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## SURVEILLANCE AND EPIDEMIOLOGIC MAPPING OF MONOCLONAL ANTIBODY-DEFINED RABIES VARIANTS IN FLORIDA

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**ABSTRACT:** Brain tissues from 128 rabid animals from Florida in 1987 and 1988 were analyzed with monoclonal antibodies and cases were mapped by species and antigenic variant. The single variant found in terrestrial animals was distinguished easily from the variety of antigenic variants identified for infected bats, and there was no evidence of transmission of rabies between bats and terrestrial animals. The raccoon (*Procyon lotor*) appeared to be the sole maintenance source for terrestrial animal rabies in Florida.

**Key words:** Rabies, wildlife reservoir, monoclonal antibody, raccoon, *Procyon lotor*, bats, fox, *Vulpes vulpes*, epidemiology, surveillance.

### INTRODUCTION

Although vaccination and control programs have made rabies in domestic animals an uncommon occurrence in the United States, the few persisting cases, and the human exposures resulting from them, focus attention on a large reservoir of sylvatic rabies. Yearly reports of 3,000 to 5,000 cases of wildlife rabies (Centers for Disease Control, 1989) force the continuation of programs of rabies prevention for humans and domestic animals at an estimated annual cost greater than \$300,000,000 (Fishbein and Arcangeli, 1987).

Recent advances in biotechnology now permit the immunization of wildlife with vaccine baits (Baer et al., 1971; Tolson et al., 1987; Esposito et al., 1988; Kieny et al., 1988; Rupprecht et al., 1986; Schneider et al., 1988; Wandeler et al., 1988) and suggest a means by which rabies control programs may be extended to include wildlife species. A successful program would greatly reduce the risk of rabies transmission to humans and may eliminate the disease from certain species. However, it would be nearly impossible to vaccinate all affected species.

The complex epizootiology of sylvatic rabies in the United States includes species of both Carnivora and Chiroptera as major

vectors, and sporadic disease occurs in many mammalian species. Since vaccination of all susceptible animals is not a reasonable goal, a control program for wildlife must be predicted on the "compartmentalization" of disease; i.e., most cases within a given outbreak area occur in a single host species that is believed to be responsible for enzootic maintenance. Other species in the area may be infected through contact with the major host species, but cases in these animals are widely dispersed, occur sporadically, and do not perpetuate the enzootic (Winkler, 1975). If these observations are correct, successful immunization of the predominant vector species within an enzootic area should stop all disease transmission.

Although these concepts are important for disease control, intra- and interspecies rabies transmission have been very difficult to study. Only recently were panels of monoclonal antibodies (MAbs) developed that can identify antigenic variants in rabies virus isolates (Wiktor and Koprowski, 1978). MAbs permit an epidemiologic study of the prevalence and distribution of different variants and their transmission between different animal species (reviewed in Smith, 1989).

Because of the successful immunization of raccoons with vaccine baits and the in-

terest in field studies of disease control in this species (Rupprecht et al., 1986; Esposito et al., 1988), we conducted a study of the variants found in rabies isolates from a raccoon rabies enzootic area. The raccoon (*Procyon lotor*) is the major host species for rabies in Florida, and accounts for as many as 80% of the cases reported in a given year (Burridge et al., 1986). Although this species is the most likely target of an immunization program, rabies cases in other species are also of interest. The gray fox (*Urocyon cinereoargenteus*) is not a major rabies vector at present but epizootics of gray fox rabies were recorded in Florida in the 1950's and 1960's (Burridge et al., 1986; Jennings et al., 1960) and it is possible that the current cases (<10/yr) represent a residual focus of disease transmission independent of the reservoir in raccoons. Recurrent enzootic cases are not observed for any other terrestrial species in Florida; however, in the last 10 yr an average of 28 rabid bats were reported each year (Burridge et al., 1986). The contribution of the bat rabies cases to enzootic maintenance in terrestrial species in Florida, which is not known, was also a focus of this study.

We analyzed virus samples from 84 (94%) of 89 terrestrial animal rabies cases in Florida in 1987 and used surveillance data from 1986, 1987, and 1988 to map the prevalence and distribution of terrestrial animal rabies. Virus samples from 44 (81%) of 54 bat cases occurring in 1987 and 1988 were also analyzed and the variant distribution by species was mapped. Our intention is to use these data to estimate the minimum number of independently maintained enzootics in terrestrial species, and to estimate the contribution of rabid bats to disease in terrestrial species.

## MATERIALS AND METHODS

### Case surveillance and sample collection

Information on the number of rabies cases reported in 1986 to 1988 and the county submitting rabies samples was obtained from the

Florida Department of Health and Rehabilitative Services (HRS) County Public Health Units and Office of Laboratory Services (State Health Office, Florida Department of Health and Rehabilitative Services, Tallahassee, Florida 32399, USA). Florida law requires that all incidents in which a person is bitten by or exposed to a known or suspected rabid animal be reported to public health authorities. Brain tissue from the suspected rabid animal is then examined at an HRS laboratory by immunofluorescent antibody (IFA) for evidence of rabies infection (Dean and Abelseth, 1973).

Brain tissue from 128 rabies-positive animals was tested at the Centers for Disease Control (Atlanta, Georgia 30333, USA) with a panel of MAbs: 102 (92%) of 111 terrestrial and bat cases in 1987 and 26 (81%) of 32 bat cases in 1988. Of the samples, 119 were from wildlife species including 68 raccoons (*P. lotor*), six gray foxes (*U. cinereoargenteus*), one striped skunk (*Mephitis mephitis*) and 44 bats which included 13 red bats (*Lasiurus borealis*), 14 Seminole bats (*L. seminolus*), 10 yellow bats (*L. intermedius floridanus*), two free-tailed bats (*Tadarida brasiliensis*), one hoary bat (*L. cinereus*), one evening bat (*Nycticeius humeralis*), one eastern pipistrelle bat (*Pipistrellus subflavus*), one Mississippi myotis bat (*Myotis austroriparius*), and one unidentified bat. Nine samples were from domestic animals including five cats, three dogs, and one horse.

### Sample preparation

Multiple impression slides were made from brain material. After acetone fixation, one slide was stained with fluorescein isothiocyanate-labeled rabies diagnostic reagents (a 1:100 dilution of BBL anti-rabies globulin, BBL Microbiology Systems, Cockeysville, Maryland 21030, USA and a 1:50 dilution of Centocor anti-rabies monoclonal globulin, Centocor, Inc., Malvern, Pennsylvania 19355, USA) to determine the amount of antigen present. Samples containing rabies specific-inclusions in 75% to 100% of the impression area were tested for reaction with the panel of MAbs.

When antigen distribution was sparse or uneven or the reactivity pattern with the panel of MAbs was inconclusive, a 10% suspension of the brain material in Eagle's minimum essential medium (GIBCO Laboratories, Grand Island, New York 14072, USA) supplemented with 10% heated fetal calf serum (Flow Laboratories, Inc., McLean, Virginia 22102, USA) was prepared and used as inoculum for mouse neuroblastoma cells (MNA) (obtained from the late T. J. Wiktor, The Wistar Institute, Philadelphia, Pennsylvania 19104, USA; Smith et al., 1984a). Cell

culture-passaged material was prepared as follows: A suspension of MNA cells ( $6 \times 10^6$  cells in a volume of 1 ml) was incubated with 0.5 ml of brain suspension for at least 1 hr at 37 C. Additional medium was then added and the cell suspension divided between one 25-cm<sup>2</sup> plastic flask and seven 8-well Lab-Tek tissue culture chamber slides (Nunc, Inc., Naperville, Illinois 60566, USA). The flask and the slides were incubated in a humidified incubator with 0.5% CO<sub>2</sub> at 37 C. Slides were fixed in acetone on day 2 and cultures containing more than five foci of infection per well (as determined by IFA) were reacted with the panel of MABs. Isolates with insufficient infectivity were passaged at weekly intervals by dispersing the cell monolayer in the flask culture and seeding a flask and slides as before. This process was repeated until cultures contained > five foci/well, usually at the second passage. Repeated passage in culture did not affect the MAB reactivity pattern.

#### Monoclonal antibodies

With the exception of MAB T, MABs were produced from BALB/c mice immunized with the ERA (Evelyn-Rokitnicki-Abelseth) vaccine strain of rabies virus (obtained from The American Type Culture Collection, Rockville, Maryland 20852, USA; Smith et al., 1984a). MAB T, the gift of Dr. Lothar Schneider (Rabies Laboratory, Federal Research Institute for Animal Virus Diseases, D-7400 Tübingen, Federal Republic of Germany), was prepared from mice immunized with a field strain of rabies (Schneider et al., 1985). The experimental panel consisted of 20 MABs reactive with the nucleocapsid protein of rabies virus and were used as ascites fluid. MABs were diluted to a concentration producing 4+ fluorescence with homologous virus. This dilution (the working dilution) and a 10× less dilute preparation were used for IFA staining of virus isolates as follows: (1) brain impressions and MNA cell cultures were reacted with the MABs for 30 min at 37 C in a humidified chamber and then washed in PBS for 10 min to remove unbound antibody; (2) samples were then reacted with fluorescein isothiocyanate-conjugated goat antibody to mouse immunoglobulin G (Cappel Research Products, Durham, North Carolina 27704, USA) with incubation and washing as in (1); and (3) a positive reaction was determined by observing fluorescing intracytoplasmic inclusions with a Zeiss Axioskop microscope fitted with vertical illumination (HBO-100W/2 mercury lamp and the standard FITC filter set, Carl Zeiss Inc., Thornwood, New York 10594, USA). Magnification was 200 to 400×. Samples were reacted first with the working dilution of the MAB panel.

Negative or weak positive reactions were confirmed by repeat tests with a 10× less dilute preparation of MAB.

#### Variant identification

Patterns of reactivity for each isolate were established according to the following criteria: (1) a positive reaction signified that the intensity of fluorescence and amount of antigen stained with the working dilution of MAB was identical to that of homologous control; (2) a weak reaction signified that the intensity of fluorescence with the working dilution of MAB was much less than that of homologous control, only large antigen accumulations stained, and brain impressions with very little antigen accumulation and cultures from initial MNA cell passage often did not react with the working dilution of MAB and reacted only weakly with a 10× less dilute preparation of MAB; and (3) a negative reaction signified that there was no specific fluorescence with the working dilution or 10× less dilute MAB regardless of cell culture passage or accumulation of antigen.

Using these criteria, we have identified characteristic reaction patterns for rabies variants associated with enzootic rabies in four carnivore species in seven outbreak areas of the United States and for enzootic rabies in four bat species in species-defined outbreaks throughout the continental United States (Smith, 1989). This work has shown that virus isolates from the raccoon rabies enzootic areas of the southeastern and the mid-Atlantic United States will react with all the antibodies in the panel with the exception of MAB 1 and that this pattern (Variant Raccoon/Southeast/Mid-Atlantic USA) is found only in raccoon rabies enzootic areas of the United States (Smith et al., 1984b, 1986). A negative reaction with MABs 15, 18, and 19 identifies isolates from bat species in the southeastern United States and separates them from the terrestrial animal variant (Smith and Baer, 1988). A negative reaction with MAB T identifies a variant common to infected *Lasius* bats, the bat species most frequently reported rabid in the southeastern United States (Smith and Baer, 1988).

## RESULTS

#### Surveillance summary, 1951 to 1988

Figure 1 is a summary of rabies cases in raccoons, foxes, and bats in Florida for the last 38 yr arranged so as to compare changes in rabies prevalence and affected species in different regions of the state and to recognize possible associations when in-

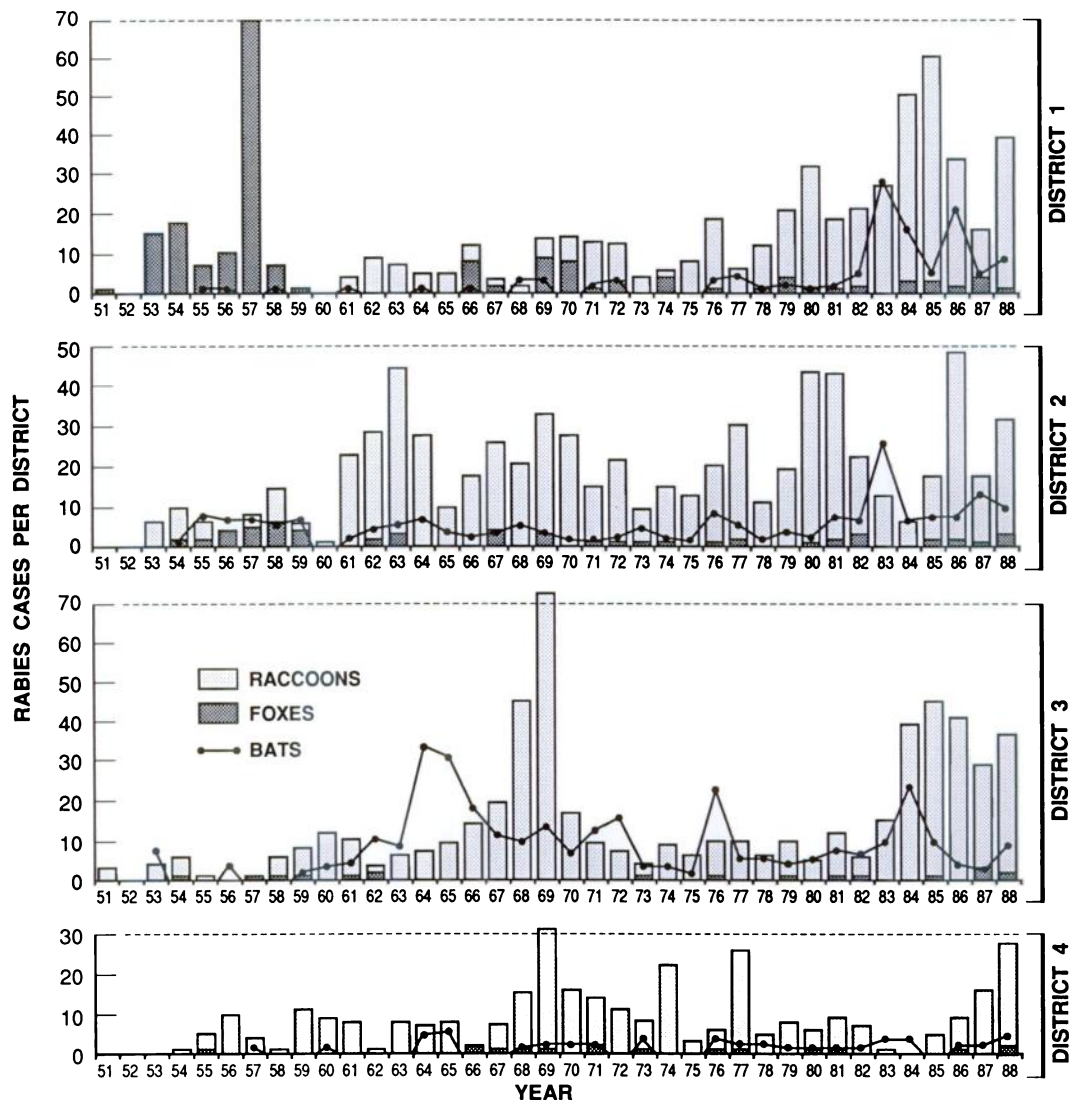


FIGURE 1. Numbers of cases of rabies in raccoons, foxes and bats in Florida by district, 1951–1988.

creased cases occur in more than one species. Although rabies surveillance is traditionally based on county (political) lines, a grouping of counties into four large districts roughly defines geographically different regions of the state (see Figs. 2–6 for the location of the districts). District 1 includes the red clay region of the Panhandle, which is good fox habitat and was the area recording most of the fox rabies cases, including the epizootic cases of the 1950's and the smaller outbreak in the late 1960's. Districts 2 and 3 recorded the first

cases of raccoon rabies in Florida in the 1950's, contain the canals and natural waterways along which raccoon rabies spread in the 1950's and 1960's, and have reported the greatest number of raccoon cases. District 3 also recorded the first case of insectivorous bat rabies in the United States and has reported the largest number of bat rabies cases in Florida. District 4 encompasses Lake Okeechobee and the Everglades and reported the least number of rabies cases of any area of Florida.

With the exception of the epizootic years

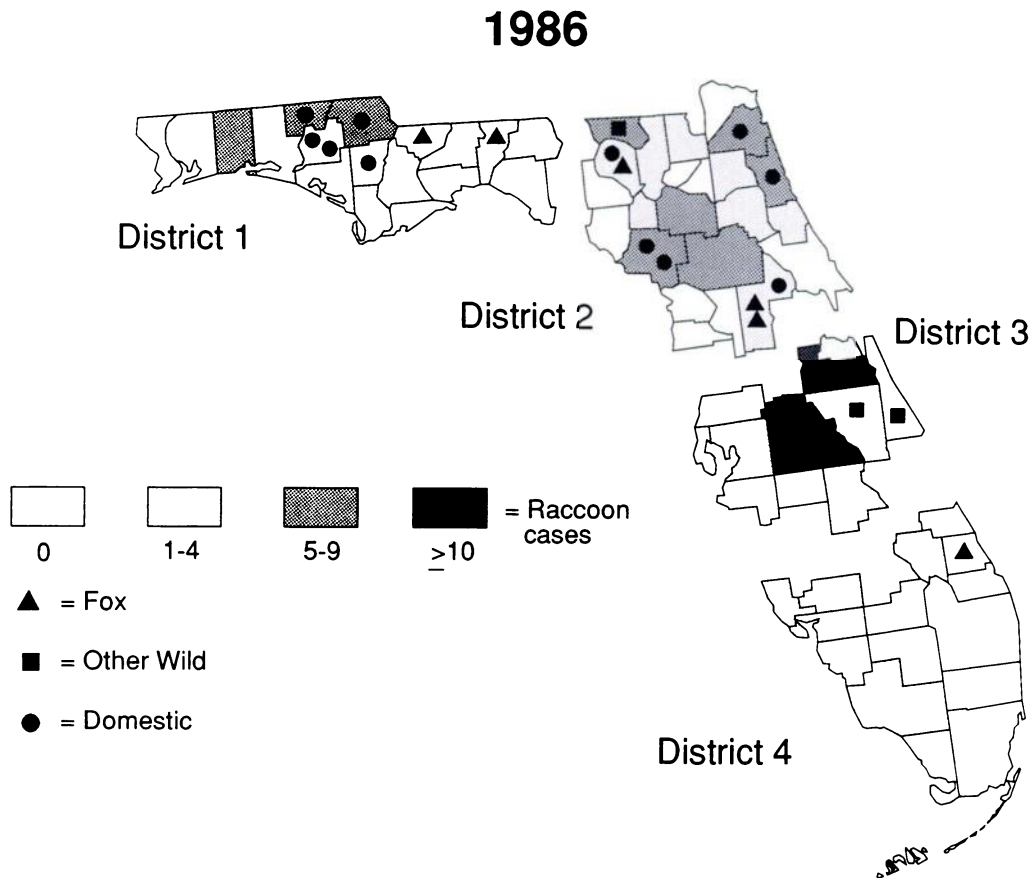


FIGURE 2. Prevalence and distribution of terrestrial rabies cases by county in Florida in 1986.

1953 to 1958 and 1966 to 1970, there was no evidence of sustained intraspecies transmission among foxes. Fox rabies cases were sporadic in occurrence and widely distributed in the state. Few fox rabies cases were recorded in any district regardless of the number of cases in raccoons or bats.

Periodic increases were observed for raccoon rabies cases in each of the districts and in several instances these increases were preceded or accompanied by increased numbers of bat rabies cases. In the 1980's in District 1, raccoon rabies cases increased gradually, and bat rabies cases increased sharply. Increased bat cases were observed in District 2 in 1983 between two peaks of raccoon rabies (1980 to 1981 and 1986). In District 3 two dramatic increases in the number of raccoon rabies cases in 1968 to 1969 and 1984 to 1988 were pre-

ceded or accompanied by increased numbers of cases of rabies in bats. Although periodic increases were observed in raccoon cases in District 4, very few bat rabies cases were reported at any time.

#### Case surveillance, 1986 to 1988

*Terrestrial animal rabies:* Although raccoons were only 16% of the total number of animals examined for rabies by public health laboratories in Florida during 1986 to 1988, the 326 positive animals accounted for 68% of rabies diagnoses (Table 1). Cases occurred throughout the state in 52 (78%) of 67 counties (Figs. 2–4) in patterns of enzootic rather than epizootic frequency. Annual reports of  $\geq 10$  cases/county were made just five times in 3 yr and 30% of the total cases were from counties reporting fewer than five cases/year.

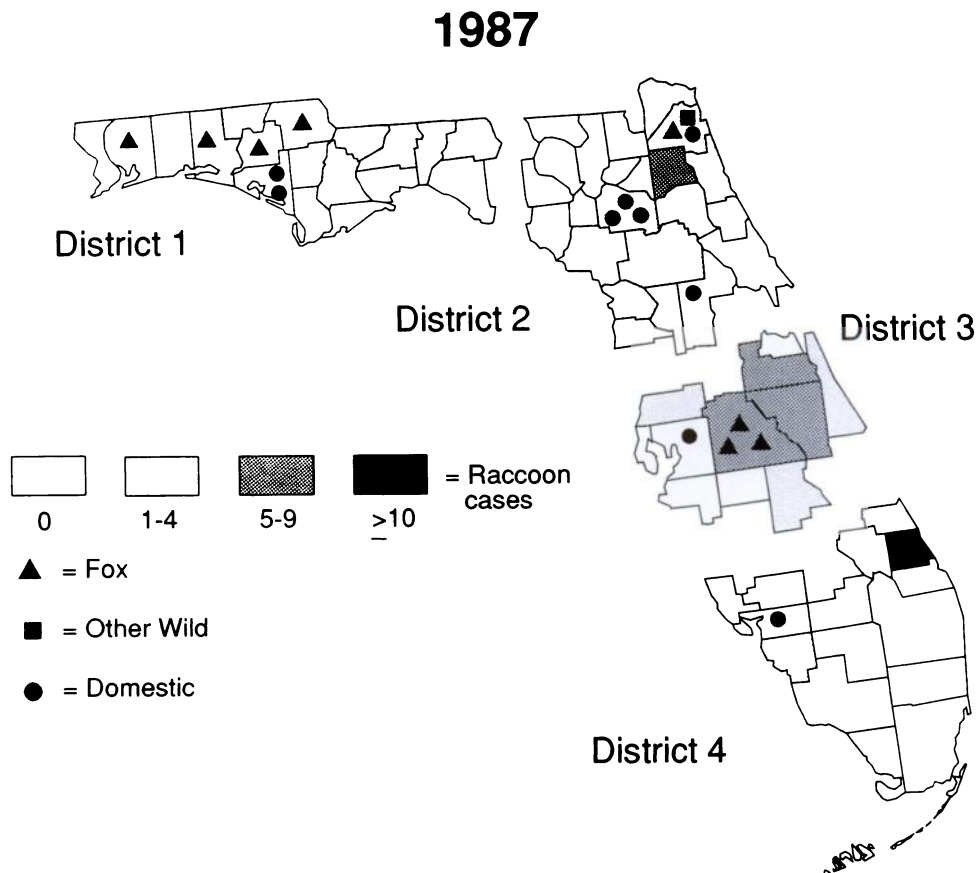


FIGURE 3. Prevalence and distribution of terrestrial rabies cases by county in Florida in 1987.

Although 14 counties reported no raccoon rabies in the 3 yr study period, the geographic distribution of cases suggested low-level endemicity throughout the state rather than an absence of disease in a particular area. This was particularly evident in southern Florida (District 4), which reported the least rabid raccoons (Figs. 2-4).

Twenty-one fox rabies cases were reported (6% of all positive samples from terrestrial wildlife) and five cases (a mean of two cases/year) were recorded for skunks (Table 1). Cases in other species of terrestrial wildlife were only occasionally found. Non-raccoon rabies cases were sparsely distributed and occurred most often in counties recording multiple cases of rabies in raccoons (Figs. 2-4). The only county that averaged more than one non-

raccoon case per year was Polk (District 3) which also reported 30 rabid raccoons during this period.

Although 52% of the animals examined for rabies were dogs and cats, they represented only 7% of total cases (Table 1). Cases occurred sporadically and were most often reported in or near foci of increased disease in raccoons (Figs. 2-4).

**Bat rabies:** Bats accounted for 7% of specimens submitted for rabies examination and 18% of cases (Table 1). In contrast to terrestrial animal rabies, which was widely distributed throughout the state, cases in bats were more clustered. Only 20 (30%) of 67 counties reported bat rabies, and nearly one-half of the cases (42/88, 48%) were reported from just two counties (Escambia County in District 1 and Duval County in District 2).



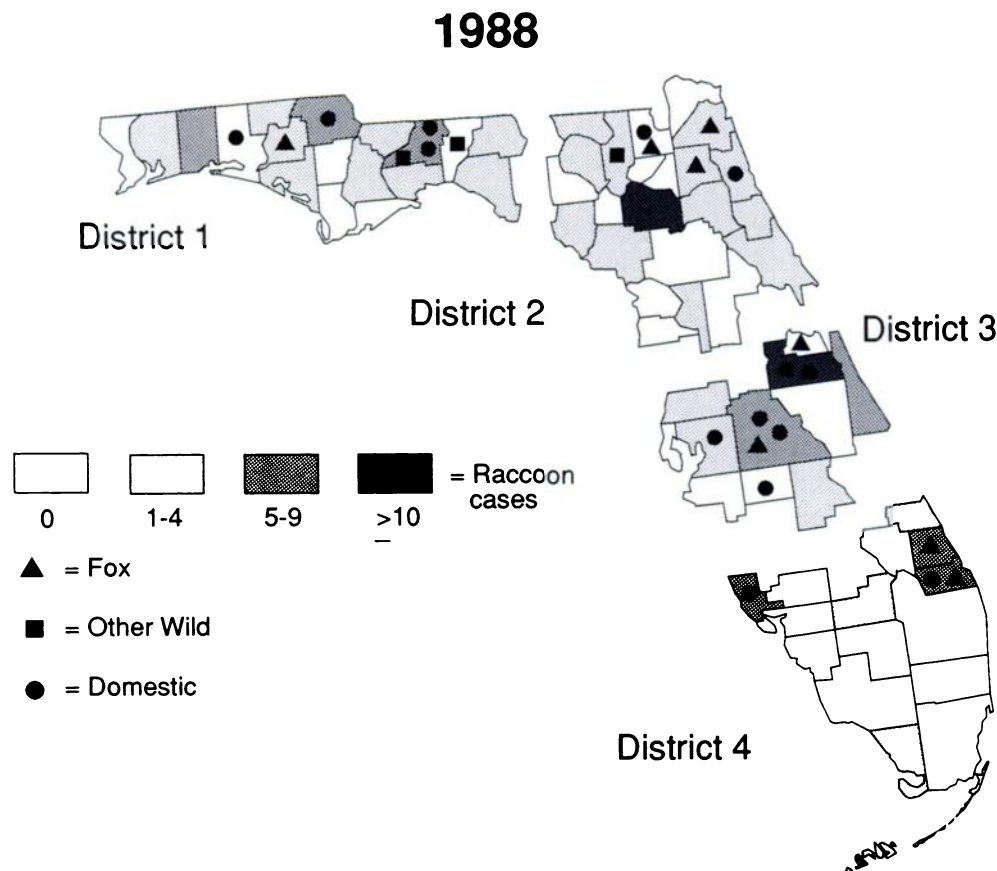


FIGURE 4. Prevalence and distribution of terrestrial rabies cases by county in Florida in 1988.

Although bats submitted for rabies examination in Florida are not routinely identified by species, 46 (85%) of 54 cases in 1987 and 1988 were identified (Table 2). Rabies was found in a variety of different bat species; no clearly dominant vector species was identified. Most of the cases were in lasiurine bats (41 of 46 or 89% of the bats identified by species) but the predominant lasiurine species varied in importance in different years (Table 2) and differed in different regions of the state (Figs. 5, 6). In 1987 *L. borealis* was the major host and most cases in this species were found in northern Florida. In this same area in 1988 only one rabid *L. borealis* was reported, and *L. seminolus* was the major host. Additionally in 1988, there was an increased number of cases in

*L. intermedius floridanus* most of which were found in Districts 3 and 4.

Cases in other bat species were reported infrequently. Two rabid *T. brasiliensis* were found in central Florida (District 3, Orange County). The single isolates from *L. cinereus* and *N. humeralis* were from northern Florida (District 1, in Escambia County), the *P. subflavus* isolate was from central Florida (District 2, Lake County), as was a single rabid *M. austroriparius* (District 3, Highlands County).

**Mab-N analysis:** Of 89 rabies cases diagnosed in terrestrial animals in Florida in 1987 (Fig. 3), 84 virus isolates (94%) were tested with the MAb panel. A single variant (Raccoon/Southeast/Mid-Atlantic USA) was identified in 83 of the 84 isolates (Table 3). The one exception, an isolate



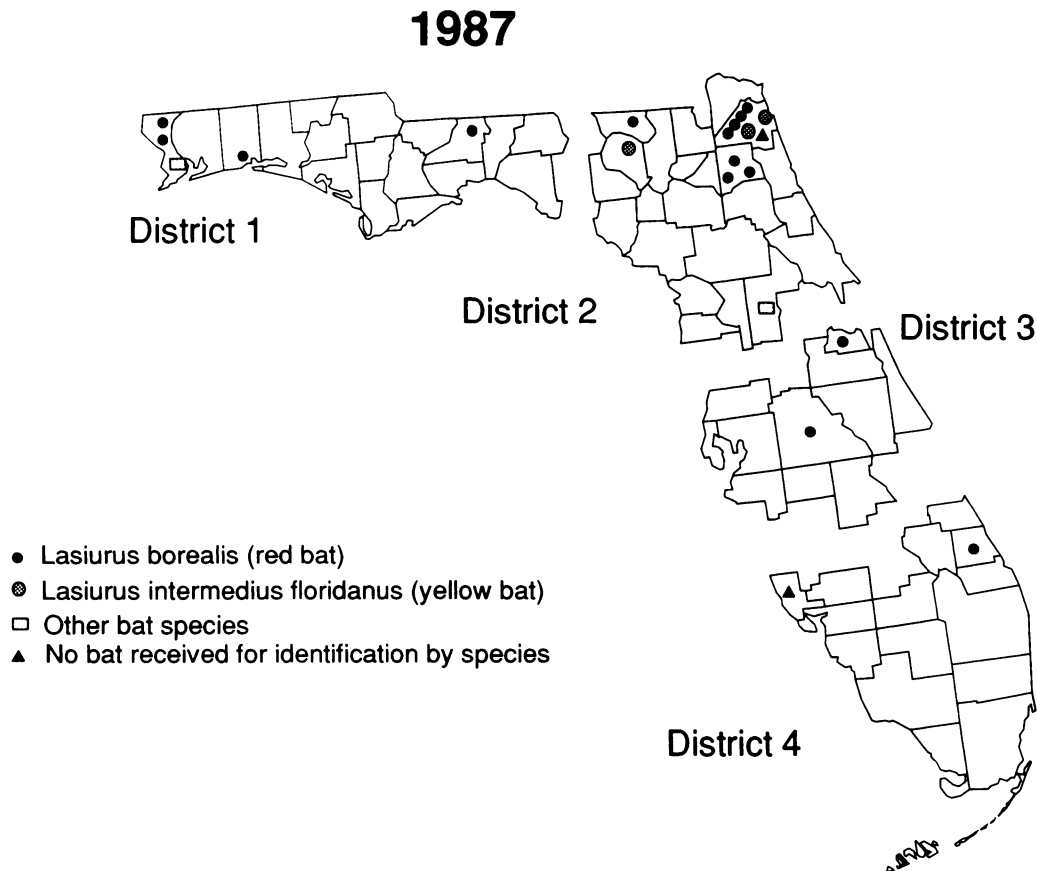


FIGURE 5. Distribution of bat rabies cases by species in Florida in 1987.

from a raccoon in District 3 (Merritt Island, Brevard County), reacted with all the antibodies in the panel, a reaction pattern of laboratory or vaccine strains of rabies virus (Smith, 1989). Laboratory contamination of this sample could not be dismissed; no additional samples from this animal were available, and no other isolate in this study reacted similarly.

The common variant in terrestrial animals was completely absent in rabies cases in bats. Virus isolates from bats could be distinguished from terrestrial animal rabies by a negative reaction with 3 MABs (MAB 15, 18, and 19, Table 3).

In contrast to the antigenic homogeneity of isolates from terrestrial species, a variety of reaction patterns was found in bat rabies isolates. The most common reaction pattern, which was characterized by a nega-

tive reaction with MAB T, was found in all isolates from lasiurine bats. Small variations in reactivity permitted the separation of the lasiurine isolates into three groups.

The first lasiurine reaction group included 26 (96%) of 27 isolates from either *L. borealis* or *L. seminolus*. The reaction pattern presented by these isolates was identical to the variant commonly found in rabid red bats elsewhere in the United States (Smith and Baer, 1988). Only one other Seminole bat rabies isolate had been tested (an isolate collected in Georgia in 1982); it also contained the red bat variant (data not shown). In Districts 1 and 2 the red bat variant was the most common variant and was found in 27 (90%) of 30 isolates; these were from 24 red or Seminole bat samples and one sample each from *N.*

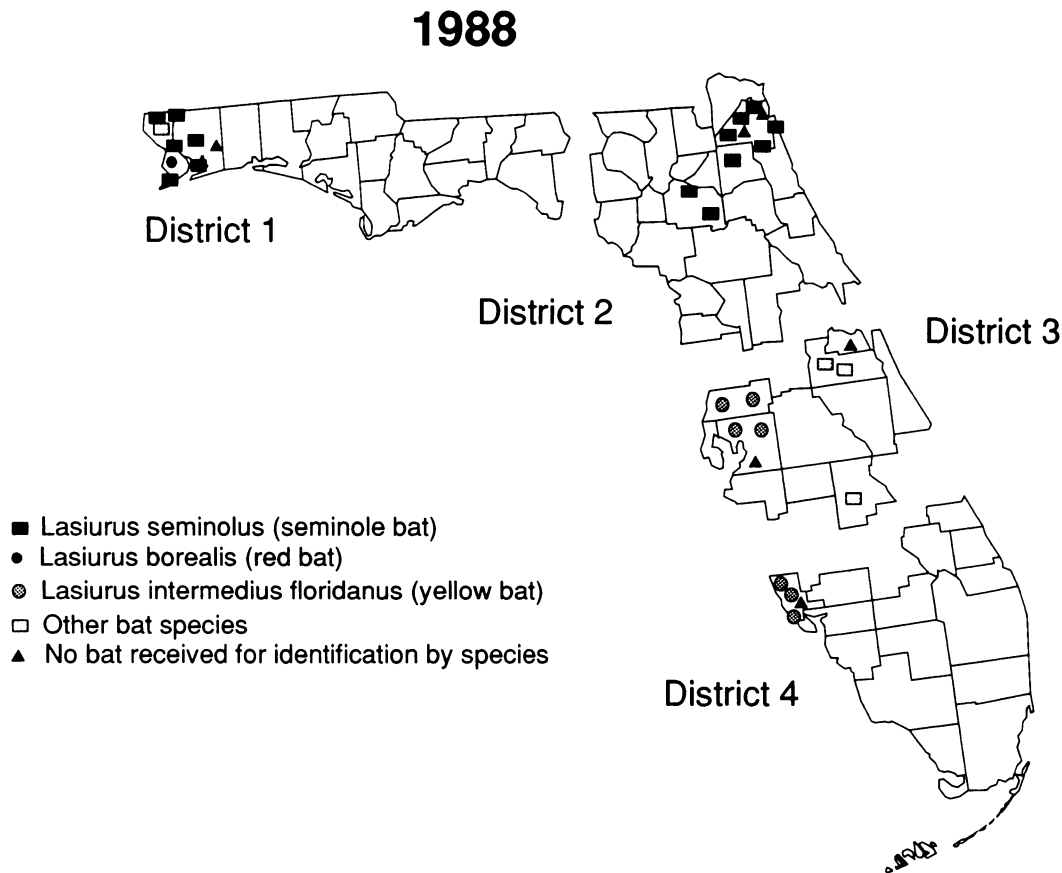


FIGURE 6. Distribution of bat rabies cases by species in Florida in 1988.

*humeralis*, *P. subflavus*, and *L. intermedius floridanus*. In contrast to its prevalence in Districts 1 and 2, this variant was found in only two (14%) of the 14 isolates from District 3 or 4. In both instances the isolates containing the red bat variant were from red bats.

The second largest reaction group within the lasiurine isolates was characterized by a diminished reaction with MAb 13 and included nine (90%) of 10 isolates from *L. intermedius floridanus*. No isolates from yellow bats outside Florida were available for comparison, but this reaction pattern was identical to that of two isolates taken from this species in Florida in 1983 and 1986 (data not shown). This variant was not found in any other bat species in Florida. It was the most common variant in Districts 3 and 4 but it was found else-

where only twice, in yellow bats collected in Duval County and Suwannee County in 1987 (District 2).

The third lasiurine group, characterized by a positive reaction with MAb 12, comprised only two isolates: a *L. cinereus* isolate from District 1 (Escambia County in 1988) and an isolate from a red bat in District 3 (Seminole County in 1987). This reaction pattern identified a variant common to hoary bats in other areas of the United States (Smith and Baer, 1988).

Two additional reaction patterns were noted in isolates from non-lasiurine bat species. Three samples contained a variant commonly associated with rabid Mexican free-tailed bats (*T. brasiliensis mexicana*) in the southwestern United States (Smith and Baer, 1988). The first sample was from a bat collected in District 4 (Sarasota Coun-

TABLE 1. Rabies diagnostic tests at the Florida Department of Health and Rehabilitative Services Office of Laboratory Services in 1986 to 1988.

	Number positive/number examined			% of positive 1986-1988	% of examined 1986-1988
	1986	1987	1988		
Wildlife					
Terrestrial					
Raccoon	128/663	71/751	127/759	68.2	15.8
Fox	5/79	8/124	8/109	4.4	2.3
Skunk	3/106	1/78	1/57	1.1	1.8
Otter	1/4	0/3	1/7	0.4	0.1
Bobcat	0/7	0/7	1/9	0.2	0.2
Others <sup>a,b</sup>	0/891	0/936	0/858	0	19.6
Total	137/1,750	80/1,899	138/1,799	74.3	39.7
Bat	34/317	22/317	32/334	18.4	7.1
Total wild	171/2,067	102/2,216	170/2,133	92.7	46.7
Domestic					
Cat	7/1,484	5/1,321	14/1,609	5.4	32.2
Dog	4/938	3/856	0/942	1.5	19.9
Horse	1/27	1/30	0/30	0.4	0.6
Others <sup>a,b</sup>	0/19	0/30	0/23	0	0.5
Total domestic	12/2,468	9/2,237	14/2,604	7.3	53.2
Grand total	183/4,535	111/4,453	184/4,737	100	100

<sup>a</sup> Others tested in 1986 included 177 opossums, 137 ferrets, 6 monkeys, 5 moles, 2 coyotes, 2 cougars, 1 kinkajou, 1 armadillo, 1 kudu, 1 shrew, 2 wild hogs, 1 elephant, 555 rodents and lagomorphs, 11 cattle, 5 goats, 3 pigs, in 1987, 145 opossums, 192 ferrets, 4 monkeys, 7 moles, 1 coyote, 1 panther, 1 hartebeest, 1 leopard, 2 armadillos, 2 wild hogs, 1 kangaroo, 579 rodents and lagomorphs, 8 cattle, 7 goats, 13 pigs, and 2 lambs, and in 1988, 145 opossums, 181 ferrets, 2 monkeys, 10 moles, 3 panthers, 2 cougars, 2 deer, 2 weasels, 1 coatimundi, 1 kinkajou, 1 armadillo, 1 bear, 507 rodents and lagomorphs, 12 cattle, 3 goats, and 8 pigs.

<sup>b</sup> Animals submitted for rabies diagnosis were identified by common name only. Information on generic taxons was available only for rabies-positive samples.

ty in 1987). Because the carcass had been destroyed, the bat could not be identified by species. However, two additional samples were from bats found in District 3 (Orange County in 1988) and identified as *T. brasiliensis*.

The reaction pattern of a single isolate from a Mississippi myotis bat differed from all other isolates in Florida. Because this variant had been identified only twice in other areas of the United States (data not shown), its association with a particular bat species is not known.

## DISCUSSION

Antigenic analysis of rabies virus from 83 rabies cases occurring in terrestrial animals in Florida in 1987 supported surveillance observations depicting a rabies transmission cycle among terrestrial species in Florida maintained solely by *P. lo-*

*tor*. The variant isolated from the 16 rabies cases in terrestrial animals other than *P. lotor* was identical to that infecting raccoons throughout the state and identical to that found throughout the raccoon rabies enzootic areas of the southeastern states. Although it is possible that other variants are circulating in Florida but are not differentiated from the raccoon variant by this MAb panel, non-raccoon rabies occurred sporadically with cases widely distributed in the state. It is unlikely that this low incidence of disease in species other than raccoons is sufficient to maintain enzootic transmission independent of the disease in raccoons. These conclusions are of course predicted on a surveillance system that can detect disease in a variety of different species at the same frequency. It is possible that differences in animal behavior, and effect of rabies on animal behavior could in turn affect the probability with

TABLE 2. Rabid bats in Florida by species,\* 1987 to 1988.

Species	Number rabid		Percentage of all rabid bats	
	1987	1988	1987	1988
<i>Lasiurus seminolus</i> (Seminole bat)	0	14	0	54
<i>Lasiurus borealis</i> (red bat)	15	1	75	4
<i>Lasiurus intermedius floridanus</i> (yellow bat)	3	7	15	27
<i>Tadarida brasiliensis</i> (Florida free-tail bat)	0 (1)	2	0	7
<i>Lasiurus cinereus</i> (hoary bat)	0	1	0	4
<i>Nycticeius humeralis</i> (evening bat)	1	0	5	0
<i>Pipistrellus subflavus</i> (eastern pipistrelle bat)	1	0	5	0
<i>Myotis austroriparius</i> (Mississippi myotis bat)	0	1	0	4

\* Includes only those rabid bats identified by species.

which a given species comes into contact with humans and is reported to the public health authorities. However, since enzootic disease in foxes and skunks is readily detected in other areas of the United States, it is not likely to go undetected in Florida.

This fairly simple single host epizootiology of terrestrial animal rabies in Florida contrasted sharply with the complex picture of bat rabies. The variety of antigenic variants found in bat rabies isolates and

their association with different species suggested that several independently maintained species-defined enzootics may contribute to Florida's chiropteran rabies. There was also a suggestion of a regional separation of bat rabies by species. In the northern part of the state, cases were reported most often in red or Seminole bats; in central Florida the yellow bat was the predominant rabid species.

In further contrast to terrestrial animal

TABLE 3. Antigenic analysis of 128 rabies samples from Florida, 1986 to 1987.

MAB-defined variant/enzootic area	Reaction pattern defining variant MAB-N number										Rabies samples tested	
	1	15	18	19	T	12	16	13	@*	Animal	Number of isolates	
1. Raccoon/Southeast-Mid-Atlantic USA	—	+	+	+	+	+	+	+	+	Raccoon	67	
	—	+	+	+	+	+	+	+	+	Fox	6	
	—	+	+	+	+	+	+	+	+	Skunk	1	
	—	+	+	+	+	+	+	+	+	Cat	5	
	—	+	+	+	+	+	+	+	+	Dog	3	
	—	+	+	+	+	+	+	+	+	Horse	1	
2. Lab?	+	+	+	+	+	+	+	+	+	Raccoon	1	
3. Lasiurine bat/migratory range	W	—	—	—	—	W	W	+	+	Seminole bat	14	
	W	—	—	—	—	W	W	+	+	Red bat	12	
	W	—	—	—	—	W	W	+	+	Evening bat	1	
	W	—	—	—	—	W	W	+	+	<i>E. pipistrelle</i> bat	1	
	W	—	—	—	—	W	W	+	+	Yellow bat	1	
	W	—	—	—	—	W	W	W	+	Yellow bat	9	
	W	—	—	—	—	+	W	+	+	Hoary bat	1	
	W	—	—	—	—	+	W	+	+	Red bat	1	
4. Tadarida bat/migratory range	+	—	—	—	+	+	—	+	+	Free-tailed bat	2	
	+	—	—	—	+	+	—	+	+	Unidentified bat	1	
5. ?/?	W	—	—	—	+	+	—	W	+	Mississippi myotis bat	1	

\* @, 12 MAbs which react with all isolates from the southeastern United States; —, no reaction; +, positive reaction; W, weak reaction detectable with 10× less dilute preparation of MAB. MAB designation is as listed in Smith, 1989.

rabies, there was very little evidence of spillover infection from a predominant rabid bat species to other less prominent species in the same area. Even though 87% of bat rabies cases occurred in just three species and the remaining 13% (seven cases) comprised five species, only two of the sporadic cases in non-lasiurine species contained a "major" variant and thus may have been infected through contact with the principal lasiurine vectors. Four samples from three other species contained variants, which although uncommon in Florida, are found in bat species-defined enzootics in other parts of the United States.

Although the apparently complex epidemiology of bat rabies suggested by this analysis is deserving of further study, the most important public health observation to be made from these data was the absence of transmission from bats to terrestrial animals. Variants identified for infected bats in Florida could be distinguished easily from the single variant circulating in terrestrial animals. We found no evidence of bat to terrestrial animal transmission in Florida, although the 1951 to 1988 surveillance reports show the distribution of terrestrial animal rabies cases temporally and geographically overlapped bat rabies cases in Florida. Although one might argue that virus transmission from bats to terrestrial animals and subsequent adaptation to a different host might result in changes in the "bat" variant such that it might then test as identical to the "raccoon" variant, this has not been true when serial transmission has been studied in the laboratory (Rupprecht et al., 1990) nor when virus has been isolated from field samples of terrestrial animals thought to have been infected through contact with rabid bats. To date, rabies variants have maintained their characteristic reaction patterns despite passage to different animal species (Smith, 1988).

In conclusion, there was no evidence in Florida of transmission of rabies between bats and terrestrial animals. Although sporadic transmission occurs in other areas of

the United States, and will probably be found in Florida eventually, such transmission is not thought to result in sustained transmission among terrestrial animals. Surveillance and laboratory analysis of rabies cases and the variants infecting them should of course continue; but, in the absence of such evidence, we concluded that *P. lotor* is the sole maintenance source for enzootic terrestrial animal rabies in Florida and that successful immunization of this species should dramatically limit rabies transmission among terrestrial animals in Florida.

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