[7] Nevertheless, dogs showing any measurable titer after vaccination were protected.

Rabies and Other Lyssavirus Infections

Chapter 20

Rabies and Other *Lyssavirus* Infections

Craig E. Greene

Etiology

Rabies

Rabies virus is the prototype of the genus *Lyssavirus* in the family Rhabdoviridae.[89] These agents are enveloped, bullet-shaped RNA viruses that usually measure 75 × 180 nm (Fig. 20-1). The single strand of nonsegmented RNA (negative-sense) encodes five structural proteins: a nucleocapsid (N) protein, a phosphoprotein (P), a matrix (M) protein, a glycoprotein (G), and an RNA-dependent RNA polymerase (L). These viruses have been isolated worldwide and were originally considered to belong to one common antigenic type. However, techniques using monoclonal antibodies (MABs) produced against viral proteins and gene-sequencing techniques have provided evidence for antigenic and genetic differences (variants) among various isolates from major wildlife and domestic animal hosts within a given geographic region. At least seven distinct genotypes of rabies virus exist. Analysis of sequences from the nucleoprotein gene of worldwide virus isolates supports studies of MAB typing, which suggest that currently circulating terrestrial-mammal rabies virus strains may have originated in domestic dogs from south of the Indian subcontinent 1500 years ago.[45] Subsequent European colonization was followed by spread to areas outside this region, such as the Americas, Asia, and Africa.[314,316] For example, dog rabies virus began circulating within the past 200 years in Africa, associated with European colonial influence; it spread from east to west over the next 100 years.[323] Because it has become well established, eradication will be difficult.[215] Infection in imported domestic dogs may have contributed to spread to sylvatic species, with establishment of enzootic foci in wildlife within these areas.[216] The regional distribution of rabies virus strains by host species and geographic location suggests that once introduced, virus spreads gradually within cohabitating animal populations.

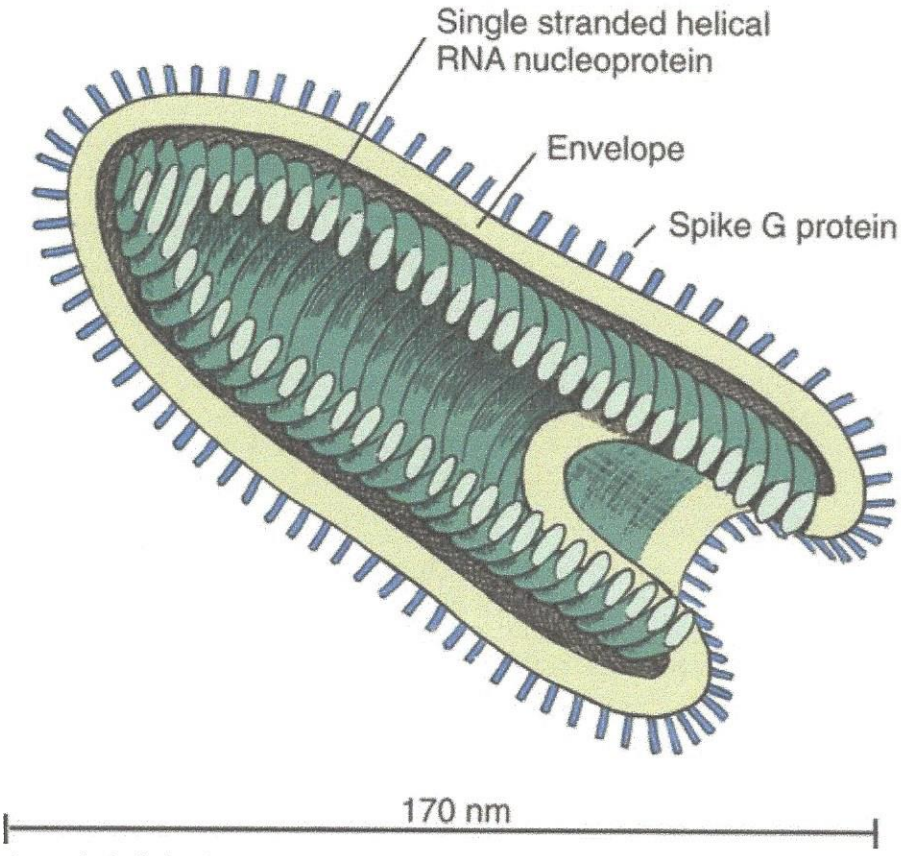


FIG. 20-1 The bullet-shaped rhabdovirus. (Art by Kip Carter © 2004 University of Georgia Research Foundation Inc.)

Rabies virus replicates by budding from the host cell membranes, and viral nucleocapsid develops in the cytoplasm. Complete viral particles may be formed at the cell surface, but more commonly, they bud from intracytoplasmic membranes. Free virus particles infect new or adjacent cells by fusing their envelopes with the host cell membrane, which allows direct entry of viral genetic material.

Rabies virus is enveloped, and inactivated by various concentrations of formalin, phenol, alcohol, halogens, mercurials, mineral acids, and other disinfectants. The virus is extremely labile when exposed to ultraviolet light and heat.

Rabies virus can remain viable in a carcass for several days at 20° C, although it may survive much longer when the body of the victim is refrigerated.[221] Immunofluorescent or genetic testing, commonly used for rabies diagnosis, does not depend on the presence of viable viral particles. Viral antigen or nucleic acid may be detected at times beyond the presence of viable virus. Virus survival for isolation by mouse inoculation can be greatly increased in unrefrigerated tissue by storing it in 50% glycerol in phosphate-buffered saline at room temperature (20° C). Preservation can also be enhanced if a 20% suspension of infected tissue or virus culture is made with a solution that is high in protein or amino acids. Storage at ultralow temperatures (−30° C to −80° C) prolongs viral activity for years in untreated fresh-frozen tissue. However, freezing samples in a household-type freezer with subsequent defrosting cycles will damage the tissue and destroy the virus for subsequent detection.

Other *Lyssavirus* Infections

Besides rabies virus, at least six other *Lyssavirus* genotypes have been described (Table 20-1); reports of more types are expected in the future as surveillance is enhanced, particularly among bats.[16] (See Other *Lyssavirus* Infections, under Epidemiology.)

TABLE 20-1

Classification of the Genus *Lyssavirus*

Genotype	Phylogroup	Serotype	Description of Strains (Abbreviation)	Geographic Location (Reservoir Hosts)
1	I	1	Classical rabies virus, including street and fixed varieties	Worldwide, terrestrial (carnivores and bats)
2	II	2	Lagos bat virus (LBV) 1, 2, and 3	Africa (bats)
3	II	3	Mokola virus (MOKV) 1, 2, 3, and 5	Africa (unknown)
4	I	4	Duvenhage virus (DUVV) 1, 2, and 3	Africa (bats)
5 and 6	I	5	European bat *Lyssavirus* (EBLV) types 1 and 2	Europe (bats)
7	I	6	Australian bat *Lyssavirus* (ABLV)	Australia (bats)
Putative	I		Aravan virus (ARAV)	Kyrgyzstan (bats)
Putative	I		Khujand virus (KHUV)	Kyrgyzstan (bats)
Putative	I		Irkut virus (IRKV)	Eastern Siberia (bats)
Putative	III		West Caucasian bat virus (WCBV)	Western Caucasus mountains (bats)

Modified from Refs. 178, 365.

Epidemiology

Susceptibility

All warm-blooded animals are vulnerable to infection with rabies virus, but mammals are the only known vectors and reservoirs in nature. Factors such as the viral variant, the quantity of virus inoculated, and the bite site affects susceptibility. In addition, the degree of species susceptibility varies considerably. Foxes, coyotes, jackals, wolves, and certain rodents are among the most susceptible animal groups. Skunks, raccoons, bats, rabbits, cattle, and some members of the families Felidae and Viverridae have a high susceptibility. Groups with only moderate susceptibility include domestic dogs, sheep, goats, horses, and nonhuman primates. Birds and primitive mammals such as the opossum may have low susceptibility. Cats are actually more resistant than dogs are to experimental infection with some canine rabies virus isolates but are

much more prone to develop infection with some field isolates from wildlife and with vaccine virus. Younger animals are usually more susceptible to rabies infection than are older ones.

Transmission

The disease is nearly always caused by the bite of an infected animal that has rabies virus in its saliva. Other modes of transmission are infrequently involved in infections of the dog and cat but may serve to maintain infection in wildlife. Transmission from exhaled or excreted virus has been suggested for spread between animals in extremely large colonies of cave-dwelling bats and by infections after laboratory exposures.[124,161] Such airborne infections probably involve large quantities of aerosolized virus under conditions of poor ventilation and a susceptible exposed host. Rabies can occasionally result from ingesting infected tissue or secretions.[132] Suspected transplacental rabies infections in skunks, bats, and a cow have been reported.[93] Environmental transmission by fomites is rarely, if ever, involved. Human rabies is usually caused by a bite, but it has been acquired by corneal transplantation. The disturbing number of human cryptic rabies cases in which no obvious source of exposure can be determined argues against complacency with this disease. Infections with salivary shedding of virus before obvious clinical signs have been observed, and thus the absence of dramatic neurologic abnormalities cannot be used to rule out absolutely the possibility of rabies infection.

Hosts and Range

More than 27,000 cases of animal rabies are reported yearly in the world. The estimated actual number of cases is many times greater. Rabid dogs are the main source of infection in people. Rabies virus transmission from dogs to people is intensified as the density of susceptible dogs exceeds 4.5 dogs/km.[2,31] Combined measures of immunocontraception and rabies vaccination have been proposed to help alleviate this zoonotic risk.[31,368]

The World Health Organization estimates that 55,000 to 100,000 human rabies cases occur annually, most in tropical countries of Asia and Africa.* This number is equal to one fatality from rabies every 10 minutes. As a result of potential exposure to rabies virus, approximately 10 million people annually receive postexposure prophylaxis (PEP). Most affected victims in these regions are children under 15 years of age who did not receive appropriate PEP after being bitten by rabid dogs. In contrast, a major reduction in the disease incidence in the Americas has been achieved by coordinated vaccination programs for dogs. For example, in the United States, where rabies vaccination for dogs is mandatory, three or fewer humans die of rabies annually, and approximately 45,000 more receive PEP. The incidence of indigenous canine rabies has become so low that the United States was officially declared canine–rabies free in 2007.[60] Canine cases that have been observed are in imported dogs. The low incidence of dog infections in North America has allowed it to be a part of the Pet Travel Scheme (PETS), similar to European Union countries, for dogs entering Great Britain.[53,180] There have been similar reconsiderations of the quarantine restrictions on dogs and cats entering Japan from the United States.[182] In Latin America, vaccination programs for dogs have intensified in the past 20 years and have resulted in a reduction in the annual dog-rabies virus fatalities in people from more than 150 to approximately 20.[52,289a,367] Many island or peninsular nations, such as Antarctica, New Zealand, Taiwan, some of the Caribbean islands, Ireland, Norway, Finland, Sweden, Iceland,

Hawaii, and Japan, are reportedly free of rabies. In western Europe, countries such as Spain, Portugal, Italy, and Greece have become free of terrestrial rabies at considerable cost through oral vaccination of wildlife, especially the red fox (*Vulpes vulpes*). However, there is a continual risk of reintroduction of rabies through importation of infected animals.[357] Most rabid animals imported in the European Union have been entering from Morocco.[252a] Compulsory parenteral vaccination and serologic testing for imported domestic animals have also been instituted in some areas to help maintain this status.

Although all mammals are susceptible to rabies virus, members of Canidae, Viverridae, and chiropteran species are the most capable vectors of the disease. Throughout the world, in most of the Northern Hemisphere, rabies is predominately a sylvatic disease of wildlife, whereas in the Southern Hemisphere, the feral dog in urban areas is the primary species involved in the transmission of the disease (Fig. 20-2). Turkey is the only country in Europe where dog rabies exceeds that in wildlife species and where urban dog-mediated rabies persists.[177a,225] Rabies virus in a given enzootic area is usually a distinct variant that adapts itself to a single dominant reservoir host. Therefore, independent host-specific enzootic cycles of infection exist among individual host species. For example, wildlife reservoir species in various geographic areas of the United States are raccoons, skunks, foxes, coyotes, and insectivorous bats (see sections on Wild Carnivores and Bats). In Europe and parts of Asia, the primary wildlife species are foxes and raccoon dogs (*Nyctereutes procyonoides*), whereas in South Africa, jackals (*Canis adustus* and *Canis mesomelas*) and bat-eared foxes (*Otocyon megalotis*) are the predominant reservoir hosts.[34,130a,260,283] In neighboring Botswana, spillover isolates in domestic animals are from jackals and the yellow mongoose (*Cynictis penicillata*).[176] Reservoirs for rabies in Mongolia include wolves (*Canis lupus*), foxes (*V. vulpes*), and manuls (*Felis manul*).[260] In certain Caribbean nations, mongooses (*Suricata suricata*) are the important reservoir host. Although bats can transmit their infection to terrestrial carnivores, the genotypes affecting bats or carnivores also remain distinct.

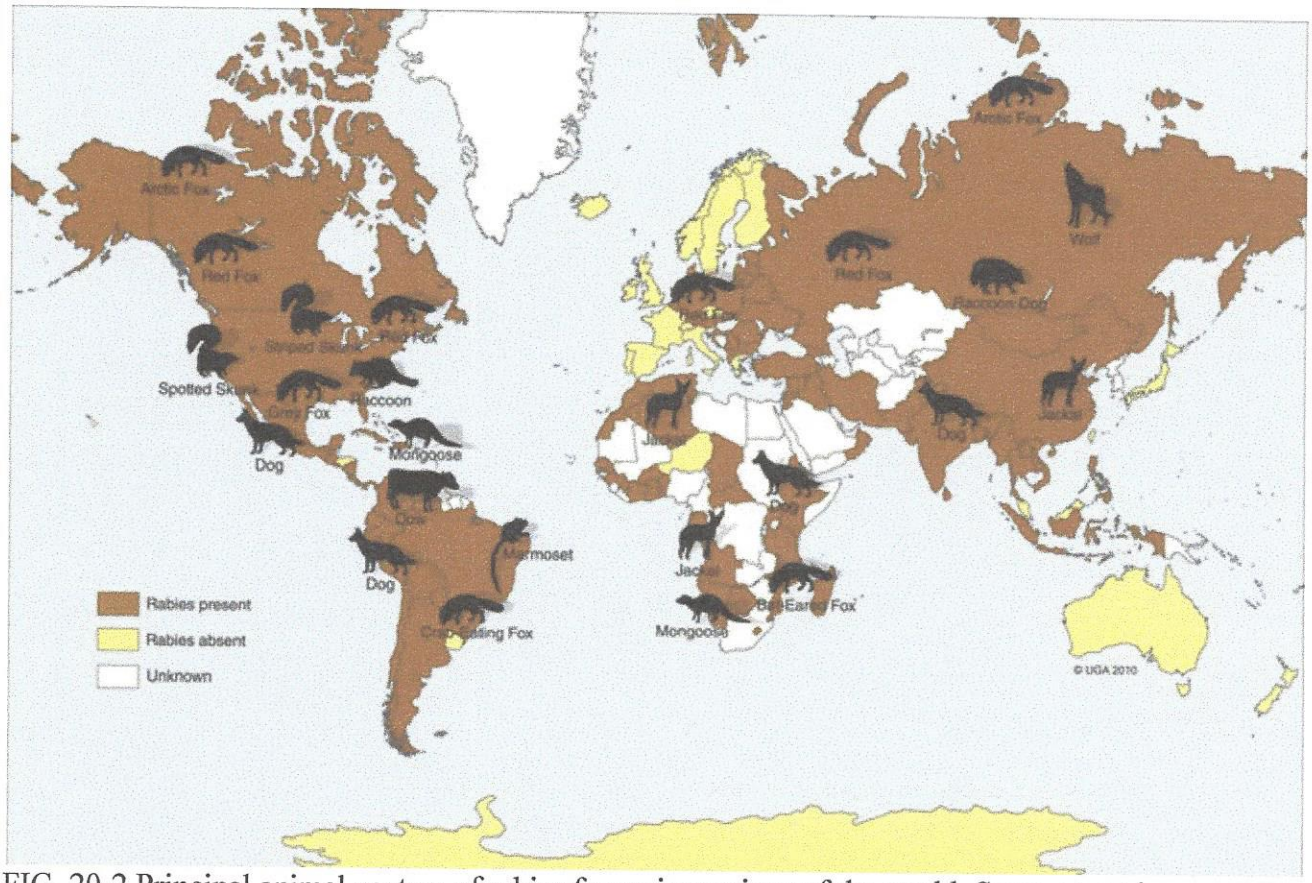


FIG. 20-2 Principal animal vectors of rabies for major regions of the world. Some countries are reportedly free of rabies (see Hosts and Range in text). (Art by Brad Gilleland © 2010 University of Georgia Research Foundation Inc.)

Rabies in enzootic areas appears to be cyclic. It spreads into unexposed, susceptible wildlife populations in a region; subsequent decreases and increases in the prevalence of disease are caused by population mortality and immunity, which periodically cycles in the wildlife population. These wild animals serve as maintenance hosts for virus transmission to dogs, cats, cattle, and horses. Most human exposures result from contact with these domestic species.

Other *Lyssavirus* Infections

As with rabies virus, other lyssaviruses are maintained in wildlife reservoir hosts while existing in independent manner from rabies virus. Eleven genotypes of *Lyssavirus* are known to exist worldwide (Fig. 20-3, and see Table 20-1): rabies virus, Lagos bat virus, Mokola virus, Duvenhage virus, two European bat *Lyssavirus* (EBLV) strains, and one Australian bat *Lyssavirus* (ABLV) type.[332] Four novel lyssaviruses, Aravan, Khujand, Irkut, and West Caucasian, have also been identified.[207–209] Although boasting a rabies-free status, Great Britain and Australia do have these *Lyssavirus* strains in bats.[113,177,177] EBLV strains occasionally infect terrestrial mammals as a "spill-over." No evidence has been found that these infections have resulted in adaptation to these inadvertent terrestrial hosts. Infected domestic animals suffer terminal illness, so the infection is not transmitted. Antibodies to EBLV-1 were detected in a domestic cat in Denmark.[335] EBLV-1 infection was documented in two cats in France; bat-to-cat

transmission was suspected.[82] Since 1987, EBLV type 2 (EBLV-2) has been identified from Daubenton's bats (*Myotis daubentonii*) in England.[113,116,143,177,360] A serosurvey conducted on Daubenton's bats in Scotland indicated between 6% and 19% of the bat sera had positive test results for antibodies to *Lyssavirus*,[15] indicating an endemic reservoir. Foxes were experimentally susceptible to EBLV-1 and EBLV-2 by intracranial but not intramuscular inoculation, suggesting resistance to natural infection.[76] Only the foxes infected intracranially with ELBV-1 developed neurologic disease. The role of these rabies-like bat *Lyssavirus* strains in the spread of infection to people is thought by some researchers to be marginal.[365] Infections of humans with EBLV-1 have been rare, with one in Russia in 1985.[178] Subsequently, a fatal human rabies caused by EBLV-2a was reported in Finland in 1985, and later another case was described in the United Kingdom in an unvaccinated bat handler.[253] This latter incident was the first indigenous case of a *Lyssavirus* infection in a person within the United Kingdom in over 100 years. The remainder of human infections in that country have been rabies, which were acquired by foreign travel.[174] Other references should be consulted for a review of worldwide *Lyssavirus* infections in humans.[178]

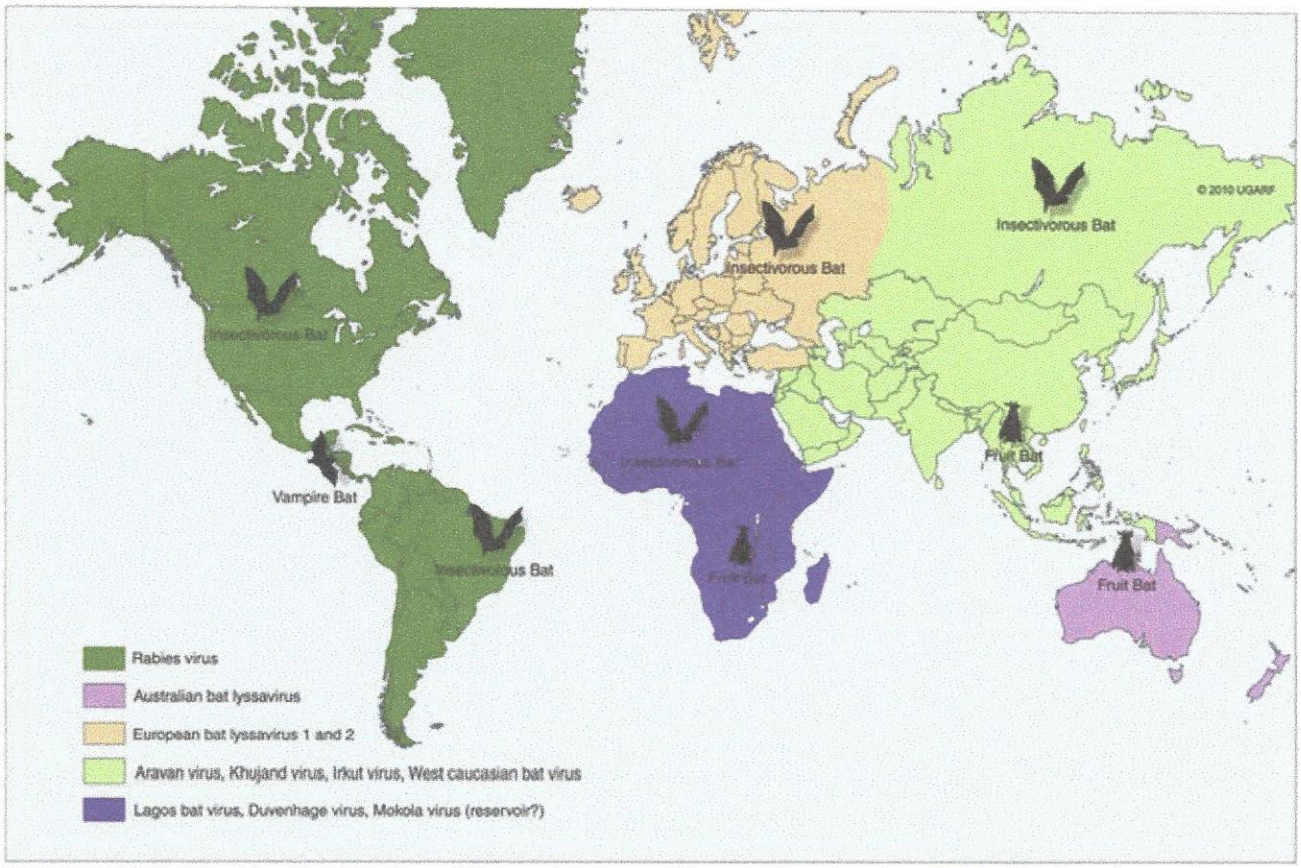


FIG. 20-3 Worldwide distribution of lyssaviruses and bat reservoirs (see Table 20-1 and text for detailed information). (Art by Brad Gilleland © 2010 University of Georgia Research Foundation Inc.)

ABLV, or *Ballina* virus, has been identified in fruit bats ("flying foxes") in Australia,[120] and in addition, it has been identified in all species of wild and some captive Australian bats.[111,353] Fatal infections in two people have been reported.[6] Serologic evidence suggests that a closely related

virus exists in the Philippines.[18] Because of the close antigenic relationship of ABLV with rabies virus, cross-protection from vaccination is presumed effective.[332]

Experimental inoculation of dogs and cats with bat-derived *Lyssavirus* strains has shown a relative insusceptibility to inoculation in comparison with terrestrial rabies virus strains.[232] Although outcomes have varied depending on the strains used and age of animals studied, transient or mild clinical signs with prolonged incubation periods have been observed. Seroconversion has been observed after inoculation; however, lesions within nervous tissue have been mild, and cultivable virus, viral antigen, or nucleic acid has been difficult to detect. These findings, which have also been observed in other carnivores and naturally infected humans, suggest differences in mammalian infections with bat-derived *Lyssavirus* compared to terrestrial strains.

Dogs and Cats

The highest incidence of dog and cat rabies in the United States generally occurs in areas where wildlife rabies is endemic. Most dogs and cats are infected with the predicted terrestrial rabies virus variant associated with the dominant wildlife reservoir host in their respective geographic region.[236] Although the prevalence of wildlife rabies has been on the increase, cases of rabies in dogs and farm animals have been decreasing (Fig. 20-4). Vaccination of dogs and animal control programs have been the main factors responsible for this decline. Although cases of dog rabies have declined, dogs account for the majority of reported animal bites in the United States (see Bite Wound Infections, Chapter 51). Many of these bites result in people seeking antirabies prophylaxis. Despite the general elimination of rabies in dogs, the dog-virus variants have been identified as having established some foci in wild terrestrial carnivores.[340] As described earlier, dog-virus variants spilled over into jackals and mongoose in Africa. In the United States, Texas had a transient spike in the occurrence of dog rabies because of infection with a particular domestic dog–coyote strain of rabies virus in coyotes (*Canis latrans*) beginning in the late 1980s (Fig. 20-5).[205] An oral vaccination program was initiated to thwart the spread of this variant; there have been no reports of its occurrence since 2004, indicating its elimination from the United States.* Worldwide, domestic and feral dogs account for most of human rabies deaths and PEP.[64] In less developed nations, where dog rabies has not been controlled, the prevalence of canine and human rabies is quite high.[72,382] Adequate vaccination of at least 50% to 70% of dogs in a given population may be necessary to block the occurrence of rabies epidemics.[78]

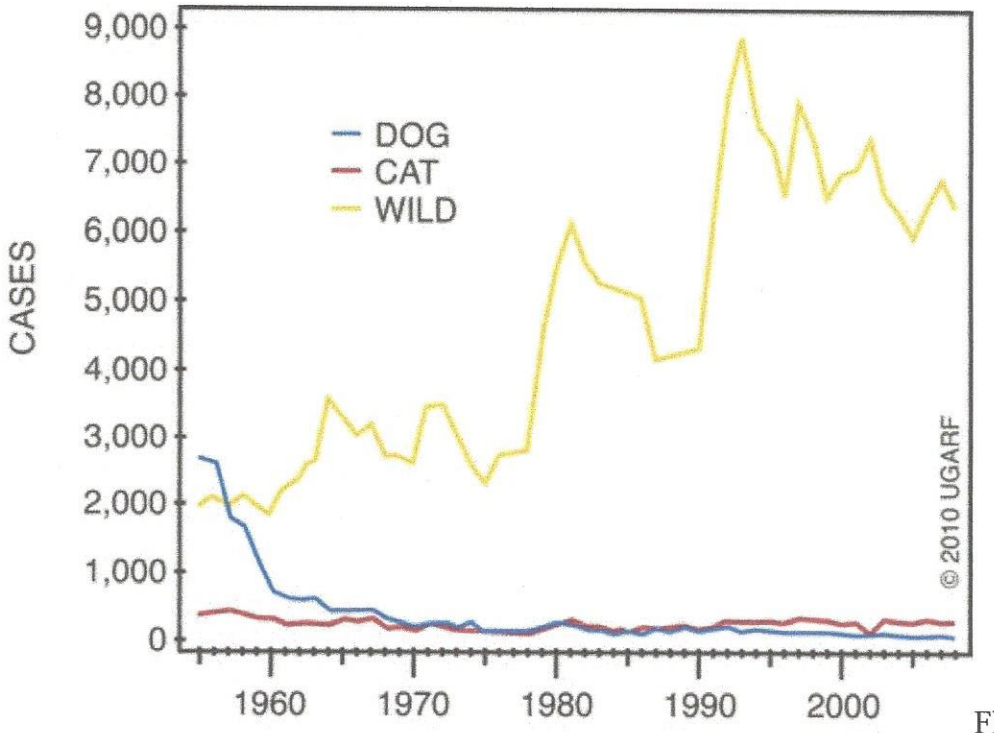


FIG. 20-4 Rabies cases in dogs, cats, and wildlife in the United States from 1955 to 2008. The prevalence in dogs has decreased because of vaccination, and cats now have a greater occurrence of rabies. (Data from records maintained at the Centers for Disease Control and Prevention, Atlanta; Art by Thel Melton © 2010 University of Georgia Research Foundation Inc.)

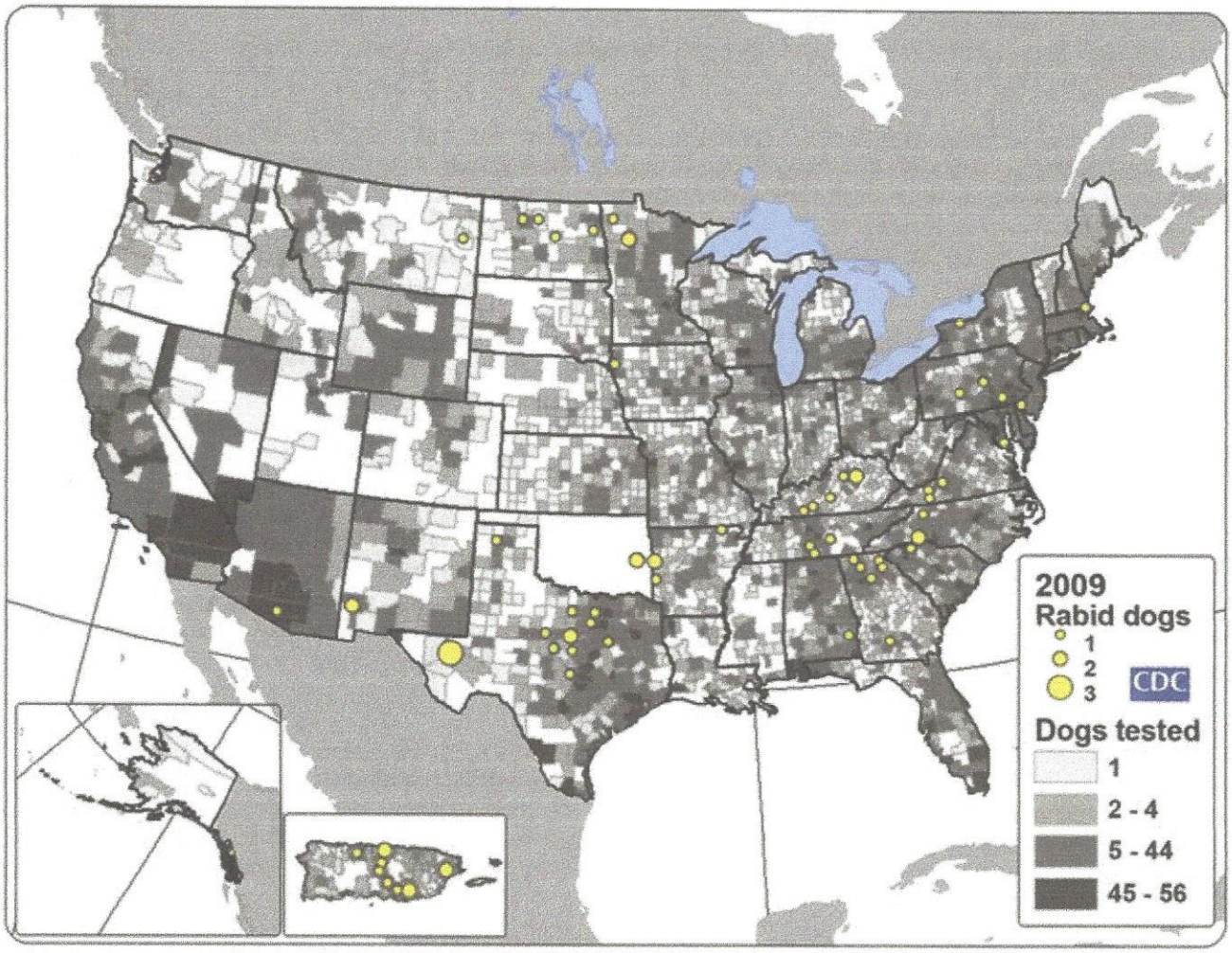


FIG. 20-5 Reported cases of rabies in dogs in the United States for 2008. (Data courtesy the Centers for Disease Control and Prevention, Atlanta, GA, as reported in Ref. 42; Art enhancement by Thel Melton, University of Georgia, Athens, GA.)

Most rabies cases in dogs in dog rabies-free countries, such as the United States or in the European Union, have been in imported pups destined for commercial sale or for humane rescue.[59,116,121,235,317] Because of the 4- to 8-week incubation period, clinically healthy dogs from dog rabies-endemic countries may be incubating infection. United States federal importation laws require vaccination of dogs 12 weeks of age or older from rabies-endemic areas; however, unvaccinated puppies must be admitted with owner confinement of 30 days. Vaccination restrictions are waived for dogs from rabies-free countries. Unfortunately, laxity in these importation policies has resulted in lack of compliance and an increasing number of documented imported rabies in young dogs within the United States.[57,58,227,235]

An increase in cases of rabies in cats usually is related to spillover of infection from wildlife because no specific virus variant attributed to cats has been reported. The relative importance of rabies in cats as a source for human exposure in a given geographic area depends on whether canine rabies is being controlled by vaccination. In the United States, since 1979, rabies in cats has shown a slight increase over the previous 7-year period. Beginning in 1981, more cases of

rabies in cats than in dogs have been reported annually. This increase probably reflects the low number of cats vaccinated for rabies, community tolerance of feral cats, and the epidemics of wildlife rabies in the mid-Atlantic and Northeast regions of the United States (Fig. 20-6). Numerically, cats have been the most important domestic animal affected since 1992, with 200 to 300 cases annually.[42,286,286] The frequency of human rabies exposures attributed to rabid cats is now increasing at a greater rate than that related to dogs. Rabid cats, which usually are reclusive, often become aggressive and may attack humans and other animals when disturbed.[100]

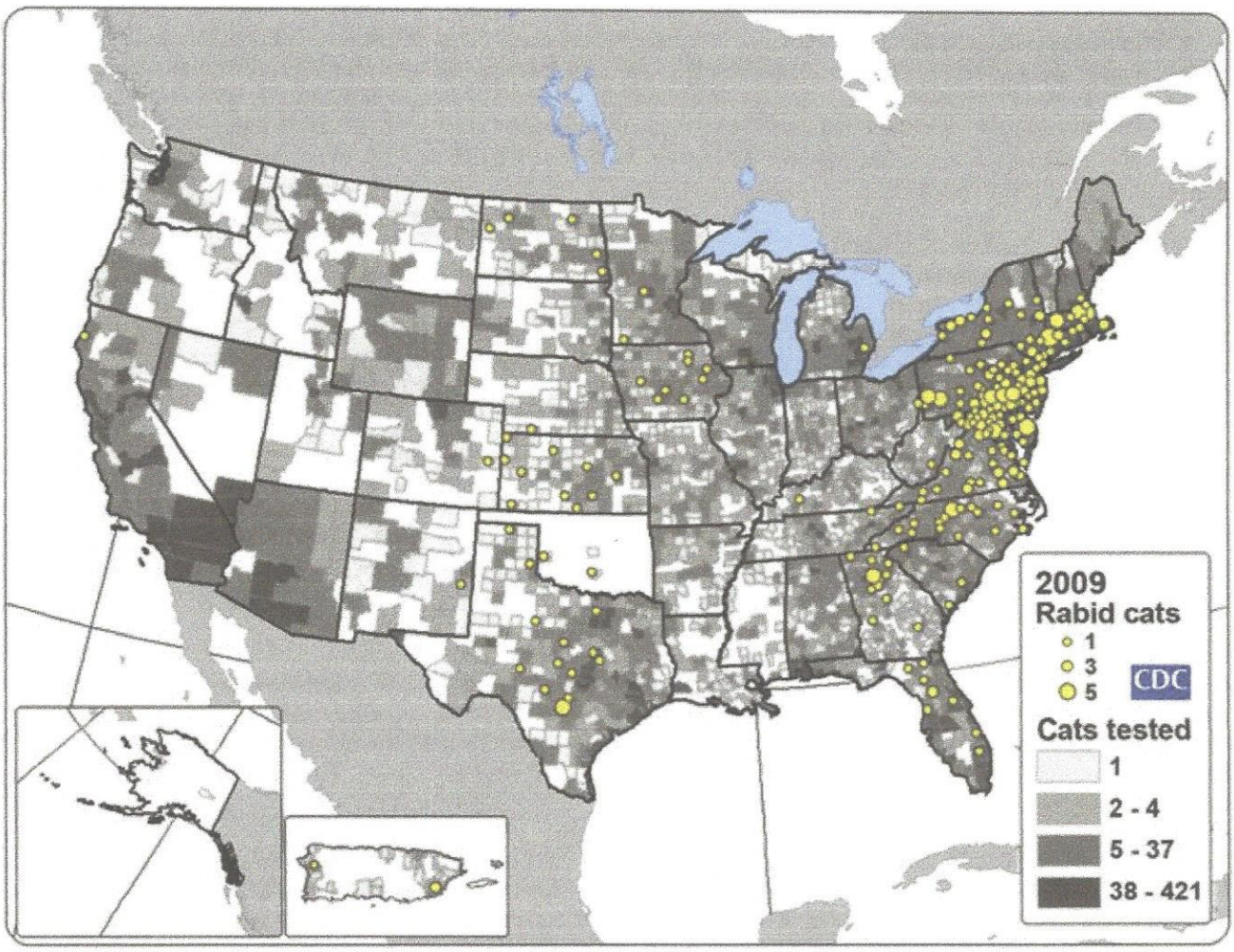


FIG. 20-6 Reported cases of rabies in cats in the United States for 2008. (Data courtesy the Centers for Disease Control and Prevention, Atlanta, GA, as reported in Ref. 42; Art enhancement by Thel Melton, University of Georgia, Athens, GA.)

Wild Carnivores

The striped skunk (*Mephitis mephitis*), the most common skunk in the United States, is one of the most important species in perpetuating wildlife rabies in the central regions of the United States (Fig. 20-7). Studies based on antigenic typing have demonstrated the existence of at least three distinct variants in skunks: one in the south central states, another in the north central states extending into Canada, and another in California. Striped skunks are commonly found to range

in urban-suburban-wildland interfaces and interact with many other carnivorous species, making them prominent risks for becoming infected and transmitting rabies to other animals and humans.[356] Most frequent behaviors reported in rabid striped skunks were appearing outside during the day, entering dog pens, and attacking pets.[261] Although the spotted skunk (*Spilogale* sp.) presented a serious rabies threat in the western United States during the 1800s, the involvement of this small, secretive animal is relatively minor today.

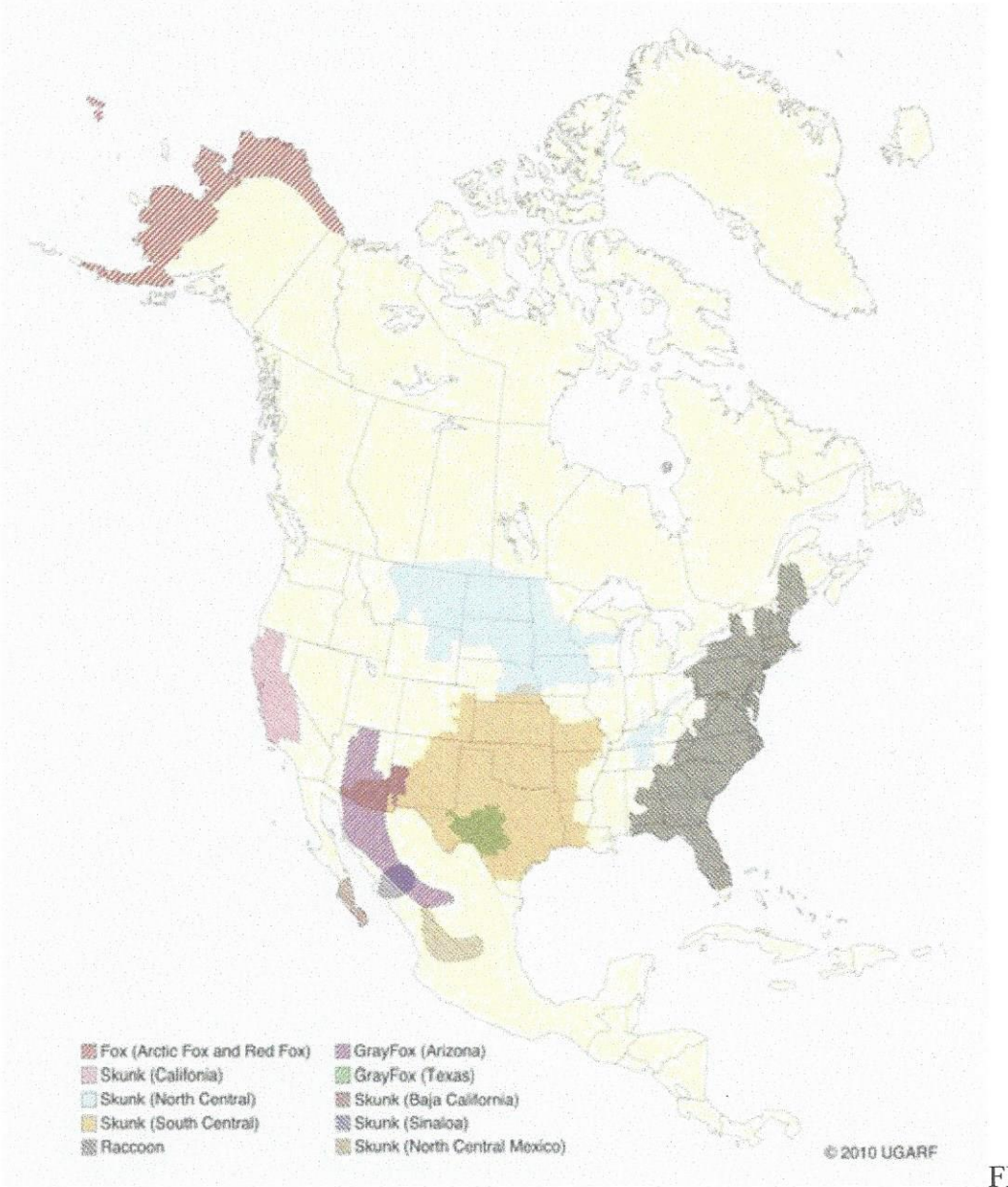


FIG. 20-7
Currently recognized areas of endemic rabies in wild terrestrial animals in the United States and Canada. (Data compiled from maps provided by the Centers for Disease Control and Prevention, Atlanta, GA, and other references cited in text under Wild Carnivores; Art by Thel Melton, University of Georgia, Athens, GA.)

The threat to humans from rabid skunks is based on the animal's susceptibility to the virus, the high prevalence of rabies infection in the population, their ability to live in proximity to humans, and the excretion of relatively large quantities of virus in their saliva during the prolonged period of clinical illness. Rabid skunks often attack anything that moves with extreme fury and frequently roam during daylight hours, which is unusual behavior for this nocturnal animal.

Foxes are important reservoirs in the ecology of wildlife rabies throughout the Northern Hemisphere, although they account for relatively few human exposures. In North America, fox rabies occurs throughout the range of the red fox (*V. vulpes*), the gray fox (*Urocyon cinereoargenteus*), and the Arctic fox (*Alopex lagopus*). An outbreak occurring among red foxes in 1990 in upstate New York was probably an extension of a Canadian fox epidemic. Coyotes have a role in the maintenance of the disease in southern Texas. A focus of another variant of rabies virus in gray foxes has been found in Arizona and Texas. The prevalence of the disease seems to decline when fox populations are reduced to levels in balance with available resources through either natural mortality or fox population-reduction programs. Oral vaccination programs, using modified live-virus (MLV) or recombinant vaccine placed in bait, had considerable success in control of red fox rabies in Europe and Ontario, Canada, and gray fox and coyote rabies in Texas.[318] Despite wildlife vaccination in Europe, the nidus of infected foxes from Eastern Europe maintains the infection cycle.[273] Emergence of dog rabies in northern Israel has been attributed to acquisition of the virus from rabid foxes.[85]

Rabid foxes may exhibit either furious or paralytic forms of the disease; however, regardless of form, the disease is invariably fatal. Despite the shorter course of clinical illness, foxes can effectively transmit the virus to other species, but the need for human PEP from direct exposure to rabid foxes has been extremely low.

Before the mid-1950s, rabies was not a serious problem among raccoons in the United States. However, from 1950 to 1970, the occurrence of rabid raccoons began to rise dramatically in Florida and Georgia and soon spread to Alabama and northward to South Carolina.

In the mid-Atlantic coast states, a major epidemic began in the late 1970s caused by the apparent translocation of infected animals from the Southeast. The mid-Atlantic outbreak spread throughout the northeastern states and southward into North Carolina by the mid-1990s. Foci from this and the Florida–Georgia–Alabama epidemic have now merged. The epidemic had spread northward along the Atlantic coastal states up into Canada.[10,42,42] Raccoons have adapted well to suburban and semiurban environments; thus the number of rabies cases in cats and dogs and other domestic animals in the Northeast has dramatically increased.[46] Rabid raccoons have even been identified in urban centers.[218] The danger of human exposure to rabid raccoons has also increased, and at least one human death directly associated with rabid raccoons has been confirmed in Virginia. Exposure of people to rabid domestic animals such as the cat, which commonly become infected by raccoon attacks, has resulted in the greatest public health risk. Oral bait vaccination of raccoons in these regions has been shown to be effective in reducing the spread of rabies within the zone of immunization.[281] Raccoons may be occasionally co-infected with canine distemper virus, which may alter the immune response within the nervous system and confuse the diagnosis as to the cause of neurologic dysfunction.[134]

Bats

Lyssaviruses have been identified in bats in the Americas, Africa, Australia, and Eurasia. Because of their mobility and the opportunity for bats to infect new areas, no geographic region can be truly considered free of lyssaviruses.[44] Rabies in North American insectivorous bats was first recognized in the early 1950s, but studies suggest that rabid bats were in this region much earlier. The range of bat rabies is widespread throughout North America. In the United States, less than 1% of randomly caught bats are infected; however, 5% to 15% of dead or ill bats submitted for examination may have rabies.[23] Bats that interact with humans are more likely to have rabies than those avoiding people; bats that bite people have the highest prevalence.[270] Rabid bats may develop paresis or paralysis. They may be disoriented and fly into obstacles. Aggression has been observed in some cases. In the United States, most wild bats are insectivorous. Subclinical infection has been suggested in fruit bats in zoologic gardens in Europe, but additional investigation is needed.[285,304]

Although multiple cocirculating variants of rabies virus can be found in insectivorous bats, most submissions and rabid bats are from only a few of the common species (primarily *Eptesicus fuscus*, *Myotis lucifugus*, *Lasiurus borealis*, and *Tadarida brasiliensis*). Phylogenetic studies have shown that bat rabies virus variants found in terrestrial animals are distinct, and variants identified in different bat species are also quite distinct. Uncommonly, bat rabies virus variants have crossed over into terrestrial mammals, with secondary transmission.[220] For example, since 2001 a bat rabies virus variant has spread into skunks and foxes in northern Arizona.[15a,66] From 1980 until 2007, 61 of the 178 total cases of human rabies in the United States and Canada were caused by the bat rabies virus variant.[90] Bat variant rabies cases have become more predominant than terrestrial animal variant cases with each successive year.[90] Brain tissues from most of them have been examined by MAB or gene-sequencing techniques, or both. Variants associated with insectivorous bats were identified most frequently and usually were associated with the silver-haired bat (*Lasionycteris noctivagans*) or eastern pipistrelle (*Pipistrellus subflavus*), but victims had no history of bite. Bats are small and cause tiny lesions, and people may not realize that exposure has occurred.[124,240,241,313] The number of humans who develop rabies from nonbite exposure is higher for contact with bats as compared with terrestrial carnivores; however, public education on nonbite transmission is needed because of the perceived greater concern and prophylaxis associated with bite lesions.[33] Despite these reports in people, few authenticated cases of rabies transmission have been reported from insectivorous bats (*E. fuscus*) to cats and even fewer cases of transmission to dogs.[268] Rabid bats seldom attack; bat bites usually occur from bats found paralyzed or semiparalyzed or from normal-appearing bats found in buildings.

Vampire bats, which feed exclusively on blood, are a major rabies threat to people and animals in Mexico, Central America, and parts of South America. More than 100,000 cases of rabies in cattle attributed to vampire bats occur annually in Latin America. The routine nightly feeding of vampires makes them extremely effective in transmitting rabies virus, and the presence of rabies in vampire bats parallels that seen in insectivorous bats and terrestrial animals. The vampire bat is not found in North America, except for Mexico. Use of the same cave by vampires and other species of bat may be a source of transmission between species. Genetic characterization of rabies isolates from vampire bats and dogs in Brazil indicated two independently maintained cycles of infection.[162] A focus in Colombia in domestic dogs and humans was found to be caused

by virus variants found in insectivorous bats.[268] In addition, an independent focus in marmosets (*Callithrix jacchus jacchus*) has been associated with human infections.[106]

Rodents and Lagomorphs

The prevalence of clinical rabies among rats, mice, squirrels, and rabbits and hares is very low.[99] Rodents and rabbits account for a high percentage of animal bites to people, but no cases of human rabies have ever been associated with these species, probably because they are extremely susceptible to infection and generally will not survive the attack by a rabid carnivore. For such reasons, these species are not routinely examined for rabies in public health laboratories (see Exposure Incident under Postexposure Prophylaxis for People). In the United States, rabies has been reported among large rodents such as woodchucks (*Marmota monax*) and beavers (*Castor canadensis*); most cases have been in the eastern states where raccoon epizootics exist.[14] Therefore, unprovoked encounters with large aggressive rodents should be considered as a possible source of rabies exposure.

Pathogenesis

The incubation period is influenced by the age of the bitten individual, the degree of innervation of the bite site, the distance from the point of inoculation to the spinal cord or brain, the variant and amount of virus introduced, PEP, and other factors. The virus may be undetectable in local tissues after the bite, and it does not enter the blood. Rabies is unique in that the incubation period, which is relatively prolonged compared with that of other infectious diseases, is primarily a result of the route of virus entry into and spread within the central nervous system (CNS) (Fig. 20-8).[305]

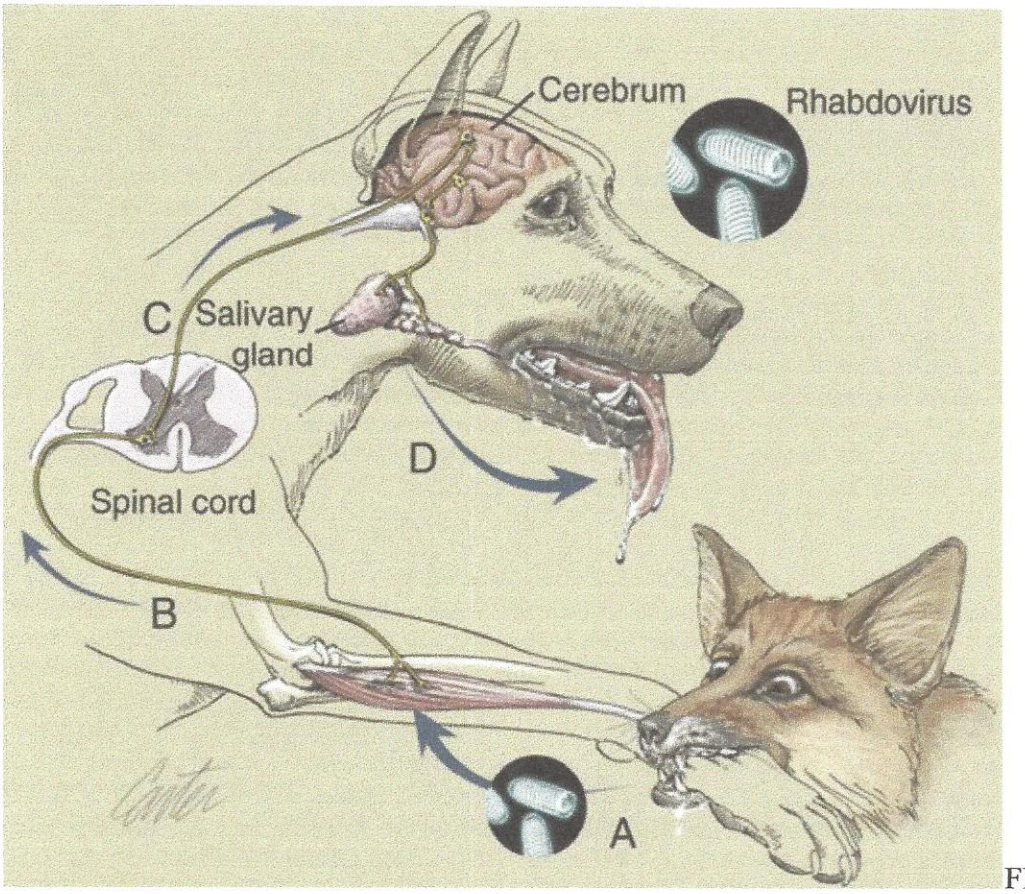


FIG. 20-8 Pathogenesis of rabies virus infection. Rabies virus enters peripheral nerves, or may replicate in myocytes and spread to motor nerve endings ***(A)***. Retrograde intra-axonal (centripetal) spread to the CNS occurs in peripheral motor nerves ***(B)***. Virus replicates in spinal cord neurons and spreads rapidly throughout the nervous system, causing progressive lower motor neuron paralysis ***(C)***. Virus enters the brain, causing cranial nerve deficits and behavioral changes. Virus spreads centrifugally in peripheral and cranial nerves, from which it enters the salivary glands and saliva ***(D)*** and other tissues. (Art by Kip Carter © 2004 University of Georgia Research Foundation Inc.)

Entry of Virus

After IM inoculation, virus may enter peripheral nerves directly or replicate locally in nonnervous tissue. Virus may enter neuromuscular junctions and neurotendinal spindles after a variable period of days, weeks, or months.[61] Rabies virus glycoprotein has homology to certain neurotoxins and attach to axon terminals through lipoprotein receptors, including those for acetylcholine. Virus spreads passively by intra-axonal flow in peripheral nerves at a rate of up to 100 (range, 10 to 400) mm per day. Both motor and sensory fibers may transport virus. The greater the degree of innervation at the site of the bite, the shorter the incubation period. In naturally occurring cases of rabies, ranges of incubation periods before CNS signs have been reported to be 3 to 24 weeks (average, 3 to 8 weeks) in dogs, 2 to 24 weeks (average, 4 to 6 weeks) in cats, and 3 weeks to 1 year or more (average, 3 to 6 weeks) in humans.

Although uncommon, infection by routes other than by bite is possible. After intranasal exposure, virus enters the trigeminal nerves and ganglia in its course to the CNS. The cribriform plate and olfactory bulbs have been suggested as a route of spread, but this is not well documented. After ingestion, the virus can infect cells of the oral mucosa, taste buds, pulmonary system (by aspiration), and intestinal mucosa. From these sites, virus migrates up branches of the cranial nerves and spreads to the brainstem.

Rabies virus variants may vary in their ability to be transmitted by nonbite means. In comparing the dog and coyote strains with silver-haired bat viruses, the latter was better able to infect epithelial cells, replicate at lower temperatures, and have neuroinvasive tendencies.[122,144,144]

Spread in the Central Nervous System

Interneuronal spread of virus corresponds to the progression of clinical signs that are noted. The virus enters the spinal cord or brainstem ipsilateral to the site of initial virus inoculation by retrograde axoplasmic flow. Once in the CNS, virus spreads by intra-axonal means to involve the contralateral neurons and ascends rapidly and bilaterally in the spinal cord or brainstem to the forebrain. In naturally infected dogs, virus in the brain preferentially localizes in the limbic areas, thalamic nuclei, reticular formation, and trigeminal and vagal nuclei.[319] Damage to the motor neurons causes progressive lower motor neuron (LMN) disease, which, in turn, produces the typical ascending flaccid (hyporeflexic) paralysis of rabies. Damage to the CNS caused by rabies virus has mainly been attributed to direct viral invasion of the nervous system. Damage to neural tissue is visibly limited in comparison to the severe degree of paralysis; inhibition of function or synthesis of neural transmitters is suspected. Apoptosis, or genetically induced premature cell death, may be important in the limited amount of observed neuronal necrosis.[165] Host immune responses to rabies virus may accentuate the inflammation and degeneration of nervous tissue. Interference with cardiorespiratory control results in death.

Spread from the Central Nervous System

After replication within the CNS, the virus moves outward to other body tissues via the peripheral, sensory, and motor nerves at a rate of 100 to 400 mm per day. Both visceral and somatic portions of cranial and spinal cord nerves become involved, including the autonomic nervous system. Virus also spreads via cranial nerves to the acinar cells of the salivary glands at this time. The presence of virus in saliva demonstrates that the brain has already been infected. Similarly, the presence of cranial neurologic dysfunction indicates that the saliva likely contains virus. Although virtually every tissue may be infected, outward spread in the peripheral nervous system does not occur in all cases. The rate (20% to 88% positive) of salivary gland infection also varies, depending in part on the species infected and the virus variant. Death may occur before salivary gland involvement.

Recovery

Recovery from rabies has been exceedingly rare.[166] In many instances, demonstrating the presence of virus may be difficult. During the early incubation period, rabies virus may be sequestered at the site of inoculation while replicating in myocytes and nerves. The long period

between exposure and clinical signs may be the result of local replication and has been associated with high titers of anti–rabies virus antibody in the cerebrospinal fluid (CSF) and CNS tissue. Adequate serum titers of anti–rabies virus antibody, acquired by active or passive immunization, have been correlated with protection against infection and restricted viral replication.[175] Effective cell-mediated immunity is essential to the eventual elimination of rabies virus. Recovery should be regarded as of extremely minor importance in the epidemiology of the disease and not relevant in public health considerations. With appropriate intensive medical management and passive immunotherapy humans have recovered (see Exposure Incident, under Postexposure Prophylaxis for People).

Excretion of Virus

Typically, virus excretion occurs for a brief period before the onset of neurologic signs and continues until the animal dies within a few days. Most public health laws require a 10-day observation period after a bite from a suspected dog or cat because the period of virus shedding before onset of neurologic signs in naturally infected animals is generally between 1 and 5 days. Dogs that develop neurologic signs and die suddenly actually may have lower concentrations of virus in their brains and salivary glands than do those that live longer. Typically, excretion of virus in experimentally infected cats has started from 1 to 2 days before to 3 days after the onset of clinical signs.

Clinical Findings

Rabies virus infection has classically been divided into two major types: furious and paralytic. The classification and progression of infection is artificial because rabies can be quite variable in its presentation, and atypical signs are commonly seen. Not all animals progress through all the clinical stages. The initial history may reveal that the pet has a wound history. Because of the severity of wounds, signs may not always be suspected as coming from a bite.

Dogs and Cats

During the prodromal phase in dogs, which usually lasts 2 to 3 days, apprehension, nervousness, anxiety, solitude, and variable fever may be noted. Friendly animals may become shy or irritable and may snap, whereas fractious ones may become more docile and affectionate. Pupillary dilation with or without sluggish palpebral or corneal reflexes may become apparent. Most animals will constantly lick the site of viral inoculation. Some dogs may develop pruritus at the site of exposure and claw and chew at the area until it is ulcerated. The behavior of cats during the prodromal period is similar to that of dogs; however, cats more typically show fever spikes and unusual or erratic behavior for only 1 or 2 days.

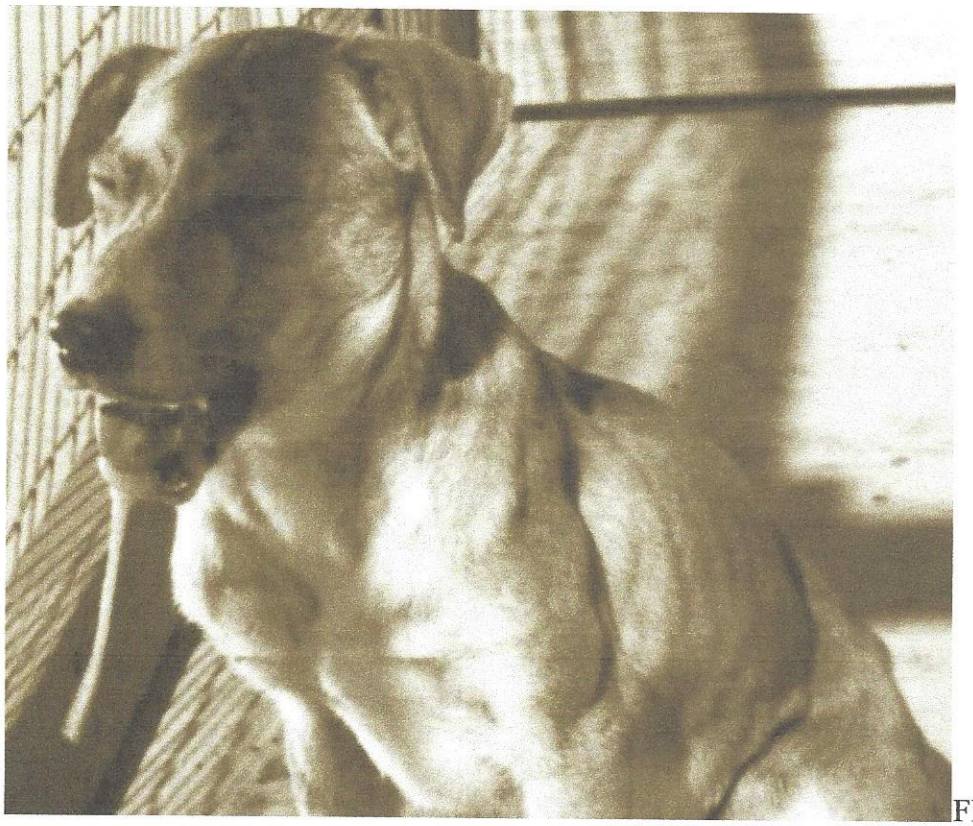
The furious or psychotic type of the disease in dogs usually lasts for 1 to 7 days and is associated with forebrain involvement. Animals become restless and irritable and have increased responses to auditory and visual stimuli. They frequently become excitable, photophobic, and hyperesthetic and bark or snap at imaginary objects. As they become more restless, they begin to roam, usually becoming more irritable and vicious. Dogs may eat unusual objects (pica), especially wood, that become gastrointestinal foreign bodies. They may avoid contact with people and prefer to hide in

dark or quiet places. When caged or confined, dogs often try to bite or attack their enclosure. They usually develop muscular incoordination, disorientation, or generalized grand mal seizures during this phase. If they do not die during a seizure, they may experience a short paralytic stage and then die (Fig. 20-9).

FIG. 20-9 Dog with paralytic stage of rabies in sternal recumbency with torticollis. (Courtesy CDC, Atlanta.)

Cats can develop more consistently the furious phase of the disease, showing erratic and unusual behavior. These cats are described as having anxious, staring, wild, spooky, or blank looks in their eyes.[112] When confined in cages, they may make vicious, striking movements and attempt to bite or scratch at moving objects. In addition, they may have muscular tremors and weakness or incoordination. Some cats may run continuously until they seem to die of exhaustion.

The paralytic or dumb type of rabies usually develops within 2 to 4 days (range, 1 to 10 days) after the first clinical signs are noted. LMN paralysis usually progresses from the site of injury until the entire CNS is involved. Cranial nerve paralysis may be the first recognizable clinical syndrome if the bite occurs on the face. When the brainstem becomes affected, a change in the tone of the bark, resulting from laryngeal paralysis, may be observed. Dogs, which more commonly show this type of disease, may begin to salivate or froth excessively as a result of the inability to swallow and the deep labored respiration that occurs. A "dropped jaw" develops as a result of paralysis of the masticatory muscles (Fig. 20-10). Dogs may make a choking sound, which convinces an owner that something is caught in the animal's throat. Owners or veterinarians may then become exposed to the virus in the saliva while attempting to remove a suspected foreign object. The course of the paralytic phase usually lasts 2 to 4 days. The animal often goes into a coma and dies of respiratory failure.

 FIG. 20-10 Dog with rabies. Note open jaw and visible tongue with excessive salivary secretions resulting from the inability to swallow. (Courtesy CDC, Atlanta.)

The paralytic disease in cats often follows the furious form of the disease and begins around day 5 of clinical illness. Although the total course of illness may last 10 days, rabid cats often die after 3 to 4 days.[295] As in dogs, initial paralysis of the bitten extremity can progress to paraparesis, incoordination, and ascending or generalized paralysis, terminating in coma and death. Mandibular and laryngeal paralysis is less common in cats. Increased frequency of vocalization is a commonly reported sign in cats, and owners often recognize a change in the pitch of the cat's voice.[112] Cats occasionally develop the paralytic form directly after the prodromal phase with few or no signs of excitement.

Atypical, abortive forms of rabies virus infection with recovery may occur but are considered very rare phenomena. Clinically healthy carriers have not been found in domestic dogs within enzootic areas.[380] Experimentally infected dogs that developed acute progressive LMN paralysis have shown clinical improvement a few days to months later. Survival with chronic infection has been reported to rarely occur after experimental rabies infection in cats, but clinical recovery from paralysis has not been observed.

People

The clinical syndrome of rabies in people is similar in duration and variability to that in dogs and cats. Genetic studies of viral isolates from human rabies indicate that clinical manifestations such as encephalitic or paralytic rabies are not determined by different virus variants but by other

factors such as the site of the inoculation and spread of virus within the nervous system.[150] Fever, headache, anxiety, nervousness, and hyperesthesia at the bite site have been reported. As the syndrome progresses to the excitable phase, clinical signs consist of excitability, restlessness, hyperkinesis, and violent behavior. Humans salivate incessantly and may refuse to drink water. They experience painful pharyngeal spasms when attempting to swallow fluids, which gives rise to the term "hydrophobia." As disorientation and excitability continue, some patients die in convulsive episodes, whereas others develop generalized LMN paralysis and respiratory arrest. Although the rare instance of recovery has been reported after extraordinary efforts, the disease is considered to be invariably fatal after the onset of clinical signs.

Diagnosis

Rabies is often suspected because of neurologic abnormalities in an affected animal. However, because of the atypical nature of the clinical signs now recognized, rabies should be considered in any animal that suddenly develops profound behavioral changes or features of LMN paralysis, or both.

For a review of the diagnosis of rabies, also see the Centers for Disease Control and Prevention (CDC) website at www.cdc.gov/rabies/. The definitive diagnostic test is the demonstration of rabies virus antigen by the direct fluorescent antibody (FA) test in suitable brain tissue.[30] Direct immunochemical methods using light microscopy have given comparable accuracy.[217] No premortem diagnostic tests are sensitive enough to be consistently reliable for rabies diagnosis in animals. Nevertheless, there may be some limited indications for testing serum, CSF, or biopsy specimens before the death of the animal. No hematologic or serum biochemical changes are characteristic or specific for rabies. Biochemical changes in CSF have been minimal in experimentally infected dogs and have been rarely reported in natural infections. Increased CSF protein (110 to 150 mg/dL) and leukocytes (120 to 1140 cells/μL), with small lymphocytes predominating, have been reported in dogs with postvaccinal rabies encephalomyelitis.[29] Cats with postvaccinal rabies also have had increased CSF protein (55 to 80 mg/dL) and increased CSF lymphocyte count (5 to 17 cells/μL).

Detection of Virus in Dermal Tissues

This method has been predominantly used to diagnose rabies antemortem in people. Because virus enters extraneural tissues via outward spread from the CNS, it arrives at nerve endings in the skin and at the salivary glands simultaneously. Because of the heavy sensory innervation, the skin at the nape of the neck (in humans) and the sensory vibrissae on the maxillary areas (in animals) may be selected for direct FA testing. Direct FA testing of a skin biopsy has a 25% to 50% probability of being positive about the time that clinical rabies develops; accuracy is increased as the disease progresses. Of all dogs and cats tested that were confirmed to be rabid by positive results of brain immunofluorescence, neurologic signs developed within 10 days of the biting incident.[328] Inactivated or recombinant rabies vaccines commonly used in dogs and cats do not give false-positive results. The skin biopsy technique should never be substituted for brain examination of an unvaccinated animal with suspect neurologic signs. This test appears to be accurate if the virus is present, but a negative test result does not rule out the possibility that the animal is infected. In humans, polymerase chain reaction (PCR) testing of skin has shown

much higher degrees of sensitivity and specificity compared to direct FA methods.[83] Use of these methods is restricted at present to human diagnostics and has not been approved for routine laboratory diagnosis of rabies in animals.

Testing of Saliva for Virus

Salivary tests for rabies virus have been used as one test to diagnose rabies in people. Rabies virus has been detected in dog saliva by slide agglutination using latex particles coated with polyclonal immunoglobulin.[184] The load of viral particles in saliva is lower than it is in brain tissue, and negative results must be confirmed by more reliable means such as the direct FA procedure on brain. Increased sensitivity and specificity of saliva testing can be achieved by using virus isolation or genetic detection methods (see later discussion).

Serologic Testing

Serologic tests are rarely used for epidemiologic surveys or for diagnosis because of the low percentage of surviving animals that have time to develop postinoculation antibody. Serologic tests are used to determine vaccine immunogenicity. Some countries require a positive antibody titer for importation.[9] Mouse inoculation was performed historically for serologic testing but has been replaced by cell culture methods. The rapid fluorescent-focus inhibition test (RFFIT), can quantify concentrations of specific rabies virus antibody in serum. Other tests for rabies virus antibodies, based on enzyme-linked immunosorbent assay (ELISA, or fluorescent antibody virus neutralization [FAVN] test), have been proposed to augment the RFFIT for serodiagnosis or support of immunization. Comparative testing under field conditions in dogs indicates that ELISA test results are higher than those with RFFIT, making ELISA methods less reliable for estimating adequate seroconversion after vaccination.[27,190a] Improvements in ELISA methods, which overcome some inaccuracies, have been described.[379]

During the incubation period of rabies, antibody responses are not usually observed, and the virus may be hidden from the immune system. After neurologic signs develop, antibodies appear in serum and later in the CSF.[276] Testing dogs or cats for serum antibodies to rabies virus to determine recent exposure to rabies virus can be ambiguous because elevated titers can result from vaccination or from past or recent exposure to virus. Therefore, a serologic response can in no way be definitively differentiated from vaccination or infection. Testing for rabies virus antibody in CSF is a possible means of documenting rabies infection because antibody is locally produced, and CSF titers may increase 2 to 3 weeks or more after the onset of clinical rabies. Because of this delay, a negative titer result does not eliminate rabies infection as a possibility.

Documentation of Rabies Immunization with Antibody Titers

The World Health Organization and the Office International des Épizooties have developed minimum guidelines as supportive evidence of rabies immunization in animals. This measurement has been adopted as one requirement for importation of animals into rabies quarantine areas (see later discussion, Quarantine and Shipment of Animals). In an update of these guidelines, the RFFIT test has been augmented by the FAVN test.[8] This latter test has become another international standard for shipment of animals. No difference has been observed

in sensitivity or specificity for either method in comparing sera of vaccinated or unvaccinated animals.[49,309] Modification of the FAVN using MABs and peroxidase conjugates has allowed automation of the procedure for dogs.[158,262] In following antibody titers after vaccination, titer levels may vary based on the degree of similarity between the rabies viral strain used in the test system and that in the vaccine.[247] Many other factors may influence the increase in antibody titer in individual dogs or cats.[231] For example, the timing of serum collection after vaccination; the breed, size, and age of the animal; the number of prior boosters; whether a monovalent versus polyvalent vaccine is used; whether a dog or cat is vaccinated; and the choice of vaccine may be important determinants of the level of titer measured.[187,242,242] A titer of 0.5 IU/mL has been used as the standard level expected for an adequate titer in people and animals. Although parenteral vaccination may more readily achieve this level, oral vaccination with the SAG2 strain did not always induce this level of seroconversion.[297] Nevertheless, dogs showing any measurable titer after vaccination were protected. A titer of 0.5 IU/mL is required for animals exported to most rabies-free areas. No "protective" titer has been found in animals. Individual interpretation is the responsibility of the veterinarian submitting the specimen and the agency requesting the test be done. See Web Appendix 5 for laboratories that perform these assays.

Pathologic Findings

Submission of Specimens

Selection and submission of proper specimens are critical for accurate rabies diagnosis. Handling live, suspected rabid animals must be done with extreme care. Heavy protective gloves must be worn, and catchpoles, cages, and other equipment often facilitate capture and transport of such animals. The animal must be euthanized by a humane method, and the brain must be protected from damage. The use of an ax or power saw should be discouraged when opening the skull, because these may create hazardous aerosols. A procedure to remove the brain of a suspected rabid animal has been published.[333] Small specimens such as mice and kittens may be submitted whole. A technique for retroorbital removal of brain specimens for collection of material for epidemiologic studies has been described.[244] Complete brain removal is still indicated when human exposure has occurred. When brain tissue has been inadvertently damaged or destroyed, the spinal cord is an alternative but less desirable substitute.

The head (or body) of an animal suspected of having rabies that has died or been euthanized should be cooled immediately and maintained chilled (wet ice) or refrigerated until examined. The head or brain should *not* be frozen because this delays examination, and the thawing process causes brain tissue damage. A complete history should accompany each specimen. Various approved shipping containers are available from public health or animal control facilities and must protect the specimen, as well as those handling the container. The container should always indicate that hazardous laboratory specimens are enclosed.

Specimens must be sent to the laboratory as quickly as possible. Postexposure treatment is often delayed while awaiting laboratory results. Recommendations are that specimens be delivered personally or by courier whenever possible to minimize delay or potential loss.

A method of submitting brain tissue, dried on filter paper, has been described that permits submission of specimens without refrigeration.[247] Brains from rabid and control dogs were spotted on filter paper that was dried and stored for up to 222 days. Analysis was performed by nucleic acid sequence-based amplification and reverse transcriptase-PCR (RT-PCR).

Gross and Microscopic Lesions

No gross lesions are detectable in the CNS with rabies infection. Despite the dramatic neurologic signs and high mortality, neuropathologic changes are mild. Pathologic changes depend on the severity and duration of infection at the time of examination. Acute polioencephalitis characterized by minimal neuronophagia, neuronal degeneration, and nonsuppurative inflammation is seen very early in the course of the disease. Necrotizing encephalitis is seen in the next phase of infection and corresponds to a gradually increasing titer in the serum and CSF. Chronic infections are characterized by focal or widespread lymphocytic and plasmacytic perivascular cuffing and focal mononuclear cell infiltrates in the CNS. Ganglioneuritis is usually present. The longer the course of illness, the more pronounced is the nonsuppurative inflammatory response in the brain and spinal cord. In some cats, spongiform lesions appear as vacuolation in the neuropile of the gray matter, most commonly in the thalamus and inner layers of the cerebral cortex.

Direct Fluorescent Antibody and Immunohistochemical Testing of Nervous Tissue

This postmortem test is both rapid and sensitive and is currently the most widely used and preferred and reliable method of diagnosing potential rabies infection in animals.[142] False-negative results are rare with this test when compared with those of mouse inoculation.[327] Thin touch impressions of the medulla, cerebellum, or hippocampus are used for this test. In decomposing canine brains at room temperature of 25° C to 29° C, the direct FA test result remains positive in most cases for up to 96 hours; the corresponding mouse inoculation test usually becomes negative by 48 hours.[5] Unless it is completely decomposed, the head should be submitted because specific fluorescence may still be detected. Rabies virus has also been detected by immunoperoxidase techniques on formalin-fixed, partially autolyzed and paraffin-embedded tissue.[20,133,212,351] Animals need not show neurologic signs at the time of examination, and all animals excreting virus in the saliva will have detectable virus in the CNS by immunohistochemical examination. In natural infections of dogs virus is usually localized to the neuronal perikaryon, extending along into the dendritic regions.[319]

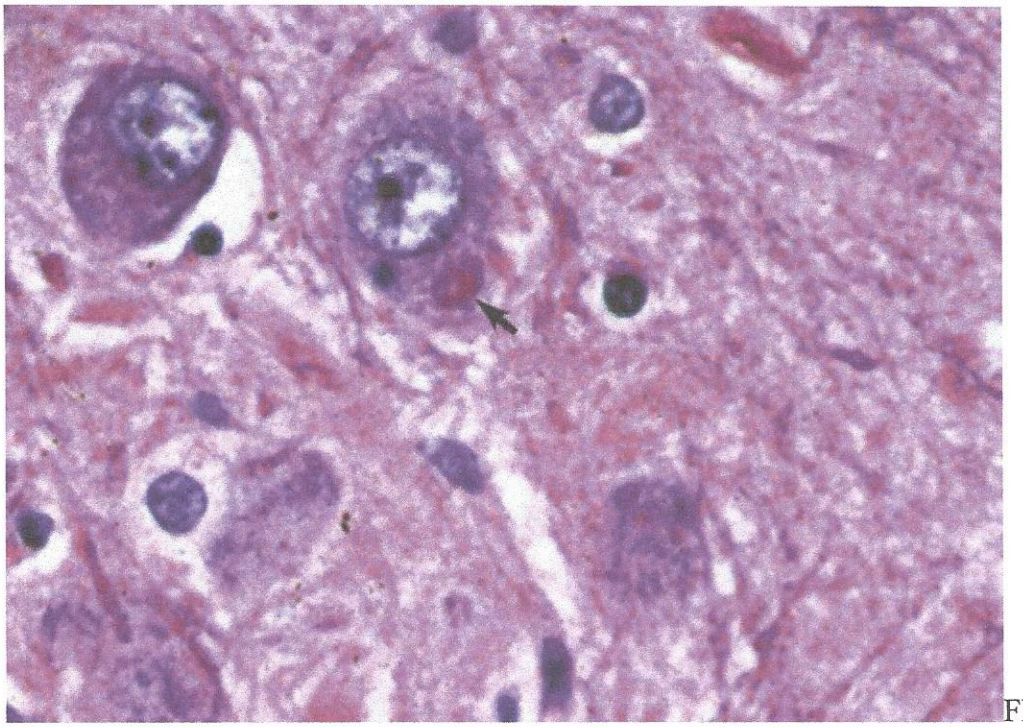
Genetic Detection of Virus and Genetic Sequencing

Rabies virus RNA has been detected in nervous tissue by RT-PCR.[131,147,163,183,351] Amplified sequences have included the phosphoprotein, nucleoprotein, and glycoprotein genes. PCR has been used as a confirmatory test in direct FA-negative samples or in decomposed brain tissue that is difficult to evaluate with direct FA methods or virus culture,[86,146,146] but the predictive ability of a negative result has not been discussed. In situ hybridization can detect rabies virus genomic RNA in paraffin-embedded brain tissues.[167,351] For detection of rabies in living human patients, RT-PCR has been used on saliva, CSF, and urine,[81,257,344,345] and in dogs,

quantitative PCR has been used on saliva and CSF[300]; however, it is not considered a desirable alternative in detecting potentially rabid animals exposed to humans, in which the brain tissue is preferred for confirmation. In humans, RT-PCR analysis of saliva has been the most accurate means of antemortem diagnosis of rabies when compared with other detection methods and is as accurate as brain biopsy.[257] Quantitative (real-time) PCR methods have been developed for this purpose.[252] Direct sequencing of PCR products along with automated gene sequencing can also help distinguish among virus variants to determine the most probable wildlife reservoir for an isolate from human or animal infection. A quantitative PCR assay has been developed to detect rabies virus in human and animal brain specimens.[346,348] Quantitative PCR has also been more accurate than other PCR assays used to detect lyssaviruses in brains from bats.[117]

Intracellular Inclusions

The classic test for the presence of rabies is to examine the brain for the presence of intracytoplasmic inclusions, known as Negri bodies, in larger neurons (Fig. 20-11).[206] They are most commonly found in the thalamus, hypothalamus, pons, cerebral cortex, and dorsal horns of the spinal cord. Negri bodies are most common in neurons of the hippocampus in carnivores and in Purkinje's cells of herbivores. Negri bodies in tissue sections or impressions of brain tissue are best demonstrated with Seller's or Van Gieson's stains, in which they stain magenta. Unfortunately, Negri bodies take time to develop and cannot be found during all stages of infection and in all infected cats. They usually cannot be detected until neurologic signs are apparent; therefore premature killing of the animal may reduce the chances of finding these inclusions. Negri bodies may be found in approximately 50% of the samples that test positive by direct FA. In some cases, nonrabid tissues have displayed inclusions that resemble Negri bodies. Cytoplasmic inclusions that are confused with Negri bodies can be found in the brains of healthy cats.[50,51] They occur in the pyramidal cells of the hippocampus and in neurons of the dorsal part of the lateral geniculate nucleus. Detection of Negri bodies is no longer used in most developed nations for routine diagnostic confirmation.

 FIG. 20-11 Negri body *(arrow)* in a neuron within the CNS (H&E stain, ×400). (Photograph by Elizabeth Howerth © 2004 University of Georgia Research Foundation Inc.)

Mouse Inoculation and Virus Isolation

Intracerebral inoculation of laboratory mice with fresh or fresh-frozen homogenized tissue is a confirmatory test for rabies but is not widely conducted for routine diagnosis of suspected rabies cases in the United States. Specific neutralizing antibody is incubated with extracted tissue before its inoculation to confirm that rabies virus is responsible for the observed neurologic signs. Brain tissues from infected mice are examined for virus by direct FA. This test does not distinguish between virulent and vaccine viruses because, regardless of attenuation, many of the virus strains produce a similar illness in mice. Replacement of mouse inoculation by viral inoculation into tissue culture is now feasible because virulent virus can now be grown in various cell lines.[288]

Monoclonal Antibody Characterization

MABs produced against specific rabies virus nucleocapsid and glycoprotein moieties are able to distinguish various antigenic variants. Strains of virus to be tested are grown in tissue culture or are found in tissue sections and are tested by direct FA to determine the antigenic composition of the virus. The pattern of staining with different MABs is compared with that of reference strains of virus. This technique is extremely valuable in distinguishing between vaccine and virulent strains of rabies virus, especially in cases of human exposure to animals with postvaccinal neurologic disease.

Use of serologic testing to assess immune status of companion animals

Ian Tizard, BVMS, PhD, and Yawei Ni, PhD, DVM

Summary: At the November 1997 meeting of the AVMA Council on Biologic and Therapeutic Agents, the Council recommended that the *JAVMA* publish an article on the current status of the use of serologic testing in an effort to assist practitioners who must make decisions regarding vaccination of companion animals (ie, dogs, cats, and horses). It is anticipated that the peer-reviewed article provided here will be of benefit to veterinarians and will facilitate their attempts to maintain animal health through the knowledgeable use of vaccines.

In recent years, use of serologic assays, primarily ELISA, to assess immunity of vaccinated animals has increased. This has become a standard procedure for the poultry and swine industries, in which repeated testing, along with use of sophisticated data management software, has enabled producers to determine the degree of immunity in their animals. Analysis of results of these tests provides a rational basis for determining whether revaccination is required as well as an early warning of susceptibility to disease. This report examines whether similar procedures could be of benefit in decision making for vaccine use in dogs, cats, and horses.

Effective vaccines induce immunity sufficient to confer prolonged protection in a vaccinated animal. Because vaccine-induced immunity does not last indefinitely, it must be boosted at appropriate intervals to ensure that vaccinated animals do not inadvertently become susceptible to disease. This is especially important in companion animals with long life spans, such as horses and many breeds of dogs and cats. If revaccination is delayed, an animal's immune response may wane such that it may become susceptible to disease. On the other hand, too frequent revaccination may be inefficient and increase the risk of adverse effects. Therefore, revaccination ideally should be timed to ensure that immunity does not wane below protective levels while concomitantly ensuring that an animal is not subjected to unnecessary booster vaccinations.

Several reasons could be used to suggest that vaccination even with good vaccines cannot always be relied on. For example, immune responses are biological phenomena. As such, they have a normal distribution in the population. Some animals that receive an effective vaccine will mount poor immune responses and will not be protected. It is for this reason that pet owners must be advised that vaccines cannot be absolutely guaranteed to provide protection. Yet, although we know that animals vary greatly in response to vaccines, vaccinated animals are rarely tested to ensure that they have mounted a protective immune response.

Another reason for vaccination failure involves time of administration of vaccines. It is critical that young animals are vaccinated at a time when passive maternal immunity will not interfere with their immune responses. Carefully conducted studies have identified the earliest time when, in general, vaccines will be effective. Nevertheless, veterinarians rarely take into account variation among animals for duration of maternal immunity. Modified-live virus vaccines rely on limited replication of the agent within a vaccinated animal to trigger a protective immune response. These vaccines may contain remarkably little antigen. This small antigenic mass makes these vaccines highly susceptible to interference from antibodies. When an animal has maternal or endogenous antibodies at the time of vaccination, the vaccine agent may be neutralized before it can replicate. A high concentration of antibodies in an animal implies that the animal is probably protected but also could indicate that it may not be possible to stimulate an additional immune response in that animal.

Finally, it is reasonable to assume the efficacy of vaccines produced by various manufacturers will vary. It cannot be assumed that the duration or degree of immunity induced by one brand of a specific vaccine will be identical for all other brands of that vaccine.

Without a comprehensive effort to monitor vaccine efficacy and duration of immunity, annual revaccination has the advantage of simplicity and ensures that owners bring pets in for regular examinations. Although duration of vaccine-induced immunity may be variable, manufacturers and veterinarians have conformed to this practice. Indeed, until recently, only rabies vaccines were evaluated for duration of immunity. Other vaccines were simply tested by challenging vaccinated animals months or weeks after administration. The USDA has altered its rules to require that new vaccines must protect animals for the period claimed on the vaccine label. However, although the minimum duration of immunity must be determined, it is not required that manufacturers establish maximum duration of immunity. Unfortunately, few studies have been

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performed to determine duration of immunity for vaccines designed for animals. Because it has been accepted that immunity will persist for most animals for at least 1 year (provided the animal is not challenge exposed to the agent), this assumption has rarely been critically assessed. In fact, given the amount of information known about the immune system, it is likely that many vaccines provide immunity for periods much longer than 1 year. In support of this, investigators examined the persistence of antibodies against panleukopenia, herpesvirus, and calicivirus in vaccinated cats and documented that antibodies against those diseases persisted at protective concentrations for at least 3 years.[1] In another study,[2] researchers documented that antibodies against canine distemper can persist for 8 to 10 years in dogs that are not challenge exposed to the agent.

Reasons to Test

Because we cannot always assume that a vaccinated animal is protected or that revaccination is absolutely necessary, many veterinarians are seeking methods for objectively determining immunity and risk of infection in animals prior to revaccination. It would be desirable to have simple, rapid, inexpensive tests available for measuring an animal's immune status. These tests could be used to differentiate between animals that are susceptible and require revaccination and those that do not. They also could be used to compare efficacy of vaccines from various manufacturers.

It is commonplace to test sera obtained from vaccinated swine or poultry to ensure that they are protected against disease. Thus, serum samples are routinely and repeatedly obtained from flocks to test for antibodies against pathogens that cause disease, such as avian influenza, Newcastle disease, or infectious bursal disease. If antibody titers are satisfactory, revaccination is not necessary; however, when antibody titers are low, the flock is reimmunized until birds achieve a protective titer. This procedure also permits managers to use the experience of others in the poultry industry when selecting the most effective brand of vaccine.

In general, detection of an antibody titer in serum obtained from an animal indicates that the animal has encountered the agent and an immune response has developed. However, the precise interpretation of a positive serologic response depends on specific circumstances. For example, a high antibody titer to parvovirus in an unvaccinated puppy suggests that vaccination must be delayed until maternal immunity has waned. On the other hand, detecting a high antibody titer to parvovirus in a mature dog after vaccination implies that vaccination has been successful.

Vaccination carries certain risks but rarely causes serious adverse effects. Thus, benefits of vaccination usually greatly outweigh risks. Nevertheless, any procedures that may improve this risk-to-benefit ratio should be welcomed. The major benefit of routine serologic testing would be to identify susceptible animals or to identify animals that do not require initial or booster vaccinations. Ideally, vaccination should be used to protect only those mature animals that require revaccination and then only for diseases for which the benefits of vaccination are clearly apparent.

Limitations to Serologic Testing

A simple test to determine an animal's immune status is a desirable goal; however, such a procedure has a number of limitations. Antibodies are not always protective, and for some diseases, antibody titers cannot serve as indicators of protection. An animal becomes immune to infection through many mechanisms. Thus, B-cell responses and subsequent antibody production are primarily protective against organisms exposed to body fluids. These include extracellular organisms, such as *Leptospira* spp, and bacterial toxins that are dissolved in body fluids, such as tetanus toxin, that are exposed to the immune system during transmission between cells. Conversely, for diseases in which organisms invade the body via the gastrointestinal or respiratory tract, surface antibodies of the IgA class, rather than serum antibodies, play a critical role in defense. Thus, high concentrations of serum antibodies against *Bordetella pertussis* in a dog cannot be used as a guarantee of protection, because serum antibody concentrations are poorly correlated with immunity for this disease.

For viral diseases, neutralizing antibodies and complement may play a less important role than T-cell–mediated immunity. Most antibodies do not penetrate cells, and persistent intracellular pathogens, such as viruses or intracellular bacteria, are not exposed to antibodies. Theoretically, antibodies are ineffective against such organisms, and high concentrations of antibodies may not be protective. These pathogens may replicate within cells regardless of the concentration of antibodies in the extracellular environment. This is well documented for herpesvirus or coronavirus infections, in which the viral carrier state is associated with persistent detection of virus despite a high concentration of antibodies.[3] In general, the higher the serum antibody titers, the greater the protection; however, the correlation between the 2 is not always absolute. It cannot always be assumed that an animal with low serum antibody titers is unprotected or that an animal with high antibody titers is resistant to infection. It must be strongly emphasized that interpretation of serologic results must account for the limitations of each assay and that results of assays must be interpreted conservatively.

For practitioners, the division between antibody- and cell-mediated immune responses is less clear. Although it is true that antibodies are not the primary protective mechanism against viruses, high antibody concentrations in the extracellular environment will serve to inhibit the spread of viruses between cells and, thus, promote host resistance. In diseases such as distemper of dogs or rhinopneumonitis of horses, many protective cell- and antibody-mediated processes contribute to host defenses. Although antibodies may not be protective against all diseases, antibody titers often are the only practical indicator available. Cellular immunity cannot be rapidly or cheaply measured, except in certain restricted circumstances.

Serologic tests are subject to error and may yield misleading results. Some insensitive serologic assays

may yield false-negative results, which would cause an animal to be vaccinated unnecessarily. This is of much less consequence than nonspecific tests that yield false-positive results, falsely implying that an animal is protected. Errors of this nature would, of course, lead to failure to vaccinate a susceptible animal. Understanding this limitation is central to the use of serologic assays for determining disease resistance.

Serologic tests measure the current status of an animal and cannot predict future protective status with accuracy. This is especially important when assessing relative risk and amount of challenge exposure. The immune system has a finite protective ability. Whether it can protect an animal will depend, in part, on the amount of challenge exposure by an infectious agent. Thus, an antibody titer sufficient to protect an animal against a low level exposure may well be insufficient to protect against an unanticipated heavy challenge. Results of serologic tests must be interpreted conservatively to minimize the risk of leaving an animal unprotected. It must be remembered that an animal that has mounted an immune response after vaccination will possess memory cells. Although antibody titers may have declined to low or undetectable concentrations, those memory cells remain. Thus, during challenge exposure to infectious organisms, that animal will mount a more rapid and efficient response, which may be sufficient to prevent development of clinical disease.

Serologic Assays

Serologic assays can be used to diagnose disease or assess immunity. Although many serologic assays (eg, **virus neutralization** [VN], immunofluorescence, and **hemagglutination inhibition** [HI] tests) have been used to measure antibody concentrations against infectious agents, the only practical assays suitable for veterinary practitioners are ELISA. Thus, a simple assay, such as a clinic-based ELISA, may be used to detect antibodies against **canine parvovirus** (CPV) in sera of pregnant bitches and their offspring to study the response of puppies to vaccination. With an easily accessible procedure for CPV antibody determination, veterinarians should be able to gauge the response of puppies after vaccination. It is not surprising that ELISA are used routinely to determine the protective status of poultry flocks or pig herds.

The crucial part of these tests is to establish a baseline or cutoff point. Animals with values greater than the cutoff point are adequately protected, whereas values less than the cutoff value would indicate animals that are not protected and are in need of revaccination. The cutoff value can be established 2 ways. First, challenge-exposure studies can be used. Vaccines are administered at various doses, and antibody titers are determined before animals are challenge exposed to infectious agents. The antibody titer of animals protected after a minimal vaccine dose serves as the reference point for the protective cutoff value. These studies are often performed by vaccine and test kit manufacturers. The second method is to monitor disease incidence in a specified population in relation to antibody titers. This is usually accomplished during a prolonged period of monitoring. Using this approach, some poultry and swine farms have established their own cutoff values instead of only relying on values provided by vaccine and test kit manufacturers. Each veterinarian could use the same method, although mass testing of herds or flocks has economic advantages and benefits for maintaining consistency of testing that might not be available for separately tested animals.

Interpretation of antibody titers, especially those determined by use of nonstandardized assays, may be difficult. Laboratories that offer these serologic tests are often reluctant to provide an interpretation for practitioners. Conversely, practitioners may have little experience in relating serologic results to protective status. In addition, test procedures are not standardized and lack quality-control standards. As pointed out previously, errors in interpretation of serologic test results may cause inappropriate decisions to be made and place animals at unnecessary risk of disease. Ultraconservative interpretation of serologic results can be modified as experience is gained. Practitioners should consult with laboratory personnel about results of serologic tests. Combining analysis of serologic test results with knowledge about an animal and its environment will enable practitioners to make the best recommendations possible for vaccination of each animal. Conversely, feedback from practitioners can assist laboratories as they refine testing techniques and interpretation of test results.

Diseases of Dogs

Canine distemper—Dogs are protected against **canine distemper virus** (CDV) by multiple immune mechanisms, including antibody- and cell-mediated responses. Dogs develop high concentrations of antibody to CDV after successful vaccination or infection. These antibody concentrations can be measured by means of ELISA, indirect fluorescence, and VN tests.[4-6] These antibodies clearly play a role in resistance to canine distemper. In a study,[7] investigators measured antibody titers to CDV in dogs with known immunization status. For nonvaccinated dogs less than 12 months old, they found that about half had a titer of ≥ 1:8. For dogs vaccinated against CDV, more than three fourths had a titer of ≥ 1:16. Protection appeared to be associated with an animal developing an IgG titer of > 1:50 (as determined by immunofluorescent antibody testing) within 3 weeks after vaccination. Other investigators[8] found that CDV-infected dogs with lesions of the nervous system appeared to have an impaired antibody response to the virus. For dogs that completely recovered from infection, antibodies were observed early after infection and antibody concentrations correlated with lack of lesions. In another study,[9] antibody titers were measured in dogs exposed to virulent CDV. Dogs surviving infection developed antibody titers of 1:100 within 14 days after exposure, as determined by use of VN tests. Dogs that failed to develop this antibody titer became ill and died. Similarly, it was reported in another study[10] that high amounts of antiviral IgG correlated with recovery from disease and that an inability to sustain substantial antiviral antibody response was characteristic of dogs that developed fatal encephalitis. Krakowka et al[11] documented that the ability of dogs to mount a vigorous antibody response

distinguished resistant from susceptible animals. Similarly, other investigators[4] reported that three fourths of dogs arriving at their laboratory without CDV antibodies developed respiratory tract disease, and a fourth of all dogs that did not have CDV antibodies at the time of arrival died. Conversely, for dogs with antibodies to CDV, only a fourth developed respiratory tract disease, and less than 5% died. The fact that passive immunization can be used successfully to protect dogs against infection with CDV also confirms the importance of antibodies for resistance to this disease.[12-14] One group of investigators[15] reported that antibodies against CDV, determined by use of VN tests, were protective and that dogs with a titer of ≥ 1:30 were protected, whereas those with a titer of ≤ 1:20 tended to be susceptible. Other investigators[16] documented that specific antibodies were highly effective for neutralizing CDV as well as preventing intercellular spread of CDV. Cells infected with CDV are lysed by antibody and complement.[17,18]

The importance of antibodies for determining whether a dog is protected against CDV is also emphasized by the fact that inactivated CDV vaccines, which are usually somewhat ineffective for providing substantial protection, stimulate an extremely low and transient antibody response.[19] It appears that successful viral replication in a dog is necessary if that dog is to develop a substantial and protective antibody response.

Persistence of antibodies to CDV after vaccination without challenge exposure was examined in dogs imported to Iceland, an island where canine distemper is not found.[2] Investigators found that two thirds of dogs vaccinated 8 to 10 years previously had antibody titers of ≥ 1:45. In the group for which mean interval since last vaccination was 6.25 years, 22 of 30 (73%) dogs had titers of ≥ 1:16. Although some dogs had low antibody titers, it is possible that these may have been inadequately vaccinated during the initial vaccination.

Although antibody titers are important when assessing immunity to CDV, it must be pointed out that cell-mediated responses also play a role in immunity to this disease.[20-25] Although multiple mechanisms are involved in resistance to CDV, detecting high antibody titers would indicate that an animal will likely be resistant to CDV.[26] Measurement of antibodies against CDV for dogs would, therefore, be a useful prognostic tool.

Canine adenovirus-1—Maternal antibodies can protect puppies against canine adenovirus-1[27]; therefore, antibodies must be protective for dogs. Antibody measurement would be useful in determining immune status of dogs to canine adenovirus-1.

CPV—Serologic assays, including ELISA[28] and **indirect fluorescent antibody (IFA)**, HI, and VN tests, have been developed for the diagnosis of CPV infection.[29] In general, the IFA test is best used for diagnostic purposes, whereas the other tests can be used for determining immunity. Disease attributable to CPV infection is associated with viral invasion and destruction of the gastrointestinal tract. Cell-mediated immune responses do not play a role in antiviral defenses within the intestinal lumen, where IgA is of critical importance. However, once CPV organisms have gained access to the body, T- and B-cell responses are important. In a study,[30] investigators found that CPV antibody titers of ≥ 1:80 were detected in 125 of 176 (71%) CPV-vaccinated dogs. Antibodies to CPV are transferred from immune bitches to puppies through the placenta and via colostrum.[31] Colostral transfer accounts for approximately 90% of the maternally derived CPV antibody concentration. After suckling, antibody titers of puppies are, in general, about half that of their dam's titer. Puppies with titers of ≥ 1:80 (determined by use of HI tests) were immune to oronasal challenge with virulent CPV organisms. Dogs with titers of ≤ 1:100 are not protected, whereas dogs with titers of 1:200 to 1:800 may be protected, and dogs with titers of ≥ 1:1,600 are protected. It is not uncommon for dogs successfully vaccinated against CPV to develop titers of > 1:25,000, as determined by use of VN tests. After parenteral vaccination against CPV, seronegative dogs developed substantial antibody titers as early as 2 days after vaccination, and maximal titers developed within a week.[32] Immunity was associated with persistence of antibody titers of > 1:80 (HI test). In contrast, puppies with titers of < 1:40 remained susceptible. Thus, measurement of serum antibody concentrations appears to be a useful tool for determining protective status and the need for vaccination against CPV.

Leptospirosis—The antibody response of dogs to leptospiral vaccines can be monitored by use of the microscopic agglutination or leptospiricidal activity tests. Protection (determined by use of the hamster passive-protection test) is directly correlated with serum antibody concentrations.[33] Given that leptospiral bacterins may be responsible for many of the adverse effects of vaccines administered to dogs, serologic testing of dogs for leptospiral antibodies could be of enormous benefit.

Borreliosis—Commercially available antibody test kits (ELISA) are currently available to test for borreliosis (Lyme disease). However, they are primarily used for disease diagnosis rather than assessing immune status. Clearly, dogs that lack antibodies to *Borrelia burgdorferi* will be susceptible to infection.

Respiratory tract infections—Similar to gastrointestinal tract diseases, locally produced surface IgA, rather than serum antibodies, is responsible for resistance to infection in the respiratory tract of dogs.[34] Because multiple etiologic agents are involved, including *B bronchiseptica*, canine parainfluenza, and canine adenovirus-2, serum antibody concentrations are not good predictors for resistance of dogs to respiratory tract infections.[35] This lack of association between serum antibody concentrations and resistance in the respiratory tract is reinforced by the observation that maternal antibodies do not interfere with development of resistance in the airways.

Rabies—Rabies vaccines licensed for use in animals have label indications for duration of protection of 1 or 3 years. Several years ago, the USDA permitted some vaccine companies to market rabies vaccines on which the efficacy was determined on the basis of antibody titers rather than on challenge-exposure studies. Unfortunately, it was found that serologic test results

were not predictive of resistance to challenge exposure, and those vaccines had to be withdrawn.[35]

Diseases of Cats

Antibody titers against common viral pathogens of cats can be determined by use of ELISA or VN tests. Measurement of serum antibody concentrations may be especially appropriate for cats that have had an adverse reaction to a vaccine. However, similar to dogs, the correlation between antibody titers and protection has not been completely analyzed. A low antibody titer may not always mean that a cat is susceptible to infection, and a high antibody titer does not guarantee resistance. Persistence of antibodies in cats after vaccination has been investigated by measuring antibody titers in a group of specific-pathogen-free cats that were vaccinated as kittens with 2 doses of an inactivated vaccine against **feline panleukopenia virus (FPV), feline herpesvirus (FHV), and feline calicivirus (FCV).**[1] The response to FPV was especially strong, with high titers developing within months after vaccination and persisting for at least 6 years. In contrast, antibody titers to FHV and FCV (determined by use of VN tests) never reached high concentrations and declined slowly during the subsequent 6 years. Titers against FHV persisted for at least 3 years, whereas titers against FCV persisted for at least 4 years. However, titers varied substantially among cats, with some cats becoming seronegative to FHV by 4 years and to FCV by 5 years.

FeLV—Kittens that had antibodies against FeLV and **feline oncornavirus-associated cell membrane antigen (FOCMA)** are protected against infection and oncogenesis by virulent FeLV.[36] All kittens with titers > 1:8 (determined by VN tests) were protected against a known FeLV challenge that was fatal to 95% of kittens that did not have detectable antibody titers.[3] Because of this, ELISA has been used as the basis for deciding whether to vaccinate against FeLV. Cats with antibodies against the FeLV gp70 protein or FOCMA are considered immune and do not need to be vaccinated.[37] Testing serum of cats prior to vaccination has proven to be a successful and feasible procedure and supports the concept that this could be successfully extended to other viral diseases.

Respiratory tract disease—Cats that are carriers of FHV may have relatively high concentrations of antibodies (titer of ≥ 1:96; VN test), whereas cats that are noncarriers may be seronegative or have decreasing titers.[3] Cats develop cell-mediated responses against FHV and FCV after vaccination.[38,39] Efficacy of vaccines against FHV, FCV, and FPV can be readily measured by serologic tests. Vaccinated cats develop substantial persistent antibody titers to all of these viruses.[40] Thus, serologic assays may work well to monitor these diseases, although their interpretation may be colored by the potential for development of a carrier state in cats with FHV infections.

FPV—Antibodies are protective against FPV and can be used to measure immune status. In general, cats with antibody titers against FPV of ≥ 1:8 (VN test) appear to be protected against clinical disease.

Feline immunodeficiency virus (FIV)—Cats infected with FIV will mount antibody responses and produce virus neutralizing antibodies. However, unlike the other diseases of cats described here, antibodies to FIV are not protective.[41] Specific-pathogen-free cats immunized with a synthetic peptide developed high antibody titers to the purified virus.[42] Immunized and control cats were challenged with FIV and then monitored for 12 months. Despite detection of antibodies, immunization with the specific peptide failed to prevent FIV infection. Therefore, results of antibody assays for FIV are not of value when assessing whether cats will be protected from infection and, thus, cannot be used to predict immune status.

Feline infectious peritonitis—Feline infectious peritonitis is a disease associated with development of immune-complex mediated hypersensitivity.[43] Attempts to correlate in vitro antibody activity (VN tests) with in vivo protection have revealed that antibodies produced during the course of this disease will not necessarily protect cats against infection and cannot be used to determine immune status. An ELISA is commercially available but is used primarily for diagnostic and prognostic purposes. Results of assays are only indicative of exposure to FCV and do not specifically detect antibody against FCV organisms responsible for disease.

Diseases of Horses

Horses should also be subjected to serologic testing to determine whether they are immune to specific infections or would benefit from vaccination. However, similar to dogs and cats, little effort has been made to correlate antibody titers with protection or the need for initial or booster vaccination.

Equine arteritis virus—After vaccination with an inactivated whole-virus vaccine, antibody concentrations detected by use of ELISA preceded development of a virus-neutralizing response.[44] After vaccination with an equine viral arteritis vaccine, horses develop titers as high as 1:5,120 (VN test). This antibody titer decreased rapidly, but revaccination 2 months after initial inoculation elicited a prompt antibody response, and titers persisted for at least 6 months. A titer of 1:43 (VN test) appeared to be the 50% protective dose, which was confirmed on the basis of clinical signs and viremia.[45]

Tetanus—Tetanus is an excellent example of a protective immune response mediated entirely by antibodies. Thus, serum antibody concentrations should provide a direct measure of protection. Tetanus antitoxin antibody concentrations were measured in ponies.[46] Results of that study revealed that protective concentrations (≥ 0.01 U/ml of serum) were maintained for at least 20 months after vaccination.

Equine influenza—Equine influenza vaccines have been regarded as having limited, short-lived efficacy. For example, Burrows et al[47] obtained serum samples from ponies vaccinated with 4 commercially available equine influenza vaccines. They found that little or no antibody was detected after the first inoculation (on the basis of results of HI tests); second and subsequent annual revac-

cinations produced peak titers between 7 and 14 days after inoculation.[47] Titers rapidly decreased between 14 and 28 days after vaccination and less rapidly thereafter. Serologic responses to 4 equine influenza vaccines were monitored in seronegative ponies, using HI and single-radial-hemolysis tests.[48] Four weeks after the initial vaccination, responses were barely detectable, using the HI test. After revaccination, responses to A/Miami/63 virus were low or undetectable, but responses to A/Prague/56 virus were higher (17/20 ponies with titers of ≥ 1:16). Using the sensitive single-radial-hemolysis test, investigators found a dose-related antibody response to both viruses. Titers after revaccination were two- to fivefold higher than after initial vaccination. They also detected a high rate of decrease in titers after vaccination, because titers in ponies with the highest antibody concentrations had declined to low or undetectable concentrations 14 weeks later. Ponies immunized with inactivated equine influenza vaccines document a clear association between antibody titers (measured by single-radial-hemolysis tests) and protection.[49,50] Therefore, given the inadequacies of current equine influenza vaccines, it appears that routine serologic testing may be of major benefit when determining the protective status of horses and the need for revaccination.

Equine herpesvirus—As pointed out previously during the discussion of FHV, antibody titers do not have a good correlation with immunity to herpesvirus infections. For example, cell-mediated and antibody responses of horses to **equine herpesvirus (EHV)**-1 were examined.[51] After vaccination and revaccination with a modified-live EHV-1 vaccine, horses had minimal increases in EHV-1 antibody titers (VN test). However, these same horses had a marked increase in the cell-mediated immune response, as measured by the lymphocyte transformation test. Samples from vaccinated foals and mares were tested to determine antibody concentrations and the degree of protection afforded.[52] Investigators found that titers against EHV-1 were 1:8 or less (VN test) in most foals, and all foals were not protected. The ability of EHV vaccines to stimulate cellular and antibody responses to EHV-1 and EHV-4 has been evaluated in healthy horses.[53] Comparison of results of lymphocyte blastogenesis tests indicated that horses given modified-live EHV-1 vaccines had substantial increases in responses to EHV-1 and EHV-4. Responses to EHV-1 and EHV-4 for horses given an inactivated-virus bivalent vaccine were less. Both vaccines induced major increases in antibody titers against EHV-1 and EHV-4 (VN test and ELISA). Serum concentrations of antibodies to EHV-1 and EHV-4 (ELISA) were significantly higher in horses that received a bivalent vaccine, compared with responses for horses that received a monovalent vaccine. Thus, vaccination with modified-live EHV-1 vaccines can stimulate cellular and antibody responses that cross-react with EHV-4.

Conclusions

Managers of poultry and swine operations have embraced widespread use of a panel of ELISA to determine whether their animals are susceptible to infection and require vaccination. They usually obtain serum samples from a statistically determined number of animals and extrapolate the results to the remainder of the flock or herd. These tests are relatively inexpensive and lend themselves to automation and computer-based analysis. They have the additional benefit of identifying infectious agents against which their animals have developed resistance. Thus, testing can save money for producers by preventing unnecessary vaccinations.

For companion animal practitioners, conditions are different, although the basic principal is the same. Thus, the unit of interest would be each companion animal rather than an entire flock or herd. Therefore, testing of each animal would be required, and decisions would have to be made on the basis of information and circumstances for each animal. Once reliable data are acquired, careful and conservative use of serologic assays for companion animals could provide veterinarians with the ability to identify animals that must be vaccinated or, conversely, animals that can be safely left unvaccinated. Routine serologic testing could also provide badly needed data on the efficacy of various vaccines, timing for administration of vaccines, passive immunity, and persistence of immunity for companion animals. Analysis of antibody titers may provide important information about the degree of protection animals have developed against numerous infectious diseases, such as those mentioned previously. However, they probably would be less useful for determining the degree of protection against diseases, such as rabies, respiratory tract infections, FIV and FCV infections, and EHV-1 and EHV-4 infections, although test results could be used to monitor responses to vaccination.

Given the success of routine serologic testing for determining protection in the swine and poultry industries, it is reasonable to assume the same techniques could be applied to companion animals. Better, more cost-effective vaccines and vaccination schedules could be established, unnecessary revaccination could be eliminated, and clients could be provided with a scientifically based rationale for use of vaccines. The end result would be an improvement in the overall health of animals.

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