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## NATURALLY OCCURRING RABIES VIRUS AND NEUTRALIZING ANTIBODY IN TWO SPECIES OF INSECTIVOROUS BATS OF NEW YORK STATE

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*Abstract:* Seven colonies of *Eptesicus fuscus*, the big brown bat, and five colonies of *Myotis lucifugus*, the little brown bat, in New York State were sampled for rabies virus and virus-neutralizing antibody. Eight of 278 *E. fuscus* were found to have virus, while 18 of 187 had antibody titers of  $\geq 1:8$ . One of 333 *M. lucifugus* yielded virus, while three of 127 had antibody. These data demonstrate the presence of rabies virus as well as immunity to rabies in some insectivorous bats of New York State. Evaluation of these findings in relation to the epizootiology of the disease in bats requires further investigation.

### INTRODUCTION

The first laboratory confirmation of rabies in a bat in New York State occurred in 1956. Since that time 249 insectivorous bats, representing all 10 indigenous species of the state, have been confirmed rabid. In the 5-year period 1972-76 an average of 20.6 bats per year have been diagnosed rabid, or 4% of the bats submitted for examination. The big brown bat, *Eptesicus fuscus*, has been by laboratory diagnosis the most frequently infected (79/136 or 58%), with the little brown bat, *Myotis lucifugus*, second (15/136 or 11%).

Bat rabies is found throughout New York State. Most cases are discovered in the Hudson River Valley region and around metropolitan areas, probably indicating that where more people live, more bats are submitted for rabies examination. The actual prevalence of bat rabies can be assumed to be widespread.

Since summer bat colonies often occur in close association to human habitation, a study was initiated to determine the extent of rabies infection and of naturally acquired neutralizing antibody in selected colonies of bats adjacent to human habitation.

### MATERIALS AND METHODS

A total of 12 colonies, 7 *E. fuscus* and 5 *M. lucifugus*, were investigated. With the exception of 2 colonies of *M. lucifugus*, all of the bats were from colonies at or adjacent to the location where a confirmed diagnosis of rabies in one or more bats had previously been made. All 611 bats were tested for virus, and 314 were examined for neutralizing antibody.

The bats were captured by one or more of the following techniques: Japanese mist nets, plastic bag traps set at exit holes, chloroform spraying within a plastic tent secured around a colony, and manual collection of individual bats with forceps.

Blood was collected from each live bat into 4 heparinized hematocrit capillary tubes by making a small incision on the ventral surface of the leg. The tubes were sealed and the contents centrifuged at 600 xG, inactivated at 56 C for 30 min, and stored at -20 C prior to testing. The micromethod for measuring rabies-neutralizing antibody (NA) was performed as previously described.<sup>1</sup> The plasma was diluted twofold, and a titer of  $\geq 1:8$  was considered a positive rabies antibody response.

Active rabies infection was determined by fluorescent antibody testing<sup>9</sup> (FAT) of brain tissue using fluorescein-labeled rabbit antirabies serum.<sup>18</sup>

Mouse inoculation tests (MIT) for virus isolation were conducted as previously described<sup>11</sup> using five 10-12 g Nya: NYLAR mice for each 10% brain suspension. In mice which died, rabies was verified by the FAT.

## RESULTS

### *Eptesicus fuscus*

A total of 278 big brown bats were collected from seven individual colonies over a 3-year period in 10 separate collections. Six colonies were sampled once and the seventh four times over a 16-month period (Table 1). Eight bats

(2.9%) were found by the FAT and MIT procedures to have rabies. In only one instance was more than 1 bat found positive for rabies during any given collection.

Demonstrable NA was found in 18 of 187 virus-negative bats (9.6%) tested from these colonies. A total of 30 bats in 3 colonies failed to reveal evidence of NA. The prevalence of NA in the remaining 4 colonies ranged from 10% to 40%, but the sample size in 2 colonies was extremely small (5 bats each).

The single colony with multiple collections between June 1974 and September 1975 had a rather constant prevalence of NA: 13% in the Spring of each year, 15% in August, 1975. One month later none of 22 bats collected had demonstrable NA.

TABLE 1. Prevalence of Rabies Virus and Rabies-Neutralizing Antibody (NA) in Colonies of *Eptesicus fuscus* and *Myotis lucifugus* in New York State.

Species	Collection site	Date	No. positive / No. tested	
			FAT <sup>a</sup>	NA <sup>b</sup>
<i>E. fuscus</i>	A	Aug 73	2/20	0/5
		Oct 75	0/2	0/2
	C	June 74	1/93	3/26
		May 75	1/55	6/53
		Aug 75	0/24	3/23
		Sept 75	1/24	0/22
	D	Aug 75	1/24	0/23
		July 76	1/7	2/5
	F	July 75	0/23	3/23
		July 75	1/6	1/5
Total			8/278	18/187
<i>M. lucifugus</i>	H	Oct 75	0/5	0/5
		May 76	0/40	0/39
	B	May 75	0/55	0/10
		Oct 75	0/10	0/10
	I	July 75	0/48	1/24
	J	June 76	0/11	0/10
	K	July 74	1/164	2/29
Total			1/333	3/127

<sup>a</sup>Fluorescent antibody test on brain tissue.

<sup>b</sup>Plasma neutralizing antibody titer  $\geq$  1:8.

### ***Myotis lucifugus***

Five little brown bat colonies were sampled over a 2-year period. A total of 333 bats were collected, and only 1 (0.3%) was rabid.

Neutralizing antibody was found in 3 of 124 bats tested (2.4%), 2 of them from the same colony (2/27). Two colonies were sampled twice, and no NA was detected on any occasion.

### **DISCUSSION**

The presence of serum NA in apparently normal animals has been reported in terrestrial mammals<sup>2,3,9,10,12</sup> and bats<sup>4,5,8</sup> with suggestions that it provides evidence of subclinical or abortive infection.<sup>7</sup> In our study NA was present in 9.6% of the big brown bats and 2.4% of the little brown bats collected. However, it is impossible to determine whether this antibody level indicates abortive, subclinical, or the incubation stage of rabies. One virus-positive *M. lucifugus* and 5 of the 8 virus-positive *E. fuscus* were tested for NA, but none had demonstrable antibody at the time of death.

Previous investigations on NA in bats have been largely confined to the vampire bat, *Desmodus rotundus*,<sup>3,8</sup> the Mexican free-tailed bat, *Tadarida mexicana*,<sup>1-5</sup> and the Mexican brown bat, *Myotis velifer*.<sup>4</sup> For *D. rotundus* a 24% prevalence of NA was found in one enzootic

area of Argentina.<sup>8</sup> One study of *T. mexicana* in Texas revealed a prevalence of NA, depending on the colony, ranging between 16.6 and 79.0%,<sup>4</sup> while a subsequent survey in New Mexico showed NA to be higher in adult bats in spring and autumn (21% for symptomatic and 25% for asymptomatic bats).<sup>5</sup>

The advent of the capillary-tube blood sample technique for virus neutralization tests<sup>1</sup> allows for blood collection without killing the bat or requiring pooled serum samples from bat colonies. This technique should enhance the opportunity for study of the epizootiology of rabies in insectivorous bats. The present limited investigation suggests that the prevalence of rabies in any given colony of *E. fuscus* or *M. lucifugus* at any particular time is low but that, especially for *E. fuscus*, demonstrable rabies NA does occur.

A large number of colonies, both with and without known rabid bats, especially of the species common to the northern portion of the United States, should be investigated. This would allow a determination of the prevalence of bat rabies, the immunologic status of the bats by sex and season, and the factors involved in the transmission of rabies to insectivorous bats. Until these facts are firmly established, public health officials will be handicapped in their attempts to control bats as disease vectors without unnecessarily decimating the native populations.

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