Studies on Rift Valley fever in some African murids (*Rodentia: Muridae*)

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SUMMARY

Brains, spleens and livers of 2212 murids, 27 shrews and 7 dormice, trapped at 7 sites in Rhodesia, were tested in 277 pools for the presence of Rift Valley Fever virus. There were no isolations of Rift Valley Fever, but 69 isolations of an unidentified virus were obtained. Sixteen out of 867 sera had low-titre haemagglutination-inhibition activity against Rift Valley Fever antigen, but only one out of 1260 sera had neutralizing antibody. The evidence suggests that murids fail to encounter infection in nature and are unlikely to play a role in circulation and dissemination of Rift Valley Fever virus. Four out of seven widely distributed species of murid, *Rhabdomys pumilio*, *Saccostomys campestris*, *Aethomys chrysophilus* and *Lemniscomys griselda*, were shown to be capable of circulating amounts of virus likely to be infective for mosquitoes.

INTRODUCTION

Rift Valley Fever (RVF) periodically causes major epizootics in sheep and cattle in East and Southern Africa. Epizootic spread is sometimes, but not invariably, associated with heavy rains favourable to mosquito vectors and during intervening years infections occur in enzootic areas. This pattern suggests that there is cryptic cycling of virus in wild reservoir hosts with incidental spread to domestic animals (Scott & Heisch, 1959). Rodents, particularly murids, have been investigated as potential reservoir hosts since Daubney & Hudson (1932) reported heavy mortality in two species, *Arvicanthus abyssinicus nairobiae* and *Rattus rattus kijabius*, on farms affected in the 1930 epizootic in Kenya. It has been overlooked that, following further investigation, it was concluded that RVF was not implicated as the cause of murid deaths in the field (Daubney & Hudson, 1932, 1933).

Mims (1956) found neutralizing (NT) antibody in one serum sample from an *Arvicanthus abyssinicus nubilans* rat out of 102 sera of murids collected in an area where RVF was prevalent in Uganda. Weinbren & Mason (1957) extended the observation by demonstrating that viraemia of an intensity up to $10^{8.0}$ mouse LD₅₀ per 0.3 ml occurred in *A.a. nubilans* rats infected intracerebrally or by peripheral routes. The rats did not readily succumb to infection by any route and therefore the species potentially constituted an ideal reservoir host. In South Africa, McIntosh (1961) found that at least two out of five species which he infected, *Saccostomys campestris* and *Aethomys chrysophilus*, developed viraemia of

an intensity sufficient to infect mosquitoes (McIntosh, Jupp, Anderson & Dickson, 1973).

Attempts to substantiate the role of murids in RRV, however, have been unsuccessful. Investigators in East Africa failed to isolate virus from 91 murids and shrews (Woodall & Williams, 1960), 281 murids (Henderson *et al.* 1972), 285 murids (Scott & Heisch, 1959) and 110 murids (Davies, 1975). They failed to detect NT antibody in sera from 106 murids (Henderson *et al.* 1972) and 285 murids (Scott & Heisch, 1959). In South Africa, investigators failed to isolate virus from an unstated number of *Praomys (Mastomys) natalensis* (Gear *et al.* 1951), 270 non-specified small mammals (Anon., 1959) and 665 murids, shrews and dormice (Anon. 1962). Histopathological lesions of RRV could not be detected in livers of 77 murids and shrews (Gear *et al.* 1955). Antibodies could not be detected in sera of an unstated number of *P. natalensis* (Gear *et al.* 1951), 77 murids and shrews (Gear *et al.* 1955), 50 murids (Davis, 1957), 112 non-specified rodents (Anon. 1958) and 245 non-specified rodents (Anon. 1961). Haemagglutination-inhibition (HAI) titres were detected in an unstated number out of 1145 sera from non-specified rodents from Kenya tested in South Africa (Anon. 1961) and there were HAI titres in 31 out of 106 pooled sera of murids, shrews and dormice (Anon. 1962), but doubt was cast on the significance of the HAI reactions by the fact that 18 of the 31 HAI-positive serum pools were tested for NT antibody without positive result.

Although individual studies have not been exhaustive, the cumulative evidence fails to implicate murids in circulation of the virus in nature. The present communication reports: (a) attempts to isolate virus from or demonstrate antibodies in 2246 murids, shrews and dormice of 17 species caught in Rhodesia and (b) studies of viraemia produced by experimental infection in several species. The investigation involved development of a cell culture NT test and a plaque technique for titration of viraemia.

MATERIALS AND METHODS

Virus and HA antigen

RRV strain 80612A, isolated from a lamb and maintained by intraperitoneal (i.p.) passage of infected liver in unweaned mice, was obtained from the Veterinary Research Institute, Onderstepoort, South Africa. It was used after an unrecorded number of mouse passages for preparation of haemagglutinating antigen by sucrose-acetone extraction (Clarke & Casals, 1958) of infected mouse liver.

RRV 763/70, isolated from an aborted sheep fetus in Rhodesia in 1970, was used after two intracerebral (i.c.) passes in mice for preparation of stock virus for use in viraemia studies and in NT tests. Unweaned mice were infected by the i.p. route and heparinized blood, collected after 24 h when the mice were sick and dying, was freeze-dried in small volumes as stock virus.

Specimens

Three main sites for trapping murids were selected at different altitudes. The first, Henderson Agricultural Research Station at Mazoe (17° 33' S, 31° E), lies at an altitude of 1300 m above sea level and is outside the area where RRV appears

to be enzootic (Swanepoel, 1976) but is within the limits of epizootic spread of the disease in 1969–70. The second location consisted of three farms close to each other in the Sinoia district (17° 15' S, 30° 5' E) at an altitude of about 1000 m. The farms are within the enzootic area and RVF was confirmed in cattle on neighbouring farms by demonstration of antibody responses or by isolation of virus during the time in which trapping took place. The third main site lay below 600 m in the Zambezi Valley close to the shores of Lake Charara (16° 35' S, 29° E). There are no domestic livestock in the area but there is evidence from tests on human sera that RVF occurs there (Swanepoel & Cruickshank, 1974).

Trapping at other sites was opportunist. The sites included the Veterinary Research Laboratory in Salisbury situated on 40 hectares in an urban setting at an altitude of 1500 m (17° 45' S, 31° 5' E), Mlezu Agricultural Institute near Que Que at an altitude of 1200 m (18° 55' S, 29° 50' E), Matopos National Park near Bulawayo at an altitude of 1200 m (20° 30' S, 28° 30' E) and Tsamhole Pan near Robins Camp in Wankie National Park (18° 45' S, 25° 55' E) at an altitude of 1000 m. There was no evidence that RVF was prevalent at any of these sites, but antibodies have been demonstrated in game sera from Wankie National Park, and Que Que district, on the northern edge of the watershed, adjoins the enzootic Hartley-Chakari-Gatooma area. Trapping took place between January 1974 and September 1975. Captured murids, shrews and dormice were anaesthetized with ether, bled by severing the main vessels in the chest and their brains, spleens and livers were collected. Sera and organs were stored at –30 °C until tested.

Isolation of virus

Organs were tested for presence of virus in pools representing up to ten individuals per species per catch site. Approximately 10% suspensions of pooled organs were prepared by homogenizing in sucrose-phosphate buffer (Storz, 1971). Suspensions were centrifuged for 20 min at 3000 g and 4 °C and the supernatant fluid inoculated i.c. in litters of day-old mice which were observed for three weeks.

Viraemia studies

It was shown recently that the multimammate mouse, *P. natalensis*, comprises separate 32-chromosome and 36-chromosome populations in Rhodesia (Lyons, Green, Gordon & Walters, 1977a; 1977b). Pregnant *P. natalensis* females captured at Mazoe were allowed to produce litters and the young were reared to the age of three months or more for use in viraemia studies. A few of the progeny were examined by Mr N. F. Lyons of the University of Rhodesia, Salisbury, and were found to have 32 chromosomes. Blood samples from all individuals used in the viraemia studies were tested by Messrs C. Green and D. Gordon of Blair Research Laboratory, Salisbury, and were found to have haemoglobin separation patterns in starch gel electrophoresis which correspond to the 32-chromosome pattern. Viraemia studies were also performed on young adults from a 36-chromosome population, caught by Mr D. Gordon of the Blair Research Laboratory in the Norton area (17° 55' S, 30° 40' E) near Salisbury. Viraemia studies on other species were performed with young adults captured at Mazoe.

Individuals were anaesthetized with ether and given 10^3 mouse i.c. LD₅₀ of virus 763/70 subcutaneously in 0.1 ml of diluent. A number of individuals of each species were killed for bleeding daily for the first 14 days after infection and thereafter a few *P. natalensis* were killed at weekly intervals. Heparinized 0.5 ml samples of blood were stored in liquid nitrogen for viraemia studies and serum samples were stored at $-30\text{ }^{\circ}\text{C}$ for antibody studies. Spontaneous deaths were investigated by examination of livers for histopathological lesions of RFV and by attempts to isolate the virus from livers by i.c. inoculation of day-old mice.

HAI tests

Sera were screened for HAI antibody by a micro-adaptation of the technique of Clarke & Casals (1958). Non-specific inhibitors of haemagglutinin were removed by acetone extraction of 0.05 ml volumes of sera. Dilutions of 1/20 and 1/40 of extracted sera were prepared in 0.025 ml volumes of diluent in Microtitre 'V' bottom agglutination trays (Cook Eng. Co., Alexandria, Va., USA) and tested with 0.025 ml of antigen containing 4–8 HA units and 0.05 ml of goose erythrocytes. Sera producing positive results were tested to end-point by the standard macro-procedure.

NT tests

Calf testis (CT) cells were derived from neonatal slaughter calves. The culture medium and diluent for virus and sera consisted of minimal essential amino acid medium (Eagle, 1959) with 10% tryptose phosphate broth plus 5% inactivated fetal calf serum and penicillin and streptomycin at concentrations of 200 i.u. and 100 $\mu\text{g}/\text{ml}$. Sera were inactivated at $59\text{ }^{\circ}\text{C}$ for 30 min and duplicate two-fold serial dilutions were prepared in 0.1 ml. volumes of medium in Microtiter cell culture plates. An equal volume of virus suspension, 0.1 ml containing 100 cell culture infective doses (TCID₅₀), was added to each well and serum-virus mixtures held at room temperature ($22\text{ }^{\circ}\text{C}$) before seeding with 2×10^4 CT cells per well in 0.025 ml medium. Plates were sealed and read after 6 days incubation at $36\text{ }^{\circ}\text{C}$. Field sera were screened for NT antibody at 1/8 and 1/16 while sera from murids used in viraemia experiments were screened from 1/2 to 1/16. Titres were recorded as the highest dilution at which a serum produced neutralization in both replicates. Virus controls were titrated in quadruplicate and $\log_{10}$ titres calculated by the method of Kärber (1931).

Plaque technique and viraemia tests

Heparinized whole blood samples were thawed from liquid nitrogen and inoculated i.c. without dilution in litters of day-old mice. Samples of blood were held at $-30\text{ }^{\circ}\text{C}$ while mice were observed and the amounts of virus present in bloods which killed mice were determined by plaque titration.

Petri dishes 50 mm in diameter were seeded with 2×10^6 CT cells in 5 ml of medium and incubated at $36\text{ }^{\circ}\text{C}$ in a 5% CO_2 atmosphere in plastic boxes sealed with adhesive tape. At 48 h confluent monolayers were washed with phosphate-buffered saline, pH 7.2, and duplicate cultures were inoculated with 0.1 ml volumes

of ten-fold dilutions of blood from 10^{-1} to 10^{-8} . Virus was absorbed for 30 min at room temperature and cultures overlaid, without washing, with medium as used in NT tests but containing agarose at a final concentration of 1 %, and with fetal calf serum content reduced to 2 %. Cultures were incubated for 5 days at 36 °C in a 5 % CO₂ atmosphere before being stained by the addition of 2 ml of 1/10000 neutral red in PBS. Cultures were read 6 h after staining, or on the following day, and the number of plaque forming units (p.f.u.) of virus per ml of whole blood recorded.

For comparative purposes, stock virus 763/70 was titrated in parallel by plaque method, microculture and i.c. inoculation of 3-week old weaned mice. Five mice were used per dilution of virus and each mouse received an inoculum of 0.03 ml.

RESULTS

The 2246 murids, shrews and dormice of 17 species captured at various sites, are recorded in Table 1. Their organs, tested in 277 pools, failed to yield RVF virus but produced 69 isolations of an unidentified virus, tentatively designated Mazoe virus (Table 2). Sixty-five of the isolates came from *P. natalensis*, the species associated with Lassa Fever in West Africa. Mazoe virus takes 10 days to kill mice following i.c. inoculation and is non-cytopathic in a variety of cell cultures. It failed to cross-react in complement-fixation (CF) tests with Lassa Fever antigen and serum from the Center for Disease Control, Atlanta, USA. It also failed to cross-react in CF tests with reference antisera to 251 rodent, arthropod-borne and related viruses from the National Institutes of Health, Bethesda, Md., USA and the Yale Arbovirus Research Unit, New Haven, Conn. USA. Mazoe virus has been isolated from specimens from domestic animals, including aborted fetuses, and its isolation will be reported more fully elsewhere.

A total of 867 sera from the field were screened against RVF antigen by the HAI method and 16 sera had antibody activity (Table 3) at minimal titres of 1/20 or 1/40. A total of 1260 sera were screened for NT antibody and only one serum was positive (Table 4) at a titre of 1/8. None of the HAI-positive sera had NT antibody.

The numbers of murids used in viraemia experiments were governed by availability. The results of viraemia studies in five species are presented in Figs. 1–5. Antibody responses are plotted in two species where sufficient observations were made. Four species developed demonstrable viraemia but in the 32-chromosome *P. natalensis* no trace of viraemia was detected in any of 18 individuals killed daily after infection. Lack of antibody in sera collected early in infection and the appearance of specific HAI and NT response in later samples confirm that individuals used in the experiment were immunologically susceptible to the virus (Fig. 2).

More limited observations on a further three murids are presented in Table 5. This shows that viraemia was not detected in any of three 36-chromosome *P. natalensis* killed daily for the first seven days after infection. Moderate viraemia was demonstrated in *Aethomys namaquensis*, but in *Tatera leucogaster* only two of

Table 1. *Rats, mice, shrews and dormice caught at seven sites in Rhodesia*

Species	Que	Mato-	Salis-	Mazoe	Sinoia	Kariba	Tsam-	Totals
Murids (rats and mice)	Que	pos	bury				hole	
<i>Praomys natalensis</i>	17	—	38	1040	620	88	28	1831
<i>Tatera leucogaster</i>	7	3	—	13	14	72	25	134
<i>Aethomys chryso-</i>								
<i>philus</i>	5	1	—	50	31	20	1	108
<i>A. namaquensis</i>	2	1	—	3	—	2	—	8
<i>Saccostomys cam-</i>								
<i>pestris</i>	21	—	—	16	—	3	1	41
<i>Rhabdomys pumilio</i>	3	—	36	2	—	—	—	41
<i>Lemniscomys griselda</i>	2	—	—	7	17	1	1	28
<i>Pelomys fallax</i>	—	—	—	5	—	—	—	5
<i>Steatomys pratensis</i>	—	—	—	1	2	—	1	4
<i>Thallomys paedulus</i>	—	—	—	2	—	—	1	3
<i>Leggada minutoides</i>	—	—	—	—	—	—	2	2
<i>Acomys spino-</i>								
<i>sisimus</i>	—	—	—	1	—	—	—	1
<i>Mus musculus</i>	—	—	3	—	—	—	—	3
<i>Rattus rattus</i>								
<i>alexandrinus</i>	—	—	3	—	—	—	—	3
Shrews								
<i>Crocidura hirta</i>	—	—	—	10	12	—	—	22
<i>Elephantulus</i>								
<i>brachyrhynchus</i>	—	—	—	5	—	—	—	5
Dormice								
<i>Graphiurus murinus</i>	—	—	—	—	2	5	—	7
Total catch								
per site	57	5	80	1155	698	191	60	2246
Trap-nights								
per site	2250	450	1650	7350	2700	1650	1050	17100
Catch per 100								
trap-nights	3	1	5	16	26	12	6	13

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19 samples exhibited minimal viraemia in which virus was recovered from whole blood only.

In isolated observations, viraemia was not detected in an *Otomys angoniensis* rat nor in a *Steatomys pratensis* mouse killed on the day after infection. Virus was detected in whole blood from a pygmy mouse, *Leggada minutoides*, killed on the day after infection while an elephant shrew, *Elephantulus brachyrhynchus*, killed on the third day of infection, had $10^{3.3}$ p.f.u. of virus per ml of blood.

Most deaths after infection occurred in species which circulated the highest concentrations of virus (Figs. 1–5, Table 5), but it was possible to diagnose RVF in only a proportion of instances by isolation of virus from liver or by demonstration of histopathological lesions. Some deaths could be ascribed to poisoning by ether or resulted from fighting in the instances of *Aethomys* species and *Lemniscomys griselda*. It was learnt that individuals of these species had to be caged separately.

Table 2. Isolation of virus from pooled organs of wild-caught rats, mice, shrews and dormice. All isolations were of the unidentified Mazoe virus

Species	Que Que	Mato- pos	Salis- bury	Mazoe	Sinoia	Kariba	Tsam- hole	Totals
<i>Praomys natalensis</i>	0/2*	—	0/5	37/109	28/67	0/9	0/2	65/194
<i>Tatera leucogaster</i>	0/1	0/2	—	1/2	1/5	0/8	0/3	2/21
<i>Aethomys chrysophilus</i>	0/1	0/1	—	0/6	0/7	0/2	0/1	0/18
<i>Aethomys namaquensis</i>	0/1	0/1	—	0/1	—	—	—	0/3
<i>Saccostomys campestris</i>	0/3	—	—	0/3	—	0/1	0/1	0/8
<i>Rhabdomys pumilio</i>	0/1	—	1/6	—	—	—	—	1/7
<i>Lemniscomys griselda</i>	0/1	—	—	0/2	0/4	0/1	0/1	0/9
<i>Pelomys fallax</i>	—	—	—	0/1	—	—	—	0/1
<i>Steatomys pratensis</i>	—	—	—	0/1	0/2	—	—	0/3
<i>Thallomys paedulcus</i>	—	—	—	0/1	—	—	0/1	0/2
<i>Leggada minutoides</i>	—	—	—	—	—	—	0/1	0/1
<i>Acomys spinosissimus</i>	—	—	—	0/1	—	—	—	0/1
<i>Mus musculus</i>	—	—	0/2	—	—	—	—	0/2
<i>Rattus rattus alexandrinus</i>	—	—	1/1	—	—	—	—	1/1
<i>Crocidura hirta</i>	—	—	—	0/1	0/2	—	—	0/3
<i>Elephantulus brachyrhynchus</i>	—	—	—	0/1	—	—	—	0/1
<i>Graphiurus murinus</i>	—	—	—	—	0/1	0/1	—	0/2
Total	0/10	0/4	2/14	38/129	29/88	0/22	0/10	69/277

* Isolates/pools tested.

Table 3. Haemagglutination-inhibition antibodies to RVF virus detected in sera of wild-caught rats, mice, shrews and dormice

Species	Que Que	Matopos	Salisbury	Mazoe	Sinoia	Kariba	Totals
<i>Praomys natalensis</i>	0/14*	—	1/31	10/290	1/343	0/58	12/736
<i>Tatera leucogaster</i>	0/1	0/3	—	—	0/8	—	0/12
<i>Aethomys chrysophilus</i>	0/5	0/1	—	0/15	2/24	—	2/45
<i>A. namaquensis</i>	0/2	0/1	—	—	—	—	0/3
<i>Saccostomys campestris</i>	1/14	—	—	0/1	—	—	1/15
<i>Rhabdomys pumilio</i>	0/1	—	0/29	—	—	—	0/30
<i>Lemniscomys griselda</i>	0/1	—	—	0/1	0/10	—	0/12
<i>Steatomys pratensis</i>	—	—	—	—	0/1	—	0/1
<i>Thallomys paedulcus</i>	—	—	—	0/2	—	—	0/2
<i>Mus musculus</i>	—	—	0/2	—	—	—	0/2
<i>Crocidura hirta</i>	—	—	—	—	1/7	—	1/7
<i>Graphiurus murinus</i>	—	—	—	—	0/2	—	0/2
Total	1/38	0/5	1/62	10/309	4/395	0/58	16/867

* Sera positive/sera tested.

Murids which died are not included in the numbers shown as having been tested for viraemia.

The mean log₁₀ titres expressed per ml and the standard errors of the mean titres recorded in comparative titrations of stock virus 763/70 by three methods were 7.6 ± 0.1 LD₅₀ in six replicate mouse titrations, 7.8 ± 0.2 TCID₅₀ in seven micro-culture titrations and 8.2 ± 0.1 p.f.u. in six plaque titrations.

Table 4. *Neutralizing antibodies to RVF virus detected in sera of wild-caught rats, mice, shrews and dormice*

	Que- Que	Mato- pos	Salis- bury	Mazoe	Sinoia	Kariba	Tsam- hole	Totals
<i>Praomys natalensis</i>	0/5*	—	0/13	1/767	0/230	0/22	0/1	1/1038
<i>Tatera leucogaster</i>	0/4	0/3	—	0/9	0/14	0/53	0/10	0/93
<i>Aethomys chrysophilus</i>	0/5	—	—	0/35	0/15	0/18	—	0/73
<i>Saccostomys campestris</i>	0/10	—	—	0/9	0/1	0/1	—	0/21
<i>Rhabdomys pumilio</i>	—	—	0/4	—	—	—	—	0/4
<i>Lemniscomys griselda</i>	—	—	—	0/3	0/9	0/1	0/1	0/14
<i>Pelomys fallax</i>	—	—	—	0/5	—	—	—	0/5
<i>Steatomys pratensis</i>	—	—	—	—	0/1	—	—	0/1
<i>Thallomys paedulus</i>	—	—	—	—	—	—	0/1	0/1
<i>Acomys spinosissimus</i>	—	—	—	—	0/2	—	—	0/2
<i>Rattus rattus alexandrinus</i>	—	—	0/3	—	—	—	—	0/3
<i>Crocidura hirta</i>	—	—	—	0/1	—	—	—	0/1
<i>Elephantulus brachyrhynchus</i>	—	—	—	0/3	—	—	—	0/3
<i>Graphiurus murinus</i>	—	—	—	—	—	0/1	—	0/1
Total	0/24	0/3	0/20	1/832	0/272	0/96	0/13	1/1260

* Sera positive/sera tested.

DISCUSSION

Mouse inoculation has long been the standard procedure for titration of arthropod-borne viruses, but cell culture techniques are finding increasing application. Boyle (1965) reported that a plaque technique and a technique relying on the cytopathic effect (CPE) of RVF virus on hamster cells in serum-free medium, were equally sensitive for titration of the virus and both techniques were more sensitive than mouse inoculation, *in vivo* titres being one 10-fold dilution higher per ml of virus than *in vivo* titres. Klein, Mahlandt, Ehler & Lincoln (1970), by contrast, found that mouse titration of RVF virus was consistently more sensitive than their plaque technique, *in vivo* titres being 0.7–1.5 log₁₀ greater than *in vitro* titres. In the systems used in the present study, titres determined by CPE and plaque titration were similar to those determined by titration in mice, differences being within limits of experimental error. The microculture and plaque techniques described here constitute economical and reproducible methods readily available to veterinary laboratories.

The results of the present survey are essentially in agreement with previous findings in that there is little or no evidence of circulation of RVF virus in wild murid populations. Examination of over two thousand murids failed to produce an isolation of the virus, even from an area where RVF virus was shown to be active in cattle. Low HAI titres recorded in 16 sera were unaccompanied by NT antibody activity and they cannot be accepted as specific evidence of RVF infection. The single NT-positive serum constitutes scant evidence of natural circulation of RVF virus in muric populations.

Twenty-eight species of murids are known to occur in Rhodesia (Smithers, 1975). Ten species, not represented in the present catches, have localized distributions

Fig. 1

Fig. 2

Fig. 1. Mean viraemia and geometric mean HAI and NT antibody responses in *Aethomys chrysophilus* infected subcutaneously with RVF virus. N = numbers killed for testing; D = total spontaneous deaths; RVF = spontaneous deaths ascribed to RVF.

Fig. 2. Viraemia and geometric mean HAI and NT antibody responses in 32-chromosome *Praomys (Mastomys) natalensis* infected subcutaneously with RVF virus. Symbols as in Fig. 1.

Fig. 3. Viraemia in *Rhabdomys pumilio* infected subcutaneously with RVF virus. Symbols as in Fig. 1.

Fig. 4. Viraemia in *Saccostomys campestris* infected subcutaneously with RVF virus. Symbols as in Fig. 1.

Fig. 5. Viraemia in *Lemniscomys griselda* infected subcutaneously with RVF virus. Symbols as in Fig. 1.

Table 5. *Viraemia studies in three species of murid*

	Day after infection											
	1	2	3	4	5	6	7	8	9	10	11	12
<i>Prionomys natalensis</i> (36-chromosome)												
Number viraemic	0/3*	0/3	0/3	0/3	0/3	0/3	0/3	—	—	—	—	—
Deaths	0	0	0	0	0	0	0	—	—	—	—	—
<i>Tatera leucogaster</i>												
Number viraemic	0/2	0/2	1/2	0/2	0/2	1/2	0/2	0/1	0/1	0/1	0/1	0/1
Viraemia titres (log ₁₀ p.f.u./ml)	—	—	T†	—	—	T	—	—	—	—	—	—
Deaths	0	0	0	0	0	0	0	0	0	0	0	0
<i>Aethomys namaquensis</i>												
Number viraemic	0/2	1/2	0/2	1/2	1/2	1/2	—	—	—	—	—	—
Viraemia titres (log ₁₀ p.f.u./ml)	—	4.6	—	T	2.9	T	—	—	—	—	—	—
Deaths	1	0	0	0	0	1	—	—	—	—	—	—
RVF deaths	0	0	0	0	0	0	—	—	—	—	—	—

* Number viraemic/number tested.

† T = trace viraemia, virus detected in whole blood only.

in the east and west of the country which do not overlap with areas where RVF is known to be enzootic. A further 9 species which are poorly represented in or absent from the present catches, are widely distributed but are never abundant (R. H. N. Smithers, personal communication). These species must be regarded as unlikely to attain population densities which would support sustained circulation of an arthropod-borne virus, but they include *A. namaquensis* which was shown to be capable of circulating moderate amounts of virus in limited observations. Two further widely distributed species, *Mus musculus* and *Rattus rattus alexandrinus*, are peri-domestic species introduced into the country and are unlikely to be involved in sylvatic RVF. This leaves seven widely distributed species which attain the greatest population densities. They include *Rhabdomys pumilio*, *S. campestris*, *A. chrysophilus*, *L. griselda* and three species which are subject to periodic population explosions: *P. natalensis*, *T. leucogaster* and *L. minutoides*. Two of these, *P. natalensis* and *T. leucogaster*, failed in the viraemia experiments to circulate enough virus to infect mosquitoes (McIntosh *et al.* 1973). *R. pumilio*, *S. campestris*, *A. chrysophilus* and *L. griselda* circulated large amounts of virus and *L. minutoides* exhibited minimal viraemia in a single observation. These four or five species could be regarded, therefore, as potential reservoirs of RVF virus, but the evidence from the present and previous surveys suggests that they do not encounter infection in nature. Moreover, their wide distribution does not coincide with the distribution of RVF in certain areas.

The observations on viraemia in *L. griselda*, *S. campestris* and *A. chrysophilus* are compatible with previous observations (Anon., 1958; McIntosh, 1961) but the failure to demonstrate viraemia in *P. natalensis* is in contrast to the finding of McIntosh (1961). He infected two individuals by intracardiac route with $10^{1.9}$ mouse LD₅₀ of low-passaged virus, bled them on alternate days and demonstrated moderate viraemia, with a maximum intensity of $10^{1.5}$ LD₅₀ per 0.03 ml. In the present study, individuals were infected subcutaneously with 10^3 LD₅₀ of low-passaged virus and no trace of viraemia was detected in any of 18 32-chromosome individuals or three 36-chromosome individuals tested daily. The difference in results could relate to differences in the strain of virus used, dose and route of administration, but may well be due to differences in *P. natalensis* in the two studies. It appears that the South African colony used by McIntosh (1961) has 36-chromosomes (Matthey, 1954). The indications that sibling species exist, stress the need for epidemiological investigations to be linked to chromosome studies, particularly where the ubiquitous multimammate mouse is concerned in Africa. Nevertheless, neither the findings of McIntosh (1961) nor the present observations indicate that *P. natalensis* could serve as a reservoir host of RVF.

It can be concluded that the weight of evidence is against murids playing a role in the circulation and dissemination of RVF virus, but no similar conclusion can be drawn with respect to shrews and dormice, the limited observations on them being merely incidental to the study of murids.

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REFERENCES

- ANON. (1958, 1959, 1961, 1962). *Annual report of the Arthropod-borne Virus Research Unit, South African Institute for Medical Research, Johannesburg, 1958, 1959, 1961, 1962.*
- BOYLE, J. J. (1965). Titration of Rift Valley Fever virus in hamster kidney cells in the absence of serum. *American Journal of Veterinary Research* **26**, 190-191.
- CLARKE, D. H. & CASALS, J. (1958). Technique for haemagglutination and haemagglutination-inhibition with arthropod-borne viruses. *American Journal of Tropical Medicine and Hygiene* **7**, 561-573.
- DAVIES, F. G. (1975). Observations on the epidemiology of Rift Valley Fever in Kenya. *Journal of Hygiene* **75**, 219-230.
- DAVIS, D. H. S. (1957). Studies on arthropod-borne viruses of Tongaland. III. The small wild mammals in relation to the virus studies. *South African Journal of Medical Sciences* **22**, 55-61.
- DAUBNEY, R. & HUDSON, J. R. (1932). Rift Valley Fever. *Lancet* **i**, 611-2.
- DAUBNEY, R. & HUDSON, J. R. (1933). Rift Valley Fever. *East African Medical Journal* **10**, 2-19.
- EAGLE, H. (1959). Amino acid metabolism in mammalian cell cultures. *Science, New York* **130**, 432-7.
- GEAR, J., DE MEILLON, B., LE ROUX, A. F., KOFKY, R., ROSE INNES, R., STEYN, J. J., OLIFF, W. D. & SCHULTZ, K. H. (1955). Rift Valley Fever in South Africa. A study of the 1953 outbreak in the Orange Free State, with special reference to the vectors and possible reservoir hosts. *South African Medical Journal* **29**, 514-18.
- GEAR, J., DE MEILLON, B., MEASROCH, V., HARWIN, R. & DAVIS, D. H. S. (1951). Rift Valley Fever in South Africa. 2. The occurrence of human cases in the Orange Free State, the north-western Cape Province, the western and southern Transvaal. B. Field and laboratory investigations. *South African Medical Journal* **25**, 908-12.
- HENDERSON, B. E., MCCRAE, A. W. R., KURIYA, B. G., SSENKUBUGE, Y. & SEMPALA, S. D. K. (1972). Arbovirus epizootics involving man, mosquitoes and vertebrates at Lunyo, Uganda 1968. *Annals of Tropical Medicine and Parasitology* **66**, 343-55.
- KÄRBER, G. (1931). Beitrag zur kollektiven Behandlung pharmakologischer Reihenversuche. *Archiv für experimentelle Pathologie und Pharmakologie*, **162**, 480-3.
- KLEIN, F., MAHLANDT, B. G., EYLER, S. L. & LINCOLN, R. E. (1970). Relationship between plaque assay and the mouse assay for titrating Rift Valley Fever virus. *Proceedings of the Society for Experimental Biology and Medicine* **134**, 909-14.
- LYONS, N. F., GREEN, C., GORDON, D. & WALTERS, C. R. (1977a). G-banding chromosome analysis of *Praomys natalensis* (Smith) (Rodentia Muridae) from Rhodesia. 1. 36-chromosome population. *Heredity* **39**, 197-200.
- LYONS, N. F., GREEN, C., GORDON, D. & WALTERS, C. R. (1977b). G-banding chromosome analysis of *Praomys natalensis* (Smith) (Rodentia Muridae) from Rhodesia. 2. 32-chromosome population. *In litt.*
- MATTHEY, R. (1954). Nouvelles recherches sur les chromosomes de Muridae: problemes de cytologie comparee et de taxonomie chez les Microt. *Revue Suisse de Zoologie* **62**, 163-206.
- MCINTOSH, B. M. (1961). Susceptibility of some African wild rodents to infection with various arthropod-borne viruses. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **55**, 63-8.
- MCINTOSH, B. M., JUPP, P. G., ANDERSON, D. & DICKSON, D. B. (1973). Rift Valley Fever. 2. Attempts to transmit virus with seven species of mosquito. *Journal of the South African Veterinary Association* **44**, 57-60.
- MIMS, C. A. (1956). Rift Valley Fever virus protection test surveys. *East African Virus Research Institute, Annual Report 1955-6*.
- SCOTT, G. R. & HEISCH, R. B. (1959). Rift Valley Fever and Rift Valley rodents. *East African Medical Journal* **36**, 665-7.

- SMITHERS, R. H. N. (1975). *Guide to the rats and mice of Rhodesia*. Trustees of the National Museums and Monuments of Rhodesia, Salisbury, 1975.
- STORZ, J. (1971). *Chlamydia and Chlamydia-Induced Diseases*. Springfield: Charles C. Thomas.
- SWANEPOEL, R. (1976). Studies on the epidemiology of Rift Valley Fever. *Journal of the South African Veterinary Association* **47**, 93-4.
- SWANEPOEL, R. & CRUICKSHANK, J. G. (1974). Arthropod-borne viruses of medical importance in Rhodesia. *Central African Journal of Medicine* **20**, 71-9.
- WEINBREN, M. P. & MASON, P. J. (1957). Rift Valley Fever in a wild field rat (*Arvicanthis abyssinicus*): a possible natural host. *South African Medical Journal* **31**, 427-30.
- WOODALL, J. P. & WILLIAMS, M. C. (1960). Work with rodents. *East African Virus Research Institute, Animal Report*, 1959-1960.