RESEARCH COMMUNICATION

Rabies and bats in a rabies-endemic area of southern Africa: application of two commercial test kits for antigen and antibody detection

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ABSTRACT

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In southern Africa, isolates of rabies-related viruses (i.e. Duvenhage virus and Lagos bat virus) have been made from insectivorous and frugivorous bats. As no recent formal bat virus survey has been reported in southern Africa, a survey of bats in rabies-endemic areas was undertaken. Five hundred and forty-seven bats (13 species) were collected from 21 localities in the Orange Free State, Lesotho and the northern Cape Province. None of the 190 bat sera tested using the "Trousse Platelia^{R} Rage" ELISA kit (Diagnostic Pasteur), had antibodies to rabies virus glycoprotein G. Rabies virus nucleocapsid antigen was also sought for in the brains of 530 bats (13 species) by means of the "Rapid rabies enzyme immunodiagnosis" (RREID)^{R} test (Diagnostics Pasteur). No positive results were obtained. These results show that bats are unlikely to play an important role as hosts of rabies in these parts of Africa, although a low rate of infection cannot be excluded.

INTRODUCTION

Little is known about the role of southern African Chiroptera as vectors of the rabies virus. A single confirmed case of human rabies (serotype 4; Duvenhage virus) has resulted from the bite of a microchiropteran bat in South Africa (Meredith, Rossouw & Van Praag Koch 1971). Three isolates of rabies-related virus (serotype 2; Lagos bat virus) have been made from fruit-eating bats, *Epomophorus wahlbergi* (Meredith & Standing 1981; Van der Merwe 1982), and a further isolate of Duvenhage virus has been made in the Transvaal (Foggin & Swanepoel 1985). The majority of wildlife cases of rabies (serotype 1) in South Africa occur in the Orange Free State (Barnard 1979), mostly implicating the yellow mongoose (*Cynictis penicillata*) (Swart 1989). In the USA and Canada 30 of 39 species of bats were found to be infected with rabies (Constantine 1979), all of serotype 1 (Bourhy, Kissi, Lafon, Sacramento & Tordo 1992). During 1986, 104 of 550 bats examined in Denmark were confirmed as being rabies positive (Gardner 1989). Rabid bats have also been found in Finland, the USSR, the Netherlands, Germany, Spain (Gardner 1989) and France (Bourhy *et al.* 1992). These

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viruses, called European bat lyassaviruses (EBL), are subdivided into two biotypes, EBL1 and EBL2 (Bourhy *et al.* 1992). As no recent formal bat virus survey has been reported in southern Africa, it was deemed necessary to examine South African bats in a rabies-endemic area to determine whether or not bats pose a risk of rabies to humans in these parts of Africa.

MATERIALS AND METHODS

Staff of the National Museum in Bloemfontein and the Cape Nature Conservation collected bats from localities in the Orange Free State, the northern Cape Province and Lesotho (Table 1) for taxonomic studies. All the persons involved had been vaccinated and had protective rabies antibodies. At night the bats were collected with the aid of mist nets and during the day they were searched for in old mines and caves, between rocks, and in the roofs of buildings. Five hundred and forty-seven individual bats, comprising 13 species, were trapped at the 21 localities (Table 1). In total, 530 individual brain and 190 individual or pooled serum samples were tested for rabies antigen and antibody, respectively.

In most cases the bats were killed with ether and bled by puncturing the heart with a 1-mℓ insulin syringe, but sometimes the bats were released after a small blood sample had been collected. In the latter instance, all the blood samples that had been collected from the same species and had originated from the same locality, were pooled. Approximately 1 mℓ of this blood was transferred to a 5-mℓ blood-collection tube and allowed to clot at 6 °C. The sera were separated by centrifuging them with Sure-sep^R at 800 *g* for 5 min, and then they were stored at −196 °C in Nunc^R cryotubes. The brains of the bats were aseptically removed by means of a syringe connected to a 15-gauge needle, and stored at −196 °C in virus transport medium. In some cases the liver, brown fat and salivary glands were also removed and stored at −196 °C for processing at a later stage. It was not possible to collect sera from all the bats, as some died during the collection procedure.

The "Trousse Platelia^R Rage" ELISA kit (Diagnostic Pasteur) was used as specified to determine the anti-glycoprotein antibody status of the bats. In this test a horseradish peroxidase-staphylococcal protein A conjugate was used. Since the validity of the test is based on the binding of protein A to the species' immunoglobulin G, such binding first had to be evaluated. Accordingly, sera from different species of bats were coupled to a solid phase by diluting the sera 1:200 in coating buffer (3,18 g Na_2CO_3 + 5,8 g $NaHCO_3$/2 ℓ; pH 9,6) and dispensing 100 µℓ into the wells of 96-well Nunc-immuno plates^R. After 60 min's incubation at 37 °C, the plates were washed three times with washing buffer (phosphate-buffered saline (PBS) + 0,5 % Tween20). Excess protein binding sites were blocked with a 5% bovine plasma albumin (BPA) suspension in PBS at 37 °C for another hour. After three washing cycles, 100 µℓ protein A conjugate was added to each well and left at 37 °C for 1 h to react with the bats' immunoglobulins. The plates were again washed three times before 100 µℓ of the substrate (ortho-phenylene-diamine-2HCl) was added to each well, and the plates were incubated

TABLE 1 Bat species collected in different areas

	Bloemfontein	Clarens	Excelsior	Ladybrand	Tweespruit	Verkeerdevlei	Ficksburg	Gumtree	Golden Gate	Sterkfontein	Warden	Zastron	Florisbad	Jagersfontein	Christiana	Vredefort	Postmasburg	Douglas	Koegelbeen Caves	Riemvasmaak	Lesotho
Eptesicus capensis	6	2	3							4	3	9	18	58	3	10		38		2	
Eptesicus hottentotus										1											
Tadarida aegyptiaca	26					16				6	5	6		13	1	1				15	
Miniopterus schreibersii										2	2	90		14					29		
Rhinolophus clivosus		8	9	6	2		2	9	7	7	5	6		8					6	3	7
Rhinolophus darlingi														1			3		3		
Rhinolophus denti														4			1		11		
Laephotis wintoni		2																			4
Myotis tricolor									2	6		1									1
Myotis leasuri									1	5	8										1
Sauromys petrophilus																				12	
Pipistrellus kuhlii															1						
Nycteris thebaica														1	1		10				

at room temperature in the dark for 30 min. The reactions were stopped by adding 50 µℓ 4N sulphuric acid, and then the optical densities at 492 nm were measured.

All specimens of the bat species of which the immunoglobulin G reacted with the conjugate (Table 2) were then tested for specific rabies virus anti-glycoprotein antibodies. Rabies virus nucleocapsid antigen was also sought for in the brains of the bats, by using the "Rapid rabies enzyme immuno-diagnosis" (RREID)^{R} test (Diagnostics Pasteur). Brain tissue and, in a few instances, brown fat, salivary glands and liver tissue, were homogenized in diluting buffer before they were tested for rabies antigen. The test was performed according to the manufacturer's instructions.

RESULTS

The staphylococcal protein-A-binding capacities of seven genera (nine spp.) were tested and are presented in Table 2. All the sera of the relevant species that could be tested (Table 3), were negative for rabies virus anti-glycoprotein antibodies.

TABLE 2 Evaluation of the binding between protein A conjugate and immunoglobulin G of different bat species

Species	Optical density			
	0–0,25	0,26–0,6	0,61–1,0	> 1,0
Eptesicus	n = 22	n = 3	n = 7	
Miniopterus	n = 77			
Tadarida		n = 3	n = 1	n = 32
R. clivosis	n = 13			
R. darlingi		n = 5		
R. denti			= 12	
Sauromys		n = 4		n = 5
Myotis tricolor	n = 2			
Laephotis			n = 5	

All the tests performed on the brain material of different bat species (Table 4) were rabies antigen negative. Tests performed on the brown fat, salivary glands and liver were also negative.

DISCUSSION

The sera of three bat species did not bind staphylococcal protein A to an extent sufficient for the application of the Trousse Platelia^{R} Rage kit to test for rabies virus anti-glycoprotein antibodies. However, the sera of some species (Table 2) readily bound to protein A, allowing this test to be used for the detection of antirabies antibodies in these species. One hundred and ninety serum samples originating from these bat species, were tested. If this kit is to be used in wildlife epidemiological studies, the binding ability of wildlife species' immunoglobulin G with the protein A conjugate, must first be evaluated to assess the validity of the test.

None of the bats collected in this survey acted abnormally or exhibited any symptoms of rabies. Asymptomatic rabies virus infections in bats have been recorded (Sulkin & Allen 1974), but the absence of specific antibodies and rabies virus antigen in the animals tested, makes this condition unlikely in southern Africa.

Unfortunately the Trousse Platelia^{R} Rage kit tests only for antibodies against the G glycoprotein, which is type specific and is therefore capable of detecting antibodies against rabies serotype 1 only, and not against rabies-related viruses. The RREID^{R} test determines the presence of rabies virus nucleocapsid antigen, which is group specific and therefore common to rabies and rabies-related viruses, all of which have been reported in southern Africa (Meredith *et al.* 1971; Meredith & Standing 1981; Van der Merwe 1982). Although this test is capable of diagnosing rabies as well as infections due to Duvenhage and Lagos bat viruses, the RREID^{R} has a lower sensitivity than the newly developed test, RREID-lyssa^{R} (Perrin,

TABLE 3 Bat species collected and tested for antibodies to serotype 1 in different areas

	Bloemfontein	Clarens	Excelsior	Ladybrand	Tweespruit	Verkeerdevlei	Ficksburg	Gumtree	Golden Gate	Sterkfontein	Warden	Zastron	Florisbad	Jagersfontein	Christiana	Vredefort	Postmasburg	Douglas	Koegelbeen Caves	Riemvasmaak	Lesotho
Eptesicus capensis	6									1				54				38		2	
Tadarida aegyptiaca	26									2				12						15	
Rhinolophus darlingi														1			3		1		
Rhinolophus denti														4			1		7		
Laephotis wintoni		1																			4
Sauromys petrophilus																				12	

TABLE 4 Bat species collected and tested for rabies virus nucleocapsid antigen in brain samples

	Bloemfontein	Clarens	Excelsior	Ladybrand	Tweespruit	Verkeerdevlei	Ficksburg	Gumtree	Golden Gate	Sterkfontein	Warden	Zastron	Florisbad	Jagersfontein	Christiana	Vredefort	Postmasburg	Douglas	Koegelbeen Caves	Riemvasmaak	Lesotho
Eptesicus capensis	6	2	3							4	3	9	18	58	3	10		27		2	
Eptesicus hottentotus										1											
Tadarida aegyptiaca	26					16				6	5	6		13	1	1				15	
Miniopterus schreibersii										2	2	90		14					29		
Rhinolophus clivosus		8	9	6	2		2	9	7	7	5	6		8					6	3	7
Rhinolophus darlingi														1			1		3		
Rhinolophus denti														4					11		
Laephotis wintoni		1																			2
Myotis tricolor									2	6		1									1
Myotis leasuri									1	5	8										1
Sauromys petrophilus																				12	
Pipistrellus kuhlii															1						
Nycteris thebaica														1	1		10				

Gontier, Lecocq & Bourhy 1992). Nevertheless, it appears that these two viruses do not occur in bats in the area under study, although a low rate of infection cannot be excluded.

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