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RABIES AND MORTALITY IN ETHIOPIAN WOLVES (*CANIS SIMENSIS*)

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ABSTRACT: Between October 1991 and February 1992, 41 of 53 known adult and subadult Ethiopian wolves (*Canis simensis*) in five adjacent packs in the Bale Mountains National Park, Ethiopia, died or disappeared. Brain smears from two carcasses were positive for rabies by the immunofluorescence test, and rabies virus was isolated from the brains by mouse inoculation. Based on monoclonal antibody tests on the mouse brains, we identified the virus as a minor variant of the serotype 1 rabies viruses found in domestic dogs and wild canids of Africa. Sera from two of 15 Ethiopian wolves had rabies virus neutralizing antibody.

Key words: Ethiopian wolf, *Canis simensis*, rabies, endangered species, case report.

INTRODUCTION

Several African canids are subject to rabies, including the bat-eared fox, *Otocyon megalotis* (Maas, 1993), the black-backed jackal, *Canis mesomelas* (King et al., 1993) and the African wild dog, *Lycaon pictus* (Fanshawe et al., 1991; Gascoyne et al., 1993). The Ethiopian wolf or Simien jackal, *Canis simensis*, is an endangered canid endemic to the Ethiopian highlands; with probably fewer than 500 survivors it is the rarest canid in the world (Gottelli and Sillero-Zubiri, 1992). The largest population (120 to 160 animals) occurs in the Bale Mountains National Park in southern Ethiopia. This, and the other six or seven isolated remaining populations, each comprising fewer than 100 adults, are vulnerable to severe losses or local extinction from epizootic disease (Dobson and May, 1986).

Rabies has been reported widely in domestic dogs (*C. familiaris*) in Ethiopia (Fekadu, 1982; Mebatsion et al., 1992a), including the Bale Mountains area (Gottelli and Sillero-Zubiri, 1992; Mebatsion et al., 1992b) and is the most immediate threat faced by wolves in Bale and possibly elsewhere (Sillero-Zubiri and Gottelli, 1994). Domestic dogs also affect wolves by direct competition and aggression and through genetic introgression (Gottelli et al., 1994; Sillero-Zubiri, 1994).

Three of the four rabies serotypes thus far identified by cross-immunization tests in animals (serotype 1, classical rabies; se-rotype 2, Lagos bat and serotype 3, Mokola) have been isolated from various terrestrial species in Ethiopia (Mebatsion et al., 1992a). The rabies nucleoprotein is produced abundantly during infection; group specificity is determined by its cross-reactivity and it has an important role in diagnosis and virus identification by antinucleocapsid monoclonal antibody (Mab-N) techniques. The Mab-N analyses of rabies viruses of South African origin, can be used to clearly distinguish between isolates from canids and viverrids; reaction patterns of viverrids accord with surveillance records and with a long-established infection in which mutation and evolution of host and parasite may have allowed the emergence of viral variants (King et al., 1993).

Recent cases of rabies in several rare and endangered canids such as Blandford's fox (*Vulpes cana*), Ethiopian wolf, and the African wild dog all surviving in small, fragmented populations, have given a new dimension to concerns about the control of rabies as a conservation issue (Macdonald, 1993). As a species becomes more endangered, its last remaining individuals are likely to be concentrated in a few relict populations and any of these populations could be eradicated by a disease epizootic.

Ethiopian wolves occur naturally at high densities (about one wolf/km²) in packs of up to 13 adults. In the Bale Mountains National Park, saturation of the sparsely available habitat has limited dispersal, resulting

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in a high degree of philopatry (Sillero-Zubiri and Gottelli, 1995). In the Web Valley, wolves coexist with humans, livestock, and domestic dogs. Wolves normally avoided direct contact with dogs; in all 34 agonistic interactions observed, dogs dominated wolves and chased them (Sillero-Zubiri and Gottelli, 1994). Direct interactions were also observed between wolves and serval cats (*Felis serval*), spotted hyenas (*Crocuta crocuta*), and honey badgers (*Mellivora capensis*) (Sillero-Zubiri, 1994).

In this paper we report two confirmed cases of rabies in Ethiopian wolves and the occurrence of two separate disease epizootics in the Bale population; the Mab-N reaction patterns of the rabies viruses isolated also were compared with those of other African rabies viruses evaluated in our laboratory. The conservation implications of rabies in this critically endangered species are also discussed.

MATERIALS AND METHODS

The demography and mortality of nine packs of Ethiopian wolves were studied between March 1988 to March 1992 in the central massif of the Bale Mountains National Park (Sillero-Zubiri, 1994). The Bale Mountains compose the largest range of Afroalpine habitat (over 1,000 km^2) in Africa. The study was carried out at two sites: the Web Valley (7°00'N, 39°40'E; 3,450 m above sea level) and the Sanetti Plateau (6°50'N, 39°50'E; 4,000 m) separated by only 15 km. Fifty-five wolves were individually marked using plastic ear tags, including 14 fitted with radio-collars, and 56 were identifiable by coat pattern differences (Sillero-Zubiri, 1996; Sillero-Zubiri and Gottelli, 1994). Observations were made from vantage points during daylight.

On 29 December 1991, two adult male wolves of the Fincha pack in Web were found dead within 200 m of each other, probably within 48 hr of death. Brain stem samples from these two wolves were collected and stored in liquid nitrogen. Rabies diagnosis of these specimens was carried out at the Central Veterinary Laboratory (CVL) Weybridge, United Kingdom. Diagnostic tests included direct immunofluorescence on brain smears (Dean and Abelseth, 1973) and mouse inoculation (Koprowski, 1973). Mice were euthanized by inhalation of excess anesthetic (halothane, Sigma, Poole, England). The Mab-N reaction patterns

of these two isolates, 520 and 521 (Table 1), were obtained from mouse brain smears by using a panel of 80 Mab-Ns consisting of 34 from the Wistar Institute, Philadelphia, Pennsylvania (USA), 29 prepared from the rabies-related viruses at the CVL and 17 from the Centers for Disease Control (CDC), Atlanta, Georgia (USA). Wistar and CDC Mab-Ns were used at the dilution recommended by the donors and the CVL Mab-Ns were used at a four-fold concentration of the end-point dilution. The test method employed was described by King et al. (1993), in which weaned mouse brain passage material from the isolates was mixed with baby hamster kidney BHK21 cells and distributed into 84-well plates. Following incubation in a CO$_2$-enhanced atmosphere to allow cell attachment and replication, the plates were fixed in acetone and air-dried; then the cells were reacted with Mab-Ns and anti-mouse serum conjugated to fluorescein isothiocyanate (FITC, Sigma, Poole, England). The reaction patterns were compared with those of eight other isolates we collected previously from various species of several African countries (Table 1).

Organs collected from the two carcasses were submitted for histological examination by Professor John Landells, Department of Pathology, Addis Ababa University, Ethiopia. Portions of liver, spleen, lung, and kidney were fixed in 10% formalin, embedded in paraffin, sectioned at 5 μm, stained with hematoxylin and eosin, and examined under a light microscope. In addition, 15 serum samples which had been collected from Ethiopian wolves trapped and released between 1988 and 1989 were evaluated for serum neutralizing antibody by Dr. Teshome Mebatsion at the Federal Research Institute for Virus Diseases of Animals, Tübingen, Germany, using an enzyme linked immunosorbent assay (ELISA) (Mebatsion et al., 1989) and rapid fluorescent focus inhibition test (RFFIT) (Smith et al., 1973).

Spearman's coefficient of rank correlation was used to compare the association of mortality and disappearance rates. For comparison of those rates between study sites and year we used the log-linear analysis of the three-way contingency table as applied by PROC CATMOD (SAS Institute, 1988). A Fisher's exact test was used to compare differential survivorship of dog/wolf hybrids (SAS Institute, 1988). When three-way interactions were significant, separate tests of independence within each level of one of the factors were made with a likelihood ratio chisquare test (Sokal and Rohlf, 1981).

RESULTS

Between 1988 and 1992, 42 (23 male and 19 female) adult and subadult wolves

TABLE 1. Anti-nucleocapsid monoclonal antibody (Mab-N) reaction patterns of 29 African rabies isolates from four countries, 1990 to 1993.

CVL[a] no.	Country of origin	Species	Mab-N reference number[b]															
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
520	Ethiopia	*Canis simensis*	+	+	+	+	+	+	+	+	+	+	0	0	0	+	+	+
521	Ethiopia	*Canis simensis*	+	+	+	+	+	+	+	+	+	+	0	0	0	+	+	+
401	Tanzania	*Lycaon pictus*	+	+	+	+	+	+	+	+	+	0	–	–	+	+	+	+
460	Kenya	*Canis familiaris*	+	+	+	+	+	+	+	+	+	+	0	0	0	+	+	+
431	South Africa	*Canis familiaris*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
405	South Africa	*Canis mesomelas*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
485	South Africa	*Otocyon megalotis*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
495	South Africa	*Cynictis penicillata*	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–

[a] Central Veterinary Laboratory.
[b] The 16 Mab-Ns, designated 1–16, are: 701-9, 102-27, 714-3, 590-2 and 377-7 from Wistar; DB11 from CVL; 364-11, 103-7 from Wistar; L4, L23, L25 and L28 from CVL and 8-2, 61-1 and 1-2 from CDC.
+ = positive, 0 = weak positive, – = negative.

died in a population of 111 known animals in nine focal packs. A further 35 (23 male and 12 female) wolves that were unaccounted for probably also died. The number of adults and subadults missing during every 6-mo period was correlated with the numbers found dead (r_s = 0.861, n = 8; P = 0.02). Mortality patterns were not constant; based on a log-linear analysis, the association between the number of individuals that survived and those that died or disappeared differed significantly by study site (Web and Sanetti) and year (likelihood ratio chi-square = 21.92, df = 3, P = 0.0001). In Sanetti, 12 of 23 known individuals among three packs died or disappeared during April to June 1990 (likelihood ratio chi-square = 15.32, df = 3, P = 0.0016). In the Web Valley a similar decline was observed between November 1991 and February 1992 (likelihood ratio chi-square = 65.64, df = 3, P < 0.0001), with a 77% loss of individuals; 25 died and 16 disappeared among five packs. Three of the Web Valley packs (Wolla, Fincha and Sodota) were decimated and eventually disintegrated. The few wolves surviving from those three packs were more likely to be dog-wolf hybrids; only one of 16 phenotypically normal wolves survived, whereas all three known dog-wolf hybrids survived (Fisher exact test, P = 0.0041).

Rabies virus was isolated from the two Ethiopian wolf brains collected. Following intracerebral inoculation, two mice inoculated with sample 521 were euthanized when sick at 9 days and two mice inoculated with sample 520 were euthanized at 21 and 28 days, respectively. Smears of all mouse brains were rabies fluorescence test strong positive.

In the determination with 80 Mab-Ns of the reaction patterns and comparisons with the reaction patterns of eight other African rabies viruses, 40 Mab-Ns were positive and 24 were negative with all isolates. Four Mab-Ns (10 to 13) had weak positive reactions with some but not all viruses. The weak reactions were consistent and reproducible and took the form of a

reduced amount of fluorescent dust-like particles, with the larger Negri-body-sized particles being fewer in number and sometimes less intensely stained.

No evidence of a long-standing disease was apparent from the histological examination of kidney, lung, liver and spleen. The liver of wolf 520 had separation of the liver cells from each other with much dissociation of blood among them. The liver of wolf 521 was clearly damaged, with liver cells swollen and containing brown pigments.

Of the 15 unvaccinated wolf blood serum samples tested for rabies antibody, two were positive by both ELISA and RFFIT. The ELISA titers were 1.2 and 2.5 IU/0.2 ml respectively, and both RFFIT titers were 60.

DISCUSSION

From our data we infer that two main population declines occurred among the Ethiopian wolf population in Bale. Close correlation between rates of known mortality and unaccounted disappearance was evidence that missing wolves died of similar causes to the ones found dead. While no definite cause was determined for the Sanetti decline in 1990, two carcasses recovered in Web Valley were positive for rabies. Within 4 mo, three Web packs collapsed and two others suffered losses due to a combination of shooting and disease (Sillero-Zubiri and Gottelli, 1993). There were some additional field observations to support rabies being the primary cause of mortality. Two subadult females died immediately after dispersing 1.2 and 7.5 km, respectively, from their ranges. Their carcasses had signs consistent with rabies virus infection. The sole survivor of another pack was resighted 70 days later 17 km away before it disappeared. At least three apparently sick females, two of which were pregnant, exhibited clinical signs of hind limb incoordination, which progressed to complete ataxia. Weakness was interspersed with violent whole body convulsions. These animals stopped foraging and

kept to a small area and two died within a week after the signs were first observed. The third disappeared and was never resighted.

Dog-wolf hybrids were seemingly more resistant to the epizootic than phenotypically normal wolves. Following hybridization, a population may be affected by a reduction in fitness known as outbreeding depression (Templeton, 1986). Perhaps diseases such as rabies exacerbate genetic introgression; following demographic bottlenecks, the generation of hybrid wolves which may be more resilient to rabies would accelerate genetic drift.

Rabies is enzootic in the Bale Region and there is evidence of previous sublethal infection in both dogs and Ethiopian wolves (Mebatsion et al., 1992b). Thus, those dogs and wolves may have recovered from either clinical rabies or exposure to a sublethal dose without showing signs (Fekadu, 1991). Serum neutralizing antibodies have been detected in other African carnivore populations in rabies enzootic areas, including golden (*Canis aureus*) and black-backed (*Canis mesomelas*) jackals and African wild dogs in the Serengeti (Gascoyne et al., 1993). In addition to rabies, domestic dogs in the Bale Mountains recently have been shown to have antibodies against canine distemper and parvovirus (H. Thompson, pers. comm.).

The rabies virus isolated from Ethiopian wolves were of serotype 1 canid origin. Although minor variations were observed between the canid viruses of northern and eastern Africa, their significance is unknown; they may be evidence of a different origin to the canid viruses of South Africa. Nevertheless, they are serotype 1 viruses consistent with domestic dog-associated rabies viruses, and these results support the view that domestic dogs are the most likely source of infection for Ethiopian wolves. In Zimbabwe, Foggin (1988) demonstrated that rabies viruses of viverrid origin differed both at the glycoprotein and the nucleoprotein level from those of canine origin. Furthermore, in

South Africa two distinct Mab-N reaction patterns were associated with serotype 1 isolates of either viverrid or of canid origin, although some interspecies transfer also occurred (King et al., 1993).

Disease epizootics can cause extinction in small, relict populations such as the black-footed ferret (*Mustela nigripes*) (Thorne and Williams, 1988). We believe all remaining populations of the rare Ethiopian wolf, including the world's largest concentration of the species in Bale, currently are vulnerable to extinction from stochastic environmental events, such as disease epizootics. Rabies is a serious risk for populations of rare canids; the epizootic among African wild dogs in the Serengeti (Gascoyne et al., 1993) and that of Ethiopian wolves emphasize the importance of disease monitoring in the conservation of endangered canids (Macdonald, 1993). In such cases, efforts to control rabies must incorporate the protection of endangered species (Macdonald, 1993).

High wolf population densities, high contact rates between and within packs, and the presence of sympatric domestic dogs all increase the likelihood and potential severity of disease epizootics. In Bale, domestic dogs travel regularly with their owners in and out of the mountain massif and then are in contact with many other dogs attracted to garbage and carrion in villages (Gottelli and Sillero-Zubiri, 1992). These dogs may provide the vehicle for pathogens to reach their wild relatives. No information is available on wolf-dog interactions in populations elsewhere in Ethiopia. However, contact between the species is greater at the lower limits of the wolf's altitudinal range, and wolf packs located at the periphery of a restricted population will therefore be more exposed to contact with domestic dogs.

Any future management of Ethiopian wolf populations aimed to secure their survival should concentrate on disease prevention and control of domestic dogs. Control could be achieved by preventing contact with infected animals, for example, by dog control (either removal or vaccination) to create buffer zones around wolf range, or by the implementation of an oral vaccination program for wolves (Ginsberg and Macdonald, 1990). The latter technique has been effective for orally immunizing red foxes (*Vulpes vulpes*) in Europe (Wandeler, 1991) and is at an early stage of field development for raccoons (*Procyon lotor*) in the USA (Hanlon et al., 1992). Direct intervention, by dart and manual vaccination, of the Serengeti wild dogs proved controversial since it was followed by an epizootic (Burrows, 1992; Burrows et al., 1994; Macdonald et al., 1992; Ginsberg et al., 1995). It is possible that vaccination may have been carried out when the disease was too well established within the population; alternatively, the wild dogs may have succumbed to a disease other than rabies such as canine distemper, which is also present in Serengeti (Morell, 1994). Extensive prophylactic vaccination against rabies and other zoonotic diseases needs further refinement and testing as part of its evaluation as a potential tool for the conservation of free-ranging populations of rare carnivores.

ACKNOWLEDGMENTS

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