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New Aspects of Rabies with Emphasis on Epidemiology, Diagnosis, and Prevention of the Disease in the United States

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INTRODUCTION AND HISTORICAL BACKGROUND

Rabies is an almost invariably fatal encephalomyelitis. The acute disease produced by rabies virus infection is characterized by such extraordinary symptoms that a presumptive diagnosis can be made from the writings of Democritus (500 BC) and Aristotle (400 BC) (59). The dog days of summer were thought of as a time when ordinarily docile and friendly animals became viciously aggressive. Months to years after an encounter with a rabid animal, human victims would begin to exhibit signs of intense anxiety and nervousness, paresthesia in the area of the bite wound, and painful pharyngeal spasms when offered water (hydrophobia). Convulsive seizures and progressive paralysis followed, and within a few days the patient died in coma. Prior to Pasteur's development of an antirabies vaccine, few words were more terrifying than the cry of "mad dog!"

Today, clinicians in the United States are unlikely to see a human case of rabies (Fig. 1). Vaccination and control programs of the 1940s and 1950s eliminated domestic dogs as a reservoir for rabies (8), and the introduction and widespread use of rabies immune globulin and potent vaccines virtually ensure successful postexposure treatment for humans (72). In this century, the number of human deaths attributed to rabies in the United States declined from 100 or more each year to an average of 1 to 2 each year.

However, as is the case with many control programs for infectious disease, the improved diagnostic and surveillance capabilities used successfully to eliminate one problem also serve to uncover other unanticipated problems. As rabies cases in dogs declined in the last 50 years from approximately 10,000 per year to the present level of a few hundred per year, cases in wild animals underwent an equally dramatic increase. In 1994, 7,632 rabies cases were reported in wild animals in the United States (36). Only Hawaii remains rabies free. The reasons for this increase are many and varied. The results are expenditures of >$300 million each year for rabies control programs in the United States (66).

Trials are under way in several states to vaccinate wild animals and create immune barriers to prevent or slow the spread of new or established outbreaks of rabies (48). Approval and licensing of oral rabies vaccines may provide the public health community with a new mechanism to deal with animal rabies.

THE VIRUS

Rabies virus is the prototype member of the genus *Lyssavirus* of the family *Rhabdoviridae*, order *Mononegavirales* (73). The genetic features of rabies virus are similar to those of other members of the *Mononegavirales* in that a nonsegmented, negative-stranded RNA genome is tightly encapsidated into ribonucleocapsid structures. Monocistronic (rarely polycistronic), 5′-capped, 3′-polyadenylated mRNAs are transcribed from genomic RNA that is organized so that the nucleocapsid and polymerase genes are encoded at the extreme 3′ and 5′ ends of the genome, respectively. The gene structure is delineated by conserved transcription initiation and termination signals, and gene transcripts may contain nontranslated regions of different lengths. The rabies virus virion contains five proteins: an RNA-dependent RNA polymerase (L protein; 190 kDa), a single surface glycoprotein (G protein; 65 to 80 kDa), a nucleoprotein (N; 58 to 62 kDa), a phosphoprotein (NS or M1; 35 to 40 kDa), and a matrix protein (M or M2; 22 to 25 kDa).

Members of the family *Rhabdoviridae* are grouped on the basis of their conical or bullet shape as visualized by electron microscopy. Their host range includes vertebrates (primarily mammals and fish), invertebrates (primarily arthropods), and plants. Three genera are recognized. The rhabdoviruses that infect mammals (*Lyssavirus*) or insects and mammals (*Vesiculovirus* and *Ephemerovirus*) share significant amino acid homology (Fig. 2) (70). The rhabdoviruses that replicate in insects share greater homology with each other than with the exclusively mammalian lyssaviruses. No significant amino acid homology exists between the insect/mammalian viruses and the three fish or plant rhabdoviruses for which sequence is available.

Initially referred to as the rabies and rabies-related virus group (53), the *Lyssavirus* genus (lyssa: from Greek "rage, rabies") is composed of six serotypes or genotypes which share high levels of amino acid and nucleotide sequence homology with rabies virus and cause a clinical disease indistinguishable

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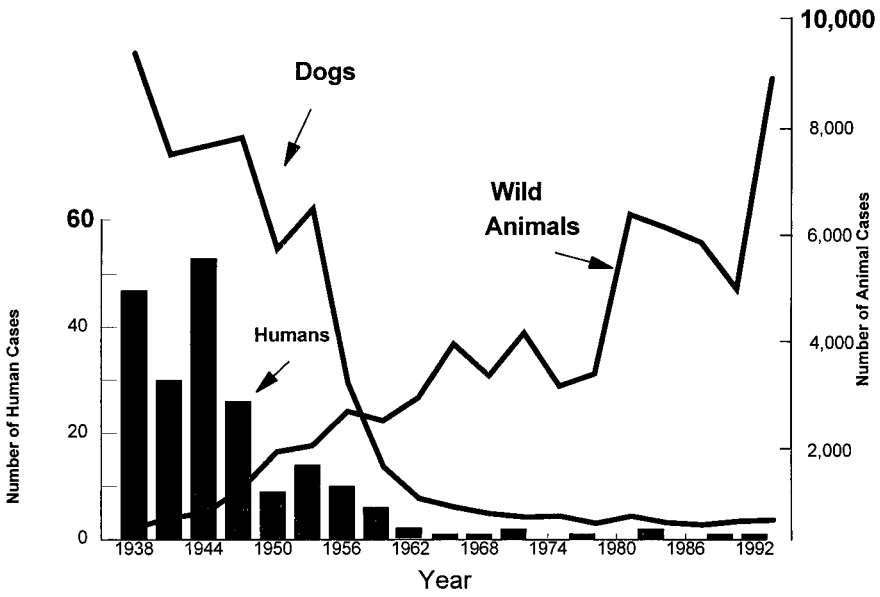

FIG. 1. Human and animal rabies cases in the United States, 1938 to 1992. Data from records maintained at the Centers for Disease Control and Prevention.

from rabies encephalitis (Table 1). No specific diagnostic test exists for the nonrabies lyssaviruses, and isolates have been identified only through weakly cross-reactive anti-N antibody in rabies virus diagnostic reagents. Specific identification is performed by using monoclonal antibodies, neutralization tests with virus-specific antisera, and, more recently, genetic typing (10, 34, 35, 51).

Reservoirs for rabies virus are found essentially worldwide; only certain insular nations and the Australian and Antarctic continents are rabies free. The virus is maintained by intraspecific transmission at endemic and epidemic levels in a wide variety of *Carnivora* and *Microchiroptera* species, and thousands of human deaths from rabies occur each year. In contrast, the nonrabies lyssaviruses have a much more limited distribution (Africa and Europe), the range of affected species is not known but appears limited to small mammals (rodents, insectivores, and insectivorous or frugivorous bats), and only endemic levels of transmission have been observed. To date, fewer than a half-dozen human deaths are attributed to infection with nonrabies lyssaviruses (Table 1); however, it is likely that a number of infections go undiagnosed. The full public health importance of the nonrabies lyssaviruses is unknown, but should their natural reservoir expand or change, new vaccines would be required. Current rabies vaccines elicit inadequate cross-protective immunity (27, 37).

Cross-reactive antigens with Mokola virus, and thereby with rabies virus, were responsible for the original inclusion of two arthropod-borne viruses in the rabies virus and rabies virus-related serogroup (7). These viruses, kotonkan and Obodhiang viruses, were isolated from pools of *Mansonia* and *Culicoides* mosquitoes in the Sudan and Nigeria, respectively. Several other viruses, including the ephemerovirus Adelaide River virus were added later because of cross-reaction with rabies antisera and/or with anti-N monoclonal antibodies (11, 70). Genetic analysis of Adelaide River virus revealed only a distant phylogenetic relationship and limited amino acid homology with rabies virus (Fig. 2). The cross-reactive epitope of Adelaide River virus and rabies virus was presumptively identified as an 11-amino-acid residue of the N protein shared by these two viruses but not other rhabdoviruses (70). These findings and the observation that kotonkan and Obodhiang viruses and other, incompletely characterized arthropod-borne rhabdoviruses which react with rabies antisera cause an acute, febrile illness with no involvement of neuronal tissue suggest that these viruses should not be included in the *Lyssavirus* genus (73).

EPIDEMIOLOGY

A concise description of the epidemiology of wildlife rabies in the United States requires data from molecular typing methods and case surveillance (58). Characteristic nucleotide substitutions permit identification of rabies virus variants associated with different outbreaks, but the presumed phylogenies of these variants are of little value without case surveillance to identify the animal reservoir, the aspects of the natural history of the animal contributing to disease maintenance, and the circumstances promoting the outbreak. On the other hand, outbreaks defined solely by case surveillance are biased toward animal species commonly found in and around human dwellings (e.g., raccoons, skunks, and house bats). Disease in more reclusive animals, such as the tree- or cave-dwelling bat species, is often overlooked or underestimated. Figure 3 presents the genetic lineages of the rabies virus variants affecting the animal species most commonly submitted for rabies tests in the United States, as well as a number of lineages for variants associated with animals, especially insectivorous bats, that are underrepresented by case surveillance.

Rabies infections of terrestrial species occur in geographically discrete outbreaks, and maps can be drawn to show areas where rabies is endemic (Fig. 4). Disease transmission within an outbreak is primarily intraspecific and involves a single, distinctive rabies virus variant which can be identified by reaction with panels of monoclonal antibodies (55) or by patterns of nucleotide substitution identified by genetic analysis (Fig. 3) (58). Spillover infection of other terrestrial animal species may occur, but these cases are sporadic and rarely initiate sustained intraspecific transmission. Once established within a particular animal population, disease transmission can persist at endemic levels for decades. The affected area gradually and sometimes

TABLE 1. Currently recognized members of the genus *Lyssavirus* of the family *Rhabdoviridae*[a]

Lyssavirus	Host(s)	Range	Human disease	History
Rabies virus	*Carnivora* (wild and domestic canids, mustelids, viverrids), *Microchiroptera* (insectivorous and hematophagous bats)	Worldwide, except for a few island nations and the Australian and Antarctic continents	>25,000 cases/yr	Disease known since antiquity. Transmission of rabies from a rabid dog to a normal dog by inoculation of saliva reported by Zinke in 1804. Pasteur attenuated the virus by serial passage and desiccation and vaccinated humans and animals in 1880s. Microscopic test for pathognomonic inclusions in nerve cells described by Negri in 1903. An immunofluorescent test for rabies viral antigen was developed in 1950s.
Lagos bat virus	Various *Megachiroptera* species (fruit bats), but few isolates available	Nigeria, South Africa, Zimbabwe, Central African Republic, Senegal, Ethiopia	None reported	Isolated in 1956 from the brains of Nigerian fruit bats (*Eidolon helvum*) at Lagos Island, Nigeria, but not characterized until 1970. Although the first isolates were made from apparently healthy bats, more recent samples are from animals showing clinical signs of encephalitis. Ten cases identified to date, including three cases in domestic animals initially diagnosed as rabies, but weak immunofluorescence led to suspicion of nonrabies lyssavirus, later confirmed by typing with monoclonal antibodies or nucleotide sequence analysis. Marginal cross-protection with rabies vaccines.
Mokola virus	Probably insectivore or rodent species, but few isolates available	Nigeria, South Africa, Cameroon, Zimbabwe, Central African Republic, Ethiopia	Two cases: a nonfatal case in 1969, and a fatal case in 1971	First isolated from *Crocidura* shrews trapped in Mokola Forest near Ibadan, Nigeria, in 1968. Characterized in 1970. Like Lagos bat virus, evidence of infection with Mokola virus recognized only by poor reaction with anti-rabies reagents. Eighteen cases identified to date, including 10 in domestic animals. Seven cases in Zimbabwe in 1981 and 1982 prompted serologic survey and identification of antibodies to Mokola virus in rodents, especially bushveld gerbils (*Tatera leucogater*). No cross-protection with rabies vaccines.
Duvenhage virus	Probably insectivorous bats, but only three bat isolates to date	South Africa, Zimbabwe, Guinea	One case in 1970	First identified in death from rabies-like encephalitis of a man bitten by an insectivorous bat near Pretoria, South Africa. Virus named after the victim. Although Negri bodies were detected in histologic examination of his brain tissue, negative immunofluorescence tests led to suspicion of non-rabies lyssavirus, subsequently confirmed by antigenic and genetic typing. Four cases identified to date, none in domestic animals. Marginal cross-protection with rabies vaccines.
European bat lyssavirus 1 (EBLV1)	Insectivorous bat (probably *Eptesicus serotinus*)	Europe	One confirmed case in 1985; suspected case in 1977	Although cases in bats in Europe were reported as early as 1954, identification of the virus was not attempted until 1985, when the first of several hundred infected bats were reported in Denmark and Germany. Almost all cases are in the common European house bat, *Eptesicus serotinus*. No cases in domestic animals. Marginal cross-protection with rabies vaccines.
European bat lyssavirus 2 (EBLV2)	Insectivorous bats (probably *Myotis dasycneme*, but few isolates to date)	Europe	One case in 1985	First identified in isolate from a Swiss bat biologist, who died of rabies in Finland. Five cases to date, almost all from *Myotis* sp. No domestic animal cases. Marginal cross-protection with rabies vaccines.

[a] Data are summarized from recent review articles on this subject (10, 34, 35, 51, 61).

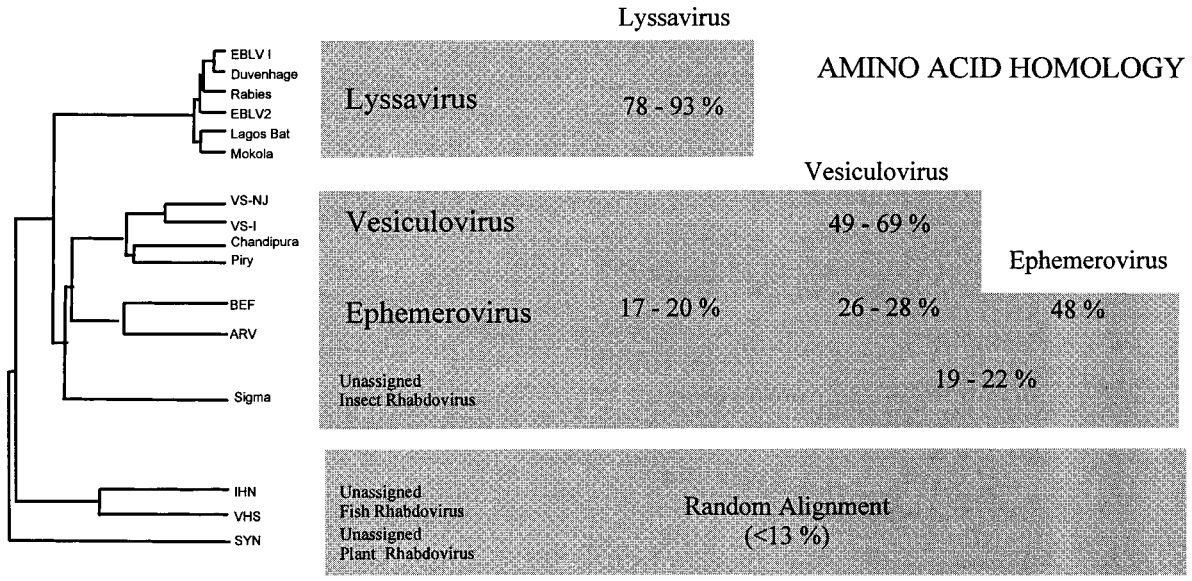

FIG. 2. Phylogenetic relationships among members of the *Rhabdoviridae* based on nucleoprotein amino acid homology. EBLV1 and EBLV2, European bat lyssaviruses; VS-NJ and VS-I, vesicular stomatitis virus from New Jersey and Indiana, respectively; BEF, bovine ephemeral fever virus; ARV, Adelaide River virus; IHN, infectious hematopoietic necrosis virus; VHS, viral hemorrhagic septicemia virus; SYN, Sonchus yellow net virus. Data from reference 70.

precipitously enlarges over time, occasionally overlapping previously existing outbreaks. For example, a newly emerging outbreak of rabies in coyotes in south Texas overlaps areas where rabies is endemic in skunks and is encroaching on an area where the main reservoir for rabies is the gray fox.

The geographic boundaries of the currently recognized reservoirs for rabies in terrestrial species are as follows.

(i) A long-standing reservoir is known for raccoons in the southeastern United States, and a more recent outbreak in the mid-Atlantic and northeastern states is probably the result of the translocation of infected animals from the southeast (49, 71). Over 4,000 cases were diagnosed in raccoons in these areas in 1994.

(ii) A long-standing reservoir has been documented in red and arctic foxes in Alaska, and the disease spread during the 1950s to include foxes across Canada as far east as Ontario, Quebec, and the New England states (42, 62). Rabies is a persistent problem in foxes in Alaska but is only intermittently present in the New England states. Usually, fewer than 100 cases are reported in foxes each year in these two areas.

(iii) Three different variants exist in striped skunks in long-standing reservoirs in California, the north central states, and the south central states (43), with several thousand cases diagnosed in skunks each year.

(iv) Two different variants are present in gray foxes in small but long-standing reservoirs in Arizona and Texas (usually 10 to 20 cases per year) (55).

(v) A recently recognized outbreak of rabies in coyotes in south Texas is the result of spillover infection from domestic dogs in a long-standing reservoir at the Texas-Mexico border (20). Prior to 1988, this area recorded only sporadic rabies infection in coyotes. In the last 8 years, however, a focus of rabies cases in coyotes and dogs in Starr and Hidalgo Counties has expanded northward to encompass most of south Texas, and the number of coyote rabies cases in Texas has increased from 6 in 1988 to 70 or more cases per year in 1990 to 1994.

While >90% of cases in wild animals in the United States in 1994 were in terrestrial species, the variants endemic in these species are found in only 3 of 16 human rabies infections acquired in the United States from 1980 to September 1995 (58). Figure 3 shows the genetic variant identified for these cases and the animal reservoir associated with maintenance of the variant. Differences in relative pathogenicity may play an as yet unrecognized role in the lack of human infections associated with terrestrial animal rabies variants, but the more likely explanation is that bite contact with a wild carnivore will rarely go unnoticed by the victim, who will then seek anti-rabies treatment. Public health education and ready access to anti-rabies biologics should keep the number of rabies deaths associated with terrestrial animal reservoirs at a very low level; however, the cost of maintaining this level of disease prevention will continue to rise.

Overlying the disease in terrestrial mammals are multiple, independent reservoirs for rabies in several species of insectivorous bats (54, 58). Like the disease in terrestrial species, distinct viral variants can be identified for different bat species (Fig. 3). Unlike the disease in terrestrial animals, however, geographic boundaries cannot be defined for rabies outbreaks in the volant, highly mobile bat species. Although some geographic orientation can be recognized for rabies virus variants transmitted within populations of resident, nonmigratory bat species, a variant transmitted by a migratory species can be found in that species throughout a migratory range that may extend over thousands of miles. For example, samples from eastern and western populations of the big brown bat, *Eptesicus fuscus*, contain distinctive variants. In contrast, rabies virus transmitted by the migratory freetail bat, *Tadarida brasiliensis*, show minimal sequence variation in samples collected in Florida, Alabama, Texas, New Mexico, Nevada, Colorado, and California. Similarly, samples from the migratory silver-haired bat, *Lasionycteris noctivagans*, in New York, Wisconsin, Washington, Colorado, and California are nearly identical. Since all areas of the United States, with the exception of Alaska and Hawaii, are home to a variety of different bat species affected by rabies, the result of these associations is that rabies is endemic in all contiguous areas of the United States in several different bat species, each transmitting a distinct rabies virus variant.

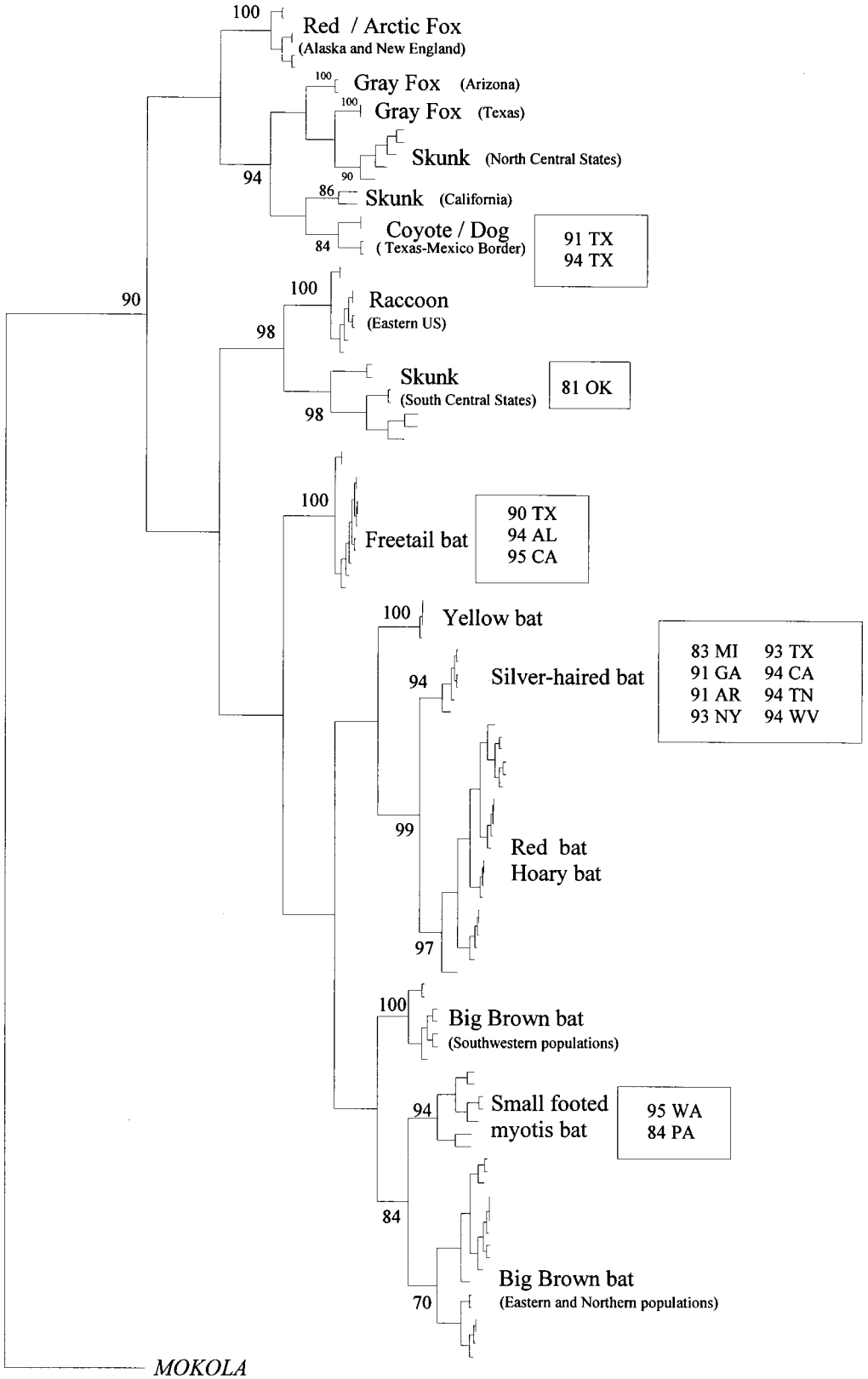

FIG. 3. Phylogenetic relationships among rabies virus isolates from wildlife reservoirs for rabies in the United States based on nucleotide homology of a 320-bp region of the nucleocapsid gene. Genetic distance was measured by the Kimura two-parameter method and neighbor-joining analysis in the Phylip 3.5 computer software (J. Felsenstein, University of California, Berkeley). Numbers at nodes indicate confidence limits greater than 70% for monophyletic groups defined by adjacent nodes (hypothetical ancestors) and were obtained by character resampling bootstrap analysis (400 iterations). RNA was extracted and analyzed as described previously (54). Boxes indicate a human case sample associated with a given wildlife rabies variant (year and state).

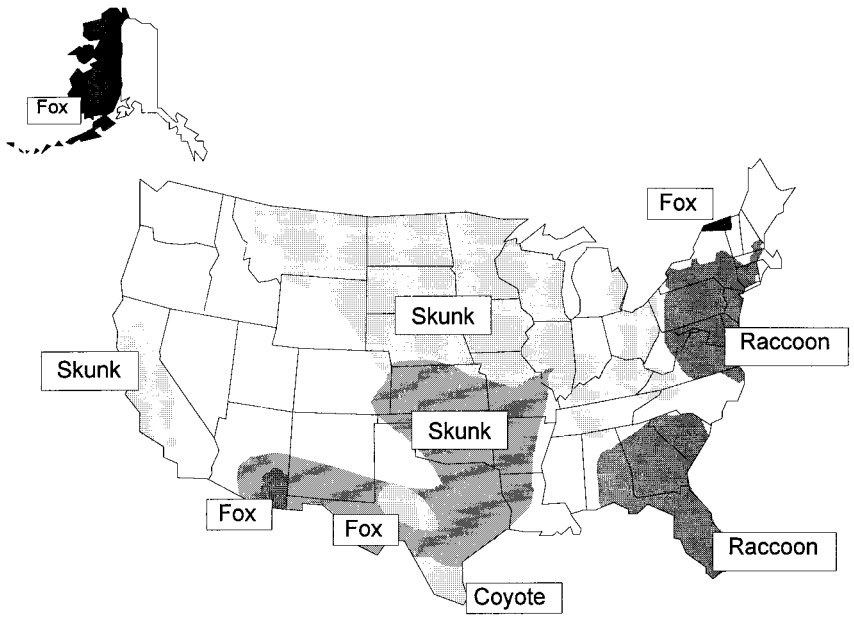

FIG. 4. Currently recognized areas where rabies is endemic in wild terrestrial animals in the United States.

Although bats are responsible for a relatively small portion of animal rabies cases in the United States (7.7% in 1994) (36), rabies virus variants from bats are associated with a disproportionate number of human rabies infections acquired in the United States (13 of 16 cases since 1980 [Fig. 3]) (58). Most striking in the investigation of these cases is the absence of a clear history of animal bite exposure. Only 2 of the 13 case histories include an account of a bite by a bat, and in one of these cases, the parents of the 5-year-old child who reported the bite could find no evidence of a bite wound and the bat could not be found. In six other cases, contact with a bat was reported by the patient, family, or acquaintances, but in no case was a bite recognized or a bite wound evident. In two of these cases, a rabies-positive bat was later found in the home or office of the patient.

Also remarkable in the investigation of human rabies deaths associated with bats was the identification in 8 of the 13 cases of a variant found almost exclusively in the silver-haired bat, *Lasionycteris noctivagans*. Silver-haired bats are a tree-living, solitary species that are not often found around human dwellings and consequently are infrequently submitted for rabies tests. In New York, for example, the death from rabies of a young girl infected with this variant prompted an analysis of bat rabies cases in the state. The study revealed that while similar percentages of the common house bat, *Eptesicus fuscus*, and the silver-haired bat submitted for testing over a 5-year period were positive for rabies infection (6.3 and 8.0% respectively), the number of submissions of the two species differed markedly (4,354 samples from the house bat and 25 samples from the arboreal bat) (19).

Although the reclusive habits of tree-living bats may make human contact less likely, this contact may be less noticeable when it does occur. Arboreal bat species may be accessible to humans when the bats use fallen trees, leaf litter, and shrubs as day roosts or use human dwellings as temporary night roosts. Additionally, rabies-infected bats may be unable to fly or to find appropriate shelter, thus increasing the opportunity for human contact. The small, sharp teeth of insectivorous bats may produce a wound as inapparent as that of the prick of a hypodermic needle. Good public health measures for the prevention of bat transmission of rabies would include education about the risks of handling downed bats, the importance of excluding bats from human dwellings, and the importance of vaccinating pets, especially cats, that may have contact with bats. Because a bite wound inflicted by a bat may not be readily visible, it may be difficult to determine with certainty whether bite contact has occurred. Persons, especially children, who have had contact with a downed bat should be questioned carefully about the need for rabies preventive treatment.

CLINICAL DISEASE

Excellent reviews of rabies pathology and clinical disease in humans and animals can be found in recent articles (4, 18, 28, 30, 31, 45, 50); therefore, this area will not be covered in great detail here. The following is a summary of aspects of the clinical effects of rabies infection that are important for diagnosis and intervention.

There are few undisputed facts in our knowledge of the pathogenesis of rabies. The lack of a good experimental model for naturally acquired rabies has hampered the study of the infectious process; also, the conclusions drawn from studies of laboratory-adapted strains of rabies often contradict observations of natural infections, and multiple virus-host adaptations make broad extrapolations across species inapplicable even when field isolates are used directly.

The initial event in a rabies infection is the introduction of virus-laden fluid (almost always saliva) into the tissues of a susceptible host. After some unknown period, the virus enters peripheral nerves and passive transport to the central nervous system (CNS) occurs. In some experimental models, virus was found to immediately enter nerves at the site of inoculation and to appear in the CNS within a very short time (52). In other systems, virus entered peripheral nerves after local replication in nonnervous tissue (40, 41). Transport to the CNS occurs by retrograde axoplasmic flow (22) at an estimated rate of 15 to 100 mm per day (64). Virus replication probably does not occur during transport, since axons do not contain ribo-

somes. Both motor and sensory fibers may be involved in viral transport (32). Spread within the CNS is intra-axonal, and infection can be widely disseminated before the onset of clinical signs (29). No specific lesion in the CNS has been correlated with the neuronal dysfunction that precipitates the increased alertness, hyperexcitability, and abnormally aggressive behavior typical of rabies infection. Neuronal necrosis is infrequent, and electroencephalogram abnormalities are lacking even at late stages of disease (reviewed in reference 18).

Although not evident in every rabies infection (25), the virus may move from the CNS via anterograde axoplasmic flow in peripheral nerves at an estimated rate of 100 to 400 mm/day (65). Virus can be detected in motor, sensory, and autonomic nerve fibers and in both myelinated and unmyelinated nerves (41). Late in the infection, virus is released from axon terminals and taken up by adjacent nonnervous tissues. The mechanism responsible for this transition is unknown. Except for salivary glands and other tissues that supply virus to oral fluids, infection of nonnervous tissue is incidental and of little or no importance in transmission or maintenance of the virus in nature. By electron microscopy, virions have been observed budding into secretory granules of mucous cells in salivary glands and other secretory tissues (6). Exocytosis of these granules into salivary ducts may contribute infectious virus to the saliva. Virions have also been observed budding on apical plasma membranes of the salivary gland mucous cells (6). Although the titer of infectious virus in saliva can be quite high for some virus-host combinations, virus may be only sporadically present in the saliva. For example, samples of saliva from a naturally infected hoary bat were positive for rabies virus on the fifth day after its capture but negative on the sixth day (21). In a more recent study, saliva was taken daily from dogs experimentally infected with graded doses of rabies virus. Virus was found in saliva taken on one or more days from 16 of 25 dogs that later died of rabies, but all 25 dogs had rabies-positive salivary glands at necropsy (26).

No evidence of an immune response to rabies infection is noted until late in the clinical course. In a recent human case in which epidemiologic data suggested an incubation period of several years (57), antibody was not detected in serum taken as late as 6 days before the patient died (14 days after the first clinical signs of rabies encephalitis) (14).

Probably as a result of neuritis, the late stages of the disease include hypoventilation, cardiac arrhythmias, and hypotension (30). Death occurs within a few days of the appearance of these clinical signs.

Many of the public health recommendations for the diagnosis of animal rabies and treatment of potential exposures are based on the observation that virus reaches salivary glands and other peripheral tissues only after prior replication in the CNS and that virus excretion in saliva is sporadic. First, the absence of rabies antigen in the brain of an animal examined by immunofluorescence (a negative diagnostic test) precludes the presence of virus in saliva, the risk of bite transmission of rabies, and the need for antirabies treatment. Second, there are no reliable intravitam tests for rabies infection. An animal suspected to have rabies must be killed, and its brain must be examined. Tests for antibody in serum and cerebrospinal fluid, viral antigen in corneal epithelium and cutaneous nerves in a skin biopsy specimen, or infectious virus in saliva can all be negative in an animal later diagnosed as rabid by the presence of virus in brain tissue (25). Third, rabies is relatively uncommon in domestic animals in the United States, but should it occur, the early signs of disease are easily recognized and the clinical progression is rapid and well characterized (i.e., virus excretion in saliva follows infection of the CNS and rarely precedes the onset of clinical signs by more than a few days) (26, 67, 68). Biting incidents involving healthy dogs or cats are very common (>1 million bites annually [39]) but have never been implicated in a human death from rabies in the United States. The public health recommendation drawn from these observations is that an apparently normal, healthy dog or cat can be confined by its owner and observed for 10 days. Unless the animal develops signs suggestive of rabies during this period, it need not be killed and tested for rabies and no antirabies biologics are required for the person bitten. In areas outside of the United States where cases of rabies in dogs are common, antirabies treatment is often begun at the time of the bite exposure but terminated if the biting animal remains healthy during the quarantine period.

Intravitam tests for rabies lack sufficient sensitivity to be useful for the diagnosis of animal rabies. The tests are useful only in human cases of viral encephalitis of unknown etiology. An early diagnosis in these instances, although unlikely to affect patient outcome, can significantly reduce the number of potential exposures to rabies virus during contact with the patient and permit the identification of persons who are candidates for rabies prophylaxis.

One of the most intriguing of the unresolved issues in rabies pathogenesis is an explanation for the long preclinical period. The incubation period in naturally infected animals (field trapped in areas where rabies is endemic and held for observation) may be 6 months or longer (9), and epidemiologic investigation of human cases has suggested incubation periods as long as 6 years (57). These observations are of more than academic interest, because they influence the design and use of antirabies biologics. As yet, none of the experimental models to study rabies pathogenesis has offered an explanation of how or where the virus persists during this period, nor at what point and for how long after entry into a wound the virus remains vulnerable to antirabies prophylaxis. Studies have shown that limb amputation up to 18 days after virus injection can prevent clinical disease in mice inoculated with a field strain of rabies with a long incubation period (3) and that postinoculation treatment with immune serum is more effective if given in the inoculated limb of mice (5). These data suggest that the virus remains near the site of entry for long periods and that postexposure treatment works by preventing infection of the CNS. An alternative view was presented by Dietzschold et al. (23), who concluded that some degree of protection is conferred even when treatment is initiated after the virus has entered the CNS. In this experiment, rabies virus genomic RNA was found in the cerebral cortex of rats 12 h after an intranasal inoculation of a fixed strain of rabies virus; however, rabies mortality was reduced in rats given 30 IU of a monoclonal antibody intramuscularly up to 24 h after the injection of virus. More remarkably, no clinical disease was evident in these animals and no rabies virus RNA was detected when the animals were sacrificed on day 30. No evidence of neurolysis was found by microscopic examination, even though some of the animals exhibited a very strong immune response to the virus infection (three animals had antibody titer of >1,000 U at day 30), leading the authors to postulate that viral clearance is accomplished by mechanisms other than antibody-mediated cytolysis. This finding conflicts with other research showing a clear involvement of antibody-mediated viral clearance in rabies pathology (46). Although current antirabies biologics are very effective, treatment is expensive (up to $2,000 per case) and efforts continue to improve or replace various components. In considering these changes, it is important to remember that we have an incomplete understanding of how postexposure treatment works.

DIAGNOSIS

One million to two million animal bites per year are treated by physicians in the United States (39). Fortunately, only a small proportion of these bites involves a risk of rabies infection, and it is the function of the rabies laboratory to rapidly and accurately identify rabies virus-infected animals. Guidelines for sample collection and testing exist (56, 63, 69); therefore, this section is limited to a discussion of test rationale and expectations.

No reliable intravitam test for rabies exists. Determination of whether to kill an animal and examine its brain for evidence of rabies is based on several factors. First, the species which are known to be reservoirs for rabies in the United States (insectivorous bats and wild carnivores) are normally reclusive, nocturnal animals that would avoid human contact if possible. An attack or bite by these animals is always considered to carry a risk of rabies, and the animal should be killed and tested. Stray and unwanted domestic dogs and cats involved in unprovoked biting incidents are also killed and tested. Because rabies is uncommon in dogs and cats in the United States and signs of disease may be easily recognized, healthy dogs and cats that can be confined by their owners and observed for 10 days are not tested unless they experience an illness compatible with rabies during the observation period. Bites by nonreservoir mammals (e.g., rodents, lagomorphs, and ungulates) are considered individually but are less likely to result in rabies testing or rabies prophylaxis.

The direct immunofluorescent-antibody (dIFA) test for rabies virus antigen in brain tissue is the preferred test for rabies diagnosis. Thin-touch impressions of medulla, cerebellum, and hippocampus are fixed in cold acetone for 1 to 4 h, air dried, and stained with fluorescein isothiocyanate-labeled anti-rabies antibody. At a magnification of $\times 400$ to $\times 1{,}000$, rabies antigen appears as dustlike particles <1 μm in diameter and/or large, round to oval masses and strings 2 to 10 μm in diameter. These intracytoplasmic inclusions appear smooth with very bright margins and a somewhat less intensely stained central area. The amount of antigen may vary from a massive infiltration of large inclusions and dustlike particles in every area of the brain to isolated small inclusions in only a few microscopic fields in only one or two areas of the brain.

Histologic stains for Negri bodies detect only 50 to 80% of dIFA-positive samples and are no longer used for rabies diagnosis in the United States (60). Immunoperoxidase tests of formalin-fixed brain material or dIFA tests of proteinase-digested fixed brain material have been developed but have not been thoroughly evaluated for sensitivity.

Virus isolation is not performed for routine diagnostic tests but is useful when the results of the dIFA are inconclusive or unusual. Since rabies virus is not cytopathic, evidence of virus growth is obtained by dIFA detection of viral antigen in acetone-fixed cell monolayers of either mouse neuroblastoma or baby hamster kidney cell lines (47).

Because the dIFA test is rapid, sensitive, specific, easy to perform, and relatively inexpensive, molecular techniques such as PCR or hybridization probes are not used for routine rabies diagnosis. Molecular techniques have been useful in antemortem diagnosis (33); however, there is no evidence that viral RNA is any more widely distributed or accessible than viral antigen until late in the clinical course. PCR has also been useful in confirming the results of dIFA tests of tissues from which virus isolation is impossible (formalin-fixed or decomposed brain tissue) (38, 44). The greatest utility of this technique has come from epidemiologic studies in which precise identification of a rabies virus variant has provided information about patterns of disease transmission (58).

PREVENTION AND CONTROL

Guidelines for animal rabies control programs and the immunization practices recommended for human rabies prevention are available from the U.S. Government Printing Office (15, 17).

Humans

The essential components of rabies postexposure prophylaxis are immediate thorough cleansing of all wounds with soap and water and the administration of anti-rabies immune globulin and vaccine. When indicated, treatment should begin as soon as possible (preferably within 24 to 48 h of an animal bite), but it should be initiated even if a lengthy delay has occurred. Globulin from hyperimmunized human plasma donors is given at 20 IU/kg of body weight. Half of the dose is infiltrated around the wound(s), and the rest is given intramuscularly in the gluteal area. A purified, cell culture-derived, inactivated rabies vaccine is given on days 0, 3, 7, 14, and 28 (1.0 ml each day by intramuscular injection in the deltoid area). No serologic test is required to detect resultant antibody levels; studies have found an excellent antibody response in all patients receiving this treatment (1).

Preexposure immunization (three 1.0-ml doses, intramuscularly, or three 0.1-ml doses, intradermally) is given to certain high-risk groups, such as those who work in rabies laboratories, animal control facilities, and veterinary clinics. Preexposure immunization is also recommended for persons traveling to areas of the world where rabies in dogs is poorly controlled and postexposure treatment may be difficult to obtain. Although almost certainly conferring some degree of protection against an inapparent contact with rabies virus, the intent of preimmunization is to eliminate the need for immune serum and reduce the number of vaccine doses to two booster injections should the worker or traveler sustain a bite or wound exposure to rabies virus.

Domestic Animals

Local programs of vaccination of dogs and cats, restriction of movement (leash laws), and removal of stray or unwanted animals are very effective measures of rabies control. More than two dozen rabies vaccines of 1- and 3-year duration of immunity are marketed in the United States, some available in combination with other animal disease vaccines. All contain inactivated rabies virus and constitute no risk for acquiring rabies. Restraint of animal movement when outdoors can eliminate most potential contact with rabies. Currently vaccinated dogs and cats bitten or otherwise wounded by contact with a wild carnivore or bat suspected of or diagnosed with rabies must be revaccinated immediately, kept under the owner's control, and observed for 45 days. Unvaccinated animals should be killed or placed in strict isolation for 6 months and vaccinated 1 month before release.

Currently available animal rabies vaccines are potent and safe, and only rarely does rabies occur in a vaccinated animal. An investigation of 280 rabies cases in dogs and cats in 1988 found only 3 cases in vaccinated animals, and all were animals given only a single dose of vaccine when they were between 3 and 6 months old (24).

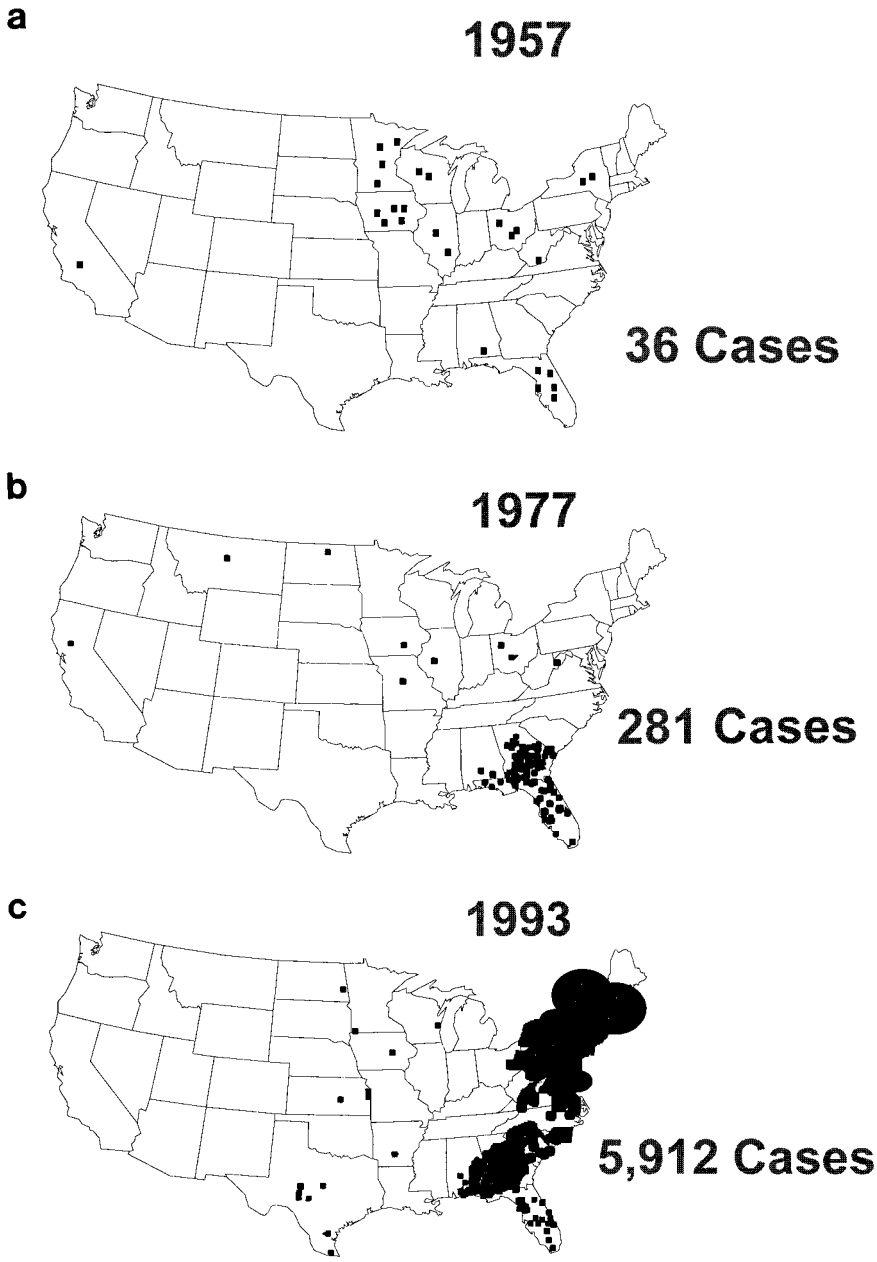

FIG. 5. Cases of rabies in raccoons in the United States in 1957 (a), 1977 (b), and 1993 (c). Data from records maintained at the Centers for Disease Control and Prevention.

Wildlife

The control of rabies in wildlife species is difficult (2, 12). Population reduction methods have not succeeded in eliminating rabies from any sylvatic reservoir. Vaccination of wildlife through oral baits has been effective in controlling or eliminating rabies in foxes from some areas of Europe, but the area covered is small in comparison with areas of the United States where the disease is endemic and only a single animal reservoir is addressed. Additionally, new outbreaks of rabies in an animal population may often go unnoticed until large numbers are affected. Once established, the virus can remain at endemic levels for decades, gradually (or sometimes precipitously) spreading to involve large geographic areas.

Perhaps the best-documented example of the risks of long-term endemicity and the difficulty of containing an outbreak is the rabies reservoir in raccoons in the eastern United States (49, 71). In the mid-1950s, a small focus of rabies was recognized in raccoons in peninsular Florida (Fig. 5a). The outbreak steadily expanded and by 1977 included areas of Georgia, Alabama, and South Carolina (Fig. 5b); however, the number of cases in raccoons remained small compared with that in other reservoirs of rabies in the United States. In 1977 for example, rabies was diagnosed in 281 raccoons, 637 bats, and 1,631 skunks (13). Unfortunately, in 1977 the outbreak also took an unanticipated jump, when rabies was found in raccoons at the Virginia-West Virginia border. This outbreak, almost certainly the result of rabies introduced with translocated animals from the Southeast, subsequently spread into

relatively dense raccoon populations in urban-suburban settings in one of the densest corridors of human population in the United States. The consequence was an explosive increase in the number of reported cases of rabies in raccoons and the number of human anti-rabies treatments administered. For example, from 1989 to 1993, terrestrial animal rabies in New York increased from a few hundred cases per year, primarily associated with a reservoir in red foxes in the more rural northern counties, to over 2,000 cases when rabies entered the raccoon populations of the more urban southern half of the state (Fig. 5c). The number of human postexposure treatments increased from 84 in 1989 to over 1,000 by 1992 (16). With few exceptions, the public health response to raccoon rabies in the last 50 years has focused on interrupting the transmission of rabies from raccoons to humans through vaccination, education, and strict enforcement of domestic animal control measures. Certainly, this approach is appropriate and has been very successful in preventing human disease. The most effective preventive measure, however, would be to eliminate or stop the spread of rabies in the raccoon population. Unfortunately, rabies is now entrenched in raccoon populations in every state along the eastern seaboard. The costs of vaccinating the animals in such a large area would be immense (66). With hindsight, a vaccination campaign targeting the original focus of disease in the mid-Atlantic could have produced substantial savings. In reality, even if such an opportunity should arise again, inadequate capability exists either to predict or respond to a similar disease introduction. This ability will come only with an increased awareness of the public health importance of zoonotic disease and strengthened epidemiologic surveillance.

FUTURE DIRECTIONS

Progress in rabies control is hampered by both economic and scientific issues. In developing countries, rabies in urban canine populations is almost entirely the consequence of an insufficiently funded public health infrastructure. While insufficient funding also inhibits sylvatic rabies control programs, elimination of rabies from the different wild species serving as reservoirs for the virus will not be possible without some innovative advances in vaccinology and a more complete understanding of how the virus is maintained and transmitted within animal populations. Epidemiologic surveillance must be strengthened to detect and hopefully predict the emergence of new disease reservoirs, because even successful urban and sylvatic control programs must anticipate a reintroduction of disease through the importation, transport, or natural movement of infected animals from outside the controlled area. Although international quarantine regulations for animal movement exist and many states have laws controlling the translocation of wild animals, the implementation of these laws is limited by insufficient funding. The trend in modern vaccinology is toward the development of defined antigens produced by either conventional or recombinant techniques with a goal of producing effective, inexpensive products. Although scientists investigating rabies were among the first to make use of these techniques, the benefits of these new vaccines have not been fully realized. In a decade when we can celebrate 100 years of research in rabies prevention, we should accept nothing short of an international effort to make rabies vaccines available worldwide for both urban and sylvatic rabies control programs.

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