Infectious Diseases of Wild Mammals

THIRD EDITION

Edited by Elizabeth S. Williams and Ian K. Barker

Iowa State University Press / Ames

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PREFACE

Nearly 20 years have passed since the publication of the second edition of *Infectious Diseases of Wild Mammals,* an important resource for, and inspiration to, a generation of wildlife biologists and veterinarians. All those concerned with diseases of wildlife are greatly indebted to the late John Davis, and to Lars Karstad and Dan Trainer, for consolidating knowledge in this field and making it so readily accessible. Their work helped establish the widespread and growing recognition of the influence of disease on populations of wild mammals, consideration of disease in the fields of wildlife management and ecology, and greater appreciation of disease as a component in the relationship among wild mammals, humans, and domestic animals.

To us has fallen the humbling task of regenerating and updating this work, in collaboration with an international field of chapter authors. Our goal has been to summarize knowledge in the field of wildlife diseases relevant to wild mammals, in a form that will be useful to students in wildlife biology or veterinary medicine; wildlife biologists and managers; veterinarians dealing with free-living and captive wildlife; and epidemiologists and public health professionals concerned with wildlife zoonoses.

The format of the third edition follows that of its predecessors, with chapters arranged by taxon of infectious agent. They deal with agents established or suspected as pathogens in wild mammals, and/or transmissible between wild mammals and domestic animals or people. Many are capable of causing devastating diseases, such as tularemia, plague, or rabies, among wild mammals; almost all of these are capable of infecting domestic animals or people, as well. Other zoonotic agents, such as those causing the rodent-borne hemorrhagic fevers and Lyme borreliosis in people, have little or no impact on the wildlife that serve as the reservoir for human infection. Some well-recognized pathogens of domestic animals, such as canine distemper virus and parvoviruses, are emerging in significance or increasingly recognized in free-ranging wildlife. Diseases such as brucellosis, bovine tuberculosis, rinderpest, and foot-and-mouth disease arguably are more strongly associated with domestic animals than with wildlife, but they pose some of the knottiest epidemiologic, management, and ethical problems where wild animals are affected. Some agents, such as the myxoma virus, rabbit calicivirus, and *Salmonella,* have been exploited as biologic agents of vertebrate pest control, with varying degrees of success and safety. Others, such as poxviruses and *Leptospira,* are functional or candidate recombinant vectors for the delivery of antigens to immunize wild mammals. The significance for wild mammals of agents such as *Chlamydia* and its relatives, and the retroviruses and mycoplasmas, seems relatively restricted, taxonomically and/or geographically, while others, such as the lyssaviruses, the poxviruses, *Salmonella,* and *Leptospira,* affect a wide array of wild mammals on most continents.

Knowledge of diseases included in the last edition has greatly expanded, and a number of new agents that fall within the scope of this volume have emerged in the past two decades. Accordingly, we have added major treatments of problems such as rodent-borne hemorrhagic fevers, Lyme borreliosis, transmissible spongiform encephalopathies, and calicivirus infections. Consideration of the implications for wildlife of agents such as

the parvoviruses, canine distemper virus, and the rickettsiae has been expanded. And, of necessity, some topics found in the second edition have been truncated, consolidated, or eliminated.

Within the limits of the space allotted, chapter authors provide an entrée to the historical literature on an agent or disease, a summary of current knowledge on the etiology, pathogenesis, immunity, and diagnosis, and discussions of implications of the agent or disease for captive or free-ranging wild mammals, domestic animals, and people. Relevant current information on the biology and epidemiology of pathogens gained by molecular techniques has been incorporated, but the rapid expansion of such knowledge, and the inevitable lag in bringing a work such as this to press, no doubt already will have dated chapters on some very active topics.

Despite the expansion in size of this volume, space limitations forced elimination of most comprehensive reviews, lists of citations, and detailed treatment of clinical signs and comparative diagnosis. Of necessity, many chapters are surveys rather than full reviews of the topic, and readers will need to consult the second edition and other sources cited for fuller historical and current information. The number of illustrations and the number and scope of tables also were limited in deference to textual material. Nonetheless, between these covers is a vast amount of information, covering a wide array of infectious agents affecting virtually all orders of mammals. Reference lists lead to the wider literature, and the index will allow the reader to find and consolidate information affecting particular families or genera of mammals, which may be scattered among several chapters. Walker's *Mammals of the World,* fifth edition, by R.M. Nowak (Johns Hopkins Press, 1991), was used as the authority for mammalian nomenclature, except where another authority is cited by chapter authors.

We gratefully acknowledge the effort that our many authors dedicated to their contributions. Selected on the basis of their expertise and familiarity with the wildlife ramifications of the topic assigned, the authors span the disciplines of wildlife biology, veterinary medicine, epidemiology, and microbiology. Years of experience in the field and in the laboratory, on all continents except Antarctica, have been distilled onto these pages. The editors found it exciting to foster international collaborations on authorship of chapters, often by means of electronic communication, and to participate as they developed and evolved. Like the editors, many authors had to cope with the effects of economic rationalism and downsizing on their workplace. Chapter authorship was an act of dedication, for little reward, often carried out to difficult deadlines under trying circumstances. Sadly, two of our contributors, Drs. Charles Seymour and Werner Heuschele, died during the preparation of their chapters. To the chapter authors goes credit for the quality of the content of this book; the editors assume responsibility for any shortcomings.

We also wish to acknowledge colleagues who read drafts of chapters or who provided technical advice on various topics. Iowa State University Press, in particular Gretchen Van Houten, has been very helpful and extremely accommodating over the course of the preparation of this edition. We greatly appreciate the tolerance and encouragement to carry this project through that were offered by our spouses, Tom Thorne and Susy Carman.

Lastly, we wish to recognize the Wildlife Disease Association and its many dedicated members, who, over the course of nearly 50 years, have shared in the study and dissemination of knowledge of diseases of wildlife, in the interests of animal and human health and welfare, and environmental conservation. The Wildlife Disease Association supported the preparation of this volume and is the beneficiary of the royalties that will accrue from its use.

Infectious Diseases of Wild Mammals

THIRD EDITION

PART I

Viral and Prion Diseases

1 RABIES

CHARLES E. RUPPRECHT, KLAUS STÖHR, AND COURTNEY MEREDITH

Synonyms: al kalabe, beshenstvo, derriengue, hari, hydrophobia (humans), kalevet, kuang cheng, lyssa, mal de calderas, oulo fato, polar madness, rabhas, rage, rabia, rabbia, raiva, thao, tollwut.

INTRODUCTION. Rabies is an acute fatal viral encephalomyelitis. The etiologic agents belong to the genus *Lyssavirus*, in the family *Rhabdovirus*. Described for at least four millennia, it is one of the oldest recognized infectious diseases. A clinical entity indistinguishable from classic rabies has been reported on all inhabited continents. Warm-blooded vertebrates are susceptible to experimental infection, but only mammals are important in the epidemiology of rabies. The etiologic agents are neurotropic viruses that primarily replicate in the central nervous system (CNS) and pass to the salivary glands and saliva. Transmission principally occurs by the bites of infected carnivores and bats. As a major zoonosis, rabies has considerable public health, veterinary, and economic impact, and it should be of concern to all professionals that deal directly with the health of free-ranging or captive mammals. Since the publication of the first edition of this book, significant advances in biotechnology have occurred, particularly in the diagnostic arena with the development of monoclonal antibodies (Mabs) and molecular typing techniques that have shed considerable light on the epidemiology of rabies. In addition, in the vaccine field, there has been substantial application of oral immunization toward wildlife rabies control.

HISTORY AND HOSTS

Africa. Circumstantial evidence indicts Africa as the cradle of lyssavirus evolution, because nowhere else are at least four serotypes or genotypes present (Rupprecht et al. 1991; King et al. 1994). Besides rabies virus, only two European bat lyssaviruses and a newly described Australian virus have become established outside of Africa, perhaps by virtue of bat flight and subsequent zoogeographic spread. Classic serotype/genotype 1 rabies virus, found mainly in dogs *Canis familiaris,* has achieved virtual worldwide distribution, primarily with human assistance (Smith and Seidel 1993), whereas the other viruses by comparison may be viewed somewhat as biologic curiosities.

Arguably, prior to the 20th century, innumerable rabies cycles recognizable in local folklore may have circulated throughout Africa. For most of the continent, however, written records to confirm this assertion hardly exist. For example, by the late 19th and early 20th centuries, numerous apparent human and animal rabies cases derived from wildlife exposure had been observed and sometimes anecdotally reported in South Africa, but actual scientific documentation did not occur until 1928. For much of the rest of Africa, confirmation of the presence of rabies occurred at approximately the same time.

It has been difficult to appraise the primary role of African wildlife realistically in maintaining the disease because of the ubiquitous domestic dog. Many human victims of rabid dogs further obscure the picture of sylvatic rabies. Dog rabies has been known in the Mediterranean littoral region of north Africa since antiquity; in sub-Saharan Africa most, if not all, of the European colonies recorded from their earliest days rabies-like diseases in dogs, other domestic animals, and humans. At this juncture it is impossible to determine whether rabies was imported, always endemic, or if wildlife played any substantial role in its maintenance.

In South Africa, the picture is somewhat clearer. Dr. E. Cluver, an assistant medical officer, published a retrospective clinical account of seven human rabies cases, the earliest of which dated from 1916, in which the biting animal was identified as either a mongoose or a genet (Cluver 1927). Three other cases occurred after dog bites. While investigating these deaths, Cluver found among the indigenous inhabitants a widespread, long-standing belief that the bite of a genet led to a fatal disease some weeks or months later. Today, using Mabs, a distinct rabies variant has been identified that is maintained among members of the subfamily Viverrinae, to which the mongooses, civets, and genets belong. The specific characteristics of this rabies variant suggest that it evolved long ago by adaptation to these particular hosts. The yellow mongoose *Cynictis penicillata* is both common and widely distributed and is probably principally involved in maintaining endemic disease in this particular locale. Although the suggestion of rabies among domestic species existed for centuries (King et al. 1994), it is otherwise difficult to document earlier involvement of African wildlife.

Eurasia. Rabies, at least among dogs, was suggested by the ancient Mesopotamians (ca. 2300 B.C.), Chinese (ca. 782 B.C.), Greeks (ca. 500 B.C.), and Romans alike, but it is uncertain when its existence in wildlife was first appreciated (Wilkinson 1988; Steele and Fernandez 1991; Baer et al. 1996). The disease was prevalent in both domestic animals and wildlife in Western and Central Europe for several centuries throughout the Middle Ages, particularly among foxes and wolves *Canis lupus* (Steck et al. 1968; Wilkinson 1988), although badgers *Meles meles* and bears *Ursus arctos* (ca. 900 A.D.) were also occasionally mentioned (Steele and Fernandez 1991). Many human cases were reported after rabid animal contact, which led to the development of vaccines for postexposure prophylaxis (PEP) in humans at the end of the 19th century (Pasteur et al. 1884; Roux 1887; Calmette 1891). Red fox *Vulpes vulpes* rabies was rampant throughout Europe in the 19th century, largely disappeared for unknown reasons during the first decades of the 20th century, but reemerged after World War II. The disease spread subsequently throughout mainland Europe (Kauker 1966; Wandeler et al. 1974; Bögel et al. 1976; Toma and Andral 1977; Steck and Wandeler 1980; Wachendörfer and Frost 1992; Steele and Fernandez 1991; Blancou et al. 1991). Rabies cases in indigenous terrestrial wildlife have since been reported in all European countries except Sweden, Portugal, Greece, the United Kingdom, Ireland, and the mainland of Norway and Spain; data from Albania are not readily available [World Health Organization (WHO) 1996]. Generally, the rabies situation has improved in Western Europe over the last decade, primarily as a result of large-scale oral vaccination campaigns against fox rabies. With few exceptions, it has been difficult to meet the criteria for rabies elimination (WHO 1990; Office International des Epizooties 1992) despite tremendous progress in rabies control in foxes.

The Americas. Whether rabies existed prior to a European presence is unknown, largely because of the paucity of a written history prior to the late 15th century, but it is likely that the present disease is a mosaic of rabies of the Old and New Worlds. Conceivably, human and livestock deaths described by the original Spanish conquistadores were attributable to indigenous rabid vampire bats *Desmodus rotundus,* but several notable reports throughout the 16th and 17th centuries mention the apparent absence of the disease throughout much of Latin America, at least among dogs (Baer et al. 1996). The apparent significance of insectivorous bats was largely unappreciated until late in the 20th century. In contrast, the ancient Bering land bridge that provided the opportunity for initial human migration may also have enabled the natural introduction of rabies into northern North American latitudes for thousands of years by dispersing Old World carnivore populations (Winkler 1975); maintenance in circumpolar areas cannot be discounted (Crandell 1991). Nevertheless, strong suggestions of the disease did not occur until approximately 200 years after the pivotal European conquest of the Americas; one of the first reports is attributable to a cleric during 1703 in Mexico (Steele and Fernandez 1991), and a prominent Mexican outbreak was recorded in 1709, with the involvement of a wolf with hydrophobia (Baer et al. 1996). In the mid-1700s, reports of rabid foxes and dogs were common throughout the mid-Atlantic British colonies, perhaps complicated by a predilection for red fox hunting and hence widespread translocation, a focus of disease that may have persisted throughout the eastern United States well into the 20th century. One of the first descriptions of the disease in Canada did not occur

until 1819, when the Duke of Richmond, then Governor General, was bitten by a pet fox and subsequently died (Steele and Fernandez 1991). By the mid-1800s, skunk rabies was reported from the American prairies and west, spreading northward into Canadian provinces by the next century; it was so prevalent that some proposed the name *Rabies mephitica* to describe skunk rabies (Steele and Fernandez 1991).

Undoubtedly, the unintentional European translocation of infected dogs during typical voyages of 4–6 weeks, which were often shorter than the incubation period of the disease, had a major influence on the global spread of rabies (Smith and Seidel 1993) particularly in the Western Hemisphere.

Another exotic introduction influenced the history and distribution of rabies in the Western Hemisphere. During the mid-1800s, importations began of the small Indian mongoose *Herpestes auropunctatus* for control of rats and snakes on sugarcane plantations in many areas (Everard and Everard 1988, 1992). Consequently, mongoose rabies has been reported on several islands, including Cuba, the Dominican Republic, Grenada, and Puerto Rico. However, the absence of the disease despite the presence of mongoose on other Caribbean islands and Hawaii is somewhat peculiar, a combination perhaps of geographic isolation and historical accident.

Although wildlife rabies may have been present in the far north for centuries (Rosatte 1988; Crandell 1991), documentation of a major outbreak among red and Arctic *Alopex lagopus* foxes did not occur until the mid-20th century, spreading into Alberta, British Columbia, Saskatchewan, Manitoba, Quebec, and Ontario by the 1950s. Remnants from this original Arctic outbreak persist in endemic foci of fox rabies in Alaska, southeastern Canada, and the northeastern United States (Krebs et al. 1997). Rabies virus variants found among gray foxes *Urocyon cinereoargenteus* in Texas and Arizona are distinct (Smith et al. 1995).

Occasional cases of wildlife rabies have been reported from throughout many portions of Latin America since at least the 19th century, but the predominance of dog rabies and its associated human fatalities historically blurred the epidemiologic significance of nondomestic species. In contrast, outbreaks of bovine rabies, and later human cases, of common vampire bat origin were clearly recognized as a fatal ascending paralysis in the late 1800s and early 1900s in Mexico, Brazil, Argentina, Bolivia, Paraguay, Guyana, and Trinidad (Acha and Alba 1988). Today, vampire rabies is widespread from Mexico to Argentina, and it responsible for the deaths of millions of livestock and hundreds of people (Constantine 1988; Schneider et al. 1996). Throughout the 20th century, a number of novel vampire control methods were developed in serious attempts at population reduction and rabies prevention (Lord 1988; Flores-Crespo and Arellano-Sota 1991), but vampire rabies persists.

DISTRIBUTION. With the exception of geographically isolated political units (e.g., the United Kingdom and Japan) that have successfully eliminated rabies through aggressive control measures or those regions that have never reported an indigenous case of the disease (e.g., much of the Caribbean and Pacific Oceania), rabies is widespread on all inhabited continents (Fig. 1.1). At least 48 countries or territories did not report rabies during 1994 (WHO 1996). However, rabies surveillance is passive and biased toward detection in humans and domestic species rather than free-ranging wildlife. It is further complicated by the mobility of infected bats that often escape surveillance efforts.

ETIOLOGY. The viruses that cause rabies belong to the Rhabdoviridae**.** This family consists of the genera *Vesiculovirus, Ephemerovirus, Lyssavirus,* and many unassigned viruses isolated from a variety of plants, invertebrates, and vertebrates. Viruses in this family are primarily assigned on the basis of their distinctive rod- or bullet-shaped structure, and all are single-stranded, negative-sense, unsegmented RNA viruses.

The genus *Lyssavirus* includes rabies virus and a group of antigenically and genetically related Old World viral species known as serotypes or genotypes with molecular weights of approximately 4.6×10^6 Da (Wunner 1991). Virions are composed of an internal ribonucleoprotein (RNP) core or nucleocapsid, containing the nucleic acid, and an outer lipid envelope covered with transmembrane protein spikes. The viral genome encodes five polypeptides, associated with either the nucleocapsid or the envelope (Tordo and Kouknetzoff 1993). The L (transcriptase), N (nucleoprotein), and the NS (nominal phosphoprotein) proteins comprise, together with the linear viral RNA, the RNP. The μ (matrix) and G (glycoprotein) proteins are associated with the envelope. The N protein comprises the group or genus-specific antigens, whereas the G protein is responsible for the classic serotype (Rupprecht et al. 1991).

Lyssaviruses are adapted to replication in mammalian neural tissue and do not persist in the external environment. Viral susceptibility to chemical and physical degradation partly depends on the source and nature of the infectious material, such as intact brain tissue or a film of saliva, and specific environmental conditions. Although infectious virus may be recovered months later in the frozen carcass of a fox on the Arctic tundra, virus can be inactivated within hours during the summer in the decomposing tissues of a road-killed raccoon *Procyon lotor* in Florida. Being enveloped, the virus is sensitive to many lipid solvents and is rapidly inactivated by exposure to fixatives such as formalin, strong acids and bases, most detergents, and ultraviolet radiation, including direct sunlight. Heat (less than 56° C), drying, and repeated freezing and thawing will usually lead to a rapid inactivation. For example, in rodent carcasses under laboratory conditions, viral infectivity became greatly diminished at 25° C–37° C within 3–4 days, although it was still present as long as 9 days later (Lewis and Thacker 1974).

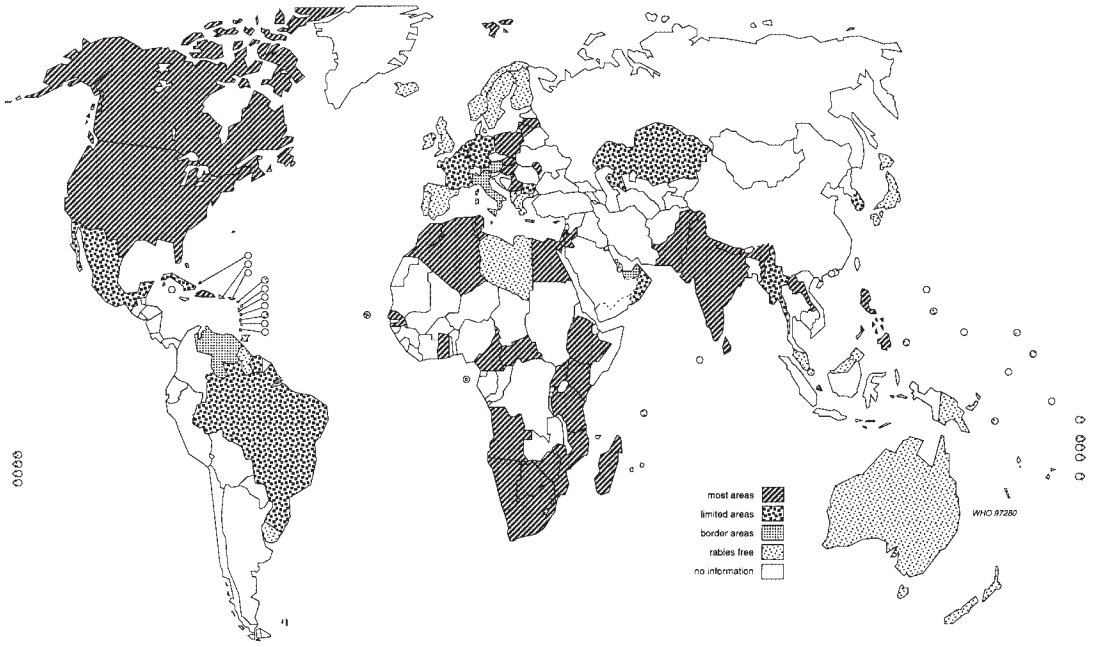

FIG. 1.1—World map depicting geographic distribution of rabies (not to scale). (The designations employed and the presentation of material on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city, or area of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines represent approximate border lines for which there may not yet be full agreement.)

Previously, rabies and the "rabies-related viruses" were defined on the basis of their morphology, serologic cross-reactivity, and their ability to cause encephalitis when inoculated intracranially into laboratory rodents (Bourhy et al. 1993). Two other rhabdovirus species isolated from insects in Africa—Obodhiang and Kotonkan—that were seemingly aligned with this group on the basis of serology await further genetic characterization. Tentatively, there are at least six other distinct lyssaviruses related to rabies, but they are easily distinguished by their characteristic antigenic and genetic properties. Within each major *Lyssavirus* serotype/genotype are numerous viral variants that exist as *quasi species,* heterogeneous mutant viral populations, subject to Darwinian evolution and punctuated equilibrium (Eigen and Biebricher 1988; Nichol et al. 1993).

***Lyssavirus* Serotype/Genotype 1: Rabies Virus.** Rabies virus is the type species and is the most significant member of the genus as concerns distribution, abundance, and relative veterinary and public health importance. Most of the relevant scientific focus has concentrated on this classic agent. Except where noted otherwise, the principal applied attributes of rabies virus and the rabies-related lyssaviruses are felt to be largely interchangeable in regard to diagnosis, pathobiology, and disease prevention, control, and management.

Besides technical differentiation from other lyssaviruses, isolates of rabies virus have also been informally categorized from the time of Pasteur as either *fixed* laboratory or vaccine strains, adapted by passage in animals or cell culture, or as unadapted street or *wild type* viruses. The use of monoclonal antibodies (Mabs) in the 1970s and the application of molecular techniques in the 1980s, particularly viral nucleic acid detection by reverse transcription and amplification of cDNA by the polymerase chain reaction (RT/PCR) with subsequent analysis of viral nucleotide sequences, have been extremely useful in the identification and differentiation of multiple viral variants originating in major reservoirs throughout the world. Such techniques have been essential in implicating the likely sources of human exposure, when a definitive history of animal bite was unavailable, and in understanding lyssavirus epidemiology and phylogeny. For example, these analyses have provided historical insights into the global dissemination of lyssaviruses and the significance of translocation upon current distribution patterns (Smith and Seidel 1993).

***Lyssavirus* Serotype/Genotype 2: Lagos Bat Virus.** Discovery of the rabies-related lyssaviruses of Africa began in 1956 when Boulger and Porterfield (1958) read reports of rabies virus isolations from bats in the United States. These reports inspired them to investigate the large straw-colored fruit bat *Eidolon helvum* living on Lagos Island, Nigeria. A virus was isolated that the authors named Lagos bat virus and that they

concluded, after serologic investigation, was not a rabies virus because it was not neutralized by rabies antiserum. This virus became the prototype of the rabies-related lyssaviruses. The presumed bat host is found on the southern half of the African continent, is remarkably widespread, and may be found anywhere from approximately 16° S to 34° S (Skinner and Smithers 1990).

The second isolation of Lagos bat virus (Sureau et al. 1980) occurred in the Central African Republic from the dwarf epauleted fruit bat *Micropteropus pusillus.* A third bat species was found when an investigation was made into widespread morbidity and mortality among Wahlberg's epauleted fruit bats *Epomophorus wahlbergi* in Natal (Meredith and Standing 1981; Crick et al. 1982).

Eidolon helvum and the epomophorine bats are tree-roosting and gregarious, which may play a role in the epidemiology of Lagos bat virus. Also significant could be the interaction between these species whose breeding grounds overlap, such as during competition for preferred fruits, particularly when in short supply during drought or seasonal variation. Alternatively, several variants may persist in different species.

In the first two recoveries of Lagos bat virus, there was no obvious indication of pathogenicity in the hosts from which they were isolated. In the case of *E. wahlbergi,* however, extensive bat mortality was observed, and the resultant media publicity resulted in recovery of a large number of dead and dying bats from which several viral isolations were made. When inoculated intracerebrally into rodents and vervet monkeys *Chlorocebus pygerythrus,* this virus produced a fatal rabies-like disease with the formation of Negri bodies in the CNS (C. Meredith unpublished). Boulger and Porterfield (1958) were unable to demonstrate Negri bodies in rodents dying of Lagos bat virus infection, but this may have been due to a lack of special stains for the detection of the sometimes rare inclusions.

A further isolation of Lagos bat virus was made from a domestic cat *Felis catus* in Natal (King and Crick 1988). The source of the infection remains unknown. Another cat showing atypical clinical signs, but suspected of being rabid, was found to be infected with Lagos bat virus in Zimbabwe (Foggin 1988). Again the source of the infection could not be determined.

Classification of 115 Ethiopian suspect rabies virus isolates by Mebatsion et al. (1992) revealed that at least one dog had been infected with Lagos bat virus. This is the only record of this viral infection in a canid. No details of this specific clinical case were published.

***Lyssavirus* Serotype/Genotype 3: Mokola Virus.** In 1968, a survey of Nigerian rodents for arboviruses resulted in the isolation of a new virus from viscera of shrews *Crocidura* spp. The virus was later named Mokola from the district of origin and shown to be a rhabdovirus related to rabies virus (Shope et al. 1970; Kemp et al. 1972). This virus became the prototype of *Lyssavirus* serotype/genotype 3. Unlike Lagos bat virus, which has not, as yet, been incriminated in any fatal human case, Mokola virus was reported by Familusi et al. (1972) as the cause of death of a young girl afflicted with a paralytic disease in Nigeria. Later that year, Familusi and Moore (1972) claimed to have recovered Mokola virus from the cerebrospinal fluid (CSF) of a child suffering from a septic meningitis, but the child recovered from the illness without adverse sequelae. The unusual circumstances of this case raised some doubts as to whether Mokola virus was actually the cause of the illness. Mokola virus was recovered from a *Crocidura* shrew in 1974 in Cameroon (le Gonidec et al. 1978) and from the brain of a harsh-furred mouse *Lophuromys sikapusi* caught in the Central African Republic (Saluzzo et al. 1984). Because Mokola virus appears to be widespread among shrews and, possibly, rodents that are common domestic commensals in west and central Africa, such animals may be sources of infection, quite unlike the case for other lyssaviruses.

Foggin (1982, 1983, 1988) described Mokola virus infection in cats and a dog in Zimbabwe. Relatively large amounts of Mokola virus, exceeding 10^3 mouse intracerebral 50% lethal dose, were required to achieve experimental infection of cats, making transmission by bite of a small rodent or shrew unlikely. Possibly, cats may become infected by predation and consumption of infected prey, although Bell and Moore (1971) found cats refractory to this route when using mice dying of serotype/genotype 1 rabies virus.

Because of Foggin's experience in Zimbabwe, all rabies-positive cat isolates in the Republic of South Africa were screened with anti-N Mabs to determine which *Lyssavirus* was involved, and cats from the eastern Cape were shown to be infected with Mokola virus (Meredith et al. 1996). Mebatsion et al. (1992) reported a case of Mokola virus infection in a cat in Ethiopia, a continent away from the South African cases. The role of cats as sentinels for rabies-related lyssaviruses in wildlife is clearly important.

***Lyssavirus* Serotype/Genotype 4: Duvenhage Virus.** The index case occurred in South Africa during 1970, when classic clinical rabies occurred in a man north of Pretoria who had been bitten on the lip by a bat while asleep, possibly Schreibers' long-fingered bat *Miniopterus schreibersi* (Meredith et al. 1971). Brain specimens from the man were negative when originally examined by the fluorescent antibody test (FAT) using a locally prepared conjugate, but Negri bodies were seen in Purkinje cells. Confirmation that this was a rabies-related virus occurred later (Tignor et al. 1977), and the original patient name was selected for viral nomenclature.

Corroboration that the Duvenhage virus originated from an insectivorous bat occurred when it was isolated from the brain of a sick bat. The general description of the bat was suggestive of *Miniopterus* sp. (Schneider et al. 1985).

A third African isolation of Duvenhage virus was made from a common slit-faced bat *Nycteris thebaica*

during a survey in Zimbabwe (Foggin 1988). This species is extremely widespread in central, east, and southern Africa. As with Lagos bat virus and Mokola viruses, the epidemiology of Duvenhage virus is not understood.

***Lyssavirus* Serotype/Genotypes 5 and 6: European Bat Virus I and II.** As reviewed by Schneider and Cox (1994), the existence of rabies among European bats was suspected as early as the 1950s. Initial reports suggested an antigenic relationship with Duvenhage virus, but later analyses demonstrated at least two unique *Lyssavirus* variants, termed European bat viruses (EBVs), subtypes I and II. The reservoirs of EBV I and II are believed to be insectivorous bats. Both subtypes are responsible for human fatalities, but no known domestic or wild mammal cases have been confirmed after active disease surveillance. More than 400 total EBV cases have been identified to date, including the first report from Great Britain (Whitby et al. 1996). Considering that many local bat populations are endangered or threatened species and that EBV subtypes appear largely restricted to those portions of Europe where dog and wildlife vaccination is widely practiced, it is hoped that these lyssaviruses will not play a significant role in European public or veterinary health.

***Lyssavirus* Serotype/Genotype 7: Australian Bat Virus.** Although there was no previous evidence of endemic rabies in Australia, a novel virus infection (Fraser et al. 1996) was diagnosed in a black flying fox *Pteropus alecto,* a megachiropteran fruit bat, during the spring of 1996. The bat was submitted to an Australian veterinary laboratory during routine surveillance of bats for another recently discovered virus, the so-called equine morbillivirus (Murray et al. 1995). The virus, hereafter referred to as Australian bat virus (ABV), possessed typical rhabdovirus morphology, was detectable by FAT using standard antirabies diagnostic reagents, and caused a nonsuppurative meningoencephalitis, consistent with lesions induced by other lyssaviruses. The ABV has since been isolated from grey-headed *P. poliocephalus* and little red *P. scapulatus* flying foxes and from a yellow-bellied sheath-tail bat *Saccolaimus flaviventris,* an insectivorous microchiropteran (Tidemann et al. 1997). Infection appears widespread; ABV has been isolated from bats in New South Wales, Queensland, Victoria, and the Northern Territory (Speare et al. 1997). Based on examination of archived fixed tissues, ABV was present in 1995, if not earlier. Antigenically and genetically, ABV isolates are similar to classic rabies viruses, as confirmed by cross-protection studies with commercial human and animal rabies vaccines (Rupprecht et al. 1996a). But they represent a distinct subtype (Gleeson 1997). Provisionally, ABV is considered to represent a new *Lyssavirus:* serotype/genotype 7. The ABV has caused at least one human death, the patient most likely infected while caring for ill bats (Allworth et al. 1996). Previously, no rabies was reported in a west Malaysian survey of 478 bats of 12 different species (Tan et al. 1969) or in a collection of more than 1000 bats in the Philippines (Beran et al. 1972). However, rabies infection was reported during 1978 from a grey-headed flying fox found dead in India (Pal et al. 1980), suggesting that some agents such as ABV may be more prevalent locally among Asian-Pacific bat populations than previously realized. Obviously, these discoveries have raised serious public health concerns (Crerar et al. 1996) on a continent believed free of the disease.

TRANSMISSION AND EPIDEMIOLOGY. A key to understanding the complex epidemiology of wildlife rabies is a basic appreciation of the agent-host-environment triad (Wandeler 1993; Wandeler et al. 1994). Unlike many etiologic agents, a productive infection by a lyssavirus usually kills its host. Thus, propagation to the next susceptible mammal usually only effectively occurs during a relatively short excretion period of the virus during the final stage of the disease, unlike previous beliefs of a long-term clinically normal carrier state. This very small window of infectious opportunity is generally concomitant with illness or in the prior 3–10 days. Virus excretion several weeks before clinical signs is unusual. Many conditions must be met to allow an efficient rabies infectious chain to be maintained without in utero transmission (Steece and Calisher 1989) or other rare transmission events. There are two primary conditions: a pertinent virus-animal interaction (e.g., virus characteristics, species susceptibility, and disease course) and appropriate host natural history (e.g., reproduction, dispersal, social interactions, population demographics, and density). The significance of human influences, predominantly on the latter conditions, should not be overlooked (Rupprecht et al. 1995).

In rabies, the term *reservoir* refers specifically to those species that maintain the disease in nature. Given more than 4000 species in the order Mammalia, all of which are believed susceptible and in theory capable of infecting another mammal, relatively few species qualify as major rabies reservoirs. An oversimplified description of an urban cycle of rabies, maintained by feral domestic dogs, and a sylvatic cycle of rabies, supported by wildlife, fails to communicate the true interactive dynamics of the disease and the frequent overlap of these general categories.

Africa. Probably no country in Africa can validly claim to be truly free of rabies. As in much of the developing world, the story of infectious diseases in Africa, especially regarding zoonoses, is one of inadequate funds, shortage of experienced personnel, lack of equipment, and largely nonexistent modern communications resulting in inadequate surveillance. As a general observation, rabies has spilled over from dogs into wild canids and, as a result, they are now major maintenance hosts in many parts of Africa.

SOUTH AFRICA. Table 1.1 lists the main wildlife species involved in rabies maintenance and transmission in southern Africa. A semi-diagrammatic map (Fig. 1.2), displays the principal rabies vectors. In most African countries, rabies can usually be found wherever there are large human populations with their associated dogs. Prior to the late 1940s, southern Africa was essentially free of dog rabies. Sporadic and isolated cases in dogs, due to wildlife exposure, occurred in South Africa, but the numbers were insignificant (Snyman 1940).

In 1947, dog rabies appeared on the northernmost border of South West Africa (now Namibia) and, by

TABLE 1.1—Wildlife species considered to be likely reservoirs of rabies virus in southern Africa

Family	Common Name	Species
Canidae	Black-backed jackal	*Canis mesomelas*
	Side-striped jackal	*Canis adustus*
	Bat-eared fox	*Otocyon megalotis*
Herpestidae	Yellow mongoose	*Cynictis penicillata*
	Slender mongoose	*Galevella sanguinea*
	Suricate	*Suricata suricatta*
	Water mongoose	*Atilax paludinosus*
Viverridae	Small-spotted genet[a]	*Genetta genetta*
Felidae	African wild cat[a]	*Felis sylvestris*

[a]Rabies in these species is no longer common.

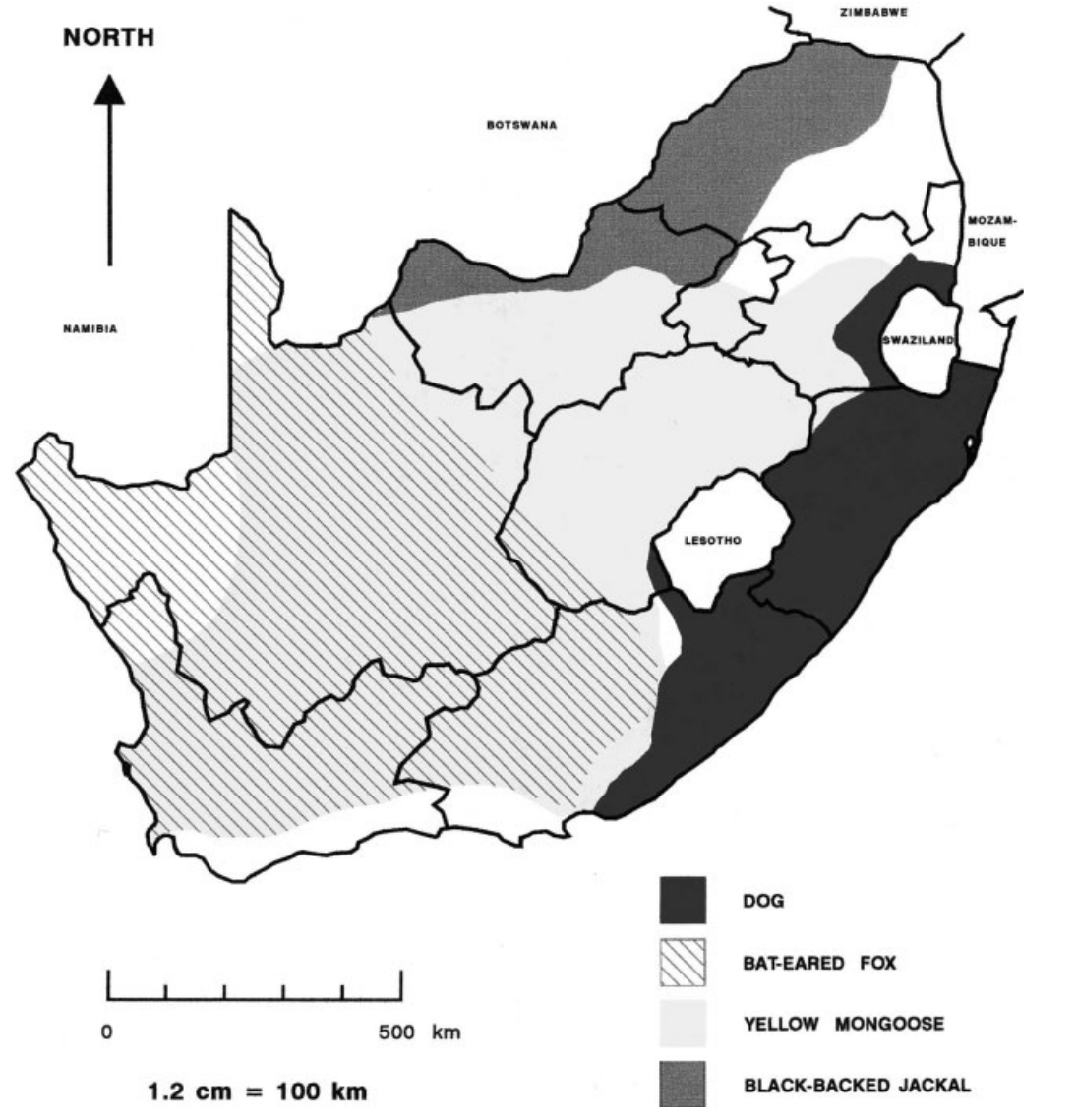

FIG. 1.2—Demonstration of the main vectors of rabies in the Republic of South Africa and the areas in which they predominate (Courtesy of G. Bishop).

1950, it had spread to the northern Transvaal province of South Africa. It reached Natal in 1961. Swanepoel (1994) showed that this epizootic followed the path of greatest human, and therefore domestic dog, population density.

The dog rabies virus variant readily transmits to wild Canidae and Hyaenidae, such as jackals *Canis* spp., bat-eared fox *Otocyon megalotis,* and aardwolf *Proteles cristatus.* Black-backed jackals *Canis mesomelas* and side-striped jackals *Canis adustus,* in Zimbabwe, and *C. mesomelas,*, in northern South Africa, now appear to be major reservoirs of the disease.

A different scenario has emerged in Namibia. As in Zimbabwe and the northern Transvaal, jackal species in the northern woodland savannah soon became major hosts. Southern Namibia is more arid and in this habitat the jackal is largely replaced by the bat-eared fox as a major host.

Sporadic cases of rabies in bat-eared fox in the Cape over the years were shown to be the viverrid variant of rabies virus (Meredith unpublished). This changed in 1970 when cases of what was probably canid strain rabies in bat-eared foxes appeared along the Orange River on the Namibian border. This infiltration continued steadily over the years in the form of a slow-moving epizootic, with substantial bat-eared fox mortality, now confirmed as being due to the canid variant of rabies virus (Thomson and Meredith 1993). Thus, the present situation in the Eastern Cape Province is now complex, with at least two variants of canid virus, that borne by domestic dogs and that derived from the bat-eared fox, superimposed on preexisting mongoose-borne viverrid rabies.

The relative importance of a particular species in any given region appears, in part, proportional to its population density. Thus, the yellow mongoose has a very wide distribution, and rabies occurs wherever it is found. However, it is of greatest importance as a rabies vector in the eastern grassland savannah where it is abundant, and it is progressively less important to the west in the arid, semidesert of the karroo where not only is food less readily available but the sparse, stony, and sandy soil does not favor burrowing. Consequently, colonies are more scattered and isolated.

In South Africa, endemic viverrid rabies is being investigated with anti-N Mabs against field isolates of virus and by nucleotide sequencing of the viral genome. Results to date have shown there to be at least four or five mongoose variants with distribution based, not on species alone, but also on geographic lines (King et al. 1994, Nel et al. 1994, von Teichman et al. 1995). These findings support a theory of genetic drift, that the endemic virus evolved over a long period, isolated in large, stable, nonmigratory mongoose populations where adaptation of both host and virus produced detectable viral variation in definable geographic areas. This situation is somewhat analogous to the phenomenon of compartmentalization seen in fox, skunk *Mephitis* spp., and raccoon *Procyon lotor* rabies in North America (Winkler 1975), but differing from that model in that apparent compartmentalization has also developed and radiated within a single species, based on distribution.

NORTH AFRICA. In Egypt, rabies viruses were reportedly isolated from three gerbils *Gerbillus gerbillus* and a fox *Vulpes* sp. (Botros et al. 1977). In a subsequent study, Botros et al. (1988) used N Mabs in an attempt to classify various rabies isolates from domestic animals and wildlife. Foggin (1988) in Zimbabwe found antibodies to Mokola virus in the sera of 13 of 141 gerbils *Tatera leucogaster* tested. Nowhere else in Africa have gerbils been implicated in serotype/genotype 1 rabies.

In another unusual finding, unreported species of rats were implicated in rabies epidemiology in Algeria (Benelmouffok et al. 1982). Support for this report came from Nigeria (Okolo 1988), where *Rattus* spp. were said to be frequently infected, but these data have not been substantiated.

Rabies virus has been isolated from the Ethiopian wolf or Simien jackal *Canis simensis*, which lives only in the Ethiopian highlands (Sillero-Zubiri et al. 1996). This endangered species consists of a population estimated to be fewer than 1000 and is severely threatened by loss of habitat, interbreeding with domestic dogs, and canine-borne diseases (Ginsberg and MacDonald 1990).

CENTRAL AND WEST AFRICA. A civet *Civettictis civetta* that died of rabies in a Nigerian zoo had been caught 14 days previously in an area reputed to be free of domestic dogs (Enurah et al. 1988). In a large survey, Bula and Mafwala (1988) found rabies in only a few wild species in Zaire: four monkeys, a hyena, and a bat. Apparently these last interesting diagnoses were not investigated further.

EAST AFRICA. The majority of rabies is in domestic dogs, though spillover into black-backed and side-striped jackal populations may have created a wildlife reservoir (Davies 1981). In Kenya, there have been cases in civet and honey badger *Mellivora capensis.* The relatively frequent occurrence of rabies in these two species demonstrates the potential influence of behavior on transmission. For example, the honey badger when molested is quite aggressive and will probably retaliate if attacked by a rabid jackal.

Rabies has also been recorded in spotted hyena *Crocuta crocuta,* bat-eared fox, and white-tailed mongoose *Ichneumia albicauda.* In recent years, African wild dogs *Lycaon pictus* have fallen victim to rabies through contact with domestic canids, with serious implications for survival of this endangered species (Gascoyne et al. 1993a,b). Both Kenyan and Tanzanian populations have been severely diminished by rabies (Kat et al. 1995). Maas (1993) confirmed rabies in bat-eared foxes in Serengeti National Park, Tanzania, in 1986.

WESTERN SOUTH AFRICA. Species involved in rabies are not significantly different than those in South

Africa (Berry 1993). Viverrid rabies, occurring mostly in yellow mongoose, is less common than in more favorable habitats where population densities are much higher. The main rabies reservoir today appears to be the black-backed jackal, as it is in Namibia. Endemic jackal rabies became such a threat to cattle ranching, with losses from a single infected jackal often amounting to ten or more head of cattle, that ranchers were often forced to immunize their livestock against rabies.

Game farming of kudu *Tragelaphus strepsiceros* and, to a lesser extent, eland *Taurotragus oryx* developed in association with cattle ranching. During 1977, high mortality from rabies was noticed in kudu (Barnard and Hassel 1981). Before this outbreak had run its course in 1985, an estimated 50,000 kudu died—approximately 20% of the total population (Hassel 1982; Hubschle 1988). The observation that sick kudu, usually salivating profusely, were the subject of licking and grooming by other animals in the herd led to experiments where it was shown that rabies could be easily transmitted by introducing saliva from infected animals into the buccal cavity (Barnard et al. 1982; Hassel 1982; Hubschle 1988). Control measures were not taken, but this unusual event subsided spontaneously, presumably once the population declined.

Despite the spectacular mammalian biodiversity for which Africa is noted, relatively few studies have implicated many indigenous wildlife species as important in the epidemiology of rabies. Notably, Felidae do not appear important as major reservoirs, although they are obviously competent vectors (Rupprecht and Childs 1996).

Asia and the Middle East. Throughout Asia, the overwhelming reservoir and principal cause of human fatalities is the domestic dog (WHO 1996, 1997). Wildlife, such as mongoose, jackal, fox, raccoon dog *Nyctereutes procynoides,* wolf, and various small mustelids, plays a comparatively much smaller role, at least as currently realized given surveillance limitations (Ahuja et al. 1985; Bahnemann 1985; Koesharyono et al. 1985; Lari 1985; Thongcharoen et al. 1985; Sama et al. 1986; Blancou 1988a). In contrast, with the possible exception of Bahrain and Qatar, wildlife rabies is fairly common in Syria, Jordan, Israel, Saudi Arabia, the United Arab Emirates, Yemen, Oman, Lebanon, Iraq, Iran, and Kuwait (WHO 1996). Rabies was not recorded from Oman and the United Arab Emirates until 1990, when the disease emerged in foxes *V. vulpes arabica* in the northeastern part of Oman and spread subsequently southward toward Salalah and to the north. Data on the number of human, domestic animal, and wildlife rabies cases are difficult to obtain (Fig. 1.3).

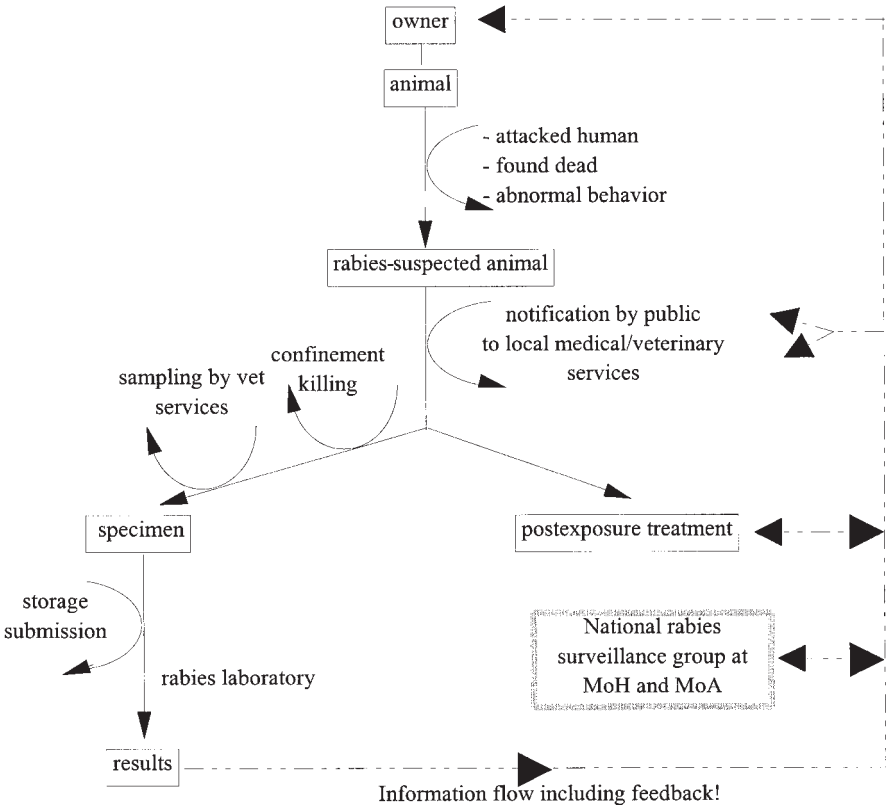

FIG. 1.3—Rabies surveillance and data flow chart. MoH, Ministry of Health; MoA, Ministry of Agriculture.

Wildlife appear to be important rabies sources in most Middle Eastern countries, with the red fox playing a major role. In particular, Israel, Saudi Arabia, Jordan, Oman, Yemen, and the United Arab Emirates reported increasing numbers of rabies cases in red foxes over the last decade. This may be related to improved surveillance, but could also be explained by an increased number and spatial spread of foxes due to habitat alterations and better availability of food and shelter. Most wildlife rabies cases in the Middle East are reported from red fox, wolf *C. lupus arabs*, and white-tailed mongoose. Rabies has also been diagnosed in numerous other wild carnivores, such as the wild cat *Felis silvestris,* fennec fox *Fennecus zerda,* striped hyena *Hyaena hyaena,* and golden jackal *C. aureus* (WHO 1996). Information on bat rabies in the Middle East is largely unavailable.

Fox rabies emerged in the Sultanate of Oman in 1990. Within 1 year, cases in foxes, wolves, mongoose, dogs, and cats were reported from several parts of the country. The disease spread throughout the fox population, which appeared to collapse in 1992, and the few rabies cases reported in foxes after 1992 suggested that the disease may have become endemic (Stöhr 1990, 1995; Novelli and Melankar 1991; Ata et al. 1993). Between 1986 and 1992, most rabies cases diagnosed in Saudi Arabia were from foxes (35%), cats (20%), and dogs (16%). Foxes appear to be a primary reservoir.

A small study of the status and biology of red and sand fox *Vulpes rueppellii* and other small carnivores in desert and semidesert environments was initiated and conducted by the National Wildlife Research Centre, Taif, in a reserve in the western part of the country (Olfermann 1995). The population density per square kilometer of *V. rueppelli* (0.25) and *V. vulpes* (0.07) was smaller than the minimum estimated as necessary to perpetuate rabies in foxes in Europe. However, population turnover was at about the same scale (50%) as found in Europe (66%). Sand foxes had a larger dispersal area and more extensive home range compared to European red fox (Lindsay and MacDonald 1986); consequently, contact may be more frequent between individuals due to overlapping ranges. Knowledge of these and related peculiarities of the fox populations of the Arabian Peninsula may help to explain the persistence of wildlife rabies in the area. Although wolf populations are reported to be high in the northern provinces and a considerable number of baboons *Papio* sp. exist in the eastern part of the country, both appear to be dead-end hosts for rabies.

Europe. As elsewhere, rabies occurs in various domestic and wild mammals in Europe, but there are only two primary reservoirs: terrestrial carnivores and insectivorous bats. Rabies is maintained rather independently in these two compartments, as substantiated by numerous virologic, genetic, and epidemiologic studies and several animal trials. This has been corroborated by the obvious success of oral immunization of foxes in Europe against rabies in areas where bat rabies is prevalent (Baer and Smith 1991; Brass 1994; Schneider and Cox 1994).

Dog rabies was controlled in western and central Europe in the early 1960s, but it still occurs in Turkey. Dogs appear also to be involved in rabies outbreaks in the European part of Russia and Romania, and to a smaller extent in Bulgaria and Lithuania (Koromyslow and Cherkasskiy 1990; Dranseika 1992; WHO 1994a; Cherkasskiy et al. 1995; Toacsen and Moraru 1995; Lalosevic 1996). In contrast, two wild mammalian species serve as major reservoirs for terrestrial rabies in Western Europe: red fox and, to a lesser extent in circumpolar areas, Arctic fox. The red fox is both the most important reservoir and victim of the disease in Europe (Fig. 1.4). Unlike most other wildlife reservoirs, this widespread, opportunistic, highly adaptable canid has

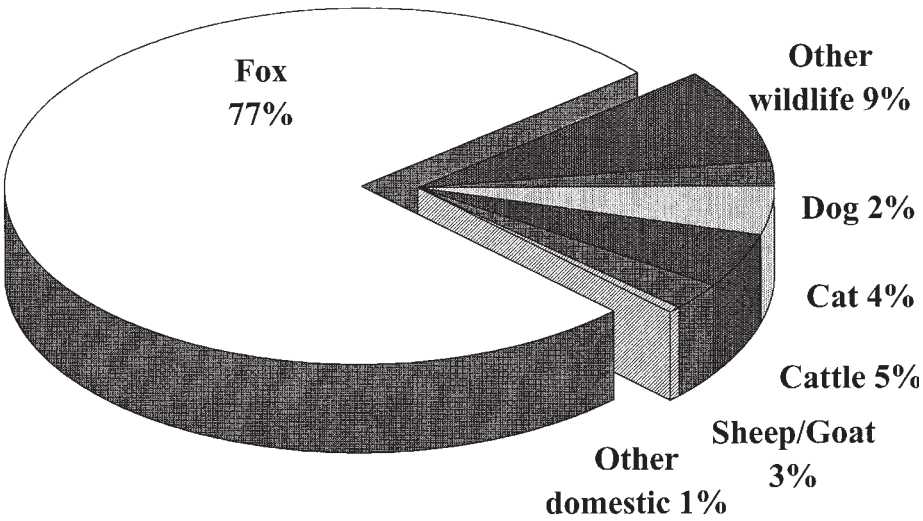

FIG. 1.4—Relative species involvement in rabies in Europe between 1977 and 1996.

been well studied. It possess flexible social structures and population dynamics that promote high conspecific contact and relatively short bursts of intensive, intraspecific transmission, making it an *ideal* rabies host (Winkler 1975; MacDonald 1980; Steck and Wandeler 1980; Anderson et al. 1981; Gessler and Spittler 1982; Blancou et al. 1991; Wandeler 1991; White et al. 1995).

Raccoon dogs are also involved in rabies dynamics, particularly in Eastern Europe and the Baltic, but little is known of the role they play in perpetuation of rabies (Cherkasskiy 1988; Blancou and Wandeler 1989; Westerling 1989; Koromyslow and Cherkasskiy 1990; WHO 1994a; Cherkasskiy et al. 1995). This highly adaptable canid originates from East Asia, but large numbers of the furbearer were released in the western part of the former Soviet Union between 1928 and 1955, and individuals gradually dispersed westward (Lavrov 1971; Nowak 1973). Approximately 100 rabid raccoon dogs were recorded annually over the last decade, representing about 5% of all rabies cases diagnosed in Poland. Similarly, in Estonia, raccoon dogs constituted about 30% of all rabies cases diagnosed in animals between 1991 and 1996. The raccoon dog was the species most frequently involved in a rabies outbreak in Finland during the late 1980s (Reinius 1992). Apparently higher population densities in eastern Russia, Finland, Latvia, Estonia, Lithuania, Ukraine, and Byelorussia, and increasingly more rabies cases diagnosed in raccoon dogs in some Eastern European countries, suggest a separate cycle of raccoon dog rabies, independent of foxes. Only limited data exist on susceptibility of raccoon dogs to the circulating rabies virus(es) in Europe (Blancou 1986, 1988b) and the corresponding susceptibility of foxes.

The first European case of rabies in a bat was reported in 1954, and an additional 14 cases were found in various insectivorous bat species by 1984 (Kappeler 1989; Selimov et al. 1989; Baer and Smith 1991; Brass 1994; Schneider and Cox 1994). Cases unexpectedly increased in 1985, with a peak in 1987, but subsequently declined; only 6–15 cases were reported annually in Europe from 1991 to 1996 (Fig. 1.5). A likely explanation for the sudden increase in bat rabies in Europe in 1985 appeared to be heightened public awareness and attention to bats as potential reservoirs (King 1991; Baer and Smith 1991; Brass 1994; Schneider and Cox 1994). Between 1954 and 1992, more than 85% of the bat rabies cases in Europe were diagnosed in *Eptesicus serotinus.* The reason for the observed higher prevalence of rabies in *E. serotinus* is unknown, but it is speculated to be related to colonial habits and roosting behavior (Brass 1994). The majority of rabid bats have been from Northern Europe, along the coast of the North Sea. Prior to 1996, rabies had not been reported in bats in the United Kingdom despite numerous examinations of bats found dead or ill (King 1991). In May 1996, however, a *Myotis daubentonii,* a species indigenous to the United Kingdom, was found near the south coast of England and was diagnosed as positive for EBV. Although there can be no certainty that the bat had not originated in England, it was suggested that the animal had flown across the English Channel from the mainland (Anonymous 1996a,b).

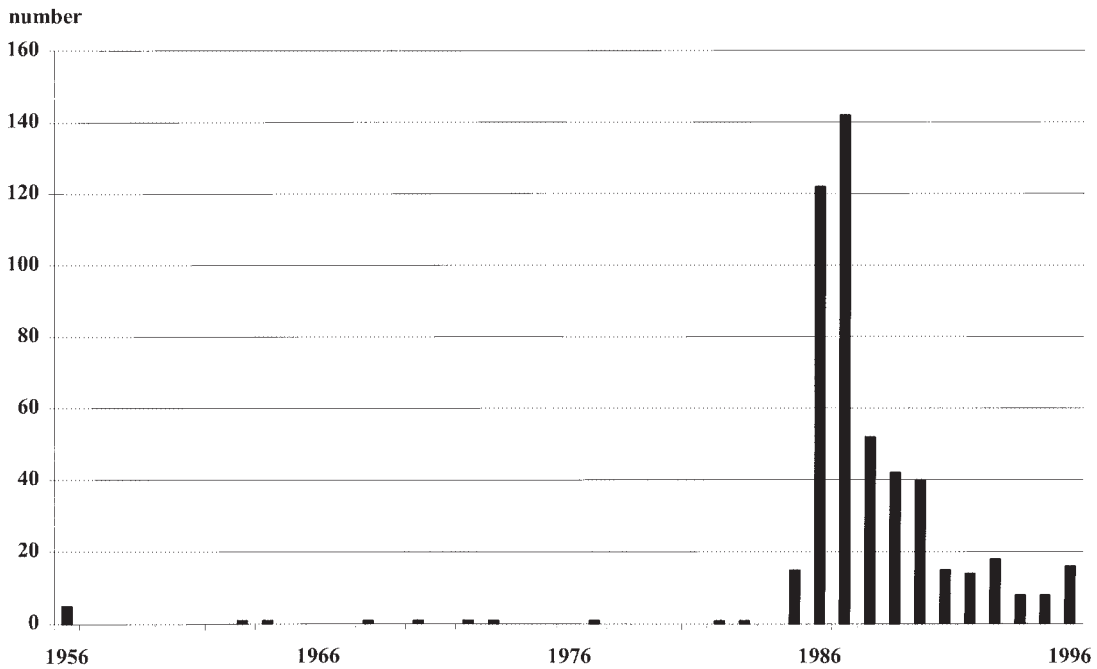

FIG. 1.5—Trends in the number of reported rabies cases in insectivorous bats in Europe.

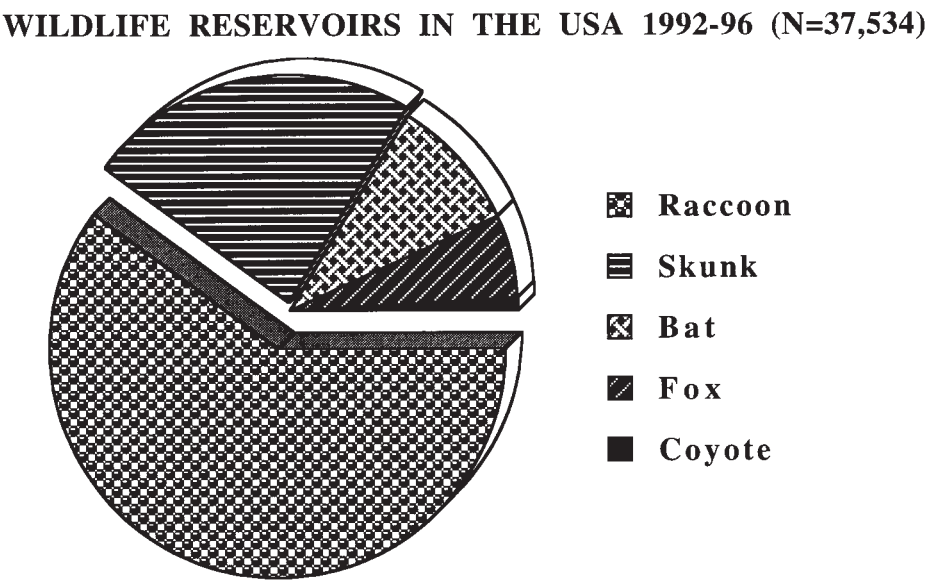

FIG. 1.6—Percent of rabies-positive specimens from various animal species, United States, 1992–96.

The Americas. The epidemiology of rabies in Canada and the United States has changed substantially in the last 100 years (Rosatte 1988; Smith et al. 1992; Rupprecht et al. 1995). Currently, more than 90% of all reported animal rabies cases occur in wildlife (Fig. 1.6), whereas for most of the century the majority of cases were reported in dogs (Krebs et al. 1997). Application of antigenic and molecular techniques has clearly demonstrated that major North American wild reservoirs are raccoons, coyotes *Canis latrans,* skunks, foxes, and bats of several species infected with many different types of viral variants (Smith et al. 1995). Wildlife rabies occurrence today is a multispecies complex, partially related to changes in human demography, animal translocation, environmental alteration, and viral adaptations over time. Epizootics are characterized by viral compartmentalization, usually to a single major host, with fairly discrete geographic boundaries, at least among the Carnivora (Smith 1996). Although many other mammals may be incidentally infected through contact with these reservoir species, such cases are quite sporadic. Once entrenched within a particular niche, viral transmission cycles may occur for decades to centuries (Tinline 1988).

In contrast to most of North America, only individual cases in several species, including fox, skunk, coyote, raccoon, coati *Nasua nasua,* puma *Felis concolor,* lynx *Felis lynx,* and a few others, have been reported throughout Latin America (Acha and Arambulo 1985; Fernandes and Arambulo 1985; Pan American Health Organization 1995), compared to the major reservoir, the domestic dog. More recent antigenic and genetic analyses of Mexican terrestrial rabies isolates demonstrate at least three distinct viral clades suggestive of long-term endemic circulation among local skunk or fox populations (de Mattos et al. 1996a), supporting the contention that a sylvatic rabies cycle may persist beyond dog-to-dog transmission (Loza-Rubio et al. 1996). This may represent a serious threat to the programmatic elimination of dog rabies in the Western Hemisphere (Cifuentes 1988; Pan American Health Organization 1995). For example, Uruguay has not reported a case of terrestrial rabies in some three decades, but the risk of viral acquisition from dogs in surrounding areas or infected bats remains, and wildlife rabies must be considered a possible mechanism for reestablishment of the disease among domestic animals.

Although several species of procyonids range throughout the Americas, endemic rabies has been associated only with raccoons and was initially reported in Florida populations during the late 1940s. This primary outbreak, recognized as a single major viral variant with uncertain origins, gradually spread over the next 30 years throughout the southeastern United States (Rupprecht and Smith 1994). Translocation of infected raccoons from the latter focus to the mid-Atlantic region during the late 1970s (Jenkins and Winkler 1987) led to an intensive raccoon rabies epizootic that continues to the present, stretching east of the Appalachians from New England to Alabama, Georgia, and Florida [Centers for Disease Control and Prevention (CDC) 1997a]. Raccoon rabies is still confined to the United States, and cases typically number between approximately 3000 and 6000 animals per year, usually peaking in the spring (Krebs et al. 1997). A new outbreak reported from eastern Ohio during the

spring of 1997 potentially threatens to engulf the entire upper Midwest, unless it can be controlled. No human rabies cases have been documented to date due to the raccoon rabies epizootic despite obvious vector competence (Winkler et al. 1985), but thousands of people receive rabies PEP due to known or presumed exposure to infected animals (Rupprecht et al. 1996b). Undoubtedly, rabies kills substantial numbers of raccoons and other animals, yet its role in population regulation, per se, and its effect on overall survivorship, are poorly understood (Brown et al. 1990).

In the United States and Canada, significant dog rabies control began during the 1940s. A portion of southern Texas bordering Mexico remains one of the few foci of dog rabies. Similar to African reports of outbreaks of jackal rabies from infected domestic dogs, during the late 1980s the number of cases of coyote rabies rose in this southern Texas border zone (Clark et al. 1994), resulting in the majority of coyote rabies reported from the United States between 1991 and 1996 (Krebs et al. 1997). The viral variant in coyotes is indistinguishable from that of infected border dogs (Smith et al. 1995). Previous observations in the United States and Canada during the early part of this century demonstrated that coyotes may support explosive rabies outbreaks (Rosatte 1988). One of the greatest dangers of this particular host is the possibility of continual spillover into the domestic dog population in a region of Texas in which companion animal immunity is less than ideal. In addition, translocation of infected Texas coyotes was thought responsible for at least one case of dog rabies in Alabama during 1993 and seven cases of apparent dog-to-dog rabies transmission in Florida during 1994 (CDC 1995). At least two human fatalities were associated with the outbreak in south Texas during 1991 and 1994, probably due to coyote-dog interactions with subsequent human exposure.

Rabies in skunks has persisted sporadically in the American plains since the last century (Gremillion-Smith and Woolf 1988; Charlton et al. 1991), extending into Canada by the late 1950s. It historically has been associated with human fatalities, perhaps because skunks may be tenacious, aggressive biters when infected. Although initial reports implicated the spotted skunk *Spilogale putorius,* the striped skunk *Mephitis mephitis* has a broader distribution, encompassing most of Canada, the United States, and northern Mexico. Although poorly documented, Greenwood et al. (1997) reported rabies may exert significant effects on skunk population characteristics, such as age structure and productivity. Little is known of the role that hooded *Mephitis macroura* or hognose *Conepatus leuconotus* skunks may play in rabies epidemiology; their northern distribution extends only into the southwestern United States. They may be more important in Mexico, but are infrequently reported rabid.

Between 1961 and 1989, skunks were the most frequently confirmed rabid animal in the United States; during 1996, 23% of the rabid animals reported in the United States were skunks (N = 1656) (Krebs et al. 1997). Annual cases range from 1000 to 4000, with peaks in spring and autumn. At least three major skunk rabies virus variants can be distinguished: north-central plains, lower Midwestern states, and California variants. Reports of rabies in skunks from southeastern Ontario, Quebec, and New England are associated with the rabies virus variant in red foxes; similarly, cases of skunk rabies in the eastern United States appear to be primarily due to spillover infection from raccoons. Skunks experimentally infected with a raccoon variant do not appear to shed virus over prolonged periods and may have decreased opportunity to infect a conspecific, compared to other rabies variants (Charlton et al. 1988).

Compared to the smaller carnivores, reports of rabies in wolves are infrequent, and the epidemiology of rabies in these large canids is poorly understood. Cases occur primarily in circumpolar regions, where human populations are generally sparse. In the United States, most cases have been reported from Alaska, averaging fewer than one case per year from 1980 to date (Krebs et al. 1997). At least in North America, it is unlikely that wolves act as a major rabies reservoir, given their disparate populations. No novel viral variants have been associated with wolves, strongly suggesting that such sporadic cases are spillover infections from rabid Arctic or red fox, as is thought to be the case for other affected taxa within this ecosystem (Taylor et al. 1991). Nevertheless, rabies may seriously alter or limit wolf populations (Weiler et al. 1995; Ballard and Krausman 1997), and rabid wolves are a public health concern. Wolf bites tend to be broad, deep, multiple, and often involve severe wounds to the head. Infected wolves have traditionally been recognized as exceptional in their ability to expose large numbers of people, often associated with significant human mortality, especially in the Old World.

Although other lyssaviruses occur in bats elsewhere, only *Lyssavirus* serotype/genotype 1, rabies virus, has been confirmed in bats from the New World. No doubt related to the prominent notoriety ascribed to vampire bat rabies during the 1930–40s, major interest in insectivorous bat rabies was delayed until the first diagnosis in a bat from Florida during 1953. Consequently, bat rabies was reported throughout the United States and Canada. The history of this early spread was recently reviewed (Brass 1994). Between 600 and 1000 cases of rabid bats are typically diagnosed annually (Krebs et al. 1997). Unlike the disease in carnivores, however, specific geographic boundaries cannot be readily defined for bat rabies. Variants associated with particular bat species often can be found throughout a species migratory range. For example, rabies virus in free-tailed bats *Tadarida brasiliensis* shows minimal variation throughout the southern United States. Most areas of the United States, with the exception of Hawaii (Sasaki et al. 1992), represent a complex mosaic of different bat species affected by distinct rabies variants (Smith et al. 1995). The proportion of rabid bats from those submitted to diagnostic laboratories typically ranges from 5% to 15% (Pybus 1986; Childs et al. 1994; Feller et al.

1997). Additionally, the occurrence of bat rabies appears largely independent of rabies in terrestrial carnivores (Rupprecht et al. 1987; Baer and Smith 1991), although viral spillover to animals besides bats (Daoust et al. 1996) occurs occasionally, such as in cats or gray fox (Smith et al. 1995).

Clearly, the public health significance of insectivorous bats as zoonotic reservoirs is small in comparison to their overall ecological utility, but bats have accounted for an increasing proportion of recent human rabies cases in the United States (CDC 1997c). In Latin America, beyond the well-studied cases in hematophagous bats, rabies has only been infrequently reported from other Chiroptera. However, there is little reason to believe that the tropical viral biodiversity present among Latin American bats will be dissimilar from that noted for North American species, once adequate surveillance has been initiated (Almeida et al. 1994; Diaz et al. 1994; Martorelli et al. 1995, 1996; Uieda et al. 1995; de Mattos et al. 1996b).

CLINICAL SIGNS. There are no definitive or species-specific clinical signs of rabies beyond acute behavioral alterations (Blancou et al. 1991; Charlton et al. 1991; Winkler and Jenkins 1991; Brass 1994); as noted by Sikes (1981) in the previous edition of this book, "the atypical is typical ... ." Severity and variation of signs may be related to the specific site(s) of the primary CNS lesion(s) or to viral strain, dose, and route of infection (Smart and Charlton 1992; Hamir et al. 1996a). At the end of the incubation period, the disease progresses through a short nonspecific prodromal stage to encephalopathy and death within days. The prodromal period usually follows a bite by several weeks; however, periods of less than 10 days to several months are well documented (Charlton 1994). Severe bites to the head and neck, and bites to highly innervated areas, may result in shorter incubation periods. Local cutaneous signs may consist of paresthesia at the site of the exposure, resulting in self-inflicted wounds, probably due to viral excitation of sensory ganglia. Nonspecific clinical signs may include restlessness, inappetance, dysphagia, vomiting, or diarrhea.

An acute neurologic period usually follows the brief prodromal period by 1–2 days. A generalized excitative increase in neurologic activity is observed that is associated with hyperesthesia to auditory, visual, or tactile stimuli and sudden and seemingly unprovoked agitation and extreme aggressive behavior (furious rabies) to animate or inanimate objects. Wildlife may lose their apparent wariness and caution around humans and domestic species, alter their activity cycles, seek solitude, or become more gregarious. Head tilt, head pressing or butting, "stargazing," and altered phonation may be observed. Profound characteristic clinical manifestations in human rabies (Hemachuda 1994), specifically true hydrophobia and aerophobia, which are exaggerated respiratory protective reflexes that result in contractions of the diaphragm and inspiratory accessory muscles triggered by attempts to swallow, the sight or sound of water, or air currents have not been reported in other animals. Cranial nerve manifestations may include facial asymmetry, trismus, choking, a lolling tongue, drooling, drooping of the lower jaw, nictitans prolapse, and anisocoria. There may be additional CNS excitation with hyperactivity, disorientation, confusion, photophobia, pica, incoordination, and convulsive seizures. Autonomic systemic excitation may also result in labile hypertension, hyperventilation, muscle tremors, priapism, altered libido, hypothermia, or hyperthermia.

A paralytic phase (dumb rabies) may follow the agitated or furious phase, or the animal may progress directly to the paralytic phase from the prodromal stage. Increasing prostration, lethargy, paresis, frequent urination or incontinence, tenesmus, constipation, tail flaccidity, or decreased spinal reflexes may be apparent. Ataxia may be observed as knuckling or swaying. Ascending, flaccid, symmetrical or asymmetrical paralysis leads eventually to respiratory and cardiac failure. Hypothalamic and hypophyseal dysfunction may contribute to acute organ wasting, such as cardiomyopathy. Ultimately, rabies most often culminates in coma and generalized multiorgan failure that leads to death 1–10 days (or rarely more) after primary signs or may result in acute death with no premonitory signs.

PATHOGENESIS. Lyssaviruses are the penultimate neurotropic agents (Jackson 1994). For at least two centuries, scholars appreciated that rabies is caused by entry of a novel agent into the body, specifically that saliva contained the infectious agent, and that nerves were the means by which the agent traveled to the CNS (Baer et al. 1996). Nerve destruction by limb amputation, cautery, or surgical sectioning and microtubule-disrupting agents can inhibit axonal transport and prevent disease, indicating that centripetal viral spread from the site of entry to the spinal cord occurs within both motor and sensory axons of peripheral nerves via primary innervation pathways (Baer 1988a).

Lyssavirus replication is believed similar to that of other negative-stranded RNA viruses that have been more intensively studied, but genus-specific peculiarities are likely to be uncovered eventually because of their relatively restricted CNS niche. After exposure, the precise mechanism of entry of virus into the nervous system is controversial. Some data support the assertion that virus binds near the acetylcholine receptor via neuromuscular junctions (Lentz et al. 1983; Spriggs 1985; Baer et al. 1990). These sites are in close proximity to unsheathed nerves at synaptic clefts where virus can gain access to the axoplasm. This hypothesis does not preclude alternative mechanisms, because other protein-based receptors can also bind virus, and acetylcholine receptors are not exclusively found at sites permissive of virus infection (Reagan and Wunner 1985).

Following viral entry, usually through a bite or less frequently via mucous membranes, attachment to cell

membranes can occur by conformational changes in the G protein during receptor-mediated fusion (Flamand et al. 1995). After adsorption, particles may penetrate the cytoplasm within an endosome and uncoat to RNP under low-pH conditions (Gaudin et al. 1993). In ribosome-rich locations, such as in the perikaryon or proximal dendrite, the viral RNP may initiate primary transcription. After synthesis, replication of the genomic RNA continues with the production of positive-stranded RNA, acting as a template for progeny negative-stranded RNA. Within the CNS, Lyssavirus replication has been associated almost exclusively with neurons, and there is little evidence to suggest replication in other cells. Viral assembly, and budding of the virion from the infected cell, may occur by the reverse process of initial viral attachment. Notwithstanding that a high proportion of some viral amino acid substitutions may be tolerated while still retaining molecular fidelity, even small alterations at certain antigenic locations may be critical in the determination of function, in part because of molecular influences on secondary protein structure (Coulon et al. 1994). In fact, as little as a single amino acid substitution can have substantial impact on viral virulence (Dietzschold et al. 1983).

The incubation period of rabies, usually 1–3 months following exposure, may vary from as little as several days to several years (Smith et al. 1991), but it is rarely more than 6 months. In nature, this sporadic, unusually slow course of infection in an individual host may assure rabies virus survival in vivo until new susceptible generations gradually reach a density supportive of efficient transmission at the population level. Longer incubation periods may also allow the selection of a specific virus population that can effectively multiply in the salivary glands and be readily excreted in the saliva (Charlton et al. 1984).

The precise location of viral residence during the incubation period is undetermined. Virus may replicate in muscle tissue during early stages of infection, prior to entry into nerves, or it may remain at the site of entry for a prolonged time (Baer 1988a). However, virus replication in muscle cells prior to entry of neurons is not required for development of CNS infection (Shankar et al. 1991). Obviously, these somewhat conflicting observations explain neither the site, the form, nor the mechanism by which virus can exist after exposure during an eclipse phase prior to CNS replication. A number of factors, alone or in combination, may be operative. For example, virus may be sequestered at the original site of entry, undergoing minimal or no replication until a stimulus triggers viral activity in proximity to a peripheral nerve. Virus may persist at the level of the dorsal root ganglia and involve viral RNP, with little replicative activity due to defects in the available biochemical milieu. In contrast, sequestration sites may be nonneural in origin (Ray et al. 1995). Additionally, if overt disease has an immunologic basis, the incubation period may equate to a dynamic stasis perhaps related to delayed immunologic surveillance via antigen-presenting cells. In theory, inocula may also contain defective interfering particles that limit neuronal access by virions until after some variable delay period. Regardless of mechanism, unusually long incubation periods tend to be the exception.

Once CNS neurons become infected, the virus disseminates rapidly along neuronal pathways (Iwasaki 1991; Charlton et al. 1996). Infection spreads to the brainstem, deep cerebellar nuclei, Purkinje cells, and neurons in the cerebral cortex. Involvement of the hippocampus may occur relatively late, primarily infecting pyramidal neurons with little involvement of the dentate gyrus. There also appears to be a predilection for the limbic system, the reticular formation, the pontine tegmentum, and the nuclei of the cranial nerves at the floor of the fourth ventricle. At the height of CNS infection, the virus may traverse back to the peripheral nerves and centrifugally invade highly innervated areas such as the cornea, skin (especially of the head and neck), salivary glands, tonsils, and buccal membranes of the oral cavity (Schneider 1991). There is pronounced secretion of the virus into saliva at a time when agitation and aggressive biting behavior may be present, increasing the odds of viral transmission.

Late in infection, rabies virus may occasionally be found in tissues other than the CNS and salivary glands, such as the adrenal medulla and nasal glands (Balachandran and Charlton 1994). Terminally, practically all innervated organs contain virus (Charlton 1994).

Rabies has one of the highest case-fatality ratios of any infectious disease and is usually considered to be invariably fatal. However, survival from rabies, usually with severe neurologic sequelae, has been documented in a few naturally or experimentally infected cases (Fekadu 1991).

A primary difference in the pathogenesis of rabies between reservoir hosts and dead-end or victim species may not be found. Rather, the explanation for the propensity of particular taxa to fall into these categories may be associated with factors other than the nature of the infection itself and the resultant probability of viral excretion in the individual animal (Wandeler et al. 1994). Some species may be more or less likely to withstand overt aggression by a rabid individual, and small prey species may not easily survive attack by larger predators. Distinct behavioral patterns of small mammal and ungulate species, such as secretive activity or wariness of and ability to escape from rabid predators, may influence the dynamics of rabies. In addition, anatomic differences can affect the ability of an animal to serve as a reservoir, including the effective penetration of virus-laden saliva deep into muscle via the canines and carnassial teeth; the extreme contrast between Carnivora and Edentata is obvious (Leffingwell and Neill 1989).

Rather than simple destruction of cellular elements, select organic dysfunction, owing to pathophysiologic disruption in critical neuronal activities and neurotransmitter imbalance (Tsiang 1993) or programmed cell death as an attempted response to limit infection (Jackson and Rossiter 1997), may ultimately explain the

underlying mechanisms of virulence. Also, recognition is needed of the functional interaction between the immune system and the CNS during health and disease. For example, during rabies virus encephalitis, brain receptors for some cytokines may decrease while local concentrations are increasing; moreover, copious amounts of nitric oxide may be produced locally in the CNS in response to viral infection (Koprowski et al. 1993).

PATHOLOGY. No in-depth clinical chemistry investigations have been reported for naturally infected wildlife, and the direct relevance of human rabies data to free-ranging wildlife is questionable. Results of routine clinical laboratory tests are usually nonspecific. Glycosuria may be detected as may prominent lymphocytic pleocytosis of the CSF (Hanlon et al. 1989).

Despite its acute nature, often bizarre clinical manifestations, and virulence, the pathologic findings associated with rabies are usually relatively mild (Rupprecht and Dietzschold 1987). Depending on the disease course, there may be evidence of multiple fresh or healed bite wounds; gross trauma from self-mutilation; foreign bodies in the mouth, esophagus, and stomach; the gastrointestinal track may be devoid of contents; and, in geographic areas where they occur, porcupine quills may be in the muzzle. Results of external examination may be normal, except for an unusual odor (Hubbard 1985), perhaps due to poor hygiene in terminal disease. Typically, few internal gross pathologic changes are visible, other than mild cerebral edema, focal meningeal congestion and thickening, or subtle suggestions of hemorrhagic myelitis.

Nonspecific inflammation of variable severity can be observed in the brain, spinal cord, and ganglia, or it may be lacking (Hamir and Rupprecht 1990a). Severity of infection and, to some extent, the location of pathologic changes may be proportional to the duration of illness. Inflammatory infiltrates, consisting primarily of lymphocytes, macrophages or, rarely, eosinophils may be in the leptomeninges (Hamir and Rupprecht 1990b). Lesions of nonsuppurative encephalomyelitis, including perivascular cuffing, focal to diffuse gliosis, neuronophagia, and rare to modest neuronal necrosis, tend to be sparse in relation to the distribution of viral antigen detected by immunofluorescence (Perl and Good 1991).

Standard light microscopy is focused on identification of Negri bodies which are eosinophilic intracytoplasmic inclusion bodies in infected neurons. Negri bodies are haloed, and may be round, ovoid, ameboid, triangular, or oblong and pink to purple, depending on the stain. For example, Seller's stain produces classic magenta bodies with dark interior basophilic granules arranged in a rosette pattern (Velleca and Forrester 1981). Negri body development is usually directly related to duration of illness. These inclusions are detected in 50%–75% of specimens positive by virus isolation, immunofluorescence, or electron microscopy. Negri bodies are seen most frequently in Purkinje cells in the cerebellum and pyramidal cells in the hippocampus, and less often in medulla oblongata, spinal cord, cerebral cortex, basal ganglia, and peripheral ganglia.

Spongiform lesions of the gray matter, affecting the neuropil and neuronal cell bodies of the thalamus and cerebral cortex, have been documented in experimental and natural rabies cases (Charlton 1984). These should not be confused with lesions of transmissible spongiform encephalopathy (Bundza and Charlton 1988).

In rabies cases, typical rod- or bullet-shaped viral particles are observed most often in CNS and salivary glands (Gosztonyi 1994). Mature virions are approximately 75–80 × 180–200 nm. Negri bodies identified by light microscopy consist of viral nucleocapsids and correspond to matrices seen en masse by ultrastructural microscopy. These matrices correspond to the site of virus replication. Neurons containing viral matrices may have large numbers of viral particles budding from membranes of the rough endoplasmic reticulum and the plasma membrane.

DIAGNOSIS. No clinical signs are pathognomonic for rabies, and postmortem laboratory examination for evidence of lyssaviruses is the only method of definitive diagnosis in animals (Smith 1995). Antemortem methods for detection of virus by skin biopsy, corneal impressions, saliva collection, etc., may be used to confirm a clinical suspicion, but a negative test result does not rule out the possibility of rabies (Blenden et al. 1983).

Given its public health, veterinary, and wildlife management implications, rabies is usually considered a reportable disease, and a rapid, accurate, and inexpensive diagnostic method is highly desirable. Suspect animals should be euthanized in a manner so as not to damage the brain. The head, brain, or carcass of small mammals such as bats should be sent under refrigeration to the diagnostic laboratory. Freezing is not recommended, because it may delay testing until samples thaw. Alternatively, brainstem cores may be sampled in place of the entire organ (Hirose et al. 1991). In field surveillance programs, particularly in tropical regions, samples may be preserved in a 50% saline-glycerine solution.

The FAT for rabies, originally developed by Goldwasser and Kissling (1958) and now refined (Velleca and Forrester 1981; Trimarchi and Debbie 1991), is a rapid, sensitive, and specific diagnostic method used globally. Slide impressions or smears of brainstem, hippocampus, and cerebellum are immersed in fixative (usually cold acetone), before the addition of either fluorescein-labeled monoclonal or polyclonal antirabies virus reagents, and are examined by direct fluorescence microscopy. Typically, reliable results can be obtained in 2–4 hours. Test sensitivity is affected by tissue decomposition and a number of other variables. Use of salivary gland instead of brain to detect viral antigen is not recommended. The FAT on brain can also be used after formalin or other chemical fixation, but it is not

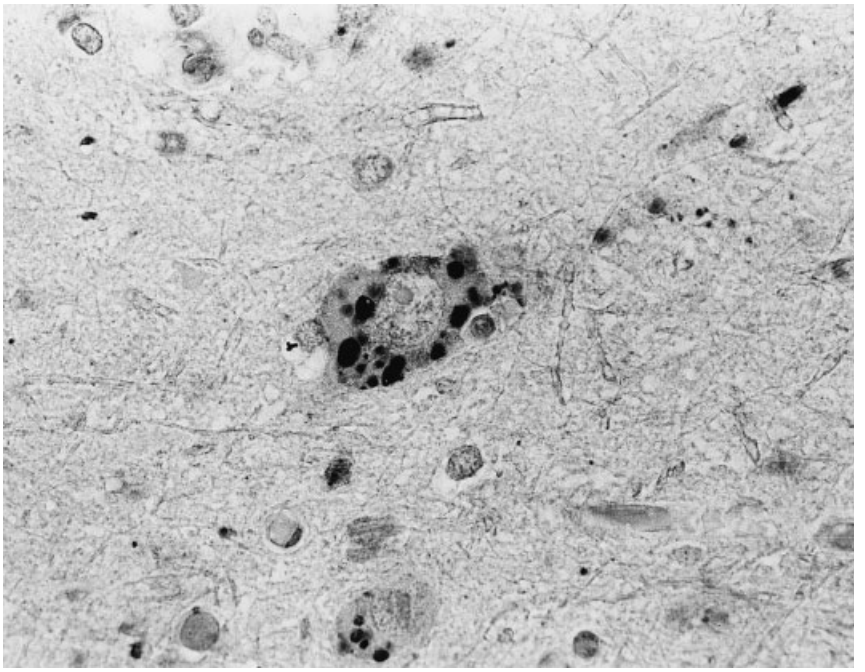

FIG. 1.7—Rabies virus intracytoplasmic inclusions (400×) in a Purkinje cell of an infected striped skunk *Mephitis mephitis,* stained by immunohistochemistry (Courtesy of M. Niezgoda).

routine, and special techniques are necessary for paraffin-embedded sections (Warner et al. 1997). Besides the FAT on formalin-fixed tissue, immunohistochemical methods of diagnosis, employing either a peroxidase or an avidin-biotin complex and Mabs (Fekadu et al. 1988; Hamir et al. 1992, 1996b), can also reliably detect rabies-specific inclusions by light microscopy (Fig. 1.7).

Routine histologic examination of paraffin-embedded brain tissues may demonstrate nonsuppurative encephalitis and Negri bodies. However, microscopic examination for Negri bodies alone may produce false-negative results because not all cases develop inclusion bodies, or false-positive results may be due to nonspecific inclusion bodies (Nietfeld et al. 1989).

Animal inoculation (Koprowski 1996) or cell culture (Rudd and Trimarchi 1987; Crick and King 1988; Sureau et al. 1991) can be used for virus isolation. These techniques are used infrequently.

A variety of serologic procedures are available for detecting antibodies to lyssaviruses (Smith 1991), such as the rapid fluorescent focus inhibition test (Smith et al. 1996) for detection of virus-neutralizing antibodies (VNAs), or enzyme-linked immunosorbent assays (ELISAs) (Barton and Campbell 1988). Demonstration of rabies VNAs in serum only indicates exposure to viral antigen. Antibodies are rarely detected until late in the clinical course of disease. Conversely, induction of VNAs may be evidence of immunity, either from natural exposure or to vaccination. Rabies VNAs are not produced in the CSF from vaccination; their presence in association with suggestive illness supports a diagnosis of rabies. The rare discovery of antibodies in the CSF of a healthy animal is strongly suggestive of prior illness and recovery (Fekadu 1991).

Modern molecular techniques, such as RT/PCR for detection of lyssavirus nucleic acid (Sacramento et al. 1991; Smith et al. 1992), may serve as an adjunct or confirmatory test to routine rabies diagnostics. However, the extreme sensitivity of these techniques greatly increases the probability of false-positive results due to laboratory contamination or technical error. Additionally, because of the diversity of lyssaviruses, false-negative results can occur if initial primer selection is inadequate to compensate for sequence heterogeneity. Moreover, universal primers for all known lyssaviruses have not been identified (Nadin-Davis et al. 1996). Considering these factors, related costs, and the research expertise necessary for proper analysis and interpretation, such molecular techniques are not recommended for routine primary rabies diagnosis at the present time (WHO 1995).

Given the aforementioned diagnostic tools, a case definition of rabies is supported by either virus isolation, detection of viral antigen or nucleic acid, or demonstration of rabies-specific antibodies in the CSF in a clinically suspect animal.

DIFFERENTIAL DIAGNOSES. Rabies should be strongly considered among the differential diagnoses

of any suspected mammalian encephalitis, especially in such high-risk taxa as the Carnivora and Chiroptera. Depending on the species in question, rabies may be clinically confused with a number of acquired conditions: viral (e.g., canine distemper, infectious canine hepatitis, pseudorabies, feline infectious peritonitis, feline panleukopenia, malignant catarrhal fever, herpes myelitis, equine encephalitides, or borna disease), bacterial (e.g., botulism, tetanus, or listeriosis), mycotic (e.g., cryptococcosis or blastomycosis), protozoal (e.g., toxoplasmosis or coccidiosis), helminth (e.g., baylisascariasis, parelaphostrongylosis, or verminous encephalitis), or others (e.g., chronic wasting disease and other transmissible spongiform encephalopathies, tick paralysis, intervertebral disc disease, degenerative encephalomyelopathy, or lymphosarcoma). Rabies may be mimicked by a multitude of other diseases whose origins may be traumatic (e.g., gunshot, hit by car, esophageal foreign body, or petrous temporal fracture), toxic (e.g., heavy metals, chlorinated hydrocarbons, or lathyrism), or physiometabolic (e.g., ketosis, polioencephalomalacia, or ischemic encephalopathy).

IMMUNITY. Immunity to rabies involves nonspecific defenses, such as intact, heavily furred, or armored skin that aids in protection against bites, and specific induced responses (Lodmell 1983; Xiang et al. 1995). Viral exposure may or may not lead to a productive viral infection, which may or may not result in detectable immune responses (Niezgoda et al. 1993). The ultimate outcome depends in part on complex interplay of viral and host factors (Nathanson and Gonzalez-Scarano 1991). During the centripetal transport of rabies virus to the brain, especially during long incubation periods, insufficient antigenic mass may be detected by the immune system to induce an appropriate response. In contrast, highly neural invasive strains might be quite immunogenic, but the host response may be slow or inadequate to prevent or clear CNS replication, given an immunologically privileged location of replication. Rabies-specific VNAs may be detected in sera at the onset of illness, but they usually are not detected, if at all, until the terminal stages of disease. These VNAs may interfere with salivary excretion of infectious virus (Charlton et al. 1987). Antibody detection in the CSF of a rabies-suspect animal is considered a reliable indication of present or past CNS infection.

The role of acquired immunity to rabies in naturally exposed wildlife is not known. Red foxes seldom have VNAs, generally less than 4%–7% of surveyed populations (Blancou et al. 1991), which is similar to the seroprevalence of 2%–10% in some insectivorous bats (Trimarchi and Debbie 1977). In epizootic areas, however, presence of VNAs varied from 1% to 3% (Winkler and Jenkins 1991) to more than 20% (Bigler et al. 1983) of raccoons sampled. In Grenada, presence of rabies VNAs ranged between 9% and 55% of surveyed mongoose, and seroprevalence was inversely proportional to the number of rabid mongoose reported. Mongoose with preexisting VNAs responded with higher titer on rabies vaccination (Everard and Everard 1988). The foregoing observations suggest that natural immunity to rabies varies (Rosatte and Gunson 1984; Black and Wiktor 1986; Orr et al. 1988; Follman et al. 1994) from essentially none to a potentially critical mechanism responsible for a robust host-parasite equilibrium (Hill and Beran 1992; Hill et al. 1992, 1993), depending in part on the species and virus variant (Niezgoda et al. 1997).

Induction of protective antiviral immunity is primarily based on response to the G protein. This protein has received considerable study because it forms protrusions that cover the outer surface of the viral envelope and is responsible for reception on the cell membrane and induction of pH-mediated endocytosis (Wunner 1991). It is the only lyssavirus protein known to induce specific VNAs. It also plays a role in eliciting cell-mediated immunity. However, there is not always a clear relationship between level of VNA and resistance to rabies, suggesting that other antigens and immune effector mechanisms are likely involved in protection against lethal virus infection.

Identification of the relative contributions of the complex lyssavirus antigens in humoral and cellular immunity has been slowly delineated and generally based on human immune responses (Celis et al. 1986, 1988; Ertl et al. 1989, 1991; Herzog et al. 1991; Perry and Lodmell 1991). Intact virus and purified viral N, G, NS, and μ proteins can induce high lymphokine secretion. Additionally, T-lymphocyte clones of the helper/inducer class may react not only with classic fixed rabies viruses but also with Duvenhage and Mokola viruses, and many recognize viral RNP determinants. Rabies cytotoxic T-cell responses occur against antigenic determinants of both RNP and G proteins. T-cell clones which react to several lyssaviruses may recognize closely situated epitopes presented in the context of the same major histocompatibility complex molecule.

Certain internal lyssavirus antigens may possess an inherent capacity to enhance immune responsiveness. These have been characterized either as superantigens (Lafon 1994) or as powerful adjuvants (Hooper et al. 1994). In the absence of other antigens, rabies RNP is capable of inducing protection against virus challenge without induced VNAs and can confer immunity against infection by heterologous lyssaviruses (Dietzschold et al. 1987).

Exploiting immunologic advances, modern rabies vaccines maximize potency, purity, safety, and efficacy and minimize cost. Although older modified-live virus vaccines were effective, did not require adjuvant, and induced long-lasting immune responses that involved a full spectrum of immune effectors, they also traditionally carried the risk of vaccine-induced disease especially in the immunocompromised host. Conversely, inactivated animal neural tissue vaccines eliminated the potential for vaccine-associated rabies, but were traditionally hampered by low potency and adverse reac-