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Rift Valley fever virus (family Bunyaviridae, genus *Phlebovirus*). Isolations from Diptera collected during an inter-epizootic period in Kenya

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SUMMARY

A total of 134876 Diptera collected in Kenya during a 3-year period were tested in 3383 pools for Rift Valley fever (RVF) virus. Nineteen pools of unengorged mosquitoes were found positive for RVF. All isolations were made from specimens collected at or near the naturally or artificially flooded grassland depressions that serve as the developmental sites for the immature stages of many mosquito species. The isolation of virus from adult male and female *A. lineatopennis* which had been reared from field-collected larvae and pupae suggests that transovarial transmission of the virus occurs in this species.

INTRODUCTION

Little overt Rift Valley fever (RVF) virus activity appears to occur in Kenya during the inter-epizootic periods (IEP). Some seroconversions have been detected in cattle during an IEP at higher-rainfall forest-edge situations, where epizootic RVF has also been encountered (Davies, 1975). The key factor determining whether there are seroconversions in these areas is now thought to be the rainfall (Davies, Linthicum & James, 1985). No seroconversion to RVF virus was detected over a 10-year IEP in sentinel cattle in the bushed and wooded grasslands, which characteristically are where epizootics are seen in cattle and sheep (Davies, 1975; F. G. Davies, unpublished observations). The occurrence of epizootics is primarily a function of an increased and prolonged rainfall throughout the epizootic areas of Kenya (Davies, Linthicum & James, 1985).

Several areas known to have been involved during recent RVF outbreaks were regularly visited and mosquitoes and other Diptera trapped throughout the year, in particular after the biannual seasonal rains. These areas were in ecological zone II in the classification of East African habitat (Pratt, Greenway & Gwynne, 1966), which is of comparatively high rainfall forest-edge or natural and forest-derived

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grasslands; or in the bushed and wooded grasslands of zones III and IV. Collection efforts were directed to determine those mosquito species present in the epizootic areas, to study their population biology in relation to the seasonal rains and their feeding preferences, and also to attempt to isolate RVF virus. Much of this work has been published (Linthicum *et al.* 1983, 1984, 1985; Linthicum, Davies & Kairo, 1984). This paper records the results of virus isolation attempts from Diptera trapped in a 3-year period from October 1981 to October 1984. One study area experienced a period of heavy and prolonged rainfall in November and December 1982 similar to that seen in the early stages of an RVF epizootic, as defined by Davies, Linthicum & James (1985).

MATERIALS AND METHODS

Collection sites

The sites at which mosquitoes have been collected are described on the basis of the classification of Kenyan eco-climatic zones (Pratt, Greenway & Gwynne, 1966). These are basically defined in terms of climate and described by reference to their vegetation.

Ecological zone II. Equatorial climates which have humid to dry sub-humid characteristics (moisture index not less than −10). This includes the coastal and high-altitude forests, grasslands and bushlands with natural or man-made clearings. The natural or derived grasslands of this zone have the greatest potential for intensive livestock production. Collections in ecological zone II were made at the Kenya Coast–Diani (4° 22′ S, 39° 34′ E; 15 m), Karen (1° 20′ S, 36° 44′ E; 1780 m), Karura (1° 14′ 30″ S, 36° 50′ E; 1700 m) and Kakamega (0° 15′ N, 34° 52′ E; 1560 m).

Ecological zone III. Dry sub-humid to semi-arid areas (moisture index −10 to −30) which have moist woodlands and bushed grasslands without forest. Typically these are *Combretum* spp. or other evergreen trees and shrubs. The potential for livestock farming is high. Collections in ecological zone III were made at Sukari Ranch (1° 12′ S, 37° E; 1500 m).

Ecological zone IV. Semi-arid (moisture index −30 to −42) with dry areas with *Acacia–Themada* bush in the dry grasslands, which have been altered in some areas by encroachment of semi-evergreen deciduous bushland. This is good rangeland for livestock production and carries a large wildlife population. Collections in ecological zone IV were made at Naivasha (0° 35′ S, 36° 20′ E; 1980 m).

Dambos. Many of the immature specimens tested in this study were collected from geomorphic structures known as dambos (Ackermann, 1936; Mackel, 1974). Dambos are shallow streamless depressions that can be seasonally waterlogged and are grass-covered. In the Nairobi area they are susceptible to flooding only after periods of heavy and prolonged rainfall which elevate the water table. Dambos serve as ideal habitats for ground-pool breeding *Aedes* spp. to lay their drought-resistant eggs.

Collection methods

Adult specimens were collected in 281 light-trap (1405 trap nights), 77 human-bait and 24 sweeping-net collections. Solid-state army miniature light traps were

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suspended 50–80 cm above the ground from 16.30 to 08.30 h and baited with CO_2 (2 kg dry ice). Collections at human bait were made at ground level from 06.00 to 09.00 h and from 17.00 to 20.00 h with a mechanical aspirator as specimens landed and started to probe. Sweeping collections were made during the day in the vicinity of the dambo. Immature stages of mosquitoes were collected in 59 larval and pupal collections from dambos, ground pools and large artificial containers. Collections from treeholes, rockholes and small artificial containers were made with a suction apparatus which removed all water in the container. Collections were concentrated at the Karen, Karura and Sukari Ranch sites because of their previous RVF epizootic history and their proximity to the laboratory in Nairobi.

Artificial flooding of dambo. A dambo on Sukari Ranch was artificially flooded to simulate heavy and prolonged rainfall, and to produce a hatch of *Aedes* spp. that had not been exposed to RVF viraemic hosts. On 4 January 1984 an 1800 m^2 area of standing water was established and maintained by pumping into it approximately 1×10^7 l of water from a nearby river during a continuous 18-day period. *Aedes* spp. specimens were collected as pupae on 16–21 January, 1984.

Specimen handling and identification

Adult specimens were placed in dry ice or liquid nitrogen in the field and taken to the laboratory. The frozen specimens were identified, sorted and pooled according to species and sex at temperatures below 0 °C. Specimens which had recently taken a blood meal were placed in separate pools. Immature mosquito specimens were placed in 2500 cm^3 plastic containers in the field and transported alive to the laboratory for identification, sorting and pooling according to species and stage. Larvae and pupae were placed in sterile plastic pans and reared to adults in holding cages (1 cu. ft). Newly emerged adults were removed from the cage and killed by freezing at -70 °C and sorted and pooled on chill tables. The pools were prepared from 50 adult or immature specimens, when available; however, on one occasion 600 larval specimens were processed in five pools. The pools were stored at either $-15°$ or -70 °C awaiting processing for virus isolation.

At least one voucher specimen was removed from each adult pool and mounted either on paper points (Culicidae, Ephydridae) or on slides in Euparal® (Ceratopogonidae, Psychodidae). Adult male genitalia of the Culicidae were removed and mounted in Euparal®. One to 5 voucher specimens were taken from mosquito larval and pupal pools and stored in 80% alcohol in vials, and 2–10 voucher specimens were reared to adult. Associated larval and pupal skins, and whole larvae and pupae were later mounted in Euparal®. Reared voucher adults were mounted on paper points.

Culicidae were identified to species using various reference keys (Edwards, 1941; Gillies & de Meillon, 1968; Hopkins, 1952; Mattingly, 1971; Tyson, 1970). Ceratopogonidae and Psychodidae were identified using the reference keys of Khamala & Kettle (1971) and Abonnene (1972), respectively. The Ephydridae were kindly identified by Dr Wayne Mathis, Department of Entomology, U.S. National Museum, Washington, D.C.

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Preparation of mosquito pools for virus isolation

The mosquitoes were triturated in sterile chilled ten Broeck tissue grinders. Single specimens were prepared in 0·5 ml volumes and larger pools in 2·5–3 ml volumes of Hanks' basic salt solution buffered with bicarbonate to pH 7·5, containing 0·75 % bovine serum albumin or 5 % of a foetal bovine or horse serum, 400 i.u. penicillin and 400 μg streptomycin sulphate per ml. The suspensions were centrifuged at 800 *g* for 10 min and the supernate used as the inoculum.

Virus isolation

Two Syrian hamsters (*Mesocricetus auratus*) were inoculated intraperitoneally with 0·1 ml of supernate from each pool. These inoculations were carried out within 1–2 h of the preparation of the specimens. They were than stored either at −70° or −15 °C according to the availability of freezers. Hamsters were examined three or four times daily after inoculation and removed when dead or moribund. Portions of the livers from dead hamsters were removed aseptically and homogenized to give a 10% suspension in the transport medium. These samples were filtered through 0·22 nm Millipore filters and re-inoculated into two additional hamsters at 10^{-3} dilutions. If these hamsters died, the second liver passage material (HL_2) was harvested and used as the inoculum for virus identification.

Virus identification

The method of virus identification has been described by Davies (1975). Baby hamster kidney (BHK21 C13) cell cultures were inoculated with 10^{-3} and 10^{-5} dilutions of the HL_2. The cultures containing flying coverslips were harvested at 18–30 h post inoculation. They were fixed in acetone and stained by a fluorescent antibody technique with a direct RVF conjugate prepared from hyperimmune horse serum. The various strains were further examined by a microserum neutralization test with a sheep RVF immune serum prepared against the Kabete strain of virus. This was carried out in Vero cell cultures against 100 $TCID_{50}$ of each of the field isolates.

RESULTS

The Diptera tested for RVF virus in Kenya from October 1981 to October 1984 are shown in Table 1. More than 134000 specimens, representing 4 families, were processed. The 73 species of Culicidae comprised 80% (108026 specimens) of the specimens tested. Nineteen pools of unengorged mosquitoes were found positive for RVF in the 3383 pools tested. The virus isolations are summarized in Table 2. Virus was isolated from nine pools of *Aedes lineatopennis* (Ludlow), one pool of *A. cumminsii* (Theobold), three pools of *Culex antennatus* (Becker), one pool of *C. rubinotus* Theobald, one pool of *C. vansomerini* Edwards, one pool of *C. zombaensis* Theobald, two pools of *Anopheles christyi* (Newstead and Carter) and one pool of *An. pharoensis* Theobald. Thirteen of the isolations came from pools of adult females and six from specimens of *A. lineatopennis* collected as pupae and reared to the adult stage.

The periods during which the isolations were made are illustrated in Fig. 1. All isolations were made from specimens collected at or near dambos in zones II and

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Table 1. *Diptera tested for Rift Valley fever virus in Kenya from October 1981 to October 1984*

Species	Ecological zone*	Specimens tested†	Pools tested	Pools positive
(Diptera, Culicidae)				
Aedes (*Aedimorphus*)				
calignosus (Graham)	II	2 (F 2)	1	—
capensis Edwards	II	82 (F 82)	11	—
cumminsii (Theobald)	II, III	2997 (M 11, F 1346, P 95, L 492, MR 181, FR 872)	116	1
dentatus (Theobald)	II, III	3238 (M 37, F 3201)	180	—
fowleri (Charmoy)	III	1 (F 1)	1	—
gibbinsi Edwards	II	8 (F 8)	2	—
hirsutus (Theobald)	III	11 (F 11)	2	—
holocinctus Edwards	II	177 (F 177)	4	—
lamborni Edwards	II	26 (M 1, F 25)	2	—
phyllolabis Edwards	II	20 (F 20)	2	—
quasiunivittatus (Theobald)	II, III, IV	5195 (M 47, F 4448, L 700)	209	—
tarsalis (Newstead)	II	2 (F 2)	1	—
tricholabis Edwards	II	145 (F 145)	5	—
vittatus (Bigot)	III	1242 (L 1242)	52	—
spp.‡	II, III	1168 (M 44, F 1084, FR 40)	90	—
(*Finlaya*)				
fulgens (Edwards)	II	391 (L 391)	15	—
ingrami Edwards	II	1 (F 1)	1	—
(*Mucidus*)				
sudanensis (Theobald)	II, III	194 (M 2, F 159, P 24, L 9)	34	—
(*Neomelaniconion*)				
albothorax (Theobald)	II	5 (F 5)	3	—
circumluteolus (Theobald)	II, III	140 (F 140)	7	—
lineatopennis (Ludlow)	II, III	59644 (M 49, F 27800, P 813, L 21681, MR 2880 FR 6421)	563	9
(*Ochlerotatus*)				
caballus (Theobald)	II	3 (F 3)	1	—
fryeri (Theobald)	II	5 (F 5)	2	—
(*Pseudarmigeres*) *natalensis* Edwards	II	50 (L 50)	1	—
(*Stegomyia*)				
aegypti (Linnaeus)	II	764 (M 1, F 19, L 744)	28	—
calceatus Edwards	II	89 (FR 5, P 5, L 79)	13	—
deboeri Edwards	II, III	429 (M 3, F 421, MR 5)	65	—
demeilloni Edwards	II	5 (F 5)	1	—
Anopheles (*Anopheles*) *coustani* Laveran group	II, III	746 (M 1, F 745)	34	—
implexus (Theobald)	II	4 (F 4)	4	—
(*Cellia*) christyi (Newstead and Carter)	II, III	827 (M 1, F 826)	78	2
demeilloni Evans	II	131 (F 131)	14	—
gambiae Giles s.l.	III	99 (F 99)	4	—
gibbinsi Evans	II	1 (F 1)	1	—
harperi Evans	II	1 (F 1)	1	—
keniensis Evans	II	9 (F 9)	4	—
natalensis (Hill and Haydon)	II	560 (M 2, F 558)	25	—
pharoensis Theobald	III	1 (F 1)	1	1
wellcomei Theobald	III	4 (F 4)	1	—
spp.	II, III	1126 (M 3, F 1123)	79	—

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Table 1 (*cont.*)

Species	Ecological zone*	Specimens tested†	Pools tested	Pools positive
Coquillettidia (*Coquillettidia*)				
aurea (Edwards)	II, III	236 (F 236)	30	—
aurites (Theobald)	II, III	16 (F 16)	5	—
chrysosoma (Edwards)	II	1 (F 1)	1	—
fuscopennata (Theobald)	II, III	32 (F 32)	8	—
spp.	II	6 (F 6)	5	—
Culex (*Culex*)				
andersoni Edwards	II	1224 (M 2, F 1222)	35	—
annulioris Theobald	II, III	1406 (M 9, F 1397, FR 7)	94	—
antennatus (Becker)	II	4988 (M 83, F 4876, FR 7, P 9, L 13)	226	3
argenteopunctatus (Ventrillon)	II	16 (F 16)	6	—
bitaeniorhynchus Giles	II, III	16 (F 16)	3	—
decens Theobald	II, III	209 (F 209)	9	—
duttoni Theobald	III	30 (F 10, L 20)	3	—
hopkinsi Edwards	III, IV	410 (L 410)	17	—
mirificus Edwards	II	152 (F 152)	6	—
musarum Edwards	II	39(F 39)	3	—
neavei Theobald	II	24 (M 1, F 23)	8	—
perfuscus Edwards	II	43 (F 43)	8	—
pipiens Linnaeus	II, III	803 (F 689, P 99, L 15)	37	—
quasiguiarti Theobald	II, III	759 (F 759)	44	—
sitiens Weidemann	II	144 (F 144)	10	—
striatipes Edwards	II	180 (F 180)	3	—
terzii Edwards	II	106 (F 106)	7	—
theileri Theobald	II, III	26 (F 26)	6	—
trifilatus Edwards	II	392 (M 1, F 391)	30	—
univittatus Theobald	II, III	2243 (M 44, F 2199)	133	—
vansomereni Edwards	II, III, IV	2476 (F 1428 L 1048)	103	1
watti Edwards	II	113 (F 113)	9	—
zombaensis Theobald	II	2326 (M 43, F 2135, FR 10, L 138)	107	1
spp.	II, III, IV	4586 (M 69, F 4354, L 163)	225	—
(*Culiciomyia*) *nebulosus* Theobald	II, III	49 (M 2, F 41, MR 3, FR 3)	22	—
(*Eumelanomyia*) *rubinotus* Theobald	II	1 (F 1)	1	1
(*Lutzia*) *tigripes* De Grandpre and De Charmoy	II, III	750 (M 1, F, 735, P 14)	31	—
Eretmapodites				
chrysogaster Graham	II	26 (F 1, L 25)	2	—
quinquevittatus Theobald	II	2660 (FR 1, L 2659)	84	—
silvestris Ingram and De Meillon	II	1957 (FR 1, P 8, L 1948)	234	—
subsimplicipes Edwards	II	27 (MR 27)	1	—
spp.	II	2 (F 2)	1	—
Mansonia (*Mansonioides*) *africana* (Theobald)	III	9 (F 9)	4	—
(Diptera, Ceratopogonidae)				
Culicoides				
brucei Austen	III	1 (F 1)	1	—
cetroneus Carter, Ingram and Macfie	III	1 (F 1)	1	—
cornutus De Meillon	III	16445 (F 16445)	57	—
milnei Austen	III	64 (F 64)	3	—
moreli Clastrier	III	925 (F 925)	23	—
nivosus De Meillon	III	6927 (f 6927)	50	—
quinquelineatus Goetghebuer	III	20 (F 20)	5	—

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Table 1 (*cont.*)

Species	Ecological zone*	Specimens tested†	Pools tested	Pools positive
schultzei (Enderlein)	III	5 (F 5)	3	—
vitshumbiensis Goetghebuer	III	2 (F 2)	2	—
spp.	III	88 (F 88)	6	—
(Diptera, Ephydridae)				
Brachydeutera munroi Cresson	III	2239 (F 2239)	2	—
Diptera, Psychodidae)				
Sergentomyia bedfordi Newstead	II	133 (M 24, F 109)	11	—
Totals		134876 (M 481, F 91045, P 1067, L 31827, MR 3096, FR 7360)	3383	19

* II, Equatorial climates of humid to dry sub-humid characteristics, forest and derived grasslands; III, sub-humid to semi-arid areas of moist woodlands and bushed grasslands without forest; IV, semi-arid dry lands with bushed grasslands of *Acacia–Themada* type.

† Number of each stage tested in brackets. M, male; F, female; MR, reared male; FR, reared female; P, pupa; L, larva.

‡ Species not determined.

Table 2. *Summary of Rift Valley fever virus isolations**

Species	Pool number	Pool size	Stage†	Location	Date collected
Aedes lineatopennis	201-6	50	FR	Sukari Ranch	20.xi.82
A. lineatopennis	201-9	90	MR	Sukari Ranch	20.xi.82
A. lineatopennis	201-14	50	FR	Sukari Ranch	20.xi.82
Anopheles christyi	204-9	1	F	Karen	23.xi.82
An. christyi	207-1	3	F	Karen	1.xii.82
Culex vansomerini	207-6	7	F	Karen	1.xii.82
C. antennatus	207-8	2	F	Karen	1.xii.82
C. rubinotus	207-9	1	F	Karen	1.xii.82
C. zombaensis	213-1	17	F	Karen	3.xii.82
A. cumminsii	215-5	14	F	Sukari Ranch	7.xii.82
C. antennatus	227–5	16	F	Sukari Ranch	15.xii.82
A. lineatopennis	242-1	8	F	Sukari Ranch	29.xii.82
C. antennatus	247-11	11	F	Sukari Ranch	31.xii.82
A. pharoensis	247-16	1	F	Sukari Ranch	31.xii.82
A. lineatopennis	337-26	23	FR	Sukari Ranch	16.i.84
A. lineatopennis	337-38	50	FR	Sukari Ranch	17.i.84
A. lineatopennis	337-42	50	FR	Sukari Ranch	17.i.84
A. lineatopennis	337-75	50	FR	Sukari Ranch	18.i.84
A. lineatopennis	337-84	50	MR	Sukari Ranch	18.i.84

* All pools consisted of unengorged specimens.

† F, female; MR, reared male; FR, reared female.

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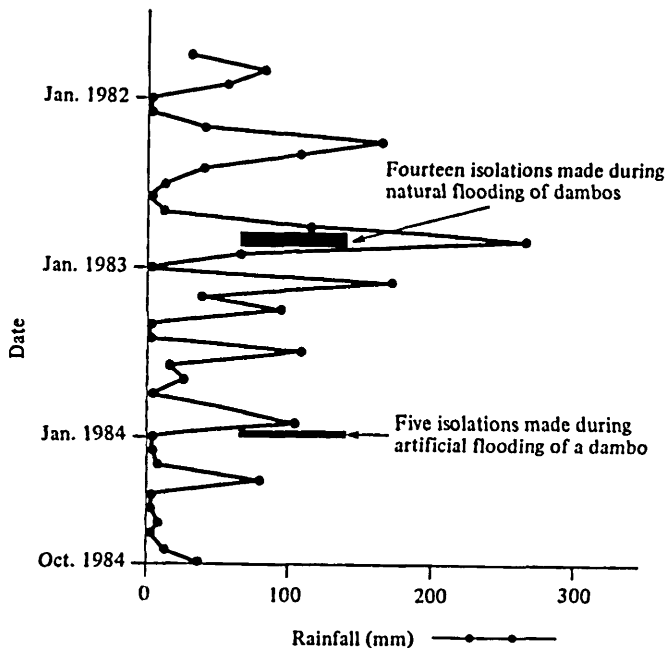

Fig. 1. The periods from October 1981 to October 1984 when Rift Valley fever virus was isolated from mosquitoes at Sukari Ranch and Karen, Kenya. Monthly rainfall is plotted on the horizontal axis.

Table 3. Aedes lineatopennis *collected as larvae and pupae after natural flooding of a dambo at Sukari Ranch, Kenya between 20 November and 5 December, 1982*

Stage	No. collected	No. pools	No. isolations	Isolation rate
Larvae	239	4	0	0:239
Pupae	1580	23	0	0:1580
Reared adult females	558	14	2	1:279
Reared adult males	731	14	1	1:731
Total	3108	55	3	

III that had been either naturally flooded by heavy rainfall (November–December 1982) or artificially flooded (January 1984). No isolation was made during the periods in which dambos did not flood.

The results of the virus isolation attempts from *A. lineatopennis* collected as larvae and pupae at a naturally flooded dambo between 20 November, 1982 and 5 December, 1982 and the three subsequent RVF isolations from laboratory-reared adults are summarized in Tables 3 and 4. Tables 5 and 6 summarize the results obtained with 5050 *A. lineatopennis* collected as pupae at an artificially flooded dambo, reared to adults in the laboratory and tested for RVF virus. Five pools of the reared adults yielded RVF virus.

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Table 4. *RVF virus isolations from laboratory-reared adult* A. lineatopennis *collected from a naturally flooded dambo at Sukari Ranch, Kenya between 20 November and 5 December, 1982*

Source	Pool	No. specimens	Hamster deaths* Primary isolation	Hamster deaths* Pass I	Re-isolation
Females	201-6	50	96/1 only	18/2	—
Males	201-9	90	20/2	18/2	$10^{5.5}$/0·1 ml
Females	201-14	50	40/1 only	18/2	—

* Hours post inoculation/number dying.

Table 5. Aedes lineatopennis *collected at an artificially flooded dambo at Sukari Ranch, Kenya on 16–21 January, 1984*

Stage	No. collected	No. pools	No. isolation	Isolation rate
Reared adult males	1600	32	1	1:1600
Reared adult females	3450	69	4	1:862
Total	5050	101	5	

Table 6. *RVF virus isolations from laboratory-reared adult* A. lineatopennis *collected at an artificially flooded dambo at Sukari Ranch, Kenya on 16–21 January, 1984*

Source	Pool	No. specimens	Hamster deaths Primary isolation	Hamster deaths Pass I	Re-isolation
Females	337-26	23	40/1	18/1	—
			56/1		
Females	337-38	50	40/1 only	18/2	—
Females	337-42	50	40/1	18/1	—
			46/1	24/1	—
Females	337-75	50	40/2	24/2	—
Males	337-84	50	40/2	20/2	—

* Hours post inoculation/number dying.

DISCUSSION

A total 3383 pools were processed for RVF virus isolation over a 3-year period. RVF virus was isolated only from mosquitoes collected when dambo formations were naturally or artificially flooded. This was restricted to a 2-month period in November and December 1982, and a period in 1984 when a small portion of a dambo formation was artificially flooded by pumping water from an adjacent river. At no other period was RVF virus obtained from the remaining 3364 pools. Davies & Highton (1980) isolated RVF virus from mosquitoes caught in the same area in 1979 when grassland depressions were flooded. Metselaar *et al.* (1974) processed approximately 146300 mosquitoes trapped in parts of Kenya where RVF occurs during epizootics. Most of these were trapped during the IEP, but some were also caught during an epizootic period. The only RVF virus isolate he obtained was

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from human serum from a patient in the coastal forest belt (ecological zone II). A total of 179000 *Culicoides* were processed for virus isolation by the inoculation of 2-day-old mice (F. G. Davies & A. R. Walker, unpublished data), most were caught in RVF epizootic areas but no RVF virus was isolated. McIntosh (personal communication) attempted RVF virus isolation from some 400000 mosquitoes trapped in known RVF areas in Natal without obtaining any isolate, other than at periods when RVF virus activity and the disease were occurring. The conclusion which may be made from this extensive work is that RVF virus appears in the mosquito populations only during those conditions prevailing when epizootic RVF occurs, or when such conditions are transiently created but not maintained at a level to allow an epizootic to generate. There would not seem to be a continuous cryptic vertebrate mosquito cycle serving as a maintenance system for RVF virus in the IEP. This is in accord with much published evidence (Scott & Heisch, 1959; Davies, Clausen & Lund, 1972; Henderson *et al.* 1972; Fagbami, Tomori & Kemp, 1973; Davies, 1975; Davies & Onyango, 1978; Swanepoel *et al.* 1978; Davies & Addy, 1979; Davies & Karstad, 1981; Prozesky, 1981; McIntosh *et al.* 1983).

The conditions predisposing to epizootics of RVF have been defined in this study as those following flooding of dambo formations in RVF epizootic areas. This flooding is followed in Kenya by the hatching of very large numbers of *A. lineatopennis* eggs which produce in turn an enormous population of adult mosquitoes. RVF virus was isolated from both male and female adult mosquitoes reared in the laboratory from larvae and pupae collected in the newly flooded dambos, which is strong evidence that RVF is transovarially transmitted by this mosquito species. The virus was isolated from wild-caught *A. lineatopennis* and also several other mosquito species encountered in the dambo biotype, but only during the brief period of November/December 1982 when there was RVF virus activity. No RVF virus isolation was made other than at this period, which must surely be a significant observation. The epidemiological and ecological association of *A. lineatopennis* with dambos in RVF epizootic areas in Kenya strongly supports a hypothesis that the virus is maintained during the inter-epizootic periods by transovarian transmission in this species. The species has been shown to feed predominantly upon domestic cattle (Linthicum *et al.* 1985). *Brachydeutera munroi* Cresson adults were tested for RVF virus because its larvae were observed to be predacious on emerging adult *A. lineatopennis* in the flooded dambo habitat (Linthicum, Davies & Kamau, 1985).

Deaths in hamsters which follow their inoculation with RVF infected material generally occur within 20–36 h. On occasion, attempting the primary isolation of virus from mosquitoes or tissues, a single hamster of a pair died at 40–56 h after inoculation. RVF virus may be demonstrated in the liver while the survivor does not contain any antibody to the virus in its serum, when assayed 2 weeks later. This observation suggests that either there was only a small quantity of virus in the original inoculum or the handling procedures allowed some inactivation of the specimens. Some 300 hamster sera were taken at random after surviving inoculation with mosquito pools in this study; none contained any neutralizing antibody to RVF virus. Field strains of RVF virus have proved to be invariably fatal for hamsters in our laboratory. Peters (personal communication) has reported late deaths in hamsters following inoculation with the Zagazig strain. The Smithburn modified live virus is not lethal for hamsters.

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The series of virus isolation attempts made from specimens obtained from the artificially flooded dambo (Table 6) were prepared in a transport medium subsequently found to be lethal for the virus. An RVF-positive liver suspension prepared in this had a titre of $> 10^7$ hamster LD50/ml, and after 3 weeks at +4 °C this had fallen to $< 10^1$ hamster LD50/ml. No re-isolation was made from this series. No RVF virus had been handled in the laboratory for a 2-month period prior to this experiment.

A tentative conclusion from this study is that RVF is maintained in the study areas throughout the IEP by a period of dormancy in the eggs of *A. lineatopennis*. The virus is thought to be transmitted transovarially by this species. Alexander (1957) reported that he had found that 'adult mosquitoes, hatched from eggs found in the dried mud of lakes in South Africa, proved to be infective for some 17 days'. No mosquito species was identified by name, however. His remarkable observation pre-dated the demonstration of transovarial transmission of a virus in mosquitoes by some 16 years (Watts *et al.* 1973; Pantuwatana *et al.* 1974).

The epizootic conditions of RVF in Kenya are related to widespread and prolonged rainfall (Davies *et al.* 1985). This raises the water table sufficiently to allow flooding of dambos which are the sites for developing immature *A. lineatopennis*. Elsewhere in Africa, other *Aedes* spp. may assume such a role. Virus has been isolated from many *Aedes* spp. (*circumluteolus* in South Africa and Uganda; *caballus* and *juppi* in South Africa; *dentatus* in Zimbabwe; *durbanensis* in Kenya; *lineatopennis* in Kenya, South Africa and Zimbabwe; *dendrophilus, tarsalis* and *africanus* in Uganda; *palpalis* in the Central African Republic) (Kenya Department of Veterinary Services, 1937; Digoutte *et al.* 1974; Meegan *et al.* 1980; McIntosh & Jupp, 1981).

The flooded dambos are rapidly utilized as breeding sites by several other species of mosquitoes (Davies, 1975; Linthicum *et al.* 1983, 1984). These appear to become infected with RVF after feeding upon domestic ruminants, and play some role in the propagation and maintenance of epizootic RVF. Virus has been isolated from *Culex neavii*, *C. pipiens*, *C. theileri*. *C. zombaensis*, *Anopheles coustani*, *Coquillettidia fuscopennata*, *Mansonia africana* and *M. uniformis* during epizootics of RVF in different African countries (Meegan *et al.* 1980; McIntosh *et al.* 1983). Many of these mosquitoes have also been shown to be capable of transmitting RVF in the laboratory (McIntosh *et al.* 1983).

A most interesting enigma remains of the role of *Eretmapodites* species in the natural history of RVF. They have been found to be infected with RVF virus in central African tropical forest (Smithburn, Haddow & Gillett, 1948) and coastal forest in Natal (McIntosh *et al.* 1980), on both occasions during an apparent IEP. Those species of the genus thus far examined do not appear to be capable of transmitting the virus transovarially (McIntosh *et al.* 1973), and 4632 larvae obtained from coastal forest in Kenya have proved to be negative when tested for RVF virus.

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