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PRESENCE OF NEUTRALIZING ANTIBODIES TO RABIES VIRUS IN STRIPED SKUNKS FROM AREAS FREE OF SKUNK RABIES IN ALBERTA

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ABSTRACT: Nine percent of 198 serum samples from striped skunks, *Mephitis mephitis* (Schreber) from five areas of Alberta were positive for rabies neutralizing antibody. Positive samples were minimal (2%) from specimens sampled in an area enzootic for rabies and occurred at greater rates in areas negative for skunk rabies. Transmission of rabies virus to skunks may have been from a source other than skunks in those areas, most probably from bats.

INTRODUCTION

The first case of rabies in striped skunks in Alberta was diagnosed in 1971 and during the 1970's most skunk rabies in the province occurred near the Alberta–Saskatchewan border area (Gunson et al., 1978). In December 1979, an outbreak of rabies in skunks was first diagnosed in southern Alberta and has been enzootic to 1983. Bats, also first diagnosed with rabies in Alberta in 1971 (Schowalter, 1980), form a large reservoir throughout the settled portions of the province and may, or may not, be involved in the skunk rabies cycle.

Detection of levels of rabies serum neutralizing antibody (NA) in sera of animals has been used by several researchers to investigate the epidemiology of rabies (Everard et al., 1974; Wandeler et al., 1974; Carey and McLean, 1978). Various species, including skunks, bats and red foxes (*Vulpes vulpes* L.), have been shown to develop NA after ingesting rabies-infected carcasses (Ramsden and Johnston, 1975), following intramuscular inoculation of virus (Sikes, 1962), and following intestinal instillation of rabies vaccine (Lawson et al., 1982). To develop NA, an animal must come into contact with the virus (Cremer, 1979a), and the immune response, or antibody formation, is produced following exposure to the pathogenic organism (Cremer, 1979b).

During this study, levels of NA to rabies virus were tested in serum samples from striped skunks to obtain possible clues to modes of rabies infection and transmission in Alberta. Specimens were collected from areas free of, or enzootic for, skunk rabies, and periods prior to, and during, the rabies epizootic.

MATERIALS AND METHODS

Sera, selected from a serum bank of about 1,500 specimens, represented striped skunks collected in five areas differing in habitat and rabies history. The Warner area, the western portion of the 1979–1983 southern enzootic (Fig. 1) consists of cultivated and uncultivated prairie habitat (Webb et al., 1967) with numerous deep-cut coulees, sloughs and irrigation canals. The Stirling area, with similar land use, is located northwest and adjacent to Warner and was free of skunk rabies until December 1982. Forty-four rabid bats (big brown, *Eptesicus fuscus* (Beauvois); little brown, *Myotis lucifugus* (LeConte); silver-haired, *Lasionycteris noctivagans* (LeConte); hoary, *Lasiurus cinereus* (Beauvois)) were taken in the general area during the period 1973–1982.

Striped skunks in the Olds, Millet and Tofield areas (Fig. 1), located in the agriculturally modified parkland of central Alberta (Bird and Bird, 1967), have been free of rabies to date. At least 75 skunks collected in these three areas and numerous other skunks from the parkland of Alberta were negative for rabies virus according to the fluorescent antibody technique during 1971–1982. Several cases of rabies in

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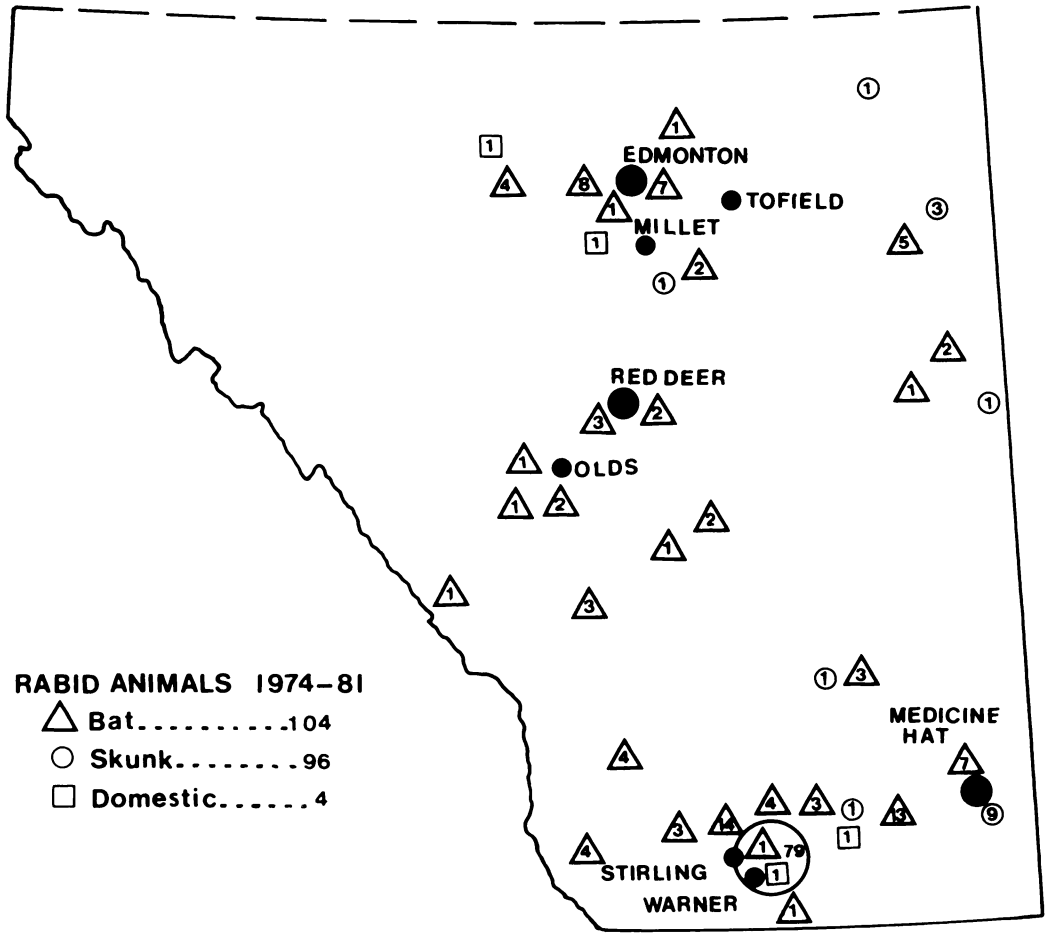

FIGURE 1. Locations in the southern half of Alberta, Canada, from which serum samples were obtained from striped skunks in relation to locations of animals diagnosed as rabid during 1974–1981.

bats have been diagnosed in or near these areas, especially Olds and Millet.

Sera from 198 striped skunks taken during 1974–1981 were analyzed for NA to rabies virus at the Department of Microbiology, University of Toronto. Samples tested included 34 from Tofield in 1974 during removal of skunks from winter dens (Gunson and Bjorge, 1979; Bjorge et al., 1981), 31 from Stirling during 1975–1976 and 42 during 1981, 24 from Olds and Millet during 1981 in surveys for studying leptospirosis, and 67 from Warner in 1981 where population reduction was in progress.

Skunks were captured using various live- and kill-traps, and night-lighting. Live animals were sexed, age was estimated in the field and confirmed in the laboratory from cemental annuli (Casey and Webster, 1975), and blood samples were collected via cardiac puncture using 10-ml Vacutainer brand collecting tubes, 20-gauge Vacutainer sample needles and holder. Samples were allowed to settle or were centrifuged. Sera were drawn off and stored in 2-ml serum provials at −23 C until analyzed. Brain tissues were tested for rabies via the fluorescent antibody test (FAT) at ADRI, Agriculture Canada, Lethbridge, Alberta.

A modified rapid fluorescent focus inhibition test (RFFIT) (Zalan et al., 1979) was employed using two-fold dilutions. Total serum NA was measured without differentiating between the various classes of immunoglobulin (Babins, 1983). Values were expressed as international units (IU). Titers less than 0.09 IU, tested prior to spring 1982, and 0.13 IU, tested after spring 1982, were considered negative. This higher ti-

TABLE 1. Positive rabies neutralizing antibody serum titer levels (IU)[a] for striped skunks in Alberta.[b]

Sample location	Age and sex[c]	Sample number	Serum collection date	Serum titer June 1982[d]	Serum titer March 7, 1983[e]
Tofield	A F	1	Dec. 1974	0.33	0.61[f]
Tofield	J F	2	Dec. 1974	0.24	Neg.
Tofield	A F	3	Dec. 1974	0.13	
Millet	A F	4	Apr. 1981	0.48	0.22[f]
Olds	A M	5	Oct. 1981	0.17	0.24
Olds	J F	6	Oct. 1981	0.33	0.26
Olds	J F	7	Oct. 1981	2.36	0.56
Olds	J F	8	Oct. 1981	1.13	0.95
Stirling	A M	9	Feb. 1976	0.17	
Stirling	A M	10	Feb. 1976	0.15	Neg.
Stirling	A M	11	Jan. 1976	0.20	Neg.
Stirling	J F	12	Sept. 1981	0.20	Neg.
Stirling	J F	13	Sept. 1981	0.24	Neg.
Stirling	A M	14	Sept. 1981	Neg.	0.26[f]
Stirling	A M	15	Sept. 1981	Neg.	0.30
Stirling	A F	16	Sept. 1981	Neg.	0.98
Stirling	A F	17	Sept. 1981	Neg.	0.28
Warner	A M	18	July 1981	0.22	

[a] International Units.
[b] Each individual skunk serum sample was tested two to four times.
[c] JM—Juvenile male; JF—juvenile female; AM—adult male; AF—adult female.
[d] Skunk sera were collected in a minimum of three different provials per specimen.
[e] Skunk sera tested were from same specimen but in a different provial than d.
[f] Toxicity was evident at serum dilutions of 1:4–1:8.

ter level increased the probability of a sample being a true positive rather than a borderline positive. Toxicity levels refer to the highest dilution of serum at which non-specific cytoplasmic effect was evident in the test. The presence of mercaptans, bacterial contamination or components of lysed red blood cells may have contributed to toxicity (Babins, 1983). Positive samples were assayed several times to ensure accuracy. Statistical analyses of data were accomplished using chi-square (Zar, 1974).

RESULTS

Of the 198 samples from skunks 18 (9%) were positive for NA with positive titers ranging from 0.13 to 2.36 IU and three of the samples tested were toxic at dilutions of 1:4–1:8 (Table 1). Sex and age of 195 of the 198 skunks were known. The sample consisted of 35% (69/195) adult females, 29% (56/195) juvenile females, 27% (53/195) adult males and 9% (17/195) juvenile males. Of the 18 positive samples, 39% (7/18) were from adult males, 33% (6/18) from juvenile females and 28% (5/18) from adult females. There were no positive sera from juvenile males. Positive titers among sex and age classes ranged from 11% (6/56) for juvenile females to 0/17 for juvenile males (Table 2).

Percent positive titers between areas of sampling ranged from two to 21 (Table 2). The Olds and Millet areas, which have been free of skunk rabies, had significantly more positive titers of 21% (4/19) and 20% (1/5), respectively, than the Warner area (2%, 1/67) which is enzootic for skunk rabies ($P < 0.025$) (Table 2). Significantly more titers positive for NA were obtained from specimens sampled in September (29%, 6/21) (Table 3), than in any other month of the year ($P < 0.05$).

Stirling was the only area where sera tested were taken in more than 1 yr. Three of 17 (18%) sera sampled in 1976 were positive for NA, 6/43 (14%) in 1981, and 0/14 in 1975, although there were no significant differences in occurrence of positive samples between the 3 yr.

Only one of the 198 skunks sampled was positive for rabies by FAT and it was negative for NA.

DISCUSSION

The presence of rabies NA seropositive animals in a given area suggests strongly that rabies virus occurs in that area (Hall, 1978). All five areas of Alberta sampled contained seropositive skunks, suggesting they came into active contact with rabies virus sometime during their life.

Skunks sampled near Olds, an area entirely free of skunk rabies to date, had the greatest number of sera positive for NA (4/19 or 21%). This area is about 200 km northwest of the nearest locality where skunk rabies has been found (Rosatte et al., 1983). However, 20 cases of bat rabies

TABLE 2. Prevalence (%) of positive serum neutralizing antibody titers to rabies virus in striped skunks in various age and sex classes in Alberta, 1974–1981.

Age	Sex	Prevalence (no. pos./no. examined)					
		Tofield area	Millet area	Olds area	Stirling area	Warner area	Totals
J	F	9.1 (1/11)	—	23.1 (3/13)	10.5 (2/19)	0 (0/13)	10.7 (6/56)
J	M	0 (0/4)	—	0 (0/2)	0 (0/4)	0 (0/7)	0 (0/17)
A	F	13.3 (2/15)	50 (1/2)	0 (0/1)	8 (2/25)	0 (0/26)	7.3 (5/69)
A	M	0 (0/3)	0 (0/3)	33.3 (1/3)	20 (5/25)	5.3 (1/19)	13.2 (7/53)
Totals		9.1 (3/33)	20 (1/5)	21.1 (4/19)	12.2 (9/73)	1.6 (1/65)	9.2 (18/195)

were diagnosed in the Olds–Red Deer area during 1971–1981 and we speculate that bats may have played a role in infecting skunks.

One of five of the skunks tested near Millet was positive for NA. Again, this area has been free of skunk rabies, except for one skunk, collected 20 km from Millet, which was positive for rabies in 1975. However, JRG has questioned that skunk rabies occurred in this area. The skunk was taken from an area of extremely low skunk density (Gunson et al., 1978). Several rabid bats have been reported from the general area (Fig. 1).

TABLE 3. Numbers of serum samples from striped skunks in Alberta which were positive for neutralizing antibody to rabies virus on a monthly basis during 1974–1981.

Month of sample	No. samples	No. positive samples
January	5	1
February	35	2
April	5	0
July	31	1
August	5	1
September	21	6
October	42	4
November	8	0
December	46	3

Wandeler et al. (1974) suggested that the potentially immune portion (measured by antibody response) of an animal population is small, and that there is no evidence to suggest that immunity plays a significant role in the survival of a great portion of a population in a rabies endemic area. Only 1/67 (1.5%) of the skunks tested from the southern skunk rabies area near Warner was positive for NA. Similarly, Ferguson and Heidt (1981) and Sikes (1962) found 1/53 and 0/53 skunks positive for NA in areas epizootic for rabies in Arkansas and Alabama, respectively. It is possible that the majority of skunks in rabies enzootic areas may not develop neutralizing antibodies. Although most of the skunks that develop clinical signs probably develop NA, very few survive. The probability of finding animals with NA may depend on the animal species and viral strain. In some instances, a viral strain of one species may be less pathogenic and/or more immunogenic. That could result in more animals having detectable levels of NA in a survey of a certain geographic area. For example, 17% of a sample of raccoons, *Procyon lotor* L. (McLean, 1971), in Florida had detectable antibody, whereas only 3% of red foxes in an epizootic area of Alabama had NA (Sikes, 1962).

Six of 43 (14%) skunks from the Stirling area in 1981 were positive for NA and several skunks diagnosed positive for rabies were taken 15–20 km from this area in early 1981. However, 3/17 (18%) skunks taken near Stirling in 1976 were also seropositive. Prior to 1976 there were only two isolated cases of skunk rabies near (50–100 km) Stirling and the nearest skunk rabies outbreak of any magnitude was in the Medicine Hat area, about 150 km northeast.

There were six rabid bats within 30 km of Stirling in 1975. As in other areas in Alberta, the skunks positive for NA in 1976 may have been exposed to rabies through bats.

Three of 34 (9%) skunks taken in the final phase of the Tofield skunk project were also positive for NA. All 60 skunks submitted from this area at that time were negative for rabies, suggesting that the positive specimens were exposed to rabies from a source other than skunks.

The greater prevalence of positive specimens in September (6/21, 29%) (Table 3) might be a result of the annual August–September peak in bat rabies in Alberta. These 2 mo are the period of greatest activity in bats in Alberta (Schowalter, 1980). Juvenile bats have recently become volant and adults and young are beginning migration. This mobility results undoubtedly in greater accidents and mortality and potential exposure to terrestrial carnivores.

Lord et al. (1975) suggested that terrestrial carnivores may become exposed to rabies from bats via ingestion or other modes of infection. They found 4/8 South American foxes, *Dusicyon gymnocercus* (Fischer), and 2/2 hog-nosed skunks, *Conepatus chinga* (Molina), in an area of Argentina to be positive for rabies NA. Vampire bats, *Desmodus rotundus* (Geoffroy), and cattle were the only rabid species in the area. In Alberta, skunks from Olds, Tofield and other areas may have developed NA to rabies virus after contact with virus from bats because rabies in skunks in those areas was not previously, or since, diagnosed.

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BOOK REVIEW . . .

A Bibliography of Parasites and Diseases of Ontario Wildlife, by L. M. Smith and E. M. Addison. Ontario Ministry of Natural Resources, Research Section—Wildlife Branch, Maple, Ontario, Canada. Wildlife Research Report No. 99. 1982. 267 pp.

The bibliography includes 768 citations on natural and experimental studies of parasites and disease of wildlife species native to the Province of Ontario, Canada, published between the late 1800's and 1981. In addition to works in primary journals, some historically valuable material appearing as research reports of the Ontario Veterinary College (now O.V.C., University of Guelph, Guelph, Ontario) and of the Ontario Department of Lands and Forests (now Ontario Ministry of Natural Resources) is also included. Titles refer to 63 species of mammals, 200 species of birds, 15 amphibians, and 14 reptiles.

Indexing by parasite and disease and by host (both scientific and common names) makes accurate and rapid search possible. The parasite and disease index is arranged by parasite taxonomic group plus the following: Anatomical Disorders, Environmental Contaminants, Genetic Disorders, Nutritional Disorders and Reproductive Disorders.

The Bibliography suffers the shortcomings of any such work limited in scope by geographic or political boundaries but constitutes a very useful reference for wildlife people working in and adjacent to the Province of Ontario. Copies are available free while supplies last from Dr. E. M. Addison, Wildl. Res. Section, P.O. Box 50, Maple, Ontario L0J 1E0, Canada.

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