Epidemiologic characteristics of rabies virus variants in dogs and cats in the United States, 1999

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Objective—To evaluate epidemiologic features of rabies virus variants in dogs and cats in the United States during 1999 and assess the role of bat-associated variants.

Design—Epidemiologic survey.

Sample Population—Rabies viruses from 78 dogs and 230 cats.

Procedure—Brain specimens from rabid dogs and cats were submitted for typing of rabies virus. Historical information, including ownership and vaccination status, was obtained for each animal. Specimens were typed by use of indirect fluorescent antibody assay or reverse transcriptase polymerase chain reaction assay and nucleotide sequence analysis.

Results—Nearly all animals were infected with the predicted terrestrial rabies virus variant associated with the geographic location of the submission. A bat-associated variant of rabies virus was found in a single cat from Maryland. More than half (53%) of submitted animals were classified as owned animals, and most had no known history of vaccination. One vaccination failure was reported in a dog that did not receive a booster dose of rabies vaccine after exposure to a possibly rabid animal.

Conclusions and Clinical Relevance—Bat-associated rabies virus variants were not a common cause of rabies in dogs and cats during 1999. Vaccine failures were uncommon during the study period. Because most rabid dogs and cats were unvaccinated and were owned animals rather than strays, educational campaigns targeting owners may be useful. (J Am Vet Med Assoc 2001;218:1939–1942)

Despite the availability of protective rabies vaccines and laws mandating pet vaccination in most states,1 dogs and cats are still affected by rabies virus every year because of lack of vaccination. Between 1994 and 1999, a mean of 280 cats and 127 dogs were reported with rabies each year in the United States.2,7 Although rabies is diagnosed in dogs and cats less commonly than in wildlife, such animals may represent a higher risk to human beings than does wildlife because of the close association between pets and their owners.

Cats and dogs are not reservoirs for rabies virus variants circulating in the United States, with the exception of a small focus of the canine variant in Texas. In contrast to many other parts of the world,8 variants of rabies virus in the United States are maintained in terrestrial wildlife reservoirs within geographically discrete clusters.2,9,10 In addition to terrestrial reservoirs, there are multiple independent reservoirs for rabies virus in several species of insectivorous bats. Disease transmission within geographic clusters is usually intraspecific and involves distinctive variants of rabies virus that can be identified by antigenic differences, as detected by patterns of reactivity with monoclonal antibodies, or through genetic analysis of viral RNA.11 Interspecific transmission occurs occasionally and is revealed by spillover rabies infections in domestic animals such as dogs and cats. However, spillover infections are uncommonly linked to secondary transmission of rabies virus to other animals.

Spillover infections sometimes occur in humans as well. Between 1990 and 2000, 32 cases of rabies in humans were reported in the United States.12-20 Variant-typing techniques helped to establish that 24 (73%) of these cases were attributed to bat-associated variants of rabies virus. Strikingly, 17 (71%) of the bat-associated cases were caused by a single unique variant normally associated with Eastern pipistrelle (Pipistrellus subflavus) and silver-haired (Lasionycteris noctivagans) bats, and most patients did not report a bat bite. Several hypotheses have been proposed to explain these findings. One suggestion is that bat-associated rabies virus variants (the Eastern pipistrelle/silver-haired bat variant in particular) may have increased virulence properties that influence virus transmission after a superficial bite.21 Another plausible explanation is that the importance of bites from species of small bats such as Eastern pipistrelle and silver-haired bats is minimized, because the patients do not appreciate the risk from bat bites, or the bite may go unnoticed and therefore untreated.20,21 An additional theory is that some of these human deaths may not have resulted from exposure to bats at all but rather from exposure to domestic animals infected with bat-associated rabies virus variants.16,20

Dogs and cats are susceptible to and occasionally infected with rabies virus variants transmitted by bats.21,22 However, a comprehensive study of rabies virus variants in dogs and cats has never been conducted to ascertain the frequency with which they are infected with bat-associated rabies virus variants. Although a few state public health laboratories routinely type rabies virus variants, most dogs and cats with rabies in the United States are assumed to have

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The authors thank the state health department personnel for sample submissions and Lillian Orciari, John McQuiston, Robert Holman, Yvonne Stifel, and Karoyle Colbert for technical assistance.
been infected with the variant of rabies virus common to terrestrial reservoirs in their geographic area. The purpose of the study reported here was to evaluate epidemiologic features of rabies virus variants in dogs and cats in the United States during 1999 and assess the role of bat-associated variants.

**Materials and Methods**

Rabies virus samples were obtained from 78 dogs and 230 cats with rabies diagnosed by use of direct fluorescent antibody (DFA) tests performed in state health laboratories between Jan 1, 1999 and Dec 31, 1999. Health departments from all 50 states and the District of Columbia participated in the study. States provided brain specimens and a history for each animal, including the species, the submitting county and state, date of animal death, ownership status, vaccination status, and reasons for rabies testing.

For each sample, a portion of brainstem was used to prepare impression smears and as a source of viral RNA. Acetone-fixed impression smears were tested by use of DFA tests, using fluorescein isothiocyanate-labeled antirabies antibodies from 3 different suppliers. Samples were examined and graded regarding the amount of antigen in the tissue impression smear. Samples that contained inclusions or dust-like particles of rabies antigen in >50% of the smear were tested by use of indirect fluorescent antibody (IFA) for reaction with a panel of 7 monoclonal antibodies as described.

If the distribution of antigen in a tissue impression smear was too sparse for reliable reactions with the antibody panel, or if the reaction pattern appeared different from that expected for animals from a particular geographic area, viral RNA was extracted. Identity of the virus was determined by use of genetic methods, as described. Briefly, these methods included reverse transcriptase polymerase chain reaction (RT-PCR) of viral RNA, using primer 1087NFdeg (5’-GAGAAA(G/A)GAACTCCGAGAGCA-3’) for reverse transcription and primers 1087NFdeg and 1312NBdeg (5’-TTGTCTCA/G/CAGCGA/AA-3’) for amplification. Because these primers did not efficiently amplify the virus from California skunks, all California samples were reamplified with primers specific for this variant of rabies virus (CA3024NF [5’-GAGAAA(G/A)GAACTCCGAGAGCA-3’] and CA2517NB [5’-TTGTCTCA/G/CAGCGA/AA-3’]). Sequence analysis of complementary DNA was performed by use of an automated DNA sequencer, manufacturer’s sequencing reagents, and professional software. For all samples, genetic analysis was conducted on nucleotide sequence for a 205-base-pair portion of the rabies virus nucleoprotein gene equivalent to nucleotides 1,112 to 1,316 of the laboratory standard rabies virus (CVS rabies virus, GenBank accession number D42112). Specific genetic variants of rabies virus were identified by phylogenetic analysis with reference virus samples. Epidemiologic data were analyzed by use of statistical software. Significance was set at P < 0.05.

**Results**

The rabies virus variant responsible for infection was determined for 308 dogs and cats during 1999; this represented 82% of rabid dogs and cats reported to the Centers for Disease Control and Prevention (CDC) during 1999. Of the 308 typed viruses, 256 were typed by use of IFA, and 52 were typed by use of RT-PCR and nucleotide sequence analysis. Almost all viruses (307/308) were the expected terrestrial rabies virus variant, as predicted by the geographic location of the submitted animal (Fig 1). The exception was a single bat-associated variant found in a cat submitted from Maryland. The rabies virus variant found in the cat was commonly associated with big brown bats (Eptesicus fuscus).

Rabies was diagnosed by state health departments throughout the year, although most rabid dogs and cats were detected during the period from May to October (Fig 2). The raccoon variant of rabies virus was responsible for infection in the highest number of animals (n = 177 [57%]), followed by the...
north-central skunk variant (68 [22%]), the south-central skunk variant (42 [14%]), the Texas fox variant (7 [2%]), the Texas dog-coyote variant (6 [2%]), the California skunk variant (4 [1%]), the Arctic fox variant (3 [1%]), and the big brown bat variant (1 [<1%]). Texas submitted the highest number of dogs and cats that were variant-typed (n = 35), followed by New York (28), Virginia (25), and Pennsylvania (20).

More than half (n = 163 [53%]) of the dogs and cats in the study were reportedly owned animals; however, dogs were significantly more likely than cats to be reported as owned (relative risk (RR) = 1.72; 95% confidence interval (CI), 1.42 to 2.07; P < 0.001). Ten percent of animals had a history of known exposure to a rabid or possibly rabid animal. Dogs were significantly more likely than cats to have had a known exposure to a potentially rabid animal preceding the onset of rabies, and this finding remained consistent when the data were stratified according to ownership status (RR = 3.09; 95% CI, 1.53 to 6.25; P < 0.001). The majority (n = 263 [85%]) of animals were submitted for rabies testing because of reported contact with a human.

Most animals (n = 289 [94%]) were reported as unvaccinated or of unknown vaccination status. Eight animals (3%) had been previously vaccinated < 30 days before onset of illness, and, therefore, their immunization status was not considered current. Ten animals (3%) had been previously vaccinated < 30 days before onset of illness, and, therefore, their immunization status was not considered current. Only 1 rabid dog from Texas that was infected with the Texas fox variant of rabies virus was considered currently vaccinated. This dog had received a single vaccine dose at 2 to 3 months of age, approximately 9 months before death. The dog had reportedly fought with a wild animal in the month before death and was not given a booster rabies vaccination as recommended in the Rabies Compendium.

Discussion

The results of this study suggest that dogs and cats are not commonly infected with bat-associated rabies virus variants in the United States. Although rabid bats accounted for 15% of wild animals detected with rabies in 1999, only 1 of 308 dogs and cats was found to be infected with a bat-associated rabies variant during the same period. We found no evidence of the Eastern pipistrelle/silver-haired bat rabies virus variant in the dog and cat population during 1999, although this variant has been associated with most of the human rabies cases during the last decade. These data suggest that dogs and cats are unlikely to play an important role in the secondary transmission of bat-associated rabies variants to humans.

Almost all of the dogs and cats in this study had the terrestrial rabies virus variant that was expected on the basis of their location, and we did not find any rabies virus variants outside their reported geographic ranges. Routine variant typing of rabies virus may be a useful diagnostic tool, especially for states that are located on the edge of an expanding rabies epizootic. For example, as the raccoon-variant rabies epizootic in the eastern United States expands westward, variant typing will be essential to help distinguish between raccoon and skunk rabies variants and may help detect the emergence of the raccoon variant in new areas.

Most of the rabid dogs and cats in this study were reported as unvaccinated owned animals. This finding is important, because these rabies infections could be easily prevented through appropriate vaccination, as recommended by national experts and required by law in many states. Pet vaccination campaigns work well to control rabies in domestic animals, as indicated by the dramatic decrease in rabies cases in the United States after widespread canine vaccination programs in the 1950s. Educational campaigns that target pet owners and emphasize the importance of rabies vacci-
nations for dogs and cats are critical to rabies control and the protection of human health.

This study, although comprehensive in scope, had several important limitations. The epidemiologic features of rabies in dogs and cats were examined for only a 1-year period, and 1999 may have been different from other years. For example, no rabies-related deaths among humans were reported during 1999, despite the fact that a median of 3 cases/year were reported between 1990 and 2000.15,16 Analysis of animal rabies data submitted to the CDC by state health departments, however, suggests that the data for 1999 were fairly representative of rabies cases reported for the previous 5 years.2,7 Another limitation of this study was that it included only dogs and cats in which rabies was diagnosed by state health departments. Many state health departments will not test potentially rabid animals unless they come in contact with humans or domestic animals; as a result, some cases of rabies in dogs and cats probably go undiagnosed each year. This practice may have also influenced the high percentage of animals that were reported as owned in this study, because owned animals may be more likely than strays to come in contact with humans. An additional limitation of this study was that it excluded specimens that states did not send for typing (n = 46) and samples that were sent but could not be typed by our laboratory (23). As a result, 18% of dogs and cats in which rabies was diagnosed during 1999 were excluded from this report; all of these animals were from states in which terrestrial rabies virus variants are expected.

Dogs and cats do not appear to be commonly infected with bat-associated rabies virus variants in the United States and are unlikely to be an important source of secondary transmission of these variants to humans. The most likely explanation for many of the recent unexplained human deaths caused by bat-associated rabies in the United States is a bat bite that was not taken seriously or was unnoticed by the person who was bitten. Although dogs and cats do not appear to be at an increased risk for infection with bat-associated rabies virus variants, they are often infected with common terrestrial rabies virus variants. Dogs and cats should be regularly vaccinated against rabies to protect them and humans they contact against infection.

References

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