The role of the yellow mongoose (*Cynictis penicillata*) in the epidemiology of rabies in South Africa—preliminary results

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ABSTRACT

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Ninety-seven yellow mongooses were captured in six different localities in South Africa and blood specimens for rabies antibody determination as well as brain and salivary glands for virus isolation were collected. No rabies virus or antibody to it was detected in any of the specimens.

Parallel to the field study, two experimental infections were undertaken in which yellow mongooses were artificially infected with serial dilutions of two different rabies isolates (one from a dog and the other of mongoose origin) in order to determine the minimal lethal dose (MLD_{50}), clinical signs, duration of illness, course of the disease, presence of virus in the saliva and salivary glands and development of antibodies to rabies virus.

A significantly higher proportion of mongooses inoculated with mongoose virus died than did those inoculated with the dog isolate. However, the clinical signs, incubation period, duration of illness and development of antibodies were independent of the dose of the inoculum. The levels of rabies virus in the saliva and salivary glands were high in all clinically affected animals infected with the mongoose isolate but only one of the two mongooses which died following inoculation of the dog isolate contained detectable levels of virus in the salivary glands. Antibodies to rabies were detected only in the terminal stages of clinical disease.

INTRODUCTION

The yellow mongoose is the major wildlife vector of rabies in South Africa accounting for up to 70 % of the total number of non-domestic rabies-positive cases diagnosed (Meredith 1982; Keightley, Struthers, Johnson & Barnard 1987). A number of studies on the biology of this animal and the influence that biological factors (i.e. reproductive cycle, low habitat specificity, etc.) may have on rabies epidemiology have been reported (Snyman 1940; Mare 1962; Zumpt 1969; 1976; Barnard 1979; Meredith 1982; Zumpt 1982; Keightley *et al*. 1987; Meredith, Smith & Smith 1988; Swart 1989). However, despite these studies and the importance of *Cynictis penicillata* in the epidemiology of rabies in South Africa, little attention has been paid to the interaction between this species and rabies virus. This paper reports initial results of an investigation aimed at redressing this situation.

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Twelve species of mongoose occur in the southern African subregion (Skinner & Smithers 1990) but, so far as is known, only the yellow mongoose is regularly involved in the endemic rabies cycle that predominates on the central plateau of South Africa (Meredith 1982).

The only other region where rabies in mongooses has been regularly reported is in the Caribbean in the Indian mongoose (*Herpestes auropunctatus*) which was imported from the Indian subcontinent into a number of West Indian islands. On the island of Grenada, it was shown that 18–43% of captured animals had circulating antibodies to rabies virus (ie. evidence of non-lethal infection), while viral recoveries were made from 0,4–3,7% of the animals sampled (Everard & Everard 1985). This finding prompted the question as to whether a similar situation occurs in the yellow mongoose in South Africa.

The investigation reported here comprised two approaches:

- A field study in which free-living *Cynictis penicillata* were captured in the known rabies endemic area. Antibody to rabies virus in the sera of these animals was measured and virus isolation from the brains and submandibular salivary glands attempted.
- A laboratory study in which serial doses of:
  (a) a rabies virus isolated from a field case of rabies in a yellow mongoose (viverrid virus); and
  (b) a rabies isolate obtained from a natural case of rabies in a domestic dog in Natal (canid virus), were inoculated into groups of captive yellow mongooses. The incubation periods, clinical signs, viral excretion and antibody responses were monitored for 120 days after infection.

The reason for inoculating the mongooses with both "viverrid" and "canid" viruses was that it has recently been demonstrated that there are at least two different rabies viruses present in South Africa (King, Meredith & Thomson 1993; Nel, Thomson & Von Teichman 1993), one associated primarily with canids and the other with viverrids. The suspicion that two different forms of rabies occur in South Africa was first mooted more than 40 years ago but hitherto there was inadequate data to support the contention (Alexander 1952).

MATERIALS AND METHODS

Field specimens

Capture of mongooses and collection of material

Ninety-seven animals were captured in six different magisterial districts (Kuruman, Potchefstroom, Kroonstad, Bloemfontein, Queenstown and Ermelo) on the central plateau of South Africa (Fig. 1). Cage traps baited with a mixture of sheep fat and fish meal or with chicken heads were used for the purpose.

Soon after capture, the animals were sedated with 10–15 mg ketamine HCl (Ketalar, Warner-Lambert SA) and 4 mg xylazine HCl (Rompun 2%, Bayer SA) given intramuscularly. Blood specimens were collected and the mongooses were euthanased with 150 mg pentobarbitone sodium (Euthatal, Maybaker SA). Immediately after death, brain and salivary glands were removed and placed in 50% glycero-saline.

Laboratory tests and specimen storage

Brain and salivary gland specimens were examined using the direct fluorescent antibody test (FAT) for rabies (Goldwasser & Kissling 1958). Suspensions of brain and salivary glands were also inoculated intracerebrally into groups of 3-week-old mice to detect the presence of rabies virus (Atanasiu 1975). Serum specimens were tested for the presence of antibodies to rabies by means of a blocking ELISA test (Esterhuysen, Prehaud & Thomson, unpublished data).

Experimental infection

Capture and care of yellow mongooses

All yellow mongooses used for experimental infections were captured from areas in which rabies had not previously been diagnosed. The method of capture was the same as for the collection of the field specimens. After capture the animals were bled, transported to the laboratory and caged individually in an isolation facility. Water was provided *ad libitum* and they were fed daily on a diet consisting of

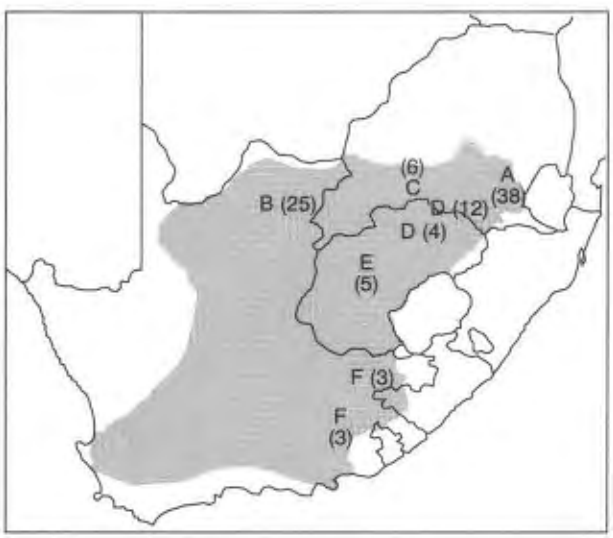

FIG. 1 Numbers of *Cynictis penicillata* captured within the yellow mongoose rabies enzootic area (shaded). Key to magisterial districts: A = Ermelo; B = Kuruman; C = Potchefstroom; D = Kroonstad; E = Bloemfontein; F = Queenstown

embryonated eggs, mince-meat mixed with fish meal, powdered milk and a mineral and vitamin supplement. Periodically, previously killed laboratory mice were also fed to the animals. All the mongooses were bled a second time for antibody determination prior to virus inoculation.

Viruses

Two salivary gland isolates of rabies virus derived from:

- A natural case of mongoose rabies in the epicentre of the mongoose rabies area (634/90).
- A domestic dog from an area in Natal where dog rabies is prevalent (CSG#3) were used to prepare 20 % suspensions.

These virus stocks were stored at −70 °C and had infectivity titres ≥ 10^5MLD$_{50}$/mℓ. The salivary gland suspensions were prepared from macerated submandibular salivary gland tissue diluted in physiological saline containing 20 % fetal calf serum, 500 IU/mℓ penicillin and 2 mg/mℓ streptomycin. Isolates 634/90 and CSG#3 were shown to be "viverrid" and "canid" viruses, respectively, using a panel of monoclonal antibodies (King *et al.* 1993).

Titration of the suspensions used were conducted by intracerebral inoculation of 3-week-old mice by standard methods (Bourhy & Sureau 1990), prior to and simultaneously with the infection of the mongooses.

Infection

Serial tenfold dilutions were made from the virus suspensions and 0,3 mℓ of each dilution was inoculated in the neck muscles of each of three mongooses. The animals were kept under observation for 120 days and the brains of mongooses that died were tested by FAT to confirm rabies. The animals were observed daily for changes of behaviour and other clinical signs.

Virus excretion

One animal of each dilution was selected and once a week a mouth swab was taken, rinsed in 0,8 mℓ of diluent (physiological saline containing 2 % calf serum, 500 IU/mℓ penicillin and 2 mg/mℓ streptomycin) and the resulting suspension immediately injected intracerebrally into five 3-week-old mice (0,03 mℓ/mouse). The mice were kept for 30 days after inoculation and brains of all mice that died were examined by FAT for rabies antigen.

The salivary glands from the mongooses that died during the experiment were macerated, and 10 % suspensions prepared and titrated in 3-week-old mice.

Antibody determination

Every two weeks, starting three weeks after infection, the animals were anaesthetized and 0,5–1,0 mℓ of blood drawn from the jugular vein. The sera obtained from these samples were tested for rabies antibodies using the blocking ELISA system which measures antibody to rabies ribonucleoprotein predominantly (Esterhuysen, Prehaud & Thomson, unpublished data). A positive control was obtained by vaccinating one animal with a commercial inactivated vaccine. Where possible, blood was collected from all animals that died—immediately before or after death.

All mongooses still alive after 120 days were euthanased and the brains and salivary glands collected and tested by FAT, and their sera tested for antibody to rabies virus.

RESULTS

Field specimens

No detectable antibody to rabies virus was found in any of the 97 mongooses captured and all brain and salivary gland specimens inoculated into mice failed to reveal any virus. No rabies virus was detected by FAT in any of the brains or salivary glands examined.

Experimental infection

Of the 18 mongooses inoculated with the mongoose virus isolate at six different levels, nine died, while with the canine isolate only two succumbed (Table 1). This difference was not related to the titre of the inoculum.

Mongooses infected with the *Cynictis* isolate, shed rabies virus in the saliva for up to seven days before death, with titres ranging between $10^{4,8}$ and $10^{8,2}$ MICLD$_{50}$/swab (Table 2). In general, rabid mongooses infected with smaller doses shed more virus in the saliva than those infected with higher doses. No correlation between the incubation period, duration of illness and the amount of virus shed was observed.

Of the two mongooses that died following inoculation with the canine virus, saliva swabs all of which were negative, were available from only one animal.

No shedding of rabies virus was detected in the saliva of any of the animals sampled that survived the challenge.

With the *Cynictis* isolate, all salivary glands of mongooses which developed rabies contained virus with titres ranging from $10^{3,8}$–$10^{8,8}$ MICLD$_{50}$/g tissue irrespective of the dose of virus inoculated (Table 3). From the two animals which died following infection with the dog isolate, the salivary glands of only the mongoose infected with the highest dose were positive, the titre being $10^{4,0}$ MICLD$_{50}$ (Table 3).

TABLE 1 Dose of rabies virus inoculated vs mortality in mongooses infected with viverrid and canid viruses

Viverrid isolate (634/90)		Canid isolate (CSG # 3)	
Virus dose	Deaths/ no. animals	Virus dose	Deaths/ no. animals
$10^{4.9}$	2/3	$10^{5.5}$	1/3
$10^{3.9}$	3/3	$10^{4.5}$	0/3
$10^{2.9}$	2/3	$10^{3.5}$	0/3
$10^{1.9}$	1/3	$10^{2.5}$	1/3
$10^{0.9}$	1/3	$10^{1.5}$	0/3
$<10^{0}$	0/3	$10^{0.5}$	0/3
$MLD_{50} = 10^{3.5}$		MLD_{50} not calculable	

MLD_{50} minimal lethal dose

TABLE 2 Dose of virus inoculated vs shedding of virus in the saliva (earliest recovery of virus prior to death) of mongooses infected with mongoose isolate 634/90

Animal no.	Virus dose	Swab titre ($MICLD_{50}$/swab)	Days prior to death
E27	$10^{4.9}$	+	7
E1	$10^{4.9}$	$10^{4.8}$	1
E16	$10^{3.9}$	$10^{7.4}$	5
E17	$10^{2.9}$	$10^{8.2}$	5

$MICLD_{50}$ mean mouse intercerebral lethal dose
\+ virus present but untitrated

TABLE 3 Dose of virus inoculated vs presence of virus in the salivary glands at the time of death of mongooses infected with viverrid and canid rabies virus isolates

Mongoose isolate (634/90)			Dog isolate (CSG # 3)		
Animal no.	Dose of inoculum	Virus titre ($MICLD_{50}$/g)	Animal no.	Dose of inoculum	Virus titre ($MICLD_{50}$/g)
E27	$10^{4.9a}$	$10^{5.4}$	E38	$10^{5.5}$	$10^{4.0}$
E1	$10^{4.9}$	$10^{3.8}$	E41	$10^{2.5}$	Negative
E13	$10^{3.9}$	$10^{8.8}$			
E16	$10^{3.9}$	$10^{8.0}$			
E14	$10^{3.9}$	$10^{4.6}$			
E17	$10^{2.9}$	$10^{7.0}$			
E18	$10^{2.9}$	$10^{6.7}$			
E19	$10^{1.9}$	$10^{5.2}$			
E12	$10^{0.9}$	$10^{6.4}$			

[a] $MICLD_{50}$ mean mouse intercerebral lethal dose

With both inocula, antibodies were detectable only in those animals showing clinical signs and which were in the terminal stages of the disease. None of the remaining animals seroconverted at any stage of the observation period.

The incubation periods in mongooses inoculated with the *Cynictis* salivary gland suspension varied between 12 and 107 days and the duration of illness between two and seven days, irrespective of the dose of virus inoculated. With the dog virus the incubation periods of the two animals that died were 18 and 24 days, and in the only animal in which clinical disease was observed, the illness lasted three days.

Clinical signs were variable and no correlation was found between them and the dose of rabies inoculated. At the onset of illness loss of fear of humans and a degree of "tameness" was observed while other animals became shy and refused to emerge from their sleeping boxes. Barking was also a common sign during this stage. As the disease progressed, incoordination, paresis progressing to paralysis and coma occurred. Aggression was observed in only one mongoose. Two animals, one infected with $10^{3.9}$ $MICLD_{50}$ of the *Cynictis* isolate and one with $10^{5.5}$ $MICLD_{50}$ of the dog isolate, failed to show any premonitory signs of rabies. These two animals were found comatose and died soon afterwards.

DISCUSSION

The major objective of this work was to establish whether or not viral maintenance in the yellow mongoose can be explained by a carrier state. The results of this admittedly limited study, indicate that a carrier state is unlikely to occur and there is no evidence for survival and seroconversion as has been reported in the case of mongooses on Grenada (Everard & Everard 1985). In this investigation, serum antibodies to rabies virus were demonstrated only in the terminal stages of clinical disease. No shedding of virus or seroconversion was detected in animals inoculated with rabies virus that survived challenge and did not develop clinical disease, despite some of them being inoculated with the same dose of virus that caused fatal infection in others. This is not uncommon in rabies and has been termed "aborted rabies infection" (Fekadu 1991). Thus animals in the wild, although exposed to the virus due to contact with rabid mongooses, may fail to develop clinical disease or to seroconvert. The possibility that animals that develop abortive infection become rabid at a later stage (i.e. after an extended incubation period) is possible but is not at present supported by any evidence. It was also observed that dogs that survived experimental challenge and that failed to develop rabies antibodies, when challenged a second time, developed high levels of neutralizing antibodies in their sera and survived (Fekadu 1991). This anamnestic response was surprising as conventional serological techniques failed to demonstrate any antibody after the first infection. Thus, it is possible that the blocking ELISA test used in this study failed to detect low levels of antibodies in mongooses that received inocula containing a potentially

infective dose of rabies virus. Repeat challenge has not as yet been conducted on yellow mongooses and it is therefore unknown whether a similar anamnestic response may occur in this species.

The longest incubation period observed in these two groups of mongooses was 107 days. Whether or not longer incubation periods occur is not known but it is reasonable to assume that this may be the case. Long incubation periods have been observed in experimentally infected foxes (Blancou, Aubert & Artois 1991) and skunks (Parker & Wilsnack 1966).

The high titres of rabies virus found in the saliva and the salivary glands of mongooses showing clinical rabies following inoculation with isolate 639/90 (viverrid virus) suggest easy transmission of the virus between mongooses since a dose as small as $10^{0.9}$ $MICLD_{50}$ was enough to produce rabies in one animal. With the dog isolate the situation was different: it was less infective for yellow mongooses than the mongoose isolate.

These preliminary findings indicate that the antigenic and genomic differences detected between "canid" and "viverrid" viruses in South Africa (King *et al.* 1993; Nel *et al.* 1993) are reflected in biological differences and that the adaption of virus biotypes to particular host species detected elsewhere in the world (Smith & Baer 1988) also occurs in the southern African subcontinent.

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