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Canid and viverrid rabies viruses in South Africa

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ABSTRACT

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Historical records suggest that in South Africa rabies was present in viverrids in the early 1800s. In the early 1950s a wave of canine rabies spread from Namibia through Botswana into the northern Transvaal and by 1961 a second front had penetrated south from Mozambique into Swaziland and northern Natal. Today, rabies is regularly confirmed in a number of canid and viverrid species in most regions of South Africa. A panel of anti-nucleoprotein monoclonal antibodies was used to examine 83 virus isolates from these species. Two major reaction patterns, one chiefly confined to viruses from canids and the other to viruses from viverrids, were obtained. In addition, some variation in the reaction patterns of viverrid viruses was observed and spill-over of viverrid virus into canids and vice versa was recorded. Rabies in South Africa appears to behave as two distinct disease entities.

INTRODUCTION

In South Africa the suspicion, based on numerous rabies-like cases in humans and animals since the early 1800s, of an indigenous strain of rabies in small carnivora of the family Viverridae, was confirmed in 1928 when laboratory tests conducted on two school children, both of whom had died after being bitten by an apparently tame yellow mongoose (*Cynictis penicillata*), proved to be positive for rabies (Snyman 1940). Since that time, with greater public awareness, improved surveillance and diagnostic techniques, the extent of the viverrid-rabies area (Fig. 1) and the role played by the various wildlife species involved has been extended. It has become obvious that *Cynictis penicillata*, the most common mongoose of the highveld plateau of South Africa is the principal wildlife rabies vector, although other viverrids, mustelids, felids and canids are involved.

Canine rabies spread to the northern Transvaal, Zimbabwe and Mozambique in 1950 via an epizootic which had entered Namibia and Botswana in the late 1940s. The infection quickly spilled over into jackals (principally *Canis mesomelas*) and by 1961 had penetrated south from Mozambique into Swaziland and northern Natal. Although early control measures applied in Natal were apparently successful, the disease reappeared in 1976 and today is a serious problem in dogs not only in Natal but also in the adjoining Transkei.

An apparently similar intrusion has developed on the west coast of southern Africa. The canine epizootic apparently also spilled over, but less obtrusively, into jackals and bat-eared foxes (*Otocyon megalotis*) and probably disseminated through the agency of both jackals and bat-eared foxes, spread through Namibia

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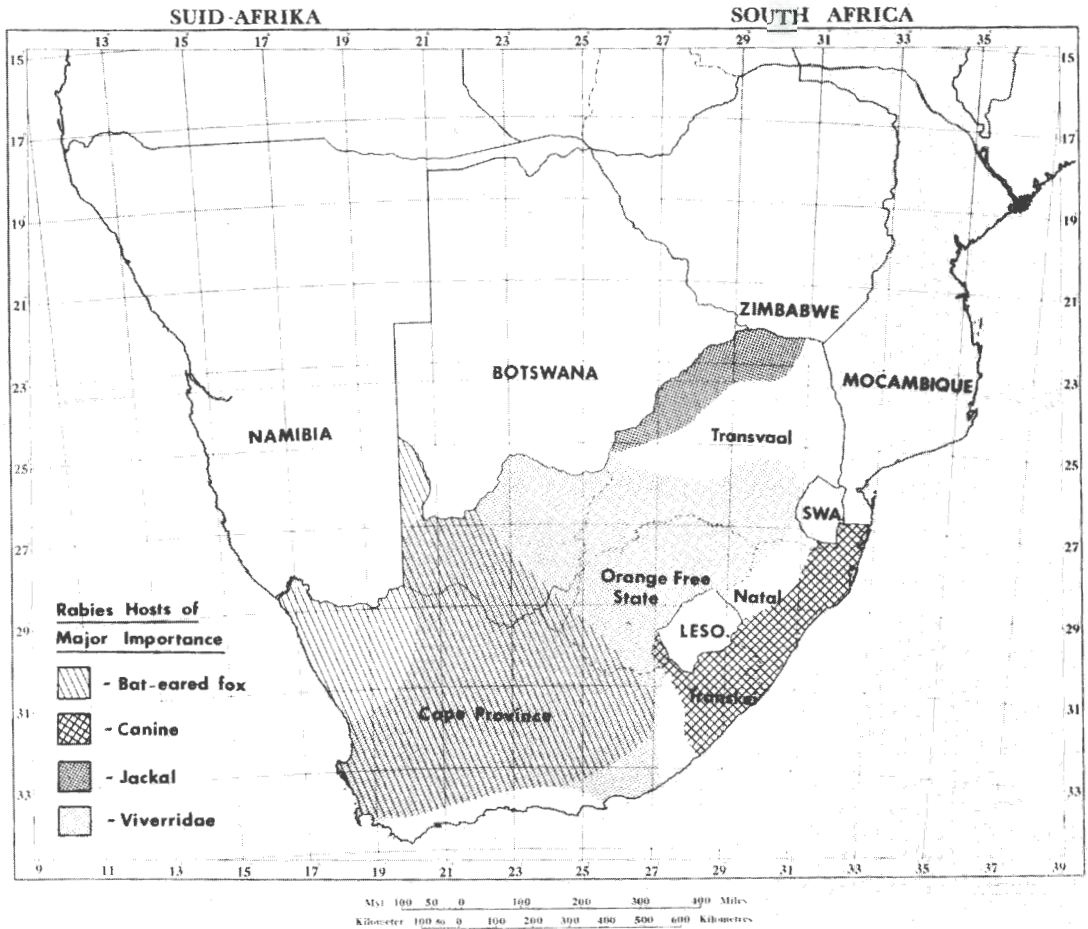

FIG. 1 Vector-species association of rabies in South Africa

TABLE 1 Animal origin of rabies viruses used in this study

Species	Common name	No. of samples
Viverridae		
Cynictus penicillata	Yellow mongoose	17 (2[a])
Galerella pulverulenta	Small grey mongoose	2
Suricata suricatta	Suricate	4
Atilax paludinosus	Water mongoose	1
Genetta genetta	Small-spotted genet	1
Civettictis civetta	Civet	1
Canidae		
Canis familiaris	Domestic dog	12
Canis mesomelas	Black-backed jackal	9
Otocyon megalotis	Bat-eared fox	14
Mustelidae		
Mellivora capensis	Honey badger (ratel)	1
Felidae		
Felis catus	Domestic cat	3
Felis lybica	African wild cat	3 (2[a])
Sciuridae		
Xerus inauris	Ground squirrel	2
Bovidae		
Bos taurus/B. indicus	Cattle	8
Capra hircus	Domestic goat	1

[a] Species not accurately identified

and western Botswana into the Cape Province in about 1970 (Thomson & Meredith 1993). Currently, rabies is regularly reported throughout the western two-thirds of this province where previously only sporadic cases, presumably from viverrid contact, occurred.

In rabies virus infection, nucleoprotein (N) antigen is produced in abundance, thus permitting detection by rabies-specific antibody conjugated to fluorescent compounds—the basis of modern diagnostic (FA) tests. In 1978 Wiktor & Koprowski produced monoclonal antibodies (Mabs) against the G (glycoprotein) and N antigens of rabies virus. Broad-spectrum Mabs prepared against the N protein (Mab-Ns) are now used in diagnosis but, in addition, panels of Mab-Ns are used to detect minor variations within the N protein of different rabies viruses, thus permitting a study of the ecology and epizootiology of rabies in a variety of species.

Foggin (1988) showed that viruses isolated from three slender mongooses (*Galerella sanguinea*) in Zimbabwe differed in their G and N proteins from

other serotype 1 viruses of Zimbabwe. In order to study the epidemiology of rabies in South Africa, we used a panel of 80 Mab-Ns to determine the reaction patterns of 83 rabies viruses isolated from a variety of indigenous viverrid and canid species.

MATERIALS AND METHODS

Preparation of plates for Mab-N analyses

Brain material from 83 fluorescent antibody-positive field rabies cases (Table 1) was passaged in suckling mice. Following necropsy, 20% suspensions of suckling mouse brain (SMB) pools from each isolate were lyophilized in 1 mℓ ampoules. For use, 0,2 mℓ of reconstituted SMB was mixed with 2 mℓ of BHK21 cells diluted to four-fold the normal seeding concentration for monolayer production and the mixtures were distributed into two 84-well Greiner plates at 8 μℓ/well. Following overnight incubation in a CO_2 incubator to allow cell attachment, the plates were fixed in 80% acetone, air-dried then stored at −20 °C.

Mab-N panel

The Mab-N panel consisted of 34 Mab-Ns from the Wistar Institute, Philadelphia, 29 Mab-Ns prepared at the Central Veterinary Laboratory (CVL), Weybridge from the rabies-related viruses and 17 Mab-Ns from the Centers for Disease Control (CDC), Atlanta. Wistar Institute and CDC Mab-Ns were used at the dilution recommended by the donors and the Weybridge Mab-Ns were used at a concentration of four-fold their fluorescence end-point.

Test procedure

One plate of each virus isolate was removed from −20 °C and air-dried. To each well was added 8 μℓ of a Mab-N. The plates were incubated at 37 °C for 30 mins, the contents discarded, then rapidly submerged in excess phosphate buffered saline (PBS) and washed for 20 mins in a PBS bath on a magnetic stirrer. After air-drying, 8 μℓ of FITC-conjugated goat anti-mouse serum was added to each well of the plates which were then incubated for a further 30 mins. Following two rinses in PBS the plates were again air-dried and examined on an incident-light fluorescence microscope using x 25 objective and periplan x 6,3 eyepiece.

Mab-Ns which were neither negative nor positive with all viruses were then retested using the second plate of each virus isolate in order to confirm the reaction pattern obtained.

RESULTS

Of the 80 Mab-Ns used, 24 were negative and 40 were positive with all isolates. The identity of each of the remaining 16 Mab-Ns, designated 1–16 (Table 2) was Wistar: 103–7; 590–2; 377–7; 102–27; 364–11; 714–3; 701–9; 802–2; CVL: DB11; L4; L23; L25; L28 and CDC: 8-2; 61–1 and 71–2 respectively.

TABLE 2 Mab-N reaction patterns of 83 rabies isolates from South Africa

Species (Reaction pattern no.)	Mab-N															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. Cyn (1)	−	+	+	+	−	+	+	+	+	−	−	−	−	−	−	−
2. Cyn (2), Gal (2), Sur (2), Jac (1)	−	+	+	+	−	+	+	+	−	−	−	−	−	−	−	−
3. Can (1)	−	+	+	+	−	−	+	+	−	−	−	−	−	−	−	−
4. Bov (1)	−	+	+	+	−	+	−	+	−	−	−	−	−	−	−	−
5. Cyn (1)	−	+	+	+	−	+	−	−	−	−	−	−	−	−	−	−
6. Cyn (1)	−	+	+	+	−	−	+	−	−	−	−	−	−	−	−	−
7. Cyn (3), Fel (1)	−	−	+	+	−	+	+	+	+	−	−	−	−	−	−	−
8. Cyn (1), Sur (1), Fel (2), Jac (1)	−	−	+	+	−	+	+	+	−	−	−	−	−	−	−	−
9. Cap (1)	−	−	+	+	−	−	+	+	+	−	−	−	−	−	−	−
10. Cyn (1), Sur (1), Fel (5), Jac (1)	−	−	+	+	−	+	+	−	−	−	−	−	−	−	−	−
11. Bov (2)	−	−	+	+	−	+	−	+	−	−	−	−	−	−	−	−
12. Cyn (1), Bef (1)	−	−	+	+	−	−	+	+	−	−	−	−	−	−	−	−
13. Cyn (1)	−	−	+	+	−	+	−	−	−	−	−	−	−	−	−	−
14. Ati (1)	−	−	+	+	−	+	+	−	−	−	−	−	−	−	−	−
15. Cyn (1)	−	−	−	+	−	+	+	+	+	−	−	−	−	−	−	−
16. Cyn (2)	−	−	−	+	−	+	+	+	−	−	−	−	−	−	−	−
17. Cyn (2)	−	−	−	+	−	+	+	−	−	−	−	−	−	−	−	−
18. Cyn (1)	−	−	−	+	−	+	−	−	−	−	−	−	−	−	−	−
19. Gen (1)	−	−	−	−	−	+	+	+	−	−	−	−	−	−	−	−
20. Cyn (1)	−	−	−	−	−	+	+	−	−	−	−	−	−	−	−	−
21. Xer (2)	−	−	−	−	−	−	+	+	−	−	−	−	−	−	−	−
22. Can (11), Bov (5), Hb (1), Civ (1), Jac (6), Bef (13)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Species key: Cyn = *Cynictis*; Gal = *Galerella*; Sur = *Suricata*; Fel = *Felis*; Xer = *Xerus*; Gen = *Genetta*; Can = Canine; Bov = Bovine; Jac = Jackal; Bef = Bat-eared fox; Hb = Honey badger; Civ = *Civetta*; Ati = *Atilax*

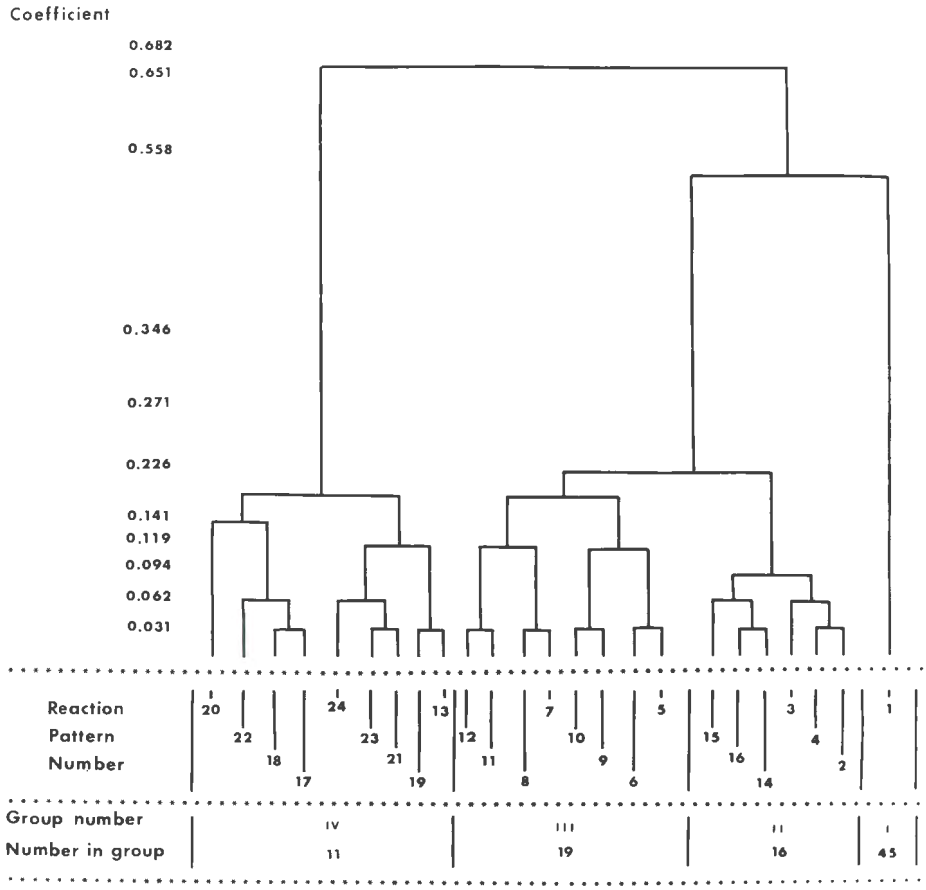

FIG. 2 "Clustan" computer programme analysis of the relationships of 83 terrestrial animal isolates from South Africa as determined by reactivity patterns with 80 Mab-Ns

The species origins of 46 viverrid-virus isolates of reaction patterns 1–21 (Groups 2–6) and of 37 canid-virus isolates of reaction pattern 22 (Group 1) are shown in Table 2

From the results given by these 16 Mab-Ns, 22 reaction patterns (Table 2) were determined and analyzed using the "Clustan" computer programme of Wishart (1987) (Fig. 2).

Two major reaction patterns, one chiefly confined to viruses from canids and the other to viruses from viverrids, were obtained. These two patterns were distinguished by their reaction with eight Mab-Ns, at least seven of which (no. 10–16, Table 2) have been shown to recognize different epitopes by reaction with a number of different serotype 1 rabies viruses (data not shown). No variation in the "canid- rabies" reaction pattern (Table 2, reaction pattern 22) was observed but considerable variation amongst the "viverrid-rabies" reaction patterns (Table 2, reaction patterns 1–21) was recorded.

The canid-rabies reaction pattern was observed in 11 of 12 dogs, six of nine black-backed jackals and 13 of 14 bat-eared foxes. The five viverrid-rabies isolates made from these three canid species are probably examples of spill-over of viverrid-rabies into canids, since they were all from canids infected within predominantly viverrid rabies areas.

The canid-rabies reaction pattern was also observed in five of eight isolates made from bovines in areas where either jackal or dog rabies predominates (Fig. 1). Of the remaining three bovine isolates, two were made in predominantly viverrid-rabies areas and one from an area near the border with Botswana where rabies in viverrids was previously shown to be present (King 1991). Canid-rabies was also found in a civet from a predominantly jackal-rabies area.

Viverrid-rabies reaction patterns were observed in isolates made from *Cynictis* (19), *Suricata* (4), *Galerella* (2), *Atilax* (1), *Xerus* (2), *Genetta* (1) and *Felis* (8) species. The felid isolates were from domestic cats (3), wild cats (3) and two cats not accurately identified, but none of these isolates were made from animals in predominantly jackal, bat-eared fox or canine rabies areas. The prevalence and distribution of felid rabies in South Africa (data not shown) suggests that *Felis* species are more typical of incidental hosts than of vector species.

DISCUSSION

The demonstration of two distinctive reaction patterns in the N protein of viruses isolated from a variety of species of South Africa not only supports the finding of Foggin (1988) in Zimbabwe that isolates of "mongoose" origin differed from other serotype 1 viruses but also confirms the suspicion that indigenous rabies has been present in viverrids of South Africa for a very long time. The variation in the reaction patterns of viruses obtained from viverrids accords with a long-established infection in which mutation and evolution of host and parasite, i.e. viverrrid and rabies virus, may have allowed the emergence of viral variants.

The spread of rabies into South Africa via dogs and wild canids including black-backed jackals and bat-eared foxes is a relatively recent occurrence and, at least with the Mab-N panel used in this work, no distinction could be made between the viruses of canid origin. These findings strongly suggest that at the present time rabies in South Africa is of two separate entities, viverrid and canid. Indeed, this picture is remarkably similar to that found in the former Soviet Union where Mab-N reaction patterns of wildlife-rabies viruses from the Asian region were clearly distinguishable from the canid viruses of the European region (King 1991).

Part of the motivation for this work was an analysis of the nature of rabies viruses within South Africa which might lead to an assessment of the regimens required in order to control and eventually eliminate the disease. Control of canid-rabies could reasonably be predicted from a combination of parenteral vaccination of dogs and by the use or adaptation of present-day oral vaccination strategies for wildlife canids. The eradication of rabies from viverrid populations presents a far more complex and difficult problem. Further research may confirm the historical opinion that viverrid-vectored rabies, although causing sporadic death in canids does not readily transmit to or between canids.

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