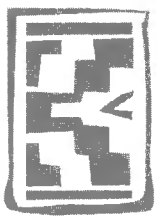

RESEARCH COMMUNICATION

Could bats act as reservoir hosts for Rift Valley fever virus?

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ABSTRACT

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The inter-epizootic reservoir host of Rift Valley fever virus (RVFV) remains unknown, although the namaqua rock rat, *Aethomys namaquensis*, as well as bats have been implicated. Bats can be asymptomatically infected with rabies, as well as several arboviruses; the possibility that they can act as host for RVFV therefore exists. To examine this possibility, 350 different samples (brain, liver, salivary glands and brown fat) obtained from 150 bats (comprising seven species) were tested for RVFV antigen using an enzyme linked immunosorbent assay (ELISA). None of the samples tested positive, but the ELISA proved to have limited sensitivity ($\geq 10^3$ $TCID_{50}$/mℓ). In order to determine whether bats could be infected with RVFV, one *Miniopterus schreibersii* and two *Eptesicus capensis* bats were inoculated by the oral or intramuscular route with 100 mℓ and 30 mℓ, respectively, of a RVFV suspension with a titre of 10^6 $TCID_{50}$/mℓ. None of the bats developed any clinical signs. A low concentration of RVFV antigen was found in the liver and urine of *M. schreibersii*, but not in brain tissue. A third *E. capensis* bat was inoculated by the intramuscular route and sacrificed on day 18. A low level of antigen was detected in the brown fat. These results demonstrate that bats can be infected with RVFV, and that further studies should be done to determine the potential of different bat species to act as reservoir hosts for RVFV during inter-epizootic periods.

Keywords: Bats, namaqua rock rat, reservoir hosts, Rift Valley fever

INTRODUCTION

Several groups of animals, both wild and domestic, have been involved in epidemics of human diseases. In order to control these diseases, steps must be taken to identify the reservoirs in the animal population, and attempts to control these natural reservoirs should be made (Sulkin 1962).

Many arboviruses are mosquito borne, but it is not clear how these viruses can survive the cold winter months in temperate zones, as the mosquito vectors are not active in these periods. Several possible theories exist:

- the involvement of migrating birds;
- transovarial transmission to a new generation of mosquitoes; and
- latent infection and recurrent viraemia in a vertebrate host (Sulkin 1962).

It has been estimated that an insectivorous bat may consume from 50–100% of its body mass in insects in a 24 h period (Sulkin 1962). Contact with arboviruses is therefore inevitable. Antibodies against more than 30 arboviruses have been found in naturally infected bats, and some, including West Nile, Chikungunya (Sulkin & Allen 1974), Sindbis (Blackburn, Foggin, Searle & Le Blanc Smith 1982) and Rift Valley fever (Boiro, Konstantinov & Numerov 1987), have been isolated from bats. It is also known that

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bats can be infected with certain arboviruses via the oral route. For example, bats can be infected with yellow fever virus following ingestion of as few as a single infected mosquito. Evidence suggesting the involvement of Chiroptera in the natural history of several human diseases, including those caused by arboviruses, Q-fever and tick-borne encephalitis (Sulkin 1962), exists. However, although bats have been associated with rabies virus, arboviruses and several fungal agents (Sulkin & Allen 1974), only rabies and histoplasmosis are recorded as having been transmitted through bats to man (Child 1994).

Aedes aegypti readily feeds on several species of bats and it was observed that on numerous occasions they took blood from bats when these were placed in cages containing unfed mosquitoes (Gordon 1922; Kumm 1932). Experiments in bats with yellow fever virus, as well as with Japanese, Venezuelan and St. Louis Encephalitis viruses demonstrated the presence of virus (with titres of up to 10^7 $TCID_{50}$) in several organs following experimental infection, while none of the bats showed any sign of encephalitis, or developed neutralizing antibodies. Mosquitoes were also seen to feed on bats at cave temperatures (10 °C) and to transmit infection under these conditions. In addition, bats that were kept at 10 °C and inoculated subcutaneously with Japanese encephalitis virus, maintained the virus during 107 d of hibernation and had a detectable viraemia 2–5 d after they were moved to room temperature conditions. In one case, a bat developed viraemia 9 d after ingestion of three infected mosquitoes (Sulkin 1962). It, therefore, seems possible that in the case of arboviruses, a natural cycle between bats and mosquitoes can exist in nature and that only after heavy rainfall causing an increase in the mosquito population overflow to other animals will occur.

The reservoir host for Rift Valley fever virus (RVFV) in inter-epizootic periods remains unknown. Although the namaqua rock rat, *Aethomys namaquensis,* has been implicated (Pretorius, Oelofsen, Smith & Van der Ryst 1997), the viraemia in these animals is of very short duration, making efficient transmission to arthropod vectors unlikely. Boiro *et al.* (1987) implicated bats as possible reservoir hosts for RVFV in Guinea. In this preliminary study to investigate bats as possible reservoir hosts for RVFV in South Africa, it is shown that some species can be infected with RVFV, without showing any clinical signs.

MATERIALS AND METHODS

Trapping of bats and sampling

The bats used in this study were collected for taxonomic studies by staff of the National Museum in Bloemfontein and the Cape Nature Conservation Department from several areas in the Free State, Northern Cape and Lesotho from 1987–1989 (Table 1). At night they were trapped with the aid of mist nets and by day they were collected from old mines and caves, between rocks and in the roofs of buildings. A total of 150 bats belonging to seven species were tested (Table 1). They were euthanazed with ether and their brains aseptically removed by means of suction using with a syringe connected to a 15-gauge needle. The brain tissue was then placed in transport medium (400 mℓ Hank's medium to which was added: 2,8 mℓ 5% $NaHCO_3$, 40 mℓ Bovine serum albumin and 4 mℓ of a suspension of Penicillin, Streptomycin and Neomycin) and stored at –70 °C until use. The liver, brown fat and salivary glands were also removed and stored at –70 °C. The liver were chosen as it is a blood-rich organ, the brown fat as it was implicated as a latent focus for viruses and the salivary gland as it could be the first place of viral multiplication in oral infection.

TABLE 1 Species and numbers of bats collected from different areas in the Free State, Northern Cape and Lesotho

Locality	Species	Number
Bloemfontein	*Eptesicus capensis*	6
	Tadarida aegyptiaca	23
Zastron	*Eptesicus capensis*	9
	Miniopterus schreibersii	49
	Myotis tricolor	1
	Rhinolophus clivosus	5
Excelsior	*Eptesicus capensis*	3
	Rhinolophus clivosus	8
Vredefort	*Eptesicus capensis*	10
	Tadarida aegyptiaca	1
Ladybrand	*Rhinolophus clivosus*	3
Clarens	*Rhinolophus clivosus*	6
	Laephotis wintoni	2
Ficksburg	*Rhinolophus clivosus*	3
Tweespruit	*Rhinolophus clivosus*	3
Gumtree	*Rhinolophus clivosus*	9
Lesotho	*Rhinolophus clivosus*	3
	Laephotis wintoni	4
	Myotis leasuri	1
	Myotis tricolor	1

TABLE 2 Numbers of samples from different organs that were tested for the presence of RVFV antigen

Brain	Liver glands	Salivary	Brown fat	Total
105	82	81	82	350

Testing for RVFV antigen

Three hundred and fifty different samples taken from the bats (Table 2) were tested for RVFV antigen using an enzyme linked immunosorbent assay (ELISA). Monoclonal antibodies directed against a non-structural protein (NS1) of RVFV (Stevens 1990) were diluted 1:10 000 in coating buffer [1,59 g of Na_2CO_3 and 2,93 g of $NaHCO_3$ in 1 000 mℓ of H_2O (pH = 9,6)] and used to coat 96 well microtitre plates (Nunc Maxisorp®) by overnight incubation at 4 °C. Following adsorption, free binding sites were blocked by incubation with 5% gelatine. Samples were added to the plates at a 1:10 dilution in phosphate buffered saline (PBS) and incubated for 60 min at 37 °C. Plates were then repeatedly washed using PBS containing 0,05% Tween-20. Polyclonal anti-RVFV serum (rabbit) (Stevens 1990) at 1:5 000 was used as secondary antibody followed by horse-radish peroxidase coated goat-anti rabbit antibody (Bio Yeda, Israel) at 1:4 000. Tetramethylene benzidine hydrochloride (TMB) was used as substrate and the colour reaction was stopped after 30 min by addition of 0,5N H_2SO_4 (Supplementary kit for Enzygnost ELISA kits, Behringwerke). The sensitivity of the ELISA was determined by testing serial tenfold dilutions of a RVFV suspension (titre 10^7TCID_{50}/mℓ) derived from a culture of RVFV (strain AN1830) grown on Vero cells. The cut-off was arbitrarily defined as an optical density value of two times that of the negative controls. Appropriate positive and negative controls were included on each plate.

Experimental infection of bats

Miniopterus schreibersii and *Eptesicus capensis* bats were trapped alive and kept in the laboratory under P3 conditions. The bats were handfed with mealworms. In order to establish experimental infection with RVFV, 30 mℓ of a 10^6TCID_{50}/mℓ RVFV suspension was injected into the wing muscle of an *E. capensis* bat, and approximately 100 mℓ of the same virus suspension was dropped into the mouths of another *E. capensis* and a *M. schreibersii* bat. Urine was collected by gently squeezing the bats against a glass plate, and the inoculated bats were euthanazed on day 4 post-inoculation in order to obtain organs for RVFV antigen testing. A third *E. capensis* bat was inoculated with 30 mℓ of a 10^6TCID_{50}/mℓ RVFV suspension via the i.m. route. This animal was euthanazed at day 18 post-inoculation and different organs were tested for the presence of RVFV antigen (Fig. 2).

RESULTS

None of the 350 samples obtained from 150 bats tested unequivocally positive for RVFV antigen using the ELISA. Four samples showed a slightly raised optical density compared to the negative controls. Although the ELISA was able to detect $\geq 10^3$ particles of RVFV per mℓ, it is possible that these samples may contain a very low level of RVFV antigen, and they will be retested using a more sensitive technique, such as the polymerase chain reaction.

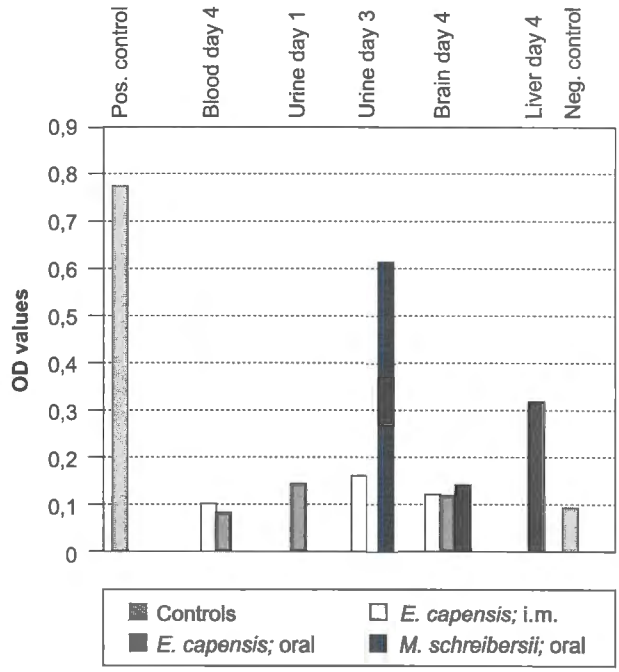

FIG. 1 Detection of RVFV antigen in the urine, blood and tissues of *E. capensis* and *M. schreibersii* following oral or i.m. inoculation with RVFV. Urine was collected on days 1 or 3 and tested for RVFV antigen using the ELISA. Following sacrifice of the animals on day 4, blood (*E. capensis*), liver (*M. schreibersii*) and brain (both species) were also tested for the presence of RVFV antigen

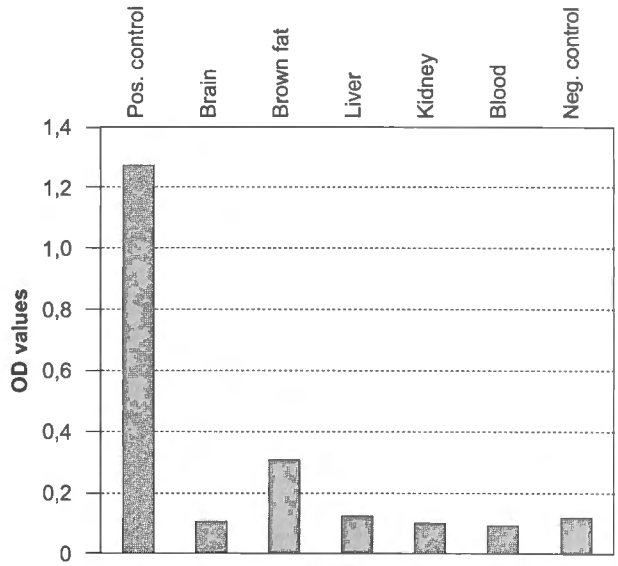

FIG. 2 Detection of RVFV antigen in the blood and tissues of *E. capensis* inoculated with RVFV via the intramuscular route and sacrificed on day 18.

None of the bats inoculated with RVFV showed clinical signs of infection. The *M. schreibersii* bat that had received the virus via the oral route excreted RVF antigen in its urine on day 3 (Fig. 1), and a low level of RVFV antigen could be demonstrated in its liver on day 4, but not in any of the other organs (Fig. 1). RVFV-antigen could not be demonstrated in the blood, urine, brain or liver from any of the inoculated *E. capensis* bats sacrificed on day 4 (Fig. 1), but the animal inoculated intramuscularly and euthanazed on day 18 post infection had a low level of RVFV in its brown fat (Fig. 2).

DISCUSSION

An ELISA for the detection of RVFV antigen was developed; the test had a sensitivity of $\geq 10^3$ particles of RVFV per ml, which is slightly more sensitive than the ELISA described by Niklasson *et al.* (1983). Using this ELISA, no RVFV antigen could be demonstrated in 350 samples obtained from 150 bats. This result could be due to the limited sensitivity of the ELISA, but more likely it reflects true absence of the virus in the bats tested. Indeed, the last cases of human RVF in South Africa occurred in 1974/75 (McIntosh, Russel, Dos Santos & Gear 1980). During 1981 a cow with haemagglutination inhibition antibodies was found, but no serological evidence of circulation of the virus was demonstrated in cattle, sheep, goats and ostriches during 1981 (van der Riet, Sayed, Barnard, van Tonder & Crouse 1985), and up to the present date. The absence of RVFV in the bats could, therefore, reflect the absence of endemic circulation of the virus in South Africa during the study period. However, 23% of *A. namaquensis* collected in the dry inland areas of South Africa from 1986–1990 had antibodies to RVFV (Pretorius, Oelofsen, Smith & Van der Ryst 1997).

On the other hand, the absence of RFVF antigen in the bats might indicate that these animals are not susceptible to natural infection with RVFV. Experimental RVFV infection of *M. schreibersii* could, however, be established in the present study, indicating that infection of bats is possible. Experimental infection of the animals did not result in any clinical disease, but the *M. schreibersii* bat did excrete RVFV antigen in its urine, and viral antigen was demonstrated in its liver, which indicates that replication of the virus had occurred. Unfortunately, no blood from this animal was available for testing, and levels of viraemia could not be established. No conclusive evidence for infection of *E. capensis* was obtained, apart from a low level of antigen in the brown fat of the animal euthanazed on day 18. Liver samples from this bat species were not tested for the presence of viral antigen on day 4, making it difficult to exclude infection of this species, as brown fat can serve as a latent focus of virus in arbovirus infections (Sulken 1962). Although these results indicate that *M. schreibersii,* at least, could possibly maintain RVFV in nature, it would be important to do further studies on RVFV infection in different species of bats, as these animals could possibly play a role, not only in the overwintering of the virus, but also in its circulation between mosquitoes and other domestic and wild mammals.

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