from *Eldana saccharina* Walker (Lepidoptera: Pyralidae) in Uganda and southern Africa respectively. – Evans (1978, *Memoirs of the American Entomological Institute* **27**: 1–332) and Gordh (1984, *Entomology News* **95**: 207–211) regard *Parasierola* and *Perisierola* as synonyms of *Goniozus*. In addition, Gordh (1986, *Journal of the Entomological Society of Southern Africa* **49**: 257–265) suggests that *Parasierola* sp. collected from *E. saccharina* larvae in Uganda (Girling, 1979, *Entomologists Monthly Magazine* **113**: 211) and *G. natalensis* collected from the *E. saccharina* in southern Africa may be the same species. However, Dr. Z. Boucek (British Museum, London), who originally identified the *Parasierola* sp., recently reaffirmed that the bethylids in question were different species.

Table 1 lists the known life-cycle characteristics of both species. Of particular note are the observations that *G. natalensis* and *Parasierola* placed eggs on different regions of the host, and even though its rearing temperature was higher, *G. natalensis* had a longer incubation and larval period than *Parasierola* sp. These differences strongly support the morphological finding that *Parasierola* sp. and *G. natalensis* are not the same species. – D. E. Conlong and D. Y. Graham, SASA Experiment Station, P.O. Mount Edgecombe, Natal, 4300.

TABLE 1: A comparison of *Parasierola* sp. (Girling, 1979) and *G. natalensis*. Unless otherwise stated, figures in brackets are 95% confidence limits.

	Parasierola sp.	*G. natalensis*
Host plants	Graminaceous crops	*Cyperus papyrus*
Rearing temperature	22 ± 4,3 °C	26 ± 2 °C
Larval presentation	Sorghum stems	Sugarcane stems
Mating period	During first 4 days	Upon emergence and anytime thereafter
Length of mating	Less than 1 minute	Less than 1 minute
Pre-oviposition period	8–9 days	2–3 days
Egg position	Dorso-laterally. On 2nd and 3rd thoracic and 1st 3 or 4 abdominal segments	Dorso-laterally. On 3rd to 6th abdominal segments
Egg shape	Elongate ovoid	Elongate ovoid
Incubation period	1 day	2–3 days
Larval period	2 days	13 days (± 1,79)
Larval morphology	Posterior visible part yellow round and smooth, without apparent segmentation	Posterior visible part cream to yellow, more pointed than round, has apparent segmentation on the larger individuals
Cocoon colour	White or brown	White or brown
Pupal period	8–15 days (mean = 10,5 days)	13 days (± 1,79)
Maternal care	For larval and pupal period	For larval period
No. of adults produced per host	6–22 (mean = 11,7)	1–40 (mean = 8 ± 1,44)
Sex ratio of offspring	7 females: 1 male	8 females: 1 male (± 1,16)
Female longevity	With host + honey: 36,8 days	With host + honey 13 (± 1,26) days. Maximum = 28 days
Male longevity	With host + honey: 22,2 days	With host + honey 6,3 (± 1,3) days. Maximum = 11 days

Life cycle of a little-known South African geometer moth, *Callioratis abraxas* Felder (Lepidoptera: Geometridae). – Although this colourful and striking geometrid moth was described by Felder in 1874 (Reise Nov. pl. c, figs 17 and 18 (1874) it was not well represented in collections until the authors reared a long series from eggs in the vicinity of East London. Superficially the adults of this genus resemble Tiger moths (Arctiids) (see Pinhey, E. C. G. 1975, *Moths of Southern Africa*. Tafelberg Press). The females of *abraxas* are diurnal and fly fairly rapidly through the bush, occasionally resting on tree trunks head downwards. Males have neither been seen on the wing during the day nor have any come to our Mercury Vapour Lamps and we assume that they are crepuscular. Females lay eggs readily in captivity but we found the correct foodplant only by offering the newly hatched larvae a sample of almost every plant and tree growing in the vicinity of where the female was caught. Eventually the larvae accepted the leaf of a cycad (*Encephalartos altensteinii* Lehm. family Zamiaceae) and a search of these cycads in Buffalo Pass near East London revealed considerable numbers of egg clusters and small larvae. The following details of their life history are based upon eggs found in October 1984 and the larvae and pupae derived from them.

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Mechanical Transmission of Rift Valley Fever Virus by Mosquitoes

A striking feature of epizootics of Rift Valley fever (RVF) among sheep on the inland plateau of South Africa is the rapidity with which infection sometimes spreads through flocks, causing heavy losses. To account for this high level of infection it seemed that conventional biological transmission by mosquitoes might be augmented by mechanical transmission mediated by various biting flies, probably most efficiently performed through interrupted blood-meals. Our field studies[1-3] have shown only low infection rates in the mosquito vectors during such epizootics, usually not greater than one infected mosquito per 1000 and, furthermore, high vector competence evidently requires high viraemia levels in the vertebrate host.[3,4] We report here the results of laboratory experiments which show that mosquitoes can transmit RVF virus mechanically.

Syrian hamsters, used to infect mosquitoes from laboratory colonies, were inoculated with the AN 1830 strain of RVF virus in its 3rd or 5th intracerebral mouse passage level and were bled for virus titration 30 hours later at peak viraemia. Titrations were done in infant mice and virus concentrations expressed as the log LD_{80} per 1.0 ml. After bleeding, the hamsters were exposed to single or groups of mosquitoes which were permitted to engorge only partially. These mosquitoes were then held for varying intervals at 80% relative humidity and 26°C before being allowed to complete their blood-meals on uninfected hamsters, which died within three days from RVF virus infection if transmission occurred. Some of the transmissions were confirmed as due to RVF virus by passage of the hamster's liver in infant mice.

The results for two experiments with *Aedes aegypti formosus* are shown in Table 1: successful transmission was achieved by groups of mosquitoes up to 25 minutes after the infective feed but not after 5.5 hours. After shorter intervals, single mosquitoes were also shown to transmit on four occasions. In further experiments, not shown in the table, two *Culex quinquefasciatus* successfully transmitted two minutes after an infective feed on a hamster with a viral titre of 7.3 logs, whereas 8 *Culex theileri* which had fed on a hamster with a titre of 9.7 logs failed to transmit 30 minutes later as did single mosquitoes in eight other separate attempts.

Table 1. Mechanical transmission between hamsters by *Aedes aegypti formosus*.

Titre of infecting hamster (logs per ml)	No. of mosquitoes	Interval after infective feed	Transmission
7.3	15	2 min	positive
	14	3 min	positive
	1	3 min	positive
	1	4 min	positive
	1	5 min	positive
	1	5 min	negative
	14	5 min	positive
	14	5 min	positive
	27	6 min	positive
	15	7 min	positive
	15	9 min	positive
	15	25 min	positive
> 10.7	17	5.5 h	negative
	16	24.5 h	negative

These tests indicate that most species of mosquito would probably transmit RVF virus mechanically following interrupted feeds, provided the infecting animal was highly viraemic and the interval between feeds was short. Since interrupted blood-meals probably occur frequently within densely packed flocks, it seems likely that mechanical transmission is an important alternative means of viral transmission during rural epizootics. Other biting flies such as *Culicoides, Stomoxys,* Simulilidae and Tabanidae are also possible vectors of RVF virus.

P. G. JUPP
B. M. McINTOSH
D. L. THOMPSON

Arbovirus Research Unit,
National Institute for Virology,
Private Bag X4,
Sandringham 2131, South Africa.

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3.8

Panveld oviposition sites of floodwater *Aedes* mosquitoes and attempts to detect transovarial transmission of Rift Valley fever virus in South Africa

THOMAS P. GARGAN, II, PETER G. JUPP* and ROBERT J. NOVAK†
Department of Arboviral Entomology, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland, U.S.A.,
*National Institute for Virology, Sandringham, Johannesburg, South Africa,
and †Illinois State Natural History Survey, Champaign, Illinois, U.S.A.

ABSTRACT. Floodwater aedine mosquito eggs were recovered from soil samples taken from grassland depressions, called pans, in the Orange Free State Province of South Africa. A sedge, *Mariscus congestus* (Vahl) C.B.Cl., was a useful indicator of *Aedes* (*Ochlerotatus*) *juppi* McIntosh oviposition areas. No transovarial transmission of virus was demonstrated by *Ae.juppi* females reared from the eggs and allowed to feed shortly after eclosion on hamsters. No virus was recovered from 557 pools of 5425 adult *Ae.juppi* that were collected as eggs and reared to the adult stage in the laboratory. Rift Valley fever virus replicated to high titres in experimentally infected *Ae.juppi* females, but horizontal transmission experiments proved inconclusive.

Key words. *Aedes juppi*, floodwater mosquitoes, South Africa, oviposition habitats, Rift Valley fever virus, *Mariscus congestus*, pans.

Introduction

Alexander (1957) considered that mosquitoes were the reservoir of Rift Valley fever (RVF) virus. He described how mosquito eggs were recovered from the dried mud of a lake in a known RVF area in South Arica, and adults reared from these eggs proved capable of transmitting RVF for a period of 17 days. The mosquito species and collection locality, unfortunately, were not identified.

Linthicum *et al.* (1985) obtained evidence that, in Kenya, RVF is maintained in nature through transovarial transmission of the virus by *Aedes (Neomelaniconion) mcintoshi* Huang, reported as *lineatopennis* (Ludlow) (Huang, 1985). This species, together with other floodwater aedine mosquitoes, breeds in grassland depressions called dambos (Linthicum *et al.*, 1983, 1984). Dambos or vleis are shallow streamless depressions associated with river drainage systems and are subject to seasonal flooding (Mackel, 1974). In South Africa, grassland depressions called pans are also important mosquito breeding areas. Pans are natural, seasonally flooded, shallow depressions with no outlets, and dry up mainly through water evaporation (Geldenhuys, 1982).

The primary objective of this study was to determine if the pans are used as oviposition sites by aedine mosquitoes and, secondarily, to

Correspondence: Dr Thomas P. Gargan, U.S. Army Medical Research Unit (WRAIR) Kenya, Box 401, APO, New York 09675, U.S.A.

determine if mosquitoes found in the pans are transovarially infected with RVF virus.

Materials and Methods

Pans and vleis were identified at the farms 'Prospect' and 'Zeta' (29°16′S, 27°03′E, altitude 5225 ft) near Tweespruit, located in the southeast part of the Orange Free State Province on the Inland Plateau of South Africa. They were sampled for mosquito eggs during the dry winter months of July and August in 1984. Tweespruit has a temperate climate with dry winters and summer rains. Rainfall had been below normal for several years, and the pans in the area had not been flooded since the summer of 1980 (R. Howell, personal communication). These farms were chosen because three isolates of RVF virus were made from female *Aedes (Ochlerotatus) juppi* McIntosh collected there during the 1974–75 epizootic (McIntosh *et al.*, 1980), when numerous sheep and cattle had died from RVF in the area. One pan (No. 1) located on farm Zeta, was studied the most extensively. This pan was mapped with elevation contours, photographed, surveyed and sampled for plant specimens and presence of mosquito eggs. Five other dry pans and two vleis in the Tweespruit area were also sampled for eggs.

Mosquito eggs were extracted from soil samples by the method of Horsfall (1956). Location and collection of the egg-bearing soil followed the method of Novak (1981). The procedure for surveying the spatial distribution of mosquito eggs involved collecting five samples of surface debris and soil (15×15×5 cm) from each plant community. Sampling in a continuous line along a transect was done across elevation contours and through the transition of different plant communities. The samples were screened, the residue was floated in a series of saturated salt and fresh water solutions, scanned microscopically and the eggs were removed. Eggs were placed on moist filter paper in petri dishes and stored at 4°C.

Egg hatching, larval rearing, and viral assays were done in two laboratories: the National Institute for Virology (NIV) in South Africa and the United States Army Medical Research Institute of Infectious Diseases (USAMRIID) in the U.S.A. The eggs were induced to hatch by biotic deoxygenation while confined in small glass tubes (Novak & Shroyer, 1978). Larvae were reared to the adult stage and then identified to species using the keys of McIntosh (1971, 1973, 1975). Voucher specimens were deposited in the United States National Museum of Natural History, Smithsonian Institution, Washington D.C., U.S.A. Female mosquitoes were allowed to feed, 2–3 days after emergence, on Golden hamsters (*Mesocricetus auratus*). Subsequently, adult mosquitoes were separated into pools of ten (USAMRIID) or twenty (NIV) specimens by species, sex and feeding status, then frozen at −70°C until tested for virus.

Mosquito pools were tested for virus either by the plaque assay technique on Vero cells (USAMRIID) or by inoculation into suckling mice (NIV). Hamsters which had been exposed to the mosquitoes were caged individually and observed for clinical signs of illness and mortality, daily for 27 days. Sera obtained from the hamsters were tested for haemagglutinating antibodies against chikungunya, Sindbis, West Nile, Wesselsbron, Middelburg and RVF viruses (NIV), or for RVF antibody by the plaque reduction neutralization test (USAMRIID).

Experimental horizontal transmission of RVF virus by the reared mosquitoes was attempted at the USAMRIID as described by Gargan *et al.* (1983). The procedure involved inoculating hamsters with the Zagazig Hospital 501 strain of RVF virus, and then allowing the mosquitoes to feed on the viraemic hamsters 24 h post-inoculation. The infective dose or the amount of virus ingested by the mosquitoes was determined by collecting three freshly fed mosquitoes and freezing them at −70°C until tested for virus. Titrations were made by plaque assay in Vero cells, and the titres expressed as plaque-forming units (PFU) per ml. All mosquitoes were maintained in a bioclimatic chamber at 26°C, 70–80% r.h., and a 15L:9D photoperiod. Following oviposition, the females were given the opportunity to refeed *individually* on hamsters after 7 and 12 days of extrinsic incubation. The mosquitoes were examined for evidence of blood feeding and then were frozen individually at −70°C until tested for virus. The viral dissemination status of individual mosquitoes was determined by separate assays of bodies and legs (Turell *et al.*, 1984). By definition, mosquitoes with a disseminated infection have virus in both the body and leg samples.

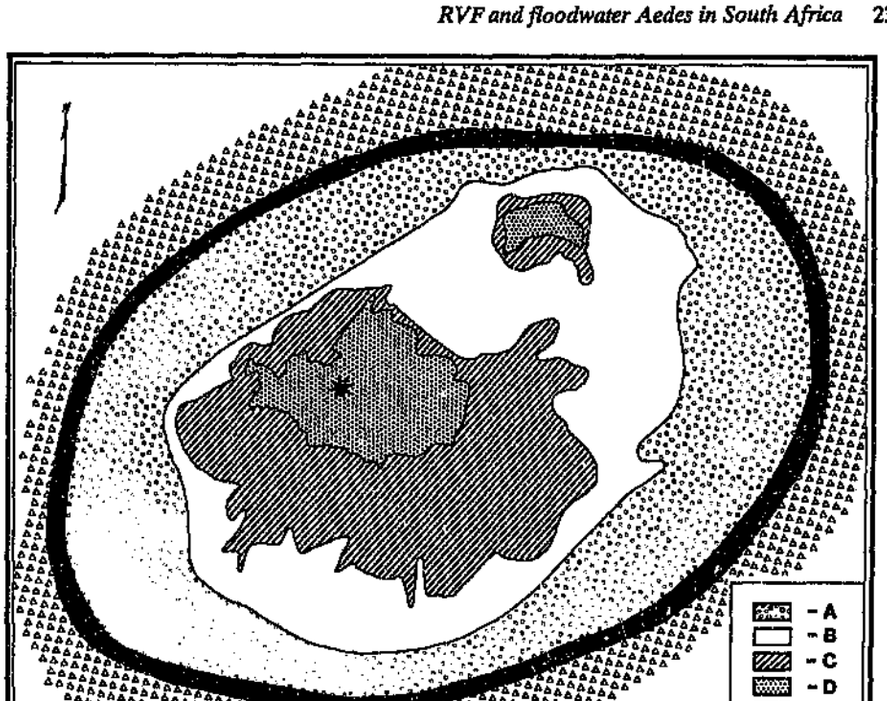

FIG. 1. Vegetation diagram of pan No. 1 located on the farm Zeta near Tweespruit in the Orange Free State Province, Republic of South Africa. Characteristic plants for the zones: A=*Eragrostis planiculmis*, *Bromus unioloides*; B=*E.planiculmis*, *Avena barbata*; C=*A.barbata*, *Mariscus congestus*; D=*M.congestus*; E=weed control area; F=cultivated field.

Results

The dry elliptical-shaped pan (No. 1) was approximately 210 m in diameter and had four major plant zones arranged in concentric circles (Fig. 1). The lowest point in the pan was within zone D and was approximately 60 cm below the outer rim. The various plant species and the number of mosquito eggs collected from each zone are shown in Table 1. The highest density of eggs was found in the low central zone D under the sedge *Mariscus congestus* (Vahl) C.B.Cl. All of the adult mosquitoes reared from these eggs were *Ae.juppi*.

The value of using the sedge *M.congestus* as an indicator of *Ae.juppi* oviposition areas was determined by collecting further soil samples adjacent to this plant in pan No. 1 and five other pans. Mosquito eggs were recovered from each of the sites (Table 2).

The results of egg hatching and larval rearing were similar for both laboratories, so the data were pooled. A hatching rate of 82% (17,002/20,666) was obtained with the eggs removed from all of the pans and vleis. Various methods were used to rear the larvae, but no single method gave consistently good results. The most successful method included continuous aeration with an infusion of either dried Kikuyu grass or dried alfalfa in distilled water with a small quantity of powdered food composed of yeast and either liver or cereal powder. The percentage of larvae that survived to the adult stage was 37% (6249/17,002). All

TABLE 1. Plant species and the number of *Aedes juppi* eggs collected from soil samples taken from the four major plant community zones in pan No. 1.

Zone	Plant species	Plant abundance	No. of soil samples	No. of eggs			
				Viable	Non-viable	Shells	Total
A	*Eragrostis planiculmis* Nees	Dominant grass	35	0	0	0	0
	Bromus unioloides Kunth	Subdominant grass					
	E.curvula (Schrad) Nees	Subdominant grass					
	Panicum schintzii Hack	Subdominant grass					
B	*E.planiculmis*	Dominant grass	10	0	0	0	0
	Avena barbata Brot	Subdominant grass					
	Tragopogon dubius Scopoli	Common herb					
	Chenopodium album L.	Common herb					
C	*A.barbata*	Dominant grass	63	517	279	270	1066
	Mariscus congestus (Vahl) C.B. Cl.	Subdominant sedge					
	T.dubius	Common herb					
	C.album	Common herb					
D	*M.congestus*	Sedge, only plant in this zone	88	3082	516	796	4394
Total			196	3599	795	1066	5460

TABLE 2. Number of *Aedes juppi* eggs collected from six South African pans when the sedge *Mariscus congestus* was used to determine the sampling sites.

	Pan No.					
	1	2	3	4	5	9
Total no. eggs	18,693	6	435	2045	300	6
Total no. samples	175	1	1	22	1	10
No. samples with eggs	157	1	1	18	1	3
No. samples without eggs	18	0	0	4	0	7

emergent adults were *Ae.juppi* except twenty-one individuals (0.3%) which were *Ae. (Neo.) unidentatus* McIntosh.

The majority (77%, 2295/2995) of the mosquitoes involved in the virus transmission experiments fed on hamsters, but neither laboratory was able to demonstrate transovarial transmission of viruses by *Ae.juppi* females fed shortly after eclosion on a total of fifty susceptible hamsters. None of these hamsters died nor were any antibodies to chikugunya, Sindbis, West Nile, Wesselsbron, Middelburg, or RVF viruses detected subsequently in the hamster sera.

No viruses were recovered from the 557 pools of 5425 adult *Ae.juppi*. At the NIV, 413 pools of twenty mosquitoes composed of 1642 male, 1848 fed female, and 676 unfed females were tested for virus by mouse inoculation. At the USAMRIID, 144 pools of ten mosquitoes composed of 788 male, 447 fed female, and twenty-four unfed female mosquitoes were tested for virus by the plaque assay technique.

RVF virus replicated and produced disseminated infections in *Ae.juppi* fed on viraemic hamsters. The results of the experimental transmission experiments, however, were inconclusive because none of the females with a disseminated viral infection re-fed a second time. Due to poor adult survival, only a single transmission attempt was made with twelve females on day-7 extrinsic incubation. The initial infective dose these mosquitoes received was $10^{7.3 \pm 0.01}$ PFU. The infection rate was 100% and the dissemination rate was 50%. Only 25% (3/12) of the females re-fed on the hamsters during the transmission attempt, and while these mosquitoes were infected with virus ($10^{4.1}$, $10^{4.3}$,

$10^{4.3}$ PFU), none of them had a disseminated viral infection.

Discussion

Apart from the unconfirmed report of Alexander (1957), to our knowledge this is the first time in Africa that aedine mosquito eggs have been collected from soil samples and subsequently reared to the adult stage. The sedge *M. congestus* proved to be an excellent indicator of *Ae. juppi* oviposition sites, and this information should assist future studies on the bionomics of this species. However, our poor yield of adult mosquitoes reared from viable eggs of *Ae.juppi* indicated that improved rearing methods need to be developed for this and other similar floodwater *Aedes* species. In similar studies in midwestern U.S.A., Novak (1981) found that certain species of plants were good indicators of oviposition sites for *Aedes vexans* (Meigen).

No virus was recovered from *Ae.juppi* and no viral transmission demonstrated to the hamsters. Possible explanations of this failure to show transovarially infected mosquitoes are either that the virus was missed, or that the numbers of mosquitoes tested may have been inadequate to include any infected specimens, because of very low filial infection rates or extremely focal distribution of infected eggs, or that *Ae.juppi* may not be involved as an enzootic maintenance vector of RVF virus in South Africa. One month following the 1974–75 RVF epizootic in the Tweespruit area the minimum field infection rate of RVF virus from wild-caught *Ae.juppi* was 1/820 (McIntosh *et al.*, 1980); RVF isolates obtained probably represented mosquitoes that became infected by feeding on viraemic sheep and cattle. In Kenya, transovarial transmission of RVF virus has been reported for *Ae.mcintoshi* (Linthicum *et al.*, 1985). It may be that the enzootic cycle of RVF in Africa involves subgenus *Neomelaniconion* species (e.g. *Ae. mcintoshi*, *Ae.unidentatus*, *Ae.circumluteolus* (Theobald)) and not subgenus *Ochlerotatus* species (e.g. *Ae.juppi* and *Ae.caballus* (Theobald)). More field data are required to resolve this important question.

Laboratory experiments showed that RVF virus replicated to high titres and produced disseminated infections in female *Ae.juppi* by day-7 extrinsic incubation. Laboratory transmission experiments reported by McIntosh *et al.* (1980) showed that this species transmitted RVF virus to hamsters at a rate of 6% (1/18). Inconclusive results were, however, obtained on the vector capabilities of this species, for none of the females with a disseminated infection re-fed in our transmission experiment.

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In conducting the research described in this report, the investigators adhered to the *Guide for the Care and Use of Laboratory Animals*, as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accrediation of Laboratory Animal Care.

The views of the authors do not purport to reflect the position of the U.S. Department of the Army or the Department of Defense.

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Table 2. Average percent cumulative mortality and percent inhibition of emergence (EI) of *Anopheles pharoensis* larval and pupal isolates from Dimilin-treated ditches (15.2 AI/acre) in Gezira, Sudan.

Ditch	Larval isolates*				Pupal isolates*		
	Larvae	Pupae	Adults	(EI%)	Pupae	Adults	(EI%)
1	20	0	0	100	20	2	90
2	20	2	1	95	20	3	85
3 (control)	20	19	17	15	20	18	10

* Based upon the average of 6 isolates, each containing 20 larvae or pupae in a cup.

Mortality of the surviving larvae and pupae isolated from the field was high. Additional mortality occurred in subsequent stages producing a very high overall percent emergence inhibition (Table 2). Similar findings were reported by Mulla et al. (1974), Mulla and Darwazeh (1975) and Schaefer et al. (1975) using Dimilin against various mosquito species.

Thus, Dimilin offers good potential for the control of various species of mosquitoes, showing a varying degree of efficacy against different species breeding in diverse habits. This compound exhibits little or no hazard to nontarget organisms in mosquito breeding sources (Miura and Takahashi 1975, Mulla et al. 1975, Steelman et al. 1975) and has the potential for rendering control of immature mosquitoes for 10 days at very low practical rates.

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THE USE OF MEPACRINE HYDROCHLORIDE TO CONTROL *VORTICELLA* ON MOSQUITO LARVAE

P. G. JUPP[1] AND A. N. SMITH[1]

One of our recent projects entailed the recovery of eggs of the flood-water mosquito *Aedes juppi* McIntosh from soil samples collected in the Orange Free State province, South Africa, and their subsequent rearing to adults. However, only 34% of the viable eggs which hatched were reared successfully to adulthood owing to mortality of the 3rd and 4th instar larvae. Examination of dying and recently dead larvae revealed a severe infestation with the ciliate *Vorticella* which appeared to be the main cause of death. No fungi or other parasitic Protozoa were seen at this time on the larvae and the possible existence of pathogenic bacteria or viruses was not investigated. It was therefore decided to carry out a series of experiments to find a chemical which could be incorporated in the water in the larval rearing trays to kill off the *Vorticella* encrustration without harming the larvae themselves. Metronidazole and chloroquine were each

[1] National Institute for Virology, Private Bag X4, Sandringham 2131, Johannesburg, and Department of Virology, University of the Witwatersrand, Johannesburg, South Africa.

tried unsuccessfully but another chemical, the anti-malarial mepacrine hydrochloride 4-(3-Chloro-7-methoxy acridin-9-ylamino)-NN-diethylpentylamine dihydrochloride dihydrate, gave promising results. It was thought likely that this would kill ciliates as it is known to kill both sporozoan and flagellate Protozoa (Bowman and Rand 1980). This article describes the results of these tests.

Mepacrine hydrochloride was dissolved in distilled water so as to provide a series of 5 concentrations from 0.0009 to 0.0045%. Infested 3rd or 4th instar larvae of *Ae. juppi*, recently dead, were placed into each of the 5 solutions. The lowest concentration at which all the *Vorticella* died was 0.0018% and the time required 21 hr. The discovery of *Vorticella* on larvae of *Ae. juppi* prompted us to examine our healthy colonies of *Culex perexiguus* Theobald and *Culex univittatus* Theobald and *Vorticella* was found on dead larvae but not on any living larvae of the 2 species. The vorticellids on these 2 mosquitoes appeared to differ morphologically and that infesting *Cx. perexiguus* also differed from the vorticellid infesting *Ae. juppi*. This may explain why less than 4 hr exposure to 0.0018% mepacrine hydrochloride was needed to kill the *Vorticella* on *Cx. perexiguus* larvae but 21.5 hr for the *Vorticella* species on *Cx. univittatus* larvae. This suggests that the *Vorticella* species on *Cx. univittatus* may have been the same as that infesting *Ae. juppi*. When dead infested *Ae. juppi* larvae were mixed with live uninfested *Cx. univittatus* 2nd instar larvae and cultured together for 2 weeks, the *Vorticella* did not spread to the *Cx. univittatus* larvae while they were alive. However, if a *Cx. univittatus* larva died from another cause, after death it swiftly became infested with *Vorticella*.

Having established the minimum concentration of mepacrine hydrochloride and the length of exposure to kill the *Vorticella*, experiments were undertaken to ascertain whether the same concentration would be detrimental to the larva itself. Since at the time of the tests no live *Ae. juppi* were available, experiments were performed using other species of mosquitoes from laboratory colonies. Exposure of mature larvae of *Cx. univittatus* for up to 8 days to mepacrine hydrochloride at concentrations greater than 0.0018% caused a 54% mortality mainly in the subsequent pupal and adult stages. The mortality rate at 0.0018% was not established although it was lower than 50%. When eggs of *Aedes aegypti formosus* (Walker) were hatched using the technique of Novak and Shroyer (1978), except that the hatching tubes contained 0.1% nutrient broth made up in 0.0018% mepacrine hydrochloride, 3 days later 10–85% of the young larvae were found dead. When the rearing of the surviving larvae was continued in dishes of mepacrine hydrochloride, 6–25% of them failed to become adults. If eggs of *Ae. aegypti formosus* were hatched in the same way but the young larvae were transferred on the first and second days to distilled water and fed in the usual way, about 87% of these larvae reached adulthood.

In conclusion, there are two possible procedures for using mepacrine hydrochloride to control *Vorticella* infestations. In the first, eggs are hatched in 0.0018% mepacrine hydrochloride made up in 0.1% nutrient broth and the first instar larvae are transferred to clean water a minimum of 21 hr later. The second procedure is to place 2nd, 3rd and 4th instar infested larvae into mepacrine hydrochloride solution for at least 21 hr and afterwards transfer them to clean water in rearing dishes.

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EVALUATION OF A SUSTAINED RELEASE FORMULATION OF *BACILLUS THURINGIENSIS* (H-14) FOR CONTROL OF WOODLAND *CULEX* MOSQUITOES[1]

C. S. APPERSON[2], E. E. POWELL[2] AND F. VAN ESSEN[3,4]

Bacillus thuringiensis var. *israelensis (B.t.i.)*, or *B. thuringiensis* (H-14) as designated by de Barjac (1978), is a bacterial agent effective against several nematocerous dipteran families, including mosquitoes (Culicidae) and blackflies (Simuliidae). During sporogenesis of the bacterium, a proteinaceous crystal or parasporal body is formed. This crystal contains a delta-endotoxin which is responsible for most of the

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[2] Department of Entomology. Box 7613, N. C. State University, Raleigh, NC, 27695–7613.

[3] Biochem Products, 3014 NW 41st Street, Gainesville, FL, 32606.

[4] Present address: Collier Mosquito Control District, P. O. Box 7069, Naples, FL, 33941.

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

P. G. JUPP AND A. J. CORNEL

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

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] $Log_{10}LD_{50}$/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	*A. 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*.