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

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The occurrence of Rift Valley fever in South Africa was first recognized in 1951.[1] In the autumn of that year an extensive and severe epizootic occurred in the Western Free State, the Southern and South-Western Transvaal, and the adjoining districts of the North-Western Cape Province.[2, 3] Many farmers lost nearly all their lambs and a large proportion of their sheep. The loss of cattle was less severe. At the same time as the occurrence of this epizootic amongst sheep and cattle there were a number of cases of illness amongst the farmers and their farm labourers. Several veterinary surgeons who had done post-mortem examinations on infected sheep or cattle also developed a similar illness. All these patients had cut open or had handled the viscera and meat of sheep and cattle. It was clear that only those individuals who handled infected tissues contracted the disease, which was proved by the isolation of virus and serological tests to be Rift Valley fever. The infection did not spread from patients to individuals coming into contact with them. Several patients developed visual defects in convalescence. This defect was found to be due to a circumscribed retinitis.[4, 5] In some, after may months, the lesions have resolved; in others the defect still persists 3 years later.

Laboratory studies, carried out towards the end of the epidemic, failed to reveal the vector amongst the mosquitos frequenting and breeding in the pans, and also failed to reveal infection amongst the few wild animals examined.

There seemed little doubt that the disease was recently introduced into this region. The oldest farmers in the affected districts recognized it as a new disease, a view endorsed by veterinary officers of many years' experience. Their opinion that it was a new and hitherto unknown infection in this region was strongly supported when, in spite of their many years' experience and their long years' residence in contact with sheep and cattle, they became ill soon after their contact with the tissues of sheep and cattle. If they had had previous contact with the infection they would have been immune. Where it came from remains uncertain. Whether it would persist in this region of South Africa was also a most important question, which has now been partly answered.

1953 OUTBREAK

In the autumn of last year, 1953, the disease once again appeared in epizootic form in the Luckhoff District of the Orange Free State. This district was not affected in 1951, but adjoins the Koffiefontein District, which was involved in the first epidemic. Within a month several hundred sheep died after an acute short illness characterized by stiff gait, weakness, and sometimes bleeding from the nose and intestine. The illness was so acute that often sheep were found dead in the morning, when the previous evening they had not appeared to be ill. Cattle were not obviously affected.

Several of the farm labourers on the affected farms were ill at the same time as the epizootic occurred amongst the sheep. The individuals affected had cut open dead sheep or handled their meat. A Native woman working in the kitchen where she handled meat from slaughtered sheep was also affected.

Most of these patients had a diphasic illness, with fever lasting 3 days, followed by one day's remission, followed by a further 3 days' fever. During the illness the patients suffered from severe headache, backache, and muscular pains. One of the patients complained in convalescence that his head still did not feel right and that he could no longer see clearly, being unable to identify objects more than 25 yards away. It will be recalled that a diphasic illness followed in some cases by defective vision was a feature of the human cases in the 1951 outbreak of Rift Valley fever. This diagnosis in the present outbreak was confirmed retrospectively by finding that the sera from these patients gave a positive mouse-protection test, as will be described later.

The nature of the new epidemic was immediately suspected by Dr. van der Linde and Dr. Dickson of the Department of Veterinary Services. In consultation with Dr. R. A. Alexander, the Director of Veterinary Services, arrangements were made to send a team from the South African Institute for Medical Research and the Medical Ecology Laboratory of the Union Health Department to study the outbreak in the field. A camp was established on Mr. P. Gouws' farm 'Legpan'. The study included the situation on this farm and the neighbouring farms 'Gannapan', 'Eldorado', and 'Wolveplaat'. These farms are about 15 miles north of Luckhoff in the South-West Free State. The veld in this area is covered by Karroo bush. There are several large pans which fill up in the rainy season, but the water soon evaporates in the dry season and leaves a dry caked and cracked surface. Gannapan, the largest of these pans, covers about 600 morgen (1,300 acres) and has a very rich and varied bird life, including spurwing geese, Egyptian geese, various species of wild duck, stilts, plover, and blue cranes, as well as large numbers of the smaller species. There are

also blesbok, springbok and steenbok on the lands adjoining the pan.

These pans form the breeding places of several species of mosquitos, particularly *Aedes caballus*, *Culex theileri* and *Anopheles squamosus*, and at dusk large numbers of these mosquitos rose to feed. It was noted that they fed actively on sheep, cattle, man and experimental mice. The sheep in the lands immediately adjoining the pans were most severely affected in the outbreak.

ISOLATION OF VIRUS FROM SHEEP

A sheep found recently dead on the veld was opened, with a sterile syringe and needles about 10 c.c. of blood was removed from the heart, and a piece of liver was excised and placed in a sterile bottle.

On return to the camp, a suspension of the liver was prepared and inoculated intraperitoneally into 8 mice. These died on the 2nd and 3rd day after inoculation. Sections of the liver showed the total necrosis typical of Rift Valley fever. Virus-neutralization tests with antisera against known strains of Rift-Valley-fever virus confirmed that this virus was the virus of Rift Valley fever.

The serum was separated from the blood and submitted to a complement-fixation test for Rift Valley fever. This test gave a negative result, which is not surprising as the disease is so rapidly fatal that there is often no time for the formation of antibodies.

The blood clot was suspended in saline and the suspension inoculated into embryonated hen eggs, and a virus, later proved to be the virus of Rift Valley fever, was successfully established in egg culture.

VECTOR STUDIES

Isolation of Virus from Arthropods

Batches of mosquitos were collected by the team at various sites in the area. Most of these batches were caught in the late afternoon on Gannapan. They included *Aedes caballus*, *Culex theileri*, and *Anopheles squamosus*.

In the evening these batches were allowed to feed on mice in a mosquito cage. It was noted that about 50% of the mosquitos fed.

The following morning the fed mosquitos were separated from the unfed. Each batch was then killed by ether or chloroform vapour. A suspension of the mosquito bodies was prepared in saline and 0·1 c.c. of this suspension inoculated in 4–8, most often 5, mice. These mice were observed for up to 14 days, when they were challenged with known Rift-Valley-fever virus. The results of these tests are given in Table I.

In the table, the feeding experiments' number is followed by the letter A, and the relation of A batches to B batches is, for example, as follows: *Batch* 2A—Mice fed to mosquitos; *Batch* 2B—Mosquitos in collection A inoculated into mice.

It will be noted that a virus pathogenic for mice was isolated from 6 batches of *Aedes caballus* and from 3 batches of *Culex theileri*. It was also shown that the infection could be transmitted by the bite of *Aedes caballus* with naturally acquired infection.

TABLE I. VIRUS ISOLATION FROM INSECTS

Date (May 1953)	*Exp. No.*	*Insect Species*	*Route of Inoc. of Mice*	*Result*
10	1	Aedes caballus	IP	negative
„	2	Aedes caballus	IP	positive
„	5	Simulium sp.	IP	negative
„	6	Aedes caballus	IP	positive
12	2 A	Aedes caballus	feeding	negative
13	2 B	Aedes caballus	IP	positive
„	3 A	Aedes caballus and Culex theileri	feeding	negative
„	3 B 1	Aedes caballus	IP	positive
„	3 B 2	Culex theileri	IP	negative
„	3 B 3	Aedes caballus	IP	positive
„	3 B 4	Culex theileri	IP	positive
„	4 A	Aedes caballus	feeding	positive
14	4 B 1	Aedes caballus	IP	positive
„	4 B 2	Anopheles squamosus ..	IP	negative
„	5	Stomoxys sp.	IP	negative
„	9	Simulium sp.	IP	negative
„	11	Culex theileri and Anopheles squamosus ..	feeding	negative
16	15	Culex theileri, fed	IP	negative
„	16	Culex theileri, unfed ..	IP	negative
„	17	Anopheles squamosus ..	IP	negative
„	18	Culex sp. (theileri) ..	IP	positive

Summary: positive virus isolations—
7 from *Aedes caballus* 1 feeding.
3 from *Culex theileri*.

Identification of Virus—Pathological Findings

The mice dying from this infection were found to have an extensive, almost total, destruction of the liver. The parenchymal cells show an eosinophilic degeneration of the cytoplasm; often the eosinophilic material is rounded up to form an inclusion body somewhat resembling the Councilman body characteristic of yellow fever in man. The nucleus of the affected cells show margination and fragmentation of the chromatin and often small intranuclear eosinophilic inclusion bodies. In addition, fragments of pyknotic nuclei are scattered through the substance of the liver and may often be found within the Kupfer cells. Similar pyknotic fragments may be seen in cells of the spleen pulp and lymph glands.

Although degeneration is so extensive there is little cellular infiltration, but neutrophil leucocytes and mononuclear cells have increased, and often there is marked congestion, red cells filling and greatly distending the sinusoids of the spaces between the degenerate and often dissolved and disappearing columns of liver cells.

This pathological picture is characteristic of Rift Valley fever in mice and sheep. A somewhat similar picture is seen in liver sections of human beings who have died of yellow fever. However, the virus of yellow fever does not produce liver lesions in mice and, unlike the virus of Rift Valley fever, is ralatively innocuous to mice when inoculated intraperitoneally. No other virus is known to produce similar liver lesions.

Immunological Findings

The viruses isolated from the sheep and the batches of mosquitos were proved by cross-immunity tests to be

the Rift-Valley-fever virus. A group of monkeys were inoculated with suspensions of infected mouse liver obtained from the mice infected with the Luckhoff sheep strain, the *Aedes caballus* strain, and also with the Ben strain isolated in 1951, and with the Smithburn neurotropic strain isolated in Uganda.

A fortnight later these monkeys were bled by intracardiac puncture and 10-20 c.c. of blood taken. The sera separated from these bloods were tested for their neutralizing power against homologous strains and

TABLE II. RESULTS OF PROTECTION TESTS WITH ANTISERA

Result = Survivors/total

Monkey Antisera		*Smithburn Strain*	*Ben* 1951 *Strain*	*A. caballus 4A strain* 1953	*Sheep* 1953 *strain*
Smithburn	N	0/5			
	I	5/5			
Ben 1951	N	0/5	1/5	1/5	1/5
	I	3/5	5/5	4/5	4/5
A. caballus	N	0/5	0/5	1/5	2/5
4A—1953	I	3/4	4/5	3/4	5/5
A. caballus	N	1/5	0/5	2/5	0/5
4B—1953	I	5/5	5/5	5/5	5/5
C. theileri	N		0/5		
3B4—1953	I		5/5		
Sheep 1953	N	1/5	0/5	2/5	
	I	4/5	4/5	5/5	
6	N	1/5	0/5		
	I	4/4	5/5		
Human Immune Serum			5/5	5/5	5/5

against the Ben (1951) and Smithburn strains respectively. The results are given in Table II. This test was repeated with some additional antisera included, with the results shown in Table III.

It was shown that the sera from the Ben and Smithburn monkeys neutralized their homologous virus and also the strains isolated in the 1953 Luckhoff outbreak,

TABLE III. MOUSE-PROTECTION TESTS WITH PREPARED ANTISERA

Antisera	*Homologous Virus*	*Virus Challenge Ben S.A.* 1951	*4 B I S.A.* 1953
Ben 1951		5/5	5/5
Smithburn	5/5	5/5	5/5
A. caballus (6) 1953	5/5	4/4	3/3
A. caballus 4*B* 1953		5/5	5/5
Sheep S.A. 1953	5/5	5/5	5/5
C. theileri 3 *B*4 1953	4/4	4/4	5/5
Human sera		0/5	
negative 1		0/5	
2		0/5	
3		0/5	
positive 1		5/5	
2		5/5	

and *vice versa*. There was thus cross-immunity confirming the identity of these strains as strains of Rift-Valley-fever virus.

More detailed studies will be undertaken to determine whether there are any antigenic differences within the group, but these minor differences do not affect the main conclusions, which are that the outbreak in the Luckhoff District in 1953 was caused by Rift-Valley-fever virus, and that the infection was harboured by *Culex theileri* and *Aedes caballus*, and further that the latter mosquito can transmit the infection whilst feeding.

Search for Animal Reservoir Hosts

The presence of the virus in sheep and mosquitos having thus been shown, arrangements were made for a second expedition to collect blood from small mammals and birds, and large numbers of ectoparasites, with the object of discovering a possible reservoir among wild animals or birds and ectoparasites capable of harbouring the virus.

A systematic collection of the animals of the veld of the affected area was made by R. Rose Innes and K. H. Schulz of the Medical Ecology Laboratory of the Union Health Department, assisted by A. C. Pelzer of the staff of the Deputy Chief Health Officer of the Union in Bloemfontein. During a period of 12 days, 100 bloods were collected from 15 species of small mammals and 14 bloods from 6 species of birds. Liver specimens were taken from most of these animals and birds. In addition, 13 large batches of ectoparasites (fleas, mites and ticks) were collected from the pooled nests of 4 different species of rodents. Twenty-nine smaller batches of ectoparasites were collected from the bodies of small mammals, more than half of which were from specimens already sacrificed for blood. Finally, 25 cows (23 infested), 300 sheep (59 infested) and 4 horses (all infested) were searched and stripped of their ectoparasites.

All the ectoparasites were identified in the Department of Entomology of this Institute before being tested for the presence of virus.

The sera from the blood specimens were separated and the serum from each animal was submitted to a Rift-Valley-fever mouse-protection test.

Portions of liver from the various animals and birds were taken and placed in 5% formol saline. These were embedded and histological sections were examined microscopically for lesions of the liver, particularly lesions which may have resulted from a previous recent attack of Rift Valley fever.

Suspensions were prepared from each lot of arthropods by grinding them up with a pestle and mortar and adding normal saline. Each suspension was then inoculated into 5 mice, which were observed for signs of illness for 1 month.

At the end of this time the surviving mice were challenged with a known Rift-Valley-fever strain of virus to determine whether they had undergone an inapparent infection during the period of observation.

Mice which became seriously ill were killed and portions of their viscera, including the brain, liver, heart and lungs, spleen and kidney, were removed. These were placed in formalin or Bouin's fixative before embedding for sectioning for histological examination.

At the same time a portion of liver was removed aseptically and stored at —20° C, pending the result of the histological and bacteriological examination. If this suggested or confirmed the suspicion of Rift Valley fever, material was available for passage and further study.

The results of the tests for Rift-Valley-fever virus in the ectoparasites of the veld animals were all negative, as is shown in Table II.

Although many of the liver sections showed pathological lesions none of these resembled the lesions of Rift Valley fever.

The Rift-Valley-Fever Mouse-protection Test

The blood sera from 4 of the farm labourers who had been ill, as well as from the animals collected by the second expedition, were submitted to a Rift-Valley-fever mouse-protection test. In this test, 0.5 c.c. of serum, or less if no more was available, was mixed with an equal quantity of a virus suspension containing 50-1,000 mlds. per 0.1 c.c. The mixture was thoroughly shaken and then incubated at 37° C. for 1 hour, being shaken at 10-minute intervals during this time.

Then each serum-virus mixture was inoculated intraperitoneally in 0.1-c.c. amounts into each of 5 mice.

These mice were observed for 7 days, a daily note being made each day of their state. The interpretation of the results was: if 4 or 5 survived = positive protection; if 2 or less survived = negative = no protection; if 3 survived = inconclusive.

The results are given in Table III. The 4 human sera were found to be protective, thus confirming the diagnosis made clinically of Rift Valley fever. The only animal giving a postive Rift-Valley-fever protection-test was a *muishond* or polecat (*Ictonyx sp.*). As this animal is known to eat dead lambs it is possible that it acquired its infection from direct contact with the infected tissues and not from an arthropod vector. Whether this animal plays any part in the ecology of the disease is unknown, but it seems unlikely that it plays an important role.

It is somewhat surprising that none of the rodents gave positive protection. Most of these are nocturnal and so would not often be exposed to *Aedes caballus*. *Rhabdomys* is diurnal but may be too small to attract mosquitos.

In 1951 it was reported that the blesbok on affected farms had lost their young. It is noteworthy then that the only two steenbok bloods tested were not protective. Of course this negative finding does not exclude the possibility that this species is susceptible to Rift Valley fever. During the day and at dusk, the steenbok were found taking shelter in the hills on the farm, relatively far removed from the pans, where the mosquitos were found in great numbers. They thus may have escaped infection but further observations are necessary to determine their susceptibility.

Thus no wild animal, except for the one polecat, was found to have had an infection with Rift Valley fever, and none of their ectoparasites was found to be harbouring the virus.

CONCLUSIONS

From this investigation it may be concluded that in 1953 Rift Valley fever still persisted in the Union of South Africa. How and where the infection is maintained has not been determined and presents an interesting problem for future study.

The important vectors are *Aedes caballus* and *Culex theileri*. The former mosquito has been shown in the present investigation to be capable of transmitting the infection while feeding. This has yet to be demonstrated in *Culex theileri*. These are both common species, indeed the predominant species in the pan veld of South Africa, which is a vast area embracing much of the North-Western Cape Province, the Western Orange Free State and the South-Western Transvaal.

Further studies are needed to define the areas in Southern Africa in which Rift Valley fever has occurred and in which it may now be endemic. Further studies

TABLE IV. RIFT-VALLEY-FEVER MOUSE-PROTECTION TESTS

Human Bloods (Initials)		*Result of Test Survivors/total*	*Interpretation*
E.W.		4/5	positive
J.W.		3/5	,,
N.M.		5/5	,,
B.M.		4/5	,,
Negative control	1	1/5	negative
	2	0/5	,,
Positive control	1	5/5	positive
	2	4/5	,,

TABLE V. RIFT-VALLEY-FEVER MOUSE-PROTECTION TESTS

Animal Bloods Species	*No. Tested*	*Result of Tests*
Rattus (aethomys) namaquensis (golden rat)	2	negative
Otomys unisulcatus (Karroo bush rat)	20	,,
Rhabdomys pumilio (striped mouse)	42	,,
Rattus (Mastomys) natalensis (multimammate mouse)	6	,,
Desmodillus auricularis (Namaqua gerbil)	1	,,
Mus musculus (house mouse)	1	,,
Elephantulus myurus (elephant shrew)	5	,,
Xerus inauris (ground squirrel)	5	,,
Cynictis penicillata (yellow mongoose)	6	,,
Ictonyx striatus (muishond)	1	positive
Suricata suricatta (suricate)	1	negative
Felis nigripes (blackfooted cat)	1	,,
Lepus capensis (Cape hare)	1	,,
Pedetes capensis (spring hare)	16	,,
Raphicerus campestris (steenbok)	2	,,

TABLE VI. RIFT-VALLEY-FEVER MOUSE-PROTECTION TESTS

Bird Bloods Species	*No.*	*Result*
Bubulcus ibis (cattle egret)	1	negative
Paccillonitta erythrorhynca (red-billed teal)	1	,,
Ardea melanocephala (heron)	1	,,
Fulica cristata (coot)	9	,,
Alopochen aegyptiarus (Egyptian goose)	1	,,

are also needed to determine what part, if any, is played by wild animals and their ectoparasites in the ecology of this infection in South Africa.

SUMMARY

Rift Valley fever, which was first recognized in South Africa in 1951, again caused an epizootic among sheep in the South-Western Orange Free State in the autumn of 1953. As in the first outbreak several cases of human infection occurred amongst the farmers and the farm labourers who handled the meat or viscera of sheep which had died or were killed when sick of this infection.

Aedes caballus and *Culex theileri*, two of the mosquitos most prevalent in the pan-veld area of this region, were found to harbour the virus. Naturally infected *Aedes caballus* mosquitos were shown to be capable of transmitting the infection whilst feeding.

A large number of the animals and birds were collected

from the affected farms. Sections of the liver from these were examined histologically and the bloods were submitted to mouse-protection tests for Rift Valley fever and their ectoparasites were tested for the presence of Rift-Valley-fever virus by the inoculation of a suspension prepared from them into mice. No lesions of Rift Valley fever were detected in the livers of these animals and birds. All the mouse-protection tests gave negative results for Rift Valley fever except the blood of one *muishond* (polecat) *Iconyx*, which gave a positive result.

Rift-Valley-fever virus was not isolated from any of several batches of arthropods from these animals and birds collected on the farms in the affected area.

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