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Aspects of rabies infection and control in the conservation of the African wild dog (*Lycaon pictus*) in the Serengeti region, Tanzania

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ABSTRACT

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Lycaon pictus is amongst the most endangered wildlife species in Africa. In 1990 rabies virus was isolated from the brain of an adult *Lycaon* found dead in the Serengeti region of Tanzania. One adult and six pups of the same pack feeding on the carcass showed clinical signs and rabies was suspected; within two days they had disappeared and are presumed to have died. Subsequently, two *Lycaon* packs in the Serengeti National Park were given inactivated rabies vaccine either by dart or by parenteral inoculation following anaesthesia. *Lycaon* sera which had been collected over the previous two years and sera collected pre- and post-vaccination were examined for the presence of rabies virus neutralizing antibody. Three of 12 unvaccinated *Lycaon* had antibody levels > 0,5 IU/mℓ; post-vaccination samples from two *Lycaon* showed increased antibody levels. Between four and ten months post-vaccination, at least four of the vaccinated animals had died from unknown causes. Issues relating to wildlife vaccination and veterinary intervention in conservation are discussed.

INTRODUCTION

Canine rabies is widespread in southern and eastern Africa (King 1993). Since 80 % of human rabies cases in Africa are attributed to dog bites (WHO 1993), canine rabies is of primary concern as a risk to human health; in addition it poses a potential threat to the survival of endangered populations of some wild carnivore species.

In August 1990, the carcass of an adult African wild dog (*Lycaon pictus*) was found in the eastern Serengeti plains after a radio-collared adult male of the same pack was located by radiotelemetry. The radio-collared male and six pups showed signs of ataxia and the pups were seen to be eating the carcass. The following day the carcass was relocated and although the pups could not be found the adult was relocated by radiotelemetry approximately 10 km from the original site. He showed signs of abnormal behaviour which included restlessness, chewing skulls

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and earth, abnormal tail and ear carriage, frequent yawning, one episode of salivation and progressive hind limb ataxia. The radio signal from the dog was lost overnight and subsequent attempts to locate him were unsuccessful. He is presumed to have died underground. None of the pups or the remaining adults from the known original pack of 20 has since been located.

In September 1990, vaccination of 29 adults of two *Lycaon* packs [Salei (16) and Ndoha (13)] was carried out. Later in the same month, five pups from the Salei pack were vaccinated separately when at five months of age. One further pack (Moru Track pack) of seven adults which was seen in December 1990 and January 1991 could not be relocated and hence was not vaccinated.

This paper describes the confirmation of rabies virus infection in this disease outbreak, how the vaccination of *Lycaon* was carried out, as well as the results of a limited serological survey for rabies-neutralizing antibodies in unvaccinated *Lycaon*.

MATERIALS AND METHODS

Brain stem samples from the carcass were collected into straws using World Health Organization (WHO) kits (Barrat & Blancou 1988). These samples remained inside the straws (Barrat 1993) during transport to the WHO Collaborating Centre, Malzeville, France. Samples were washed in phosphate-buffered saline and processed using the same protocol as for fresh samples. A direct fluorescent antibody test (Diagnostic Pasteur polyclonal conjugate ref. 72112) was carried out. The supernatant of a 10% homogenate was inoculated into five OF1 mice (Kaplan & Koprowski 1973) and into a murine neuroblastoma cell line (Barrat, Barrat, Picard & Pubert 1988). The brains of inoculated mice that had died of rabies were pooled and homogenized in serum-free cell culture medium and then freeze-dried. The rabies virus isolated was later characterized at the Central Veterinary Laboratory, (CVL) UK using a technique (King 1991) of mouse brain passage smears on Teflon-coated multi-spot slides and a panel of antinucleocapsid monoclonal antibodies (Mab-Ns).

A commercial inactivated rabies vaccine (Madivak, Hoechst) was administered to each of the 29 adult *Lycaon* of the Salei and Ndoha packs, either by dart or by hand-injection of dart-anaesthetized animals. Five pups of the Salei pack were dart-inoculated at five months of age when they were considered old enough to withstand darting; four pups of the Ndoha pack were not vaccinated. The rationale for intervention in an attempt to control rabies in this population and the protocol for this field vaccination are described elsewhere (Gascoyne, Laurenson, Lelo & Borner 1993a; Gascoyne, Laurenson & Borner 1993b).

Each of the two packs was intensively observed (Burrows, personal communication) for between 15 and 48 h after vaccination. Radio-collared *Lycaon* from the Salei pack were located by telemetry on an approximately monthly basis for eight months following the vaccination programme. The Ndoha pack was located from the air in November 1990 (approximately two months after vaccination) but it was not until January 1991 (4–5 months after vaccination) that it could be followed on the ground and its status assessed. The alpha male was missing and five new pups were present (Burrows, personal communication).

Fifteen sera collected from 13 *Lycaon* of seven packs were tested for rabies virus neutralizing antibody. These samples included eight sera from *Lycaon* of five packs anaesthetized for fitting, replacing or removing radio-collars during the previous two years. In addition four other sera from unvaccinated *Lycaon* were collected as part of the vaccination programme. On either the 28th or the 59th day after vaccination, three *Lycaon* were anaesthetized for collection of post-vaccination blood samples to assess antibody response to vaccination. One of these *Lycaon* had been vaccinated by intramuscular injection and the other two had been vaccinated by darting.

Serological testing for rabies neutralizing antibody in these sera was carried out at the CVL. The test employed was a modification of the RFFIT (Smith, Yager & Baer 1973) regularly used to determine the vaccinal status of UK rabies personnel and others. Results were expressed in International Units (IU)/mℓ, determined by comparison of test serum titre with that of the International Standard antiserum followed by reference to a statistical table.

RESULTS

All tests on the carcass brain sample were positive for rabies and the virus isolated was identified as of serotype 1. The Mab-N reaction pattern of mouse-brain passage smears was consistent with that of a serotype 1 rabies virus and indistinguishable from the virus isolated from a domestic dog in an area adjacent to the Serengeti National Park.

The response of animals to dart vaccination was fairly consistent. The impact of the dart usually caused the animal to jump, occasionally to yelp, and then to run for a few seconds. Once the dart had dropped or been pulled out, the animal usually settled down quickly. No signs of lameness, injection-site reaction or systemic illness were observed in any *Lycaon* during the immediate post-vaccination monitoring period. Vaccination of adults within each pack was carried out on one day; the packs appeared not to be disrupted as a result of the procedure.

Between one and four months after vaccination, one vaccinated adult disappeared from the Ndoha pack,

but no adverse signs were seen in other vaccinated adults of either pack; the Ndoha pack was not seen again after January 1991. In February 1991 three females from the Salei pack and three males from the Ndoha pack formed a new unit. In May 1991, two males, one of which was radio-collared, disappeared from the unit and signs of lethargy were observed in others (Burrows, personal communication). By July 1991 two of these *Lycaon* had died (death was confirmed by retrieval of radio-collars). Also in July 1991, death of two other adults, one from the Salei and one from the Ndoha pack, was confirmed; no samples from these four *Lycaon* could be retrieved for diagnosis.

Thus, between four and ten months after vaccination, at least four radio-collared of the 34 vaccinated *Lycaon* died. Subsequent curtailment of radio-collaring and radio-tracking prevented long-term monitoring of the other vaccinated animals. Since June 1991 there have been no sightings confirmed by photographic identification of any vaccinated or unvaccinated *Lycaon* of the Salei or Ndoha packs, or of *Lycaon* from the (unvaccinated) Moru Track pack.

No rabies virus-neutralizing-antibody was detected in the pre-vaccination sera of four *Lycaon* held at Frankfurt Zoo. Five weeks after vaccination, three *Lycaon* had antibody titres of 1:40 and the fourth had a titre of 1:80. Table 1 summarizes the results of the serological analyses of the Serengeti *Lycaon*. One of five *Lycaon*, from five packs (Table 1 group A), had a level of rabies neutralizing antibody > 0,5 IU/mℓ. This *Lycaon* (LEGS) was alive at least five months after the blood sample was taken. In the Salei and Ndoha packs (Table 1 group B) two *Lycaon* (M and LIMP) had antibody levels > 0,5 IU/mℓ before vaccination. These dogs were alive at least five months after sampling and LIMP survived for at least 2,5 years. In both *Lycaon* from which paired serum samples were tested (SF and M), a rise in antibody level post vaccination was recorded; the increase was greater in the hand-vaccinated animal (M) than in the dart-vaccinated animal (SF).

DISCUSSION

Often, wildlife populations are able to survive perterbations such as disease. However, in relatively isolated populations, disease epidemics have the potential to reduce numbers to levels where stochastic events may lead to extinction. Of the pathogens that may infect carnivores, rabies is of particular concern for endangered canids. Firstly, the disease has the potential to cause high mortality and secondly, the virus can infect and be transmitted by a wide range of carnivores. Thus, small populations are unlikely to be capable of independent maintenance of the disease and they are at risk from "spill-over" transmission through contact with other species. Macdonald (1993) describes three such endangered canid populations that have been affected by rabies—Blanford's fox (*Vulpes cana*) in Israel, the Ethiopian wolf (*Canis*

TABLE 1 Serum-neutralizing antibody levels against rabies virus in *Lycaon* in the Serengeti National Park, Tanzania

Pack	*Lycaon*	Date sampled	Pre-vac. (IU/mℓ)	Date sampled	Post-vac. (IU/mℓ)
Unvaccinated *Lycaon*					
Naabi/Salei	LEGS	22.05.1989	0,55		
Ndoha/Ndutu	D583	17.07.1989	< 0,21		
Border	DBGM	17.02.1990	< 0,21		
Mountain	DMDM[1]	24.05.1990	< 0,21		
Hill	DHPM	26.02.1990	0,32		
Salei and Ndoha packs					
Salei	SF[2]	16.01.1990	< 0,21	29.09.1990	0,55
	M[3]	01.09.1990	0,55	29.09.1990	5,00
	LIMP	14.05.1988	0,55		
	MBILI	01.09.1990	< 0,21		
Ndoha	VY	11.09.1990	< 0,21		
	N188	19.01.1991	< 0,21		
	FLEUR	11.09.1990	0,32	09.11.1990	
	N685[2]	NB[4]			0,96

[1] Radio-collared male believed to have died of rabies
[2] Inoculated by dart
[3] Inoculated intramuscularly by hand
[4] NB = Not bled

Salei pack adults were vaccinated 01.09.1990 and pups on 20.09.1990
Ndoha pack adults were vaccinated on 11.09.1990

simensis) in the Bale mountains National Park in Ethiopia and the African wild dog (*Lycaon pictus*) in the Serengeti-Mara ecosystem of Tanzania and Kenya.

Rabies was confirmed in a *Lycaon* carcass in the Serengeti region of Tanzania in August 1990, a year after the disease had been identified as the cause of mortality in a pack in the Masai Mara National Reserve in Kenya, part of the Serengeti ecosystem (Alexander 1993) and 3 to 4 years after rabies had caused high mortality in Serengeti bat-eared foxes (*Otocyon megalotis*) (Maas 1993).

The social organization and behaviour of *Lycaon*, described in detail elsewhere (Goodall & Van Lawick 1970; Skinner & Smithers 1990; Mills 1993), suggests that intra-pack rabies transmission may occur more readily than inter-pack transmission. In the infected Serengeti pack described herein, rabies was confirmed in only one *Lycaon*, but clinical signs consistent with a CNS disorder, including ataxia, were observed in another adult and ataxia in six pups of the same pack and on the same day (Burrows, personal communication). These signs were similar to those observed in a rabies outbreak in a pack of *Lycaon* within the Masai Mara, except that individuals with grossly swollen heads and necks were seen in the Masai Mara but not in the Serengeti. The observation of several *Lycaon* clinically affected on the same day was also reported in the rabies outbreak in *Lycaon* in the Masai Mara (Alexander *et al*. 1993) and in a wolf pack (*Canis lupus*) in Alaska (Chapman 1978). In the latter case, six wolves died within a ten-day period four weeks after contact with the first clinical case. It may be that an infected *Lycaon* is able to infect other pack members, e.g. through exchange of saliva. An alternative explanation for the simultaneous occurrence of rabies in other pack members is that several *Lycaon* may be concomitantly infected by a rabid animal of another species.

The primary source of infection for *Lycaon* in the Serengeti is unknown. Since over 80% of confirmed rabies cases in Tanzania have been reported in domestic dogs (Magembe 1985) it may be that the domestic dog is the principal host of the disease in the Serengeti region. Isolation of a serotype 1 rabies virus from the *Lycaon* carcass, with a Mab-N reaction pattern consistent with canid-associated rabies, lends support to this view. Although contact rates between domestic dogs and *Lycaon* are not known, these species have the potential to interact in pastoralist land adjacent to the Serengeti National Park. The role of wildlife species, such as the bat-eared fox, in rabies dissemination in the Serengeti remains to be elucidated.

Results from this serological study should be treated with caution. The specificity of the test for *Lycaon* sera has not been established and few samples were collected. Serological surveys have been carried out in several other wildlife populations and rabies serum neutralizing antibodies have been detected in healthy individuals of a range of species, notably the raccoon *(Procyon lotor)* (McLean 1975; Winkler & Jenkins 1991), striped skunk *(Mephitis mephitis)* (Rosatte & Gunson 1984; Charlton, Webster & Casey 1991) and Indian mongoose *(Herpestes auropunctatus)* (Everard, Baer, Alls & Moore 1981). Rabies antibodies have also been detected by use of an ELISA technique in Ethiopian wolves (*Canis simensis*) and golden jackals (*Canis aureus*) in Ethiopia (Mebatsion, Sillero-Zubiri, Gotelli & Cox 1992). In contrast, in rabies endemic areas, only a few foxes in Europe (Wandeler, Wachendorfe, Forster, Krekel, Schale, Muller & Steck 1974; Baradel, Barrat, Blancou, Boutin, Chastel, Dannacher, Delorme,Gerard, Gourreau, Kihm, Larenaudie, Le Goff, Pastoret, Perreau, Schwers, Thiry, Trat, Uilenberg & Vannier 1988) and jackals in Zimbabwe (Foggin 1988) have detectable serum neutralizing antibodies.

The significance of serological findings in wildlife populations is therefore far from clear. Results of most rabies-serological surveys of these populations have been presented as seroprevalence data. In other studies criteria used to define the threshold between seronegative and seropositive animals were poorly defined and information from a negative control population has often not been available. Moreover, methods of calculation of antibody levels and conversion to International Units are not standardized throughout all laboratories. For example, in some laboratories, the serum dilution used in the test is considered as the finite dilution, whereas in others the addition of an equal volume of virus is considered to be a further 0,5 dilution of the serum (and the virus) (Atanasiu 1973). At the Centres for Disease Control, Atlanta, in the past 20 years, no pen-raised non-vaccinated study animal (including beagle dogs, skunks, raccoons and foxes) has exhibited rabies-neutralizing activity at a 1:12,5 dilution (effectively a 1:25 dilution when challenge virus is added); an animal serum is considered to be antibody positive if a 1:12,5 dilution completely neutralizes 32-100TCID50 of rabies virus; this titre is approximately equivalent to 0,5 IU/mℓ when compared to the reference serum standard (Smith, personal communication). On this basis, three (LEGS, LIMP and M) of the 12 pre-vaccination *Lycaon* sera have been recorded as having antibody levels of > 0,5 IU/mℓ. As has been reported elsewhere (Gascoyne *et al*. 1993b) using calculations based on finite dilutions, five (LEGS, LIMP, M, FLEUR and DHPM) of the 12 pre-vaccination *Lycaon* sera would have antibody levels > 0,5 IU/mℓ.

If a rabies antibody level of > 0,5 IU/mℓ is also specific for *Lycaon*, then the three *Lycaon* (LIMP, LEGS and M) in this study may be considered to have been previously exposed to rabies virus. However, in the

absence of results from a negative control population or of experimental challenge infection data for the species, it is not possible to accurately define the number of seropositive *Lycaon* in this study, or the significance of antibody levels in terms of protection against infection.

Vaccination of wildlife species has usually been attempted only where wild animal vectors or reservoirs of disease threaten man or livestock. From a conservation perspective, wildlife vaccination may also offer a solution for protecting rare species from diseases and has been suggested as a means of safeguarding endangered canids from the threat of rabies (Ginsberg & Macdonald 1990). Vaccination of wildlife has been adopted as a conservation measure, for example, to protect an endangered population of mountain gorillas *(Gorilla gorilla berengei)* in the face of a possible measles epidemic (Hall & Harwood 1990), and to protect chimpanzees *(Pan troglodytes schweinfurthi)* against poliomyelitis (Van Lawick-Goodall 1971).

Similarly, the rationale for the Serengeti *Lycaon* vaccination was to minimize the threat of rabies to the survival of an endangered population. Innocuity for *Lycaon* of the inactivated vaccine was proven in four animals during the vaccine trial at the Frankfurt Zoo; seroconversion could be taken as a measure of vaccine efficacy, although protection was not measured by challenge. There are no data to show that the *Lycaon* vaccinated in the Serengeti died of rabies.

Intervention to control a "natural" process, such as disease, is a departure from the more traditional conservation approaches of habitat protection. However, human activity may cause perturbations of the environment which predispose towards disease outbreaks, heightening the risk of extinction to small populations. In the Serengeti, for example, the likelihood of transmission of rabies between domestic dogs and wildlife is probably increasing as the human population (and that of their dogs) is expanding and encroaching into protected wildlife areas.

Healthy discussion surrounding the *Lycaon* vaccination programme was generated by the hypothesis (Burrows 1992) that handling of *Lycaon* for vaccination and radio-collaring was correlated with the emergence of rabies-associated mortality. It is considered unlikely, however, that vaccination four to ten months previously was causally related to their mortality or disappearance (Creel 1992; Macdonald, Artois, Aubert, Bishop, Ginsberg, King & Perry 1992). In four *Lycaon* populations, including the Masai Mara, no link has been found between patterns of mortality or disappearance, and handling animals for fitting radio-collars (Ginsberg *et al.*, unpublished data).

The Serengeti *Lycaon* vaccination programme has raised important questions regarding veterinary intervention in conservation management. Little is yet known about the long-term dynamics of disease or of the impact of disease control measures in most wildlife populations. However, wildlife managers are increasingly confronted with critical situations in which decisions to intervene have to be made in the absence of satisfactory data. Vaccination programmes in endangered populations have frequently been carried out as crisis management and in these circumstances long-term consequences are often not fully evaluated. However, long-term monitoring following intervention should be considered an integral component of project design.

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