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Author: CAREY, ANDREW B.

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RABIES ANTIBODY PREVALENCE AND VIRUS TISSUE TROPISM IN WILD CARNIVORES IN VIRGINIA

ANDREW B. CAREY,[1] Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061, USA.
ROBERT G. MC LEAN,[2] Rabies Control Unit, Center for Disease Control, Public Health Service, U.S. Department of Health, Education, and Welfare, Lawrenceville, Georgia 30245, USA.

Abstract: Carnivores trapped in a rabies control program in Virginia were examined for rabies virus and serum neutralizing antibody. Local antibody prevalence ranged from 0% to 29% in gray foxes *(Urocyon cinereoargenteus)*. Rabies virus was pantropic in naturally infected gray foxes and a bobcat *(Lynx rufus)*.

INTRODUCTION

Rabies is enzootic in gray foxes *(Urocyon cinereoargenteus)* in western Virginia.[2] In 1972, during the course of an investigation into the landscape epidemiology of rabies in the enzootic area,[2] we decided to determine the prevalence of rabies virus, prevalence of antibody, and tissue tropism in carnivores in an area of an ongoing epizootic of rabies to obtain more information about the nature of the host-parasite relationship between the gray fox and rabies. In previous studies, non-neural tissue tropism of rabies virus in the laboratory[3,11,13] and pantropism in free-ranging red foxes *(Vulpes fulva)* were demonstrated.[5]

MATERIALS AND METHODS

Carnivores (primarily red and gray foxes) trapped in a rabies control effort in Rockbridge, Botetourt, and Bedford Counties in Virginia were bled and examined at necropsy. The necropsy procedure included the preparation of slide impressions from the hippocampus of the brain and collection of tissue sections from some major body organs, all of which were kept at -70 C until tested. Fluorescent antibody (FA) tests[4] were performed on the slide impressions to detect rabies virus by the Health Department Laboratory in Abingdon, Virginia. The Rabies Control Unit, Center for Disease Control (CDC), Lawrenceville, Georgia, performed mouse serum neutralizing (SN) antibody tests[16] using 40 MLD_{50} of rabies virus (CVS-27) on the sera of all animals collected. Virus titrations of four types of tissue from the two foxes diagnosed as having rabies on the basis of FA test results were performed by intracerebral (IC) inoculation of weanling white mice.[8] The isolation of virus from five other tissues from these foxes was determined by inoculating 2-4 day-old suckling mice IC and FA testing of the brains of the mice which became moribund.

RESULTS

The rabies virus and SN antibody results for the five species of mammals trapped are presented in Table 1. The gray fox had a substantially higher prevalence of rabies virus activity than the red fox (9.6% virus and antibody positive versus 2.1%). The reported case data for 1972 showed 98% of the 83 cases

[1] Present Address: Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, Connecticut 06510, USA.

[2] Present Address: Vector-Borne Diseases Division, Center for Disease Control, Ft. Collins, Colorado 80522, USA.

TABLE 1. Species collected from Rockbridge, Botetourt, and Bedford Counties, Virginia, in 1972 and tested for rabies virus and serum neutralizing (SN) antibody.[1]

Species	Virus			SN Antibody		
	Sample Size	Number Positive	Percentage Positive	Sample Size	Number Positive[3]	Percent Positive
Gray Fox *(Urocyon cinereoargenteus)*	40	2	5.0	94	7	7.45
Red Fox *(Vulpes fulva)*	25	0	0.0	47	1	2.13
Other Species[2]	6	0	0.0	15	0	0.0

[1]In addition, a bobcat *(Lynx rufus)* from Craig County, Virginia, was found to be rabid.
[2]Other species include opossum *(Didelphis marsupialis)*, striped skunk *(Mephitis mephitis)*, and raccoon *(Procyon lotor)*.
[3]Sera were considered positive when none of five mice inoculated with the serum-virus (CVS-27) mixture died during the mouse serum neutralization (SN) antibody test.

of fox rabies in Virginia and 91% of the 80 cases of animal rabies in the tri-county region were among gray foxes.[2] The distribution of rabies virus activity in this enzootic region was quite focal. Within individual sampling units, the prevalence of antibody ranged from negative to 29% in units where antibody-positive foxes were found. Differences among counties were also evident; 9.0% of the gray foxes collected in Rockbridge were virus positive, and 9.8% were SN antibody positive, 8.3% were antibody positive and none were virus positive in Botetourt and none were virus or antibody positive in Bedford County. Both virus-positive gray foxes were collected in areas where antibody-positive foxes were collected. Neither virus-positive fox had measurable rabies antibody. Both appeared normal in behavior and were postpartum yearling females. One was lactating and had five placental scars; the other was not and had six scars.

The tissue tropism of the virus in the two positive foxes is presented in Table 2. The quantity of virus found in the adrenal gland of one fox and oral mucosa tissue of both foxes was greater than previously reported.[5,18] Small quantities of virus also were isolated from the lung, heart and kidney tissues of these foxes. The virus titers of the brain and salivary gland tissues were similar to those reported by other investigators[18] and were high enough to permit the virus to be transmitted by bite to other foxes.[12]

In addition, a bobcat *(Lynx rufus)* killed while attacking dogs in Craig County (southwest of the study area) was examined. Its brain was FA-positive, and rabies virus was isolated from salivary glands, lungs, kidneys, adrenals, and spleen. The virus titer of the salivary gland was $10^{4.4}$ $MICLD_{50}$.

DISCUSSION

These field data on rabies in foxes of Virginia confirm the recent case-reported data which identified the gray fox as the

TABLE 2. Tissue tropism of rabies virus in rabid gray foxes collected from Rockbridge County, Virginia, 1972.

Gray Fox Number	Tissues Tested and Tests: $MICLD_{50}$[1], 3-Wk-Old-Mice				Fluorescent Antibody of Moribund Suckling Mice				
	Brain	Salivary Gland	Adrenal Gland	Oral Mucosa	Lung	Heart	Kidney	Liver	Spleen
204	$10^{-4.5}$	$10^{-4.2}$	$10^{-2.0}$	$>10^{-1.0}$[2]	+[3]	+	+	-	NT
309	$10^{-3.0}$	$10^{-4.6}$	$10^{-0.2}$	$>10^{-1.0}$[2]	-	+	-	-	-

[1]50% mouse intracerebral lethal dose/0.03 ml of 20% suspension of original tissue.
[2]No end point was determined.
[3]+ = positive; - = negative; and NT = not tested for virus.

major host species of wild carnivore rabies in Virginia. The prevalence of antibody in gray foxes was higher than previously reported[12,17,18] and further supports the concept that rabies is not a uniformly fatal disease in wild animals.[9,10,18]

The significance of the broad tissue tropism of the street virus in gray foxes and the bobcat is twofold. First, it is significant in nonbite transmission of the virus. The presence of the virus in the kidneys suggests that the virus may be excreted in urine, which has been reported for red foxes.[5,18] Virus excreted in urine and the large amounts found in the salivary glands, coupled with the behavior of the gray fox (communal denning, sniffing, licking, and scent-station marking with urine), support Kauker's hypothesis[7] of nonbite transmission. Infection by nasal and rectal instillation and by inhalation have been experimentally induced.[11] The occurrence of the virus in tissues throughout the body cavity provides ample opportunity for animals to be infected by eating infected tissues from the carcasses of dead rabid animals.[3,6,11,15] When red foxes were vaccinated with live rabies virus orally, the virus invaded the oropharyngeal mucosa.[1]

Even though end points in titrating oral mucosa of the two gray foxes were not determined, the amount of virus present (10^{-1} dilution of 20% suspension of tissue killed all five weanling mice inoculated) suggest infection by the non-neural tissue and not just the presence of the virus in nerve fibers. Although it is not known whether foxes consume other dead foxes (or bobcats), skunks and scavenging animals probably would. These alternative modes of transmission would be of greatest potential importance in rabies spread when fox contacts are highest, i.e., during times or in places of high fox population density and during seasons of greatest fox movements. Second, the occurrence of the virus in non-neural tissues is significant for individuals who perform postmortem examinations on animals which are hosts for rabies. Biologists and students in rabies enzootic areas should be informed of the potential hazard.

The existence of presumably immune gray foxes suggests that rabies may have a traditional host-parasite relationship with the gray fox. McLean[10] found a similar relationship with raccoons *(Procyon lotor)* and rabies. The initiation and duration of an epizootic would depend not only on the density of the fox population (i.e., the rate of contact between individuals) but also upon the proportion of immune to susceptible foxes. Smart and Giles[14] constructed a model of this relationship. The existence of an immune population of foxes (along with the susceptible population) is of special significance in instituting fox population reduction programs to control rabies, especially in terms of timing and intensity of control. Foxes could be removed after an epizootic is reported, which would be well after the epizootic is actually initiated, the first suspect animals are submitted, and an epizootic is declared. Immune animals also might be removed at the same time animals immigrate or when juvenile animals join the population. Thus, the proportion of immune to susceptible animals might be lowered, resulting in a prolonged rather than shortened epizootic. Likewise, if the population reduction were conducted after the epizootic had spread through and beyond an area, removing immune animals could be detrimental because it would lower the overall population immunity and create conditions favorable to a recurring epizootic. Such an outbreak could be caused by wide-ranging rabid foxes or could spread from small undetected epizootic or enzootic areas (nidi). Both situations outlined above depend upon whether control measures are extensive or sporadic (as is often the case) and whether susceptible foxes immigrate to occupy the vacated habitats, which would be likely since rabies epizootics most commonly occur during the period of juvenile fox dispersal.[2]

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