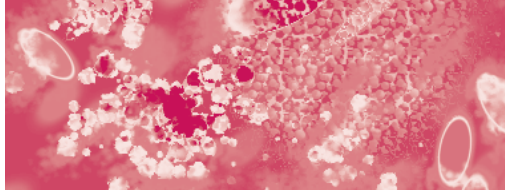

Rabies re-examined

Charles E Rupprecht, Cathleen A Hanlon, and Thiravat Hemachudha

Rabies is an acute, progressive, incurable viral encephalitis. The causative agents are neurotropic RNA viruses in the family Rhabdoviridae, genus Lyssavirus. Mammalian reservoirs include the Carnivora and Chiroptera, but rabid dogs still pose the greatest hazard worldwide. Viral transmission occurs mainly via animal bite, and once the virus is deposited in peripheral wounds, centripetal passage occurs towards the central nervous system. After viral replication, there is centrifugal spread to major exit portals, the salivary glands. The epidemiological significance of any host "carrier" state remains highly speculative. Although incubation periods average 1–3 months, disease occurrence days or years after exposure has been documented. Rabies should be suspected in patients with a concomitant history of animal bite and traditional clinical presentation, but a lack of such clues makes antemortem diagnosis a challenge. Pathogenetic mechanisms remain poorly understood, and current care entails palliative measures only. Current medical emphasis relies heavily on prevention of exposure and intervention before clinical onset. Prophylaxis encompasses thorough wound treatment, vaccine administration, and inoculation of rabies immunoglobulin. Although it is a major zoonosis, canine rabies can be eliminated, and application of new vaccine technologies permits significant disease control among wildlife species. Nevertheless, despite much technical progress in the past century, rabies is a disease of neglect and presents a modern public-health conundrum.

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Despite continued attempts at medical intervention, rabies retains the dubious distinction of being the infectious disease with the highest case–fatality ratio.[1] The population at risk ranges from children to old people, from wealthy to poor, from Argentina to Australia, and from New Delhi to New York. Everyone is at risk—status, national borders, occupation, hobby, religious persuasion, and political affiliation are no barrier.[2–5]

Compared with AIDS, tuberculosis, and malaria, does rabies really claim substantial numbers of victims? Even by rudimentary surveillance, one person dies from the disease each 15 minutes, and more than 300 others are exposed. There is a substantial threat to residents and travellers alike, primarily in tropical and subtropical regions, from an entity not thought to be a primary human contagion. Breakthroughs in rabies-vaccine development may have abated the fear surrounding the disease, and lowered its apparent medical impact nowadays, at least to the ignorant and to the privileged few in the more developed countries. However, viral evolution ensures survival among a plethora of hosts. Every mammal studied to date is susceptible, with the domestic dog as the main reservoir. Rabies continues to re-emerge and is often exacerbated despite our best intentions. From a global perspective, given the widespread distribution, public-health concerns, veterinary implications, and economic burdens, rabies is the most important viral zoonosis.[6] Why do we not destroy the offending reservoirs? Rather than use outdated and simplistic methods designed solely to kill animals non-specifically, in the name of disease control, some national authorities have turned to programmes focusing on the creation of herd immunity among species reachable by parenteral immunisation and the strategic distribution of vaccine-laden baits for free-ranging animals; such approaches can serve as integrative models for successful disease intervention.[7]

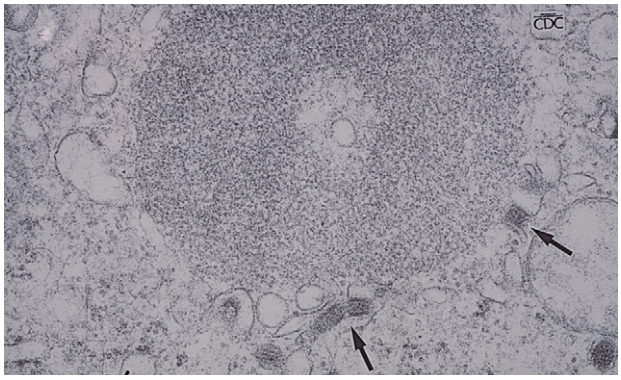

Figure 1. Electron micrograph of an Australian bat virus isolate (family Rhabdoviridae, genus Lyssavirus), showing the typical rod-shaped morphology (arrows) of virions in the cell cytosol.

The aim of this review is a re-examination of rabies, its epidemiology, diagnosis, prevention, and control in the more and less developed countries, placed in historical context. It accompanies a review in *The Lancet Neurology* on the neurological approach to patients with suspected rabies.[1]

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A companion review of the neurological aspects of rabies appears in the June issue of *The Lancet Neurology* (2002; **2:** 101–09)

Table 1. Notable events in the 20th century history of rabies

Period	Events	References
1900s	Intracytoplasmic inclusions observed	Negri (1903)
	Demonstration as a filtrable agent	Remlinger (1903)
	Neural spread to salivary glands recognised	Bartarelli (1904)
	Nervous-tissue vaccine preparation improved	Fermi (1908)
1910s	Primary tissue-culture techniques applied	Noguchi (1913); Levaditi (1913)
	Nervous-tissue vaccine production stabilised	Semple (1919)
	Bat rabies in New World suspected	Carini (1911)
1920s	Mass rabies vaccination of dogs in Japan	Umeno and Doi (1921)
	Disease eliminated from UK	Ritchie (1969)
	Staining of Negri bodies improved	Sellers (1927)
1930s	Passages maintained in embryonic explants	Stoel (1930); Webster and Clow (1936)
	Mouse inoculation in diagnosis and serology	Hoyt and Jungeblut (1930); Webster and Dawson (1935)
	Active and passive immunisation used	Shortt et al (1935); Covell et al (1936)
	Outbreaks of vampire-bat rabies in Trinidad	Pawan (1936)
	Relative approximate size determined	Galloway and Elford (1936)
1940s	Mouse test standardised for vaccine potency	Hable (1940)
	Adaptation to routine avian-embryo cultivation	Bernkopf and Klinger (1940); Koprowski and Cox (1948)
	Protective importance of serum emphasised	Hable (1945)
1950s	Initial attempts at electron microscopy	Reagan and Brueckner (1950)
	Successful expansion of US urban dog rabies control	Tierkel et al (1950)
	Insectivorous-bat rabies diagnosed in New and Old World	Scatterday and Galton (1954); Mohr (1957)
	Suckling-mouse-brain vaccine developed	Fuenzalada and Palacios (1955)
	Preventive activity of serum and vaccine	Koprowski and Black (1954); Baltazard et al (1955)
	Duck-embryo vaccine with lower reactogenicity	Peck et al (1956)
	Serial passage of fixed and street virus in tissue culture	Kissling (1958)
	Cultivation of fixed and street virus in tumour cell lines	Atanasiu and Lepine (1959)
	Fluorescent-antibody test described	Goldwasser and Kissling (1958)
	First rabies-related virus, Lagos bat, obtained	Boulger and Porterfield (1958)
1960s	Morphology defined by electron microscopy	Almeida et al (1962); Matsumoto (1962); Atanasiu et al (1963); Davies et al (1963)
	Virus shown to have RNA	Sokolov and Vanag (1962); Kissling and Reese (1963)
	Infection by the non-bite route	Constantine (1962)
	Pathogenesis refined with animal models	Dean et al (1963a); Baer et al (1965); Schneider (1969a)
	Focus on local wound treatment	Dean et al (1963b)
	Development of human-diploid-cell vaccine	Wiktor and Koprowski (1965)
	Structural, propagative, and biochemical studies advance	Sedwick and Wiktor (1967); Hummeler et al (1967); Sokol et al (1968; 1969)
	Disinfection with alcohol, iodine, soap, etc	Kaplan et al (1966)
	Virus isolation from the air in bat caves	Winkler (1968)
	Corneal test for antemortem diagnosis	Schneider (1969b)

Table continued on next page

History

Rabies is an ancient, if not one of the oldest recognised, infectious disease of people, with a rich and fanciful history, related partly to the rise of civilisation, the growth of cities, the gradual demise of superstition, and the domestication and movement of animals.[8–12] Other diseases such as smallpox are comparable in age, but a myriad of vesicular diseases prevents more precise conjecture for smallpox and other possible candidates. Suggestions of the consequences after bites by "vicious" or "mad" dogs are found among early writings from Mesopotamia and Egypt thousands of years ago.[11] Even without the conceptual benefit of an infectious-disease framework, few or no severe illnesses were otherwise causally associated with an unprovoked bite from a notably abnormal animal, leading to death after unmistakable manifestations of madness. The tendency to violence or rage provides the basic root of the modern words for this disease. Most major cultures continue to have specific terms pertinent to this precise syndrome. Medical Chinese (about 500 BC) and Indian (about 100 BC) texts describe what seems to be hydrophobia. Greek and Roman civilisations were also probably familiar with rabies, as reflected in works by Euripides, Homer, and Aristotle, for example. Coining one of the usages of the term "virus" in the first century, Celsus may have intended to differentiate the "slimy liquid or poison" of rabid dogs from the venom of toxic animals.[8] The Hebrew Talmud warns of the danger of exposure and questions the veracity of those who claim survival after being bitten by an affected dog. In medieval times, causal associations of disease with affected animals were recorded in various Islamic writings, such as those by Rhazes and Avicenna. The Italian Fracastoro planted a seed towards the germ theory as early as the 1500s, with reference to contagion or "seminaria" of rabies, and coinage of the "incurable wound'. Many philosophers reasoned that rabies was due to seasonal or spontaneous generation, related to animal stress from starvation, thirst, heat, or celestial events (dog days of summer), ideas that persisted for some into the early 20th century. During the age of discovery in the 15th and 16th centuries, Europeans not only sailed to distant lands, but also imported rabies in the form of incubating animals that survived shipboard journeys. In the 18th century, during the

Table 1. Notable events in the 20th century history of rabies (continued)

Period	Events	References
1970s	Structural and antigenic properties defined	Schlumberger et al (1970); Wiktor et al (1973); Sokol et al (1971); Cox et al (1977); Dietschold et al (1978)
	Standard production of human rabies immunoglobulin	Cabasso et al (1971)
	Oral vaccination developed	Baer et al (1971); Steck et al (1982)
	Peroxidase staining procedures in histology	Atanasiu et al (1971)
	Interferon role in prophylaxis	Atansiu et al (1970); Turner (1972); Wiktor et al (1972); Baer et al (1977)
	First human recovery	Hattwick et al (1972)
	Canine observation period based on excretion in saliva	Vaughn et al (1973)
	Models of virus transit from periphery to brain	Murphy et al (1973)
	Cell-culture technique for virus neutralising antibodies	Smith et al (1973)
	Airborne transmission to a laboratory worker	Winkler et al (1973)
	T-lymphocyte recognition in immune defence	Turner (1976); Wiktor et al (1977)
	Virus neutralising antibodies assessed	Miller et al (1978); Turner (1978); Nilsson (1979)
	Combined human-diploid-cell vaccine and serum in prophylaxis	Bahmanyar et al (1976)
	Monoclonal antibodies define antigenic variants	Wiktor and Koprowski (1978)
	Infection via corneal transplant	Houff et al (1979)
	Retrograde intra-axonal transport described	Tsiang (1979)
1980s	Cloning and determination of genomic sequences	Anilionis et al (1981); Tordo et al (1986); Bourhy et al (1989); Conzelmann et al (1990)
	Intradermal vaccination	Nicholson et al (1981); Bernard et al (1982); Warrell et al (1983)
	Specific receptors suggested	Lentz et al (1982)
	Correlation of genotypic and phenotypic changes	Dietschold et al (1983); Coulon et al (1982)
	Major raccoon rabies epizootic recognised in USA	Smith et al (1984)
	Purified chick/duck-embryo and Vero-cell vaccines	Barth et al (1983); Gluck et al (1984); Montagnon (1989)
	Recombinant vaccines produced	Yelverton et al (1983); Wiktor et al (1984); Prehaud et al (1989); Preverc et al (1990)
	Protective immunity induced by ribonucleoprotein	Dietschold et al (1987)
	Experimental prophylaxis with monoclonal antibodies	Schumacher et al (1989)
	Classification within genus Lyssavirus	Matthews (1982)
1990s	Rise in use of PCR for diagnosis	Ermine et al (1990); Sacramento et al (1991); McColl et al (1993); Kamolvarin et al (1993)
	Human monoclonal antibodies developed	Lafin et al (1990); Gebauer and Lindl (1990); Dietschold et al (1990)
	Incubation periods in excess of 6 years	Smith et al (1991)
	Enzootic bat rabies in Australia recognised	Fraser et al (1996)
	Rabies-virus cloning and use as a recombinant vaccine	Schnell et al (1994); Mebatsion et al (1996)
	DNA vaccines developed	Xiang et al (1995)
	Apoptosis recognised	Marcovistz et al (1994); Adle-Biassette et al (1996)
	Elimination of red fox rabies in western Europe	Wandeler (2000)

The references listed here are given with the review on the journal's website at http://image.thelancet.com/extras/02ID2008webfr.pdf .

French Revolution, the inventor of the guillotine is thought to have proposed experimental exposure of condemned prisoners to rabid dogs, to investigate potential therapeutic options.[9] 19th century cures ranged widely—purges, cauterisation, immersions, chemicals, and folk remedies of plant and animal parts—whereas others blamed rabies solely on hysteria originating from an overactive imagination. Though the English surgeon Hunter had earlier discussed the possibility, in 1804 the German scientist Zinke defined the transmissible nature of infectious saliva from rabid dogs, when sprinkled into wounds. Galtier experimentally adapted the disease to the rabbit during 1879, and this model was used by Pasteur in his substantial contributions to investigation of rabies during the late 1800s, the monumental human trials stemming from the use of dried spinal cords from infected rabbits. His work in vaccine production was continued by colleagues such as Roux, among others. Although surmised or attempted earlier, improvements in the definition, visualisation, pathological confirmation, taxonomic affiliation, and intervention of rabies, caused by neurotropic viruses transmitted via the bite of infected mammals, were left largely until the second half of the 20th century (table 1).

Causative agents

Viruses are differentiated on the basis of objective characteristics, such as type of nucleic acid, replication strategy, genomic organisation, relative size, and morphology (figure 1). The single-stranded, negative-sense, non-segmented RNA viruses form the order Mononegavirales (http://www.ncbi.nlm.nih.gov/ICTV/viruslist/-strandedssrna_viruses.pdf), currently consisting of four families: Filoviridae (eg, Marburg virus, Ebola virus), Paramyxoviridae (eg, respiratory syncytial virus, Nipah virus), Bornaviridae (eg, Borna disease virus), and Rhabdoviridae. The last family, of rod-shaped viruses, contains many unclassified members isolated from plants, invertebrates, and vertebrates, as well as members in four recognised genera from animals: Vesiculovirus (type species, vesicular stomatitis virus), Ephemerovirus (type species, bovine ephemeral fever virus), Novirhabdovirus (type species, infectious haemopoietic virus), and Lyssavirus (type species, rabies virus). Lyssaviruses are a collection of genetically related viruses, adapted to replication in the mammalian central nervous system.[13–18] Only one viral species was believed to cause rabies, until serological, antigenic, and genetic methods showed at least seven putative representatives or genotypes (figure 2).

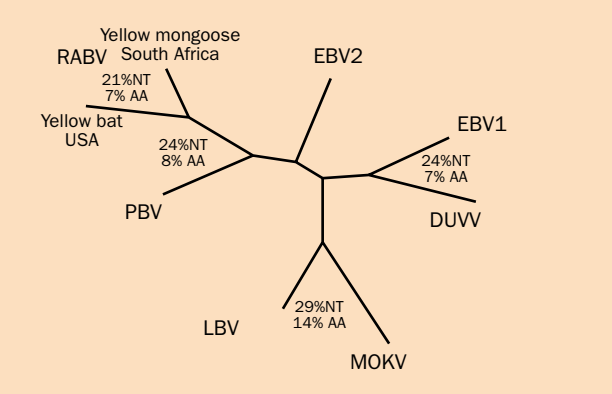

Figure 2. Representation of the seven putative genotypes in the genus Lyssavirus. Based on a comparison of sequences obtained from the nucleoprotein gene, comparing rabies virus (RABV, isolated from a yellow bat in the USA and a yellow mongoose from South Africa) with its most closely related member, a representative of Australian bat virus (PBV) and the relative distance of Mokola (MOKV) and Lagos bat viruses (LBV), to Duvenhage (DUVV) and European bat viruses (EBV1, EBV2).

Rabies-virus particles maintain a bulleted structure of about 75 nm by 200 nm, with a helical nucleocapsid surrounded by a thin protein-studded membrane (figure 3). Basic organisation and reproduction are simple compared with many other viruses. Five monocistronic genes relate to five viral proteins: the N gene codes for a nucleoprotein that encapsulates the viral RNA; the P gene produces a phosphoprotein, which is important not only in transcription and replication, but also for interactions with cellular protein components during axoplasmic transport; the M gene codes for a matrix protein; the G gene produces a single glycoprotein, a membrane-bound moiety that mediates reception and fusion at cell surfaces and serves as a target for the induction of virus neutralising antibodies; and the L gene encodes a polymerase for RNA synthesis.[19–22] Lyssaviruses face at least three primary hurdles once deposited in vivo: to gain access and enter a particular host cell; to transcribe, translate, and replicate their products in the cytoplasm; and to reassemble their base components into virions and leave the cell (figure 4). These viruses can use various surface components to penetrate a cell, including nicotinic acetylcholine and low-affinity nerve-growth-factor receptors and gangliosides.[1,23] Neurotropic by nature, lyssaviruses can be adapted under laboratory conditions to propagate in cultures of insect, reptile, or avian cells, as well as in vitro in several mammalian tissues, such as baby hamster kidney, neuroblastoma, and Vero cells.

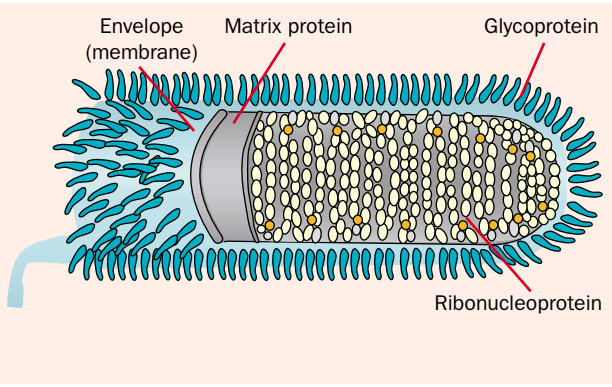

Figure 3. Diagram of a typical bulleted lyssavirus, showing a stylised view of the genome and viral antigens.

Different terms have been assigned to lyssaviruses on the basis of their characteristics. Over time, terminology changed as technical methods improved. These terms tend to be used interchangeably, but they are not synonymous. Operationally defined, an isolate is a virus originating from nature (eg, Pasteur's "street virus" or "wild-type" virus), obtained in the laboratory after primary isolation in animals or cell culture. An isolate can be obtained from a rabid patient, but isolation is not a prerequisite to a successful laboratory diagnosis. By contrast, a strain is a well-characterised isolate (perhaps most akin to Pasteur's term "fixed virus") with fairly predictable phenotypic properties after continued animal or cell-culture passages, such as a defined incubation period, altered virulence, and so on. All current commercial human and animal rabies vaccines are based on a limited number of rabies-virus strains, adapted to propagate in a particular animal species or cell type. A serotype is an artificial grouping of viruses based on their degree of serological cross-reactivity observed in virus-neutralisation or related assays, with hyperimmune serum. For example, whichever strain is used for production, animals inoculated with a potent veterinary vaccine will develop antibodies that cross-react with all true rabies viruses found in nature. This is not the case for all other lyssaviruses, which may be of different serotypes, in which protective immunity may be lacking after vaccination. With advances in molecular biology, the term genotype was coined to refer to a collection of viruses with various degrees of similarity, based on compared genetic sequences. A variant is a designated assemblage of viruses within a serotype or genotype that differs in defined antigenic or genetic properties, useful in epidemiological or phylogenetic analyses. Although disease-causing viruses are a reality, serotypes, genotypes, and so on exist only as concepts in virology, in an attempt to impose order on an often bewildering ecosystem.

Hosts and transmission

Rabies is distributed on all continents except Antarctica (figure 5). Lyssaviruses are fairly fragile and do not persist in the environment. Various mammals serve as major hosts in different parts of the world, primarily in the orders Carnivora and Chiroptera.[24] Rabies virus has been isolated from nearly all mammalian orders. The dog is the major reservoir and vector; dogs cause the majority of the roughly 35 000 human deaths each year (figure 6).[25] Cats are very effective vectors of transmission, but neither domestic nor wild cats seem to serve as reservoir hosts. Foxes of various species maintain the disease from Arctic areas to temperate and tropical latitudes. Other important canid reservoirs include coyotes in the New World, jackals in the Old World, and raccoon dogs in Eurasia. Rabid wolves are associated with severe bites and human deaths, but these are rare and wolves do not serve as true reservoirs. Several types of mongoose and related species

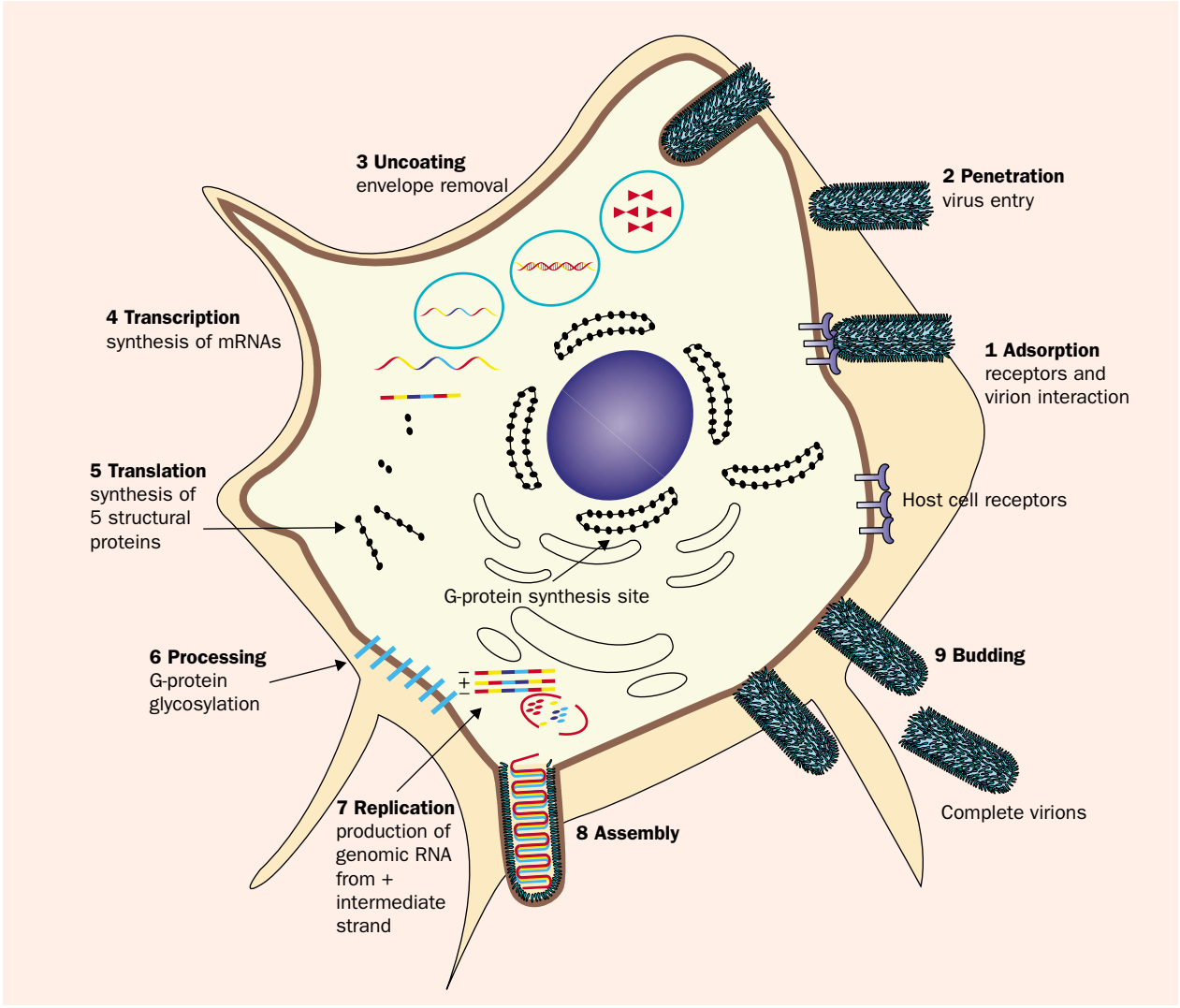

Figure 4. Conceptual flow of lyssavirus reception, entry, transcription, translation, replication, and exit from a generalised host cell.

are important in parts of Africa, Asia, and the Caribbean (where they were transported from Asia with the aim of snake and rodent control in sugar-cane plantations), as are raccoons and skunks throughout North America.[26] Species differ greatly in their susceptibility to infection. For example, opossums are somewhat refractory, whereas other species are generally merely victims, and result in dead-end infections (including human beings). Rodents and lagomorphs, although used heavily as laboratory models, are not important in the epidemiology of the disease, except in the public-health resources devoted to consultation or prophylaxis after routine contact with these ubiquitous small mammals.[27] Birds can be infected experimentally, but no field cases have been documented during the past 20 years or more, as global surveillance and diagnostic methods have improved. With the luxury afforded from disease control in domestic animals, new rabies-virus-species associations are likely to be discovered.[28]

To date, rabies virus is the only lyssavirus identified from the New World. Three other lyssaviruses, Lagos bat virus, Mokola virus, and Duvenhage virus, seem to be confined to sub-Saharan Africa, presumably among bats and insectivores, but their epidemiology is poorly defined. European bat viruses I and II are distributed among insectivorous bats in Eurasia. The most recently discovered member of the genus, Australian bat virus, was found in 1996 during surveillance for a paramyxovirus, Hendra virus; it has been associated with both *Microchiroptera* spp and pteropid ("flying foxes") representatives.[29] Irrespective of first isolation, all lyssaviruses have shown capacity as human or animal pathogens, and for practical purposes should be treated as equivalent. Basic pathogenesis, clinical signs, diagnostic techniques, prevention strategies, and control methods are the same, irrespective of lyssavirus terminology.[30] The bite route is still regarded as the most important means of transmission (figure 7). Non-bite exposures, either transdermal or across mucous membranes, rarely result in disease. Virus may be shed in the saliva concomitantly with, before, or after the development of clinical signs. Adequate laboratory and

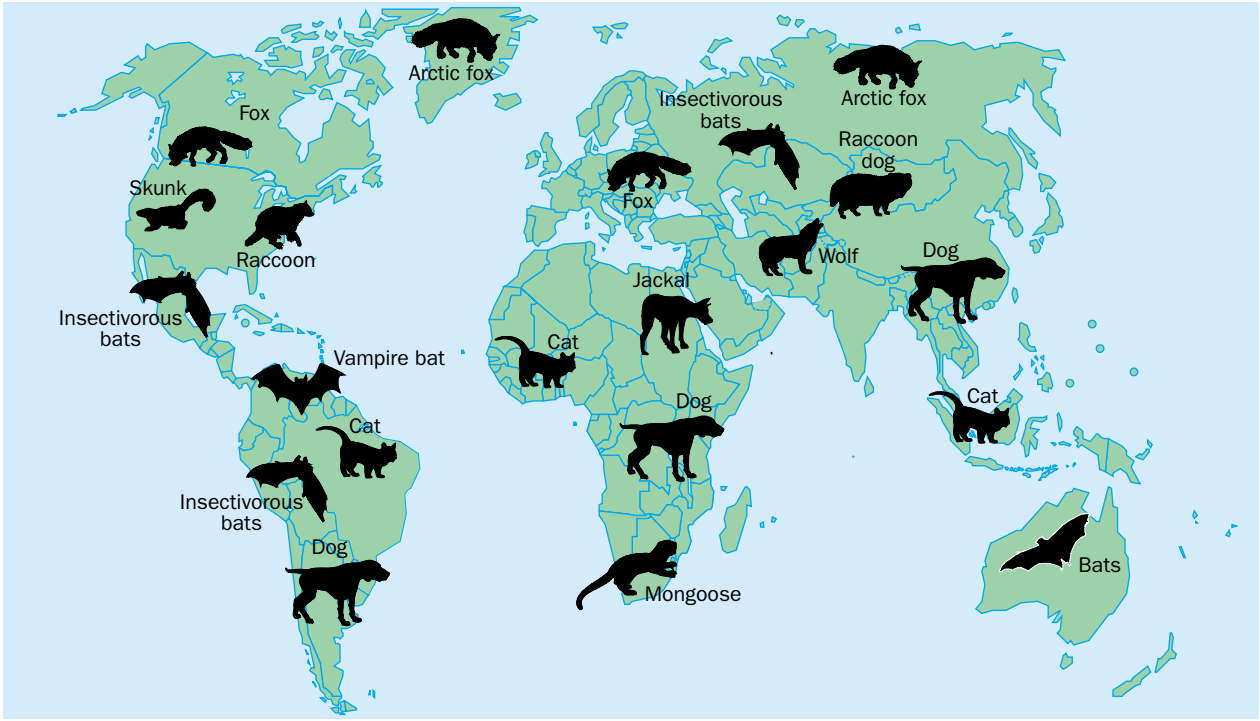

Figure 5. Global distribution of mammalian rabies reservoirs and vectors.

epidemiological studies of virus shedding relevant to clinical onset have been limited to a few domestic carnivores. Definitive periods of viral excretion are not known for other domestic species or wildlife. Incubation periods in rabies can be long, but the idea of true latency is best reserved for viruses with the ability to integrate with host genome, such as retroviruses and herpesviruses. Similarly, the concept of a carrier state, in which animals remain healthy but actively shed virus over long periods, seems of limited epidemiological significance, and is in need of further study.[31,32]

Rabid animals will not only transmit virus among the same species but also can infect dissimilar taxa. Despite such "spill-over" occurrences, compartmentalisation is a concept whereby specific virus variants within a genotype tend to perpetuate among particular hosts in different geographic areas (figure 8). Such associations may last for decades or longer.[33] In general, lyssaviruses are fairly stable entities constrained by genetic bottlenecks, although the opportunity for selection and adaptation may overcome such stasis. In the absence of proofreading enzymes to correct errors in replication, genetic drift occurs gradually over time from a limited accumulation of spontaneous mutations, rather than recombination. Certain geographic features, such as mountains and rivers, may create physical barriers to animal movement and promote localised viral evolution in specialised host niches.[34] Movements of infected animals to new unaffected areas have the potential to produce explosive, sustainable outbreaks (figure 9).[35] Occasionally, although unpredictably, less frequent but more rapid emergence of viral variants may occur, possibly extending host range.[36]

Bats and rabies

Bats are primary reservoirs of rabies on all inhabited continents.[16,17,19,24,26,37,38] This mammalian order hosts six of the seven lyssavirus genotypes described so far. To date, the only lyssavirus that has not been isolated from bats is Mokola virus. As would be predicted from their distribution, abundance, and the number of species described (nearly a thousand, second only to rodents), a greater diversity of lyssaviruses has been discovered among bats than among other mammals. True rabies viruses (genotype 1) have been recovered only from bats indigenous to the New World.[33,39,40] Records of deaths among Spanish conquistadors and their animals during the 15th century, allegedly from contact with small flying animals, suggest that bat rabies was present in America before European colonisation.[12] Vampire-bat rabies has persisted from Mexico to Argentina.[41,42] Reported from Latin America in the early 20th century, rabies in insectivorous bats was not diagnosed in the USA until the summer of 1953, when a yellow bat bit a child in Florida. One of the first human deaths, with hindsight, from bat rabies occurred in 1951, when a 43-year-old Texas woman died from rabies after a bat bite. In all likelihood, other isolated human cases previously went unrecognised, owing to general ignorance about the disease in bats, deaths attributed to other vectors because of the widespread distribution of canine rabies before World War II, and the insensitivity of diagnostic techniques at the time.

Bat rabies is widespread throughout North America, and improved surveillance suggests a similar situation in Latin America.[43,44] Infection has been diagnosed in most species that are commonly encountered and have been adequately sampled. The prevalence of rabies among bats varies with

Figure 6. A rabid dog displaying the classic form of paralytic rabies, including cranial-nerve deficits and hypersalivation.

species, sex, and region, but reported rates are greatly affected by local submission criteria and surveillance methods. Rabies infection may not be very common among apparently healthy free-ranging bats; however, human beings generally encounter bats that are ill or otherwise incapacitated, and infection is not uncommon among such animals. Depending on the surveillance criteria used, the infection rate varies from about 4% to more than 15%. Non-rabid bats may enter homes because of unrelated illnesses, inexperience, accident, disorientation, exploration, and other causes.

Surveillance in the USA has so far documented at least 39 human cases associated with bat rabies over the past 50 years, on the basis of the patient's history or viral characterisation.[26,45,46] Only nine (23%) of these human cases had a definite history of bat bite, but 20 (51%) had known or likely contact with bats. Such observations are not restricted to the USA.[44,47–49] Cases of human rabies associated with non-haemophagous bats (but without definitive evidence of a bite by a rabid bat) have also occurred in residents of Finland, Australia, Mexico, Chile, and Canada. Specific circumstances surrounding each case make spill-over infection from a rabid bat to another mammal, and then to a human being, rather than direct contact with a bat, extremely unlikely as the precipitating chain of events. Although possible, there has been no documentation of any human death after exposure to a known rabid animal, such as a dog, cat, or cow, in which a bat rabies-virus variant was identified. Most human cases in the USA have been related to rabies viruses associated with silver-haired and eastern pipistrelle bats, species not commonly submitted for testing or found to be rabid. Currently, a bite is considered the most likely route of transmission of bat rabies viruses to human beings, even in individuals with no documented history of a bite. People may not recognise the risk of rabies acquisition from bat bite. Bats are small, and the wounds they inflict may not be appreciated as an animal bite, or as a potential rabies exposure, especially compared with bites from larger mammals (figure 10). Different bat species vary in their degree of human interaction, and their reclusive habits can create rather unusual exposure circumstances.[50] Furthermore, bat rabies viruses vary in their virulence properties, and even minor lesions should not be ignored or trivialised.[51]

Given the observations of human deaths associated with bat rabies in the USA, some public-health guidelines have become more conservative for management of potential human exposures during encounters with bats.[52] Capture and testing of bats in human dwellings can alleviate more intensive epidemiological investigation, preclude unnecessary prophylaxis, and ensure proper management when documented exposure to a rabid bat is diagnosed. There is a continuing need for effective health communications for the public, entreating them not to handle bats and to seek prompt and proper prophylaxis if exposed. Some individual researchers working with bats

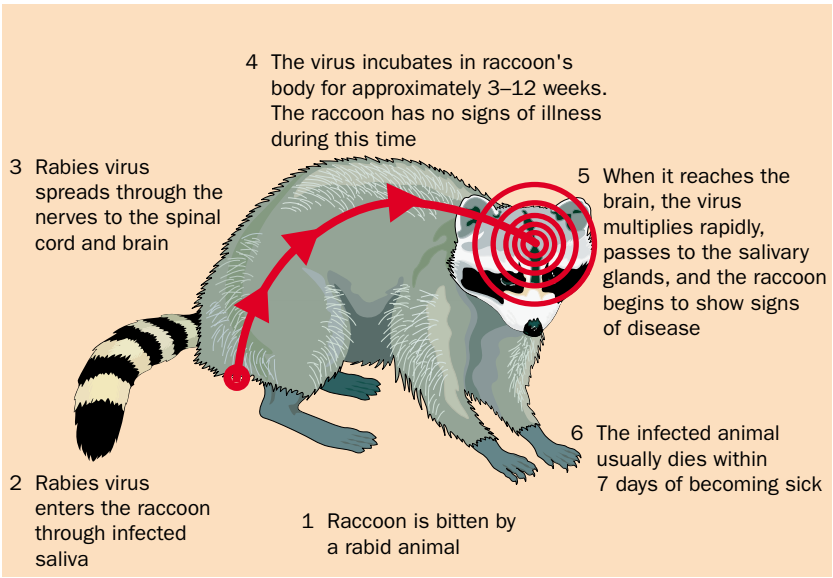

Figure 7. A productive pathogenesis cycle of animal rabies, encompassing virus entry into peripheral nerves via a bite, movement to the central nervous system resulting in encephalitis, and transit to the salivary glands, mediating infection of another host.

Table 2. Postexposure treatment recommendations of the Advisory Committee on Immunization Practices

Animal type, assessment, and disposition	Recommended treatment
Dog, cat, ferret	
Healthy and available for 10 days observation	None unless the animal develops signs of rabies; the animal should then be killed and tested
Rabid or suspected rabid	Start postexposure prophylaxis
Unknown (eg, escaped)	Consult public-health officials
Skunks, raccoons, foxes, and most other carnivores; bats	
Regard as rabid until proven negative by laboratory tests	Consider immediate vaccination
Livestock, small rodents, lagomorphs (rabbits and hares), and other mammals	
Consider individually	Consult public health officials; bites of squirrels, hamsters, guineapigs, gerbils, rats, mice, and other small rodents almost never require postexposure prophylaxis

In the USA, rabies postexposure prophylaxis for naive individuals consists of local wound cleansing, five intramuscular doses (1·0 mL in the deltoid) of rabies vaccine on days 0, 3, 7, 14, and 28, and one dose of human rabies immunoglobulin (20 IU/kg) on day 0 (or may be added up to and including day 7) administered as much as possible at the bite site. Rabies postexposure prophylaxis for previously immunised individuals consists of local wound cleansing and two doses of vaccine inoculated in the deltoid (1·0 mL) on days 0 and 3.

should consider pre-exposure vaccination. On the whole, health risks and conservation needs should be balanced by placing bat rabies in context. Veterinary surgeons should promote responsible pet ownership and vaccination to break an indirect link from wildlife exposure, and all health professionals have to diminish hysteria that may surround extremely unfortunate but thankfully rare human cases.

Is bat rabies fundamentally different from the disease in carnivores? At the time of the first descriptions of vampire-bat rabies in the early 20th century, some experimental observations suggested the notion of a carrier state. However, later laboratory and field research did not support these observations. If rabies truly were a silent condition in bats, there would have to be a mechanism for active infection without involvement of the central nervous system, in lieu of disease.[53,54] In addition, the historical lack of any cases resulting after known bites from bats submitted to the diagnostic laboratory but found negative for evidence of viral antigen would have to be explained.

Diagnosis

The widespread availability of specific laboratory tests, coupled with compatible history and signs, aids the diagnosis of rabies for the astute clinician. Any country in which the disease is endemic should have at least one national reference centre to support diagnosis, with analysis before or after death. Suspicion should begin with presentation of any encephalopathy that takes a rapid downward course, in which other more common infectious and non-infectious disorders have been ruled out (figure 11).[1,55–57] The diagnostic process is similar in both human and veterinary medicine, with the objective identification of sudden neurological illness in a patient with a known or likely exposure to a rabid animal, in the previous few weeks to months. Certainly, the lack of this critical bit of information does complicate the process, particularly during the non-specific prodrome, before the acute neurological phase. Rabies is an unpredictable disease—the only characteristic feature is that it is uncharacteristic in its presentation. For practical purposes, whatever the species, major cardinal signs are similar and may include: low-grade fever, lack of appetite, paraesthesias, ataxia, anxiety, altered mentation, and, inevitably, paralysis, coma, and death. Specific symptoms such as hydrophobia and aerophobia are thought to be limited to human beings. However, this belief may reflect subjective interpretation permitted by same-species communication. Furthermore, during the terminal course of a disease, euthanasia (rather than outright murder as was occasionally practiced before the 20th century) is rarely posited as a serious option (versus the humane and ethical tactic in veterinary medicine).

As elsewhere in microbiology, laboratory tests for rabies diagnosis should be rapid, sensitive, specific, and economical.[58,59] Before the development of modern laboratory testing, animals with suspected rabies were confined for observation, to see whether they died of the disease in an expected short time. With the development of microscopy and histological methods, observations were

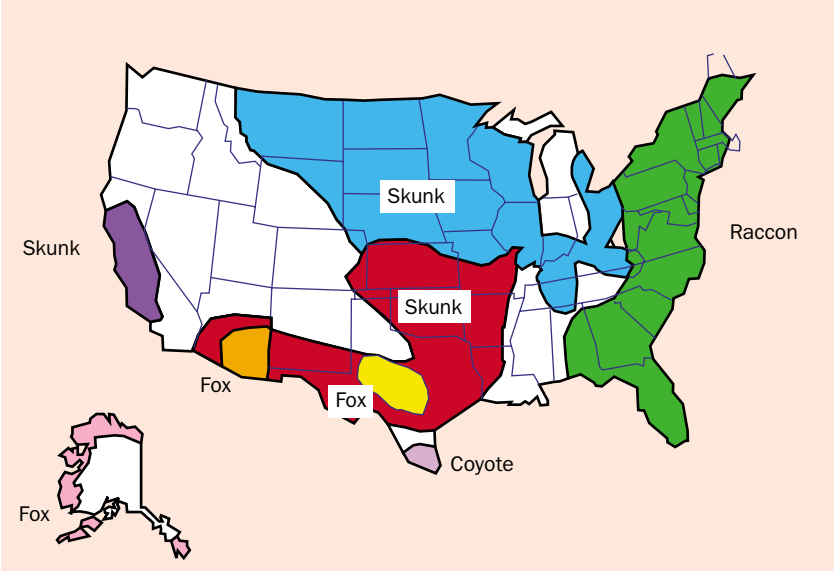

Figure 8. Compartmentalisation of rabies virus into different variants associated with specific hosts throughout the USA.

based on examination of brain tissue for supportive evidence of inflammation and inclusion bodies (figure 12). Developed during the late 1950s, the direct fluorescent antibody (DFA) test evolved and remains as the gold standard in rabies diagnosis.

Fluorescent microscopy makes use of the property of molecules, such as fluorescein isothiocyanate (FITC), to absorb light of one wavelength and to emit it at longer wavelengths. Filters separate radiation produced by a light source from these emissions, and by further selective suppression, allow safe passage of a wavelength to an observer's eyes within a defined range suitable for FITC excitation. Laboratories undertaking rabies diagnosis should optimise their technique for sensitive and specific fluorescent microscopy. There are many differences in the type, quality, and cost of commercial fluorescent microscopes. Optical components of the microscope are especially important, and the primary aim is to obtain a high-resolution diagnostic image with as little loss of brightness as possible. Suitable light sources include mercury or xenon gas arc lamps, to provide light at wavelengths appropriate for FITC excitation, in the 450–495 nm range. Ideally, an objective lens with the greatest numerical aperture should be used, in combination with an ocular to produce a total magnification between 200 and 400 times.

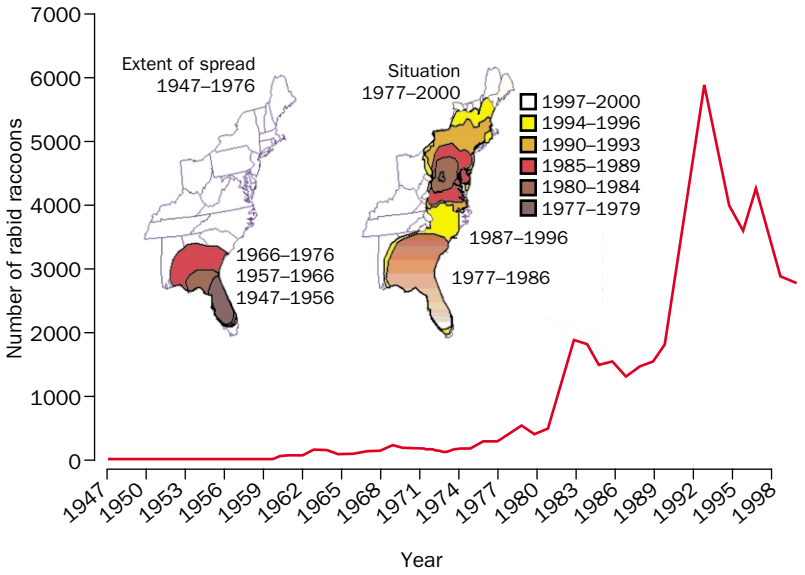

Figure 9. Gradual evolution, translocation, and establishment of raccoon rabies virus throughout the eastern USA, 1950–2000.

Touch impressions of brain tissue, obtained by biopsy or at necropsy, are typically made on glass microscope slides. These impressions are fixed in cold acetone and stained with FITC-labelled polyclonal or monoclonal antibodies directed at lyssavirus antigens. Observed by DFA, virus present in brain impressions appears as inclusions of various shapes, ranging from dust-like particles of less than 1 μm in diameter to oval masses 2–10 μm in diameter. When specifically stained with an FITC-labelled rabies antibody, these microscopic inclusions appear bright apple-green in the cytoplasm of infected cells (figure 13).

Rabies diagnosis is based on an understanding of viral pathogenesis, and a definitive test involves examination of brain tissue. Rabies develops when virus passes from the inoculation site to the spinal cord and brain. Virus is present in saliva only after replication in the central nervous system. Virus may not spread to all salivary glands, and it may be present intermittently in saliva, so routine diagnostic tests for rabies need to focus on brain tissue. A reliable negative test of a valid sample of brain tissue will affirm that exposure to a suspect biting animal could not have transmitted the disease. Known patterns of viral spread within the central nervous system suggest that examination of the brainstem and medulla is essential to optimise the chance of detection. The hippocampus was once included as a key tissue because of its historical role in the histolological demonstration of Negri bodies, but examination of this region is of limited additional value when brainstem and cerebellum are examined. Fresh, unfixed brain samples are central to a rapid and accurate diagnosis of rabies. Tissue alteration by chemical fixation, such as formalin, may make a sample unsuitable for routine DFA testing.[60] Other methods for testing fixed tissue have been developed, such as immunohistochemistry, but they require specialised training and will delay test results (figure 14). Sensitivity may be decreased after formalin fixation in the use of commercial conjugates, and not all monoclonal-antibody preparations are suitable, particularly if activity depends on conformational epitopes.

Specimen submission depends on the epidemiology of rabies in the area. Foremost among the reasons should be the prevention of human rabies by testing of suspect animals, such as biting dogs, involved in an exposure. Similarly, animals involved in the exposure of domestic species should be killed and examined, for proper veterinary management. The predictive value of a negative diagnostic test by a competent rabies laboratory approaches 100%, and postexposure concerns are pre-empted.

By contrast with postmortem rabies diagnosis in animals, antemortem testing is used in suspected human cases.[45] Useful laboratory specimens include serum, cerebrospinal fluid, saliva, and tissue, such as skin, from highly innervated locations. The appearance of antibodies to specific viral antigens, either via serum binding or neutralisation tests, is diagnostic in a patient with encephalitis, with no history of previous vaccination. Viral isolates can be obtained by animal inoculation or cell-culture passage from saliva or oral swabs. These samples can be probed for viral nucleic acid. Viral antigen can be detected by the DFA test on brain biopsy material, corneal touch impressions, or a full-thickness nuchal skin biopsy, from the haired nape of the neck (figure 15).

The advent of RT-PCR and other molecular assays in the 1980s provided a useful adjunct in rabies diagnosis, particularly as a confirmatory test.[61,62] However, compared

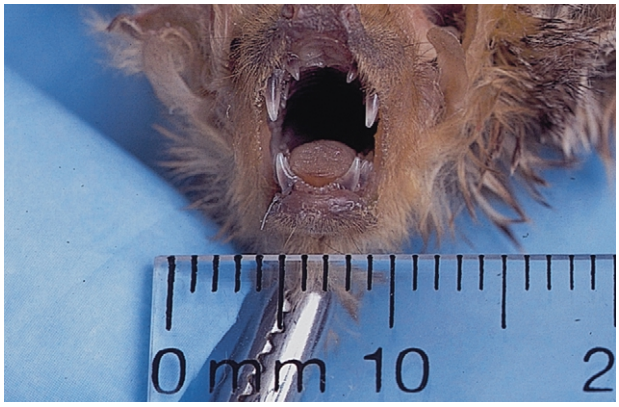

Figure 10. A rabid red bat, showing the small teeth.

with the DFA test, the benefits of RT-PCR as a routine primary assay in most situations are limited by the need for universal primers for all lyssaviruses, the need to sequence amplicons (rather than reliance on the detection of suspicious bands alone), the inherent delay compared with other tests, the cost of the equipment, and the need for maintenance of the expertise.

The usefulness of a definitive rabies diagnosis lies neither in a favourable prognosis nor in a clear therapeutic option. Rather, it allows the institution of public-health measures to limit contacts with the patient and permits reconstruction of a history in which others may have been exposed to the same infective source. Obviously, a definitive rabies diagnosis allows closure in the case of a positive finding. Faced with a genuine negative finding, other causes of encephalitis can be sought, some of which may be treatable. Rabies should be included in the differential diagnosis of any acute progressive encephalitis, even in the absence of a history of a definitive exposure. All endemic countries should have a goal for improvement in the overall quality of rabies diagnosis by formulation of basic guidelines and minimum standards for suitable equipment, reagents, protocols, quality assurance, clinical training, and

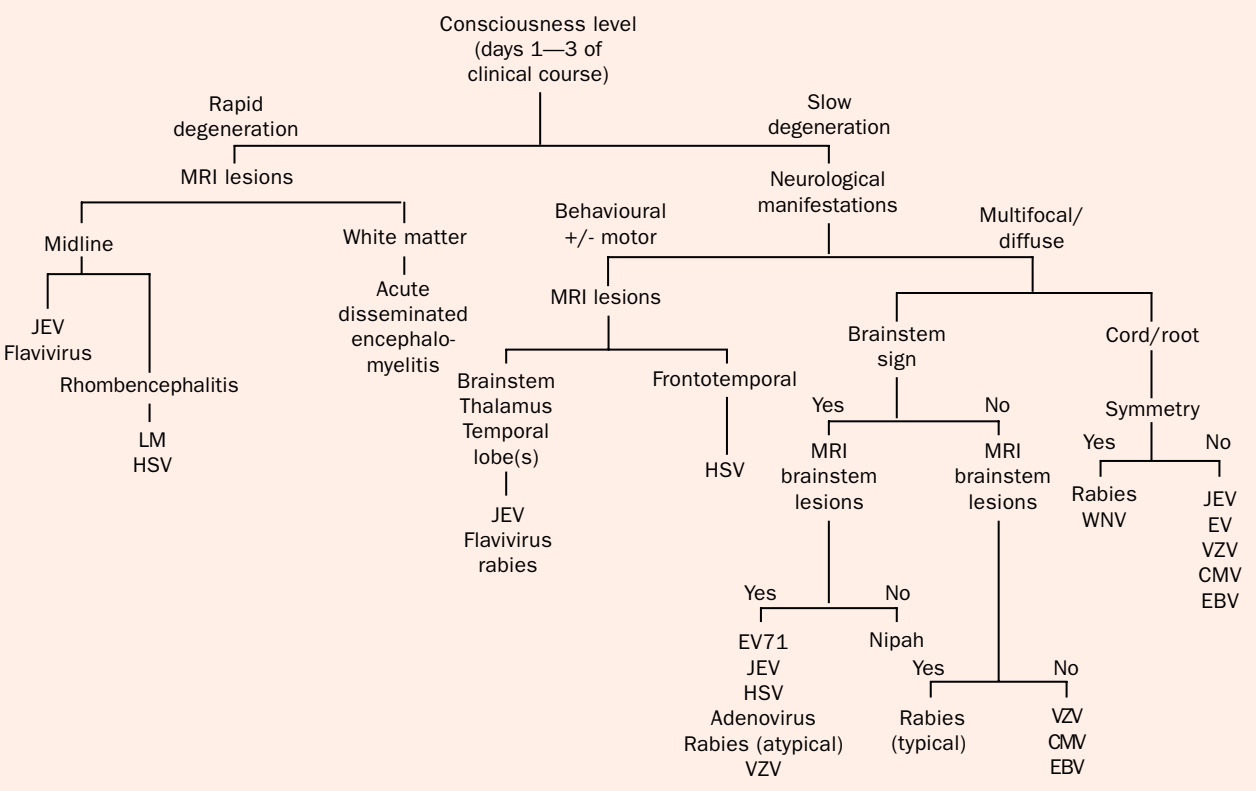

Figure 11. Algorithm for differential diagnosis of rabies. Most rabies patients remain alert and able to communicate during the first 3 days after clinical onset. Those who deteriorate rapidly have brainstem, thalamus, or midline structure involvement shown on magnetic resonance imaging (MRI), which may be associated with Japanese encephalitis virus (JEV), other flaviviruses or arboviruses, or rhombencephalitis caused by Listeria monocytogenes *(LM) or herpes simplex virus (HSV). In immune or postinfectious encephalitis or acute disseminated encephalomyelitis, there is widespread involvement of white matter of both cerebral hemispheres. Rabies patients may or may not have behavioural changes (in the form of fluctuating consciousness, increasing agitation, and so on) and phobic spasms. Patients with JEV or HSV encephalitis also can show behavioural changes. MRI may suggest rabies, of the typical or atypical form. A predilection for the brainstem, thalamus, and temporal lobe may readily separate rabies from other viral infections such as enterovirus (EV) 71, JEV, HSV, adenovirus, and varicella zoster virus (VZV), particularly by the absence of enhancing lesions during the early phases and less intense signal in T2-weighted images. Swelling and foci of haemorrhages, as seen in encephalitis caused by JEV or other arboviruses, are absent in rabies. Most patients with typical rabies manifestations (commonly associated with canine rabies virus variants) lack brainstem signs on examination. Brainstem symptoms and signs can be observed in bat-related rabies, as well as in Nipah-virus infections. Patients with paralytic rabies, in addition to the presence of mild encephalopathic symptoms, have an ascending weakness of symmetrical distribution, and should be carefully differentiated from patients with West Nile virus (WNV) infection. Asymmetrical involvement of poliomyelitis or radiculitis-like features is also noted in cases of cytomegalovirus (CMV), JEV, EV, VZV, and Epstein-Barr virus (EBV) infections.*

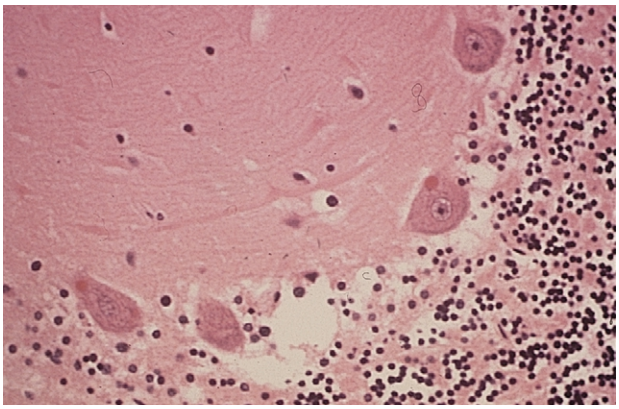

Figure 12. Histological section of human brain from a patient with rabies. There are several pale pinkish, oval intracytoplasmic inclusions in neurons. Stained with haematoxylin and eosin.

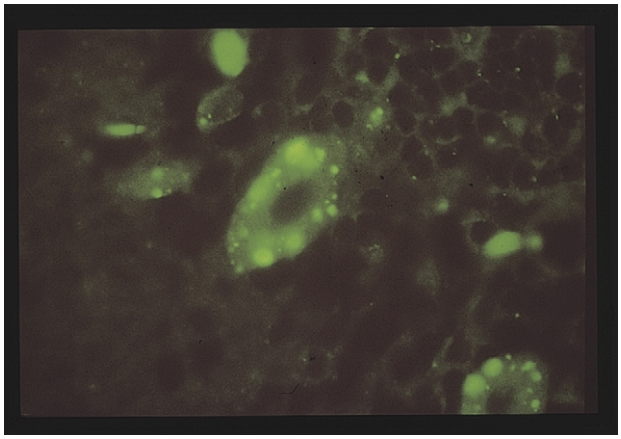

Figure 13. Section of rabid human brain processed by the DFA test, showing widespread viral inclusions, staining apple-green in colour.

laboratory safety. Rabies-free countries that do not have the necessary diagnostic expertise should maintain collaborative arrangements with international agencies.

Prevention of rabies

With directed continuing education, common sense, first aid, and the availability of modern biological agents, human rabies is nearly always preventable.[1,52,63,64] Unfortunately, there is an obvious dichotomy between the more and less developed countries. In many less developed countries, where most human cases occur because of canine rabies, deaths occur mainly because of a lack of access to affordable biological agents needed for effective postexposure prophylaxis. By contrast, in more developed countries, these agents are both available and affordable, and the few otherwise preventable human deaths happen largely through ignorance or lack of recognition. Thus, even in North America, educational efforts require continued improvement to minimise the effect of wildlife rabies, so that exposed individuals will seek appropriate treatment (table 2). Despite the ability to prevent rabies if the exposure is recognised and appropriate postexposure prophylaxis is administered, no effective treatment has been successful once clinical signs occur.

Rabies prevention begins with education of both the general public and health professionals.[65] Decisions about treatment of an individual start with a basic understanding of transmission cycles among animals and the definition of an exposure. The most important route of exposure is a bite from a rabid animal. Contamination of an open wound or mucous membranes with infectious material constitutes a non-bite exposure. In general, non-bite routes of exposure present a lower risk than an overt bite. For example, if the rare circumstances of corneal transplantation or laboratory accidents are excluded, the last documented death after non-bite exposure in the USA occurred well before World War II. In addition, as discussed by current review boards, such the Advisory Committee on Immunization Practices in the USA, mere touching or petting of a rabid animal is not considered an exposure.[52] Similarly, beyond an infected carcass, the normal environment is not a source of concern. Rabies virus is subject to rapid inactivation once outside a host. Hence, potential fomites, such as bodies of water and inanimate objects, do not have a role in rabies. No cases have ever been attributed to indirect, non-bite exposure.

Table 3. Rabies exposure categories and treatment recommendations of WHO

	Status of animal causing a potential exposure		
Nature of exposure	**At time of exposure**	**During next 10 days***	**Recommended treatment**
Animal contact but no lesion; indirect contact; no contact	Healthy	Healthy	None
	Rabies-suspect†	Rabid‡	None
Licks on the skin; scratches, abrasions; minor bites (on clothed areas of arms, trunk and legs)	Healthy	Healthy	None
		Rabid	Start vaccination§
	If unprovoked, suspect as rabid	Healthy	Start vaccination—stop treatment if animal remains healthy
		Rabid	Continue vaccination treatment.
	Rabid; wild mammal; animal unavailable for observation		Give complete course of vaccine Give complete course of vaccine Give complete course of vaccine
Major bites (multiple, or on face, head, finger, or neck); licks of mucosa	Suspect or confirmed rabid animal;‖ animal unavailable for observation		Vaccination plus rabies immune globulin Stop treatment only in the case of an observed dog or cat that remains healthy for 5 days.

*The 10-day observation period applies only to dogs and cats. In the USA, the observation period has been extended to include ferrets due to research detailing a limited shedding period similar to that described for dogs and cats. †Considered a rabies-suspect due to unprovoked contact or other abnormal clinical presentation. ‡If an animal under observation displays signs of rabies, it should be killed and tested for rabies. §There are no vaccine-only regimens approved for use in the USA. Outside the USA, rabies immune globulin is often unavailable, and hence its use is restricted to the most severe exposures. ‖In general, exposure to rodents, rabbits, and hares seldom, if ever, requires post-exposure prophylaxis.

Because there are insufficient resources for modern human postexposure prophylaxis in many less developed countries, the WHO recommendations include a triage regimen for the classification of likely exposures (table 3). Category III exposures consist of single or multiple transdermal bites or contamination of scratches or mucous membranes with saliva. Recommended postexposure prophylaxis consists of wound cleansing and rabies immunoglobulin plus vaccine, similar to protocols used in the USA. This form of exposure is the most serious and is considered a medical urgency in application of postexposure prophylaxis. Category II exposures consist only of "nibbling" on uncovered skin, "minor" scratches or abrasions without bleeding, and licks on broken skin. Recommended treatment includes wound disinfection and administration of vaccine only. By contrast, recommendations on human postexposure prophylaxis in the USA include rabies immunoglobulin and vaccine after any exposure, because of the subjective nature of this second category, and the difficulty in ascertaining risk. There is no vaccine-only schedule for the naive patient. WHO category I situations that consist of touching or feeding a potentially rabid animal, or involve licks on intact skin, are not exposures and require no treatment.

An appreciation of basic viral pathogenesis, local rabies epidemiology, and access to diagnostic facilities simplifies human treatment decisions. For example, in the USA, a cat, dog, or ferret that bites a human being should be confined and observed for 10 days, irrespective of vaccination status. Typically, postexposure prophylaxis is not initiated in the interim because of the rarity of rabies in domestic animals. If the biting animal dies or develops signs compatible with rabies during the 10-day observation period, it is submitted for prompt rabies diagnosis. The test result determines whether human postexposure prophylaxis is necessary. In many developing countries, by contrast, because of the frequency of dog rabies, animal observation may not be relevant. The WHO guidelines defer treatment pending the outcome of laboratory diagnosis only if the species is unlikely to be infected with rabies, and when the laboratory diagnosis can be made effectively within 48 h, or if an exposure involves a dog more than a year old with a current vaccination. In such cases, the healthy dog may be observed for 10 days. If the dog shows any sign of illness during the observation period, the patient immediately begins full postexposure prophylaxis rather than awaiting test results. Provocation by itself may not influence a treatment decision, because of subjective human interpretation of what defines an unprovoked situation from the perspective of the animal (eg, territoriality, fear).[66]

Reliable prevention of human rabies after an exposure requires immediate and vigorous wound cleansing with copious amounts of water and soap. Initial reduction of the viral load at the wound site by this combination of physical and chemical means is essential for maximum effect of postexposure prophylaxis.

With the gradual development of cell-culture propagation, the potency and safety of rabies vaccines have greatly improved in the past 20 years. Nonetheless, in some countries the only available vaccine is of nerve-tissue origin from sheep, goats, or suckling rodents. Suckling-mouse-brain vaccines are typically administered subcutaneously in seven daily doses with additional doses on days 10, 20, and 90, but schedules and potency vary. In line with WHO recommendations, the trend is continuing in many less developed countries toward replacement of vaccines of nerve-tissue origin with cell-culture vaccines, in various schedules.[64]

A standard cell-culture vaccination regimen (eg, the Essen schedule) consists of a vaccine on days 0, 3, 7, 14, and 28 administered in the deltoid or in the anterior thigh for children. In the USA, human rabies postexposure prophylaxis consists of this five-dose intramuscular vaccine schedule with a single dose of human rabies immunoglobulin on day 0. The dose of human rabies immunoglobulin is 20 IU/kg and the solution is infiltrated in the wounds at the site of the exposure. If all of the volume cannot be administered in and around the exposure site, the remainder can be administered at a distant site, such as the deltoid opposite the vaccine dose or the anterior thigh. The rabies immunoglobulin should not be administered in the gluteal muscles because of possible deposition in fatty tissue and potentially lower systemic distribution.

Attempts to reduce costs of postexposure prophylaxis by lowering vaccine volume and clinic visits have produced several variations on the standard vaccine regimen.[67] For example, one method uses an intramuscular regimen (2-1-1) in which two doses (1·0 mL or 0·5 mL depending on the vaccine) are given on day 0 and one intramuscular dose on days 7 and 21 is administered in the deltoids (table 4). However, perhaps one of the most substantial improvements in rabies postexposure prophylaxis has been the use of the intradermal route of vaccination.[68] One intradermal regimen (8-0-4-0-1-1) consists of 0·1 mL vaccine administered at eight sites (over the right and left deltoids, lateral thighs, lower quadrant of the abdomen, and suprascapular area) on day 0, followed by four 0·1 mL intradermal inoculations on day 7 over the deltoids and thighs, and 0·1 mL vaccine at one site (deltoid) on days 28 and 90. Another protocol is a two-site intradermal regimen (2-2-2-0-1-1) with vaccine administered at two sites (deltoids) on days 0, 3, and 7, and one additional intradermal dose (deltoid) on days 28 and 90.[68] Vaccines used for intradermal postexposure prophylaxis have included: human-diploid-cell vaccine, Vero-cell rabies vaccine, purified chicken-embryo-cell vaccine, and purified duck-embryo-cell vaccine. All abbreviated schedules should include a dose of human (20 IU/kg) or equine (40 IU/kg) rabies immunoglobulin on day 0, at the least for all WHO category III exposures. When rabies immunoglobulin is not available, the clinician is faced with a serious difficulty in treatment, making wound care and multisite intradermal vaccination some of the precious few options.[69]

Human rabies immunoglobulin is the only type of product licensed for use in the USA. However, it is expensive and its availability is severely restricted throughout the world. When mass human exposures occur,

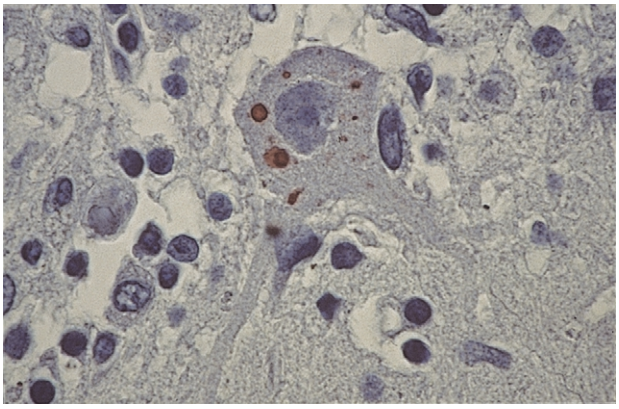

Figure 14. Close-up of a neuron from a formalin-fixed section of a brain from a patient with rabies, showing many, reddish-brown viral inclusions in the cytoplasm. Processed by immunohistochemistry.

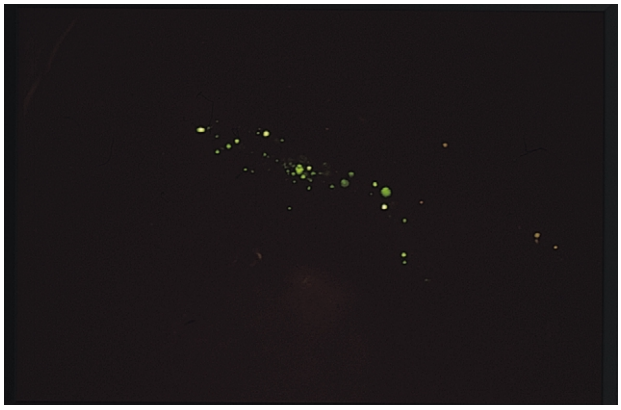

Figure 15. Immunofluorescent viral inclusions in a peripheral nerve in a cryostat section from a patient with rabies, obtained via an antemortem nuchal skin biopsy.

supply can fall to critically low levels. Equine rabies immunoglobulin is an alternative commonly used in less developed countries. After extensive modification and purification, modern equine rabies immunoglobulin is a purified, heat-treated, Fab2 product with a much lower rate of adverse effects than crude equine serum. In an effort to reduce real and hypothetical risks of adventitious agents, heat-treatment or additional detergent-treatment steps are being incorporated into the manufacturing process of rabies immunoglobulin. Although these steps are important from a safety perspective, concerns about potential effects on efficacy have been raised.[70] Local production of rabies immunoglobulin may be necessary to meet practical needs.

Rabies pre-exposure vaccination is recommended for anyone at increased risk of exposure to rabies, for example, laboratory staff working with rabies, vaccine producers, veterinary surgeons, animal and wildlife control personnel, and zoologists. Three doses of vaccine are administered on days 0, 7, and 21 or 28. According to the WHO recommendations, the intramuscular vaccination route can use one full dose (1·0 mL or 0·5 mL) of cell-culture rabies vaccine and the intradermal route 0·1 mL of any of the cell-culture vaccines. At present, the only licensed vaccines and regimens in the USA require a 1·0 mL dose administered intramuscularly. The Imovax-ID vaccine formulated for pre-exposure intradermal vaccination is no longer available for the US market, despite continued expansion of the intradermal route with this and other vaccines for both pre-exposure and postexposure prophylaxis in other countries. Incorporation of pre-exposure rabies intradermal vaccine into expanded childhood immunisation schedules has been proposed for less developed countries.[71]

Many other promising immunobiologicals are being developed, but they are more than a decade away from any direct application to human beings. DNA-based vaccines

Table 4. Rabies post-exposure vaccination schedules for the rabies-naive patient

				Days			
	0	3	7	14	21	28	90
Standard WHO schedule*	1 IM dose deltoid†	1 IM dose deltoid	1 IM dose deltoid	1 IM dose deltoid	··	1 IM dose deltoid	··
Reduced multi-site IM (2-1-1)	2 IM doses; right and left deltoid	··	1 IM dose deltoid	··	1 IM dose deltoid	··	··
8 site ID regimen (8-0-4-0-1-1)	8 x 0·1 mL ID	··	4 x 0·1 mL ID	··	··	0·1 mL ID	0·1 mL ID
2 site ID regimen (2-2-2-0-1-1-)	2 x 20% IM‡ ID	2 x 20% IM ID	2 x 20% IM ID	··	··	20% IM ID	20% IM ID
				Days			
		0–7			10	20	90
Suckling-mouse-brain vaccine§	1 dose each subcutaneously on abdomen				1 dose	1 dose	1 dose

Day 0 is the day of initiation of treatment. IM=intramuscular; ID=intradermal. Human rabies vaccines licensed for use in the USA: human diploid cell vaccine—"movax" Rabies (1·0 mL intramuscular); "Imovax" Rabies I.D. (0·1 mL ID) (only for pre-exposure and no longer available in the USA); rabies vaccine adsorbed—Rabies Vaccine Adsorbed (1·0 mL IM); purified chick embryo cell—"RabAvert" (1·0 mL IM). Rabies vaccines widely available outside the USA: purified chick embryo cell—"Rabipur" (1·0 mL IM); purified vero cell vaccine—"Verorab" (0·5 mL IM), "Imovax-Rabies Vero" (0·5 mL IM), "TRC Verorab" (0·5 mL IM); human diploid cell vaccine—"Rabivac" (1·0 mL IM); purified duck embryo vaccine—"Lyssavac N" (0·5 mL IM). For severe exposures outside the USA and all exposures in the USA, human rabies immune globulin (rabies immune globulin, human—"mogam" Rabies-HT [20 IU/kg]) should be administered at the site of the bite(s). Where human rabies immune globulin is not available or too expensive, equine rabies immune globulin (serum antirabique Pasteur [40 IU/kg]) may be used.

*The only schedule used in the USA is the standard WHO schedule and must be combined with human rabies immune globulin administered on day 0 (or up to an including day 7) at the site of the bite. †The intramuscular dose is administered in the deltoid of adults or the anterior thigh of small children and infants. ‡20% of IM dose administered ID—0·1 mL for vaccines with a volume of 0·5 mL/dose and 0·2 mL for vaccines with a volume of 1·0 mL/dose. §Volume of dose (up to 5 mL), route, and quality of vaccine varies according to production practices used by various countries.

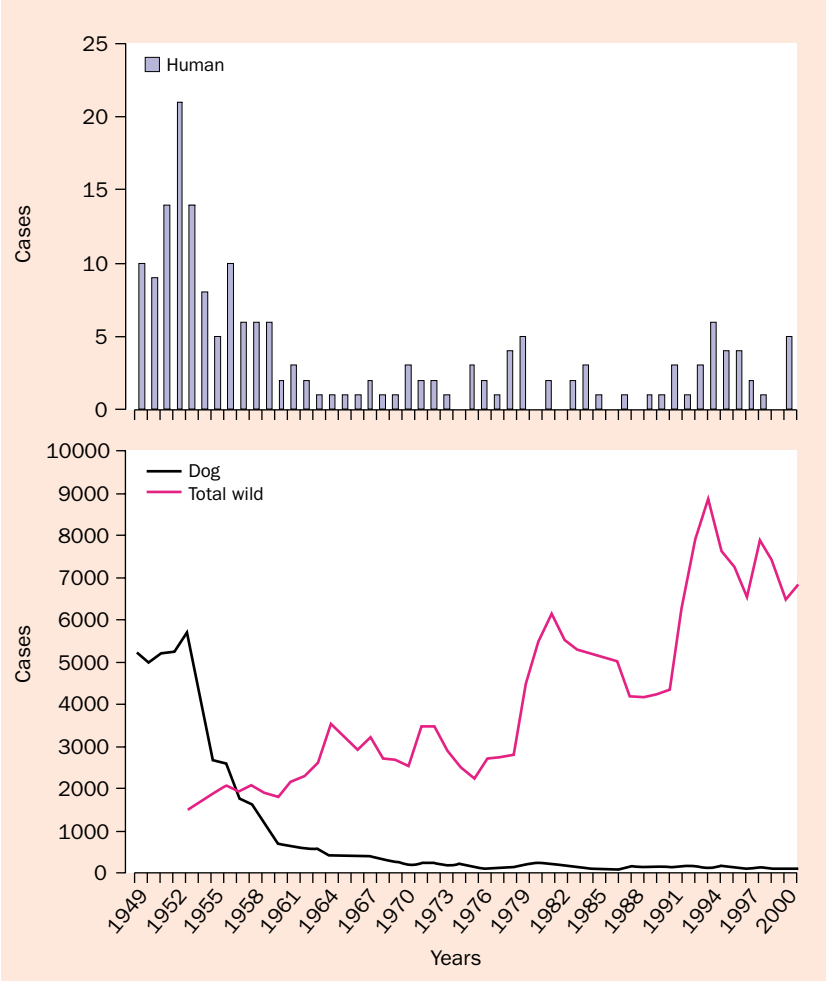

Figure 16. Surveillance of annual human cases with domestic animal and wildlife rabies cases reported in the USA.

can expand the range of lyssavirus cross-reactivity and have shown promise in animal models, including non-human primates, but they generally require at least a primary inoculation, followed by administration of a booster dose for any efficacy, and may lack the rapid kinetics needed for postexposure application.[72,73] Recombinant rabies vaccines permit the incorporation of additional genes into non-translated portions of the viral genome and have been touted as a means to produce exogenous antibodies and as a way to deliver foreign products to the central nervous system, if attenuated, modified live vaccines are ever found acceptable.[74,75] Plant biotechnology offers the premise of inexpensive antigen production and potentially "edible" rabies vaccines, but problems of yield and practical delivery remain.[76] Neutralising monoclonal antibodies are one possible alternative to rabies immunoglobulin, provided that they can be produced cost-effectively.[77] Since pure, potent, safe, and effective veterinary vaccines exist now, at a small fraction of the cost of human vaccines, rabies prevention needs to remain focused clearly on disease control in the animal reservoir (figure 16).[78]

Control of rabies

In the past, various lethal techniques have been attempted to control rabies, including habitat destruction, trapping, shooting, institution of bounties, gassing of dens, and distribution of poisons.[79] Population reduction, as the sole technique in disease abatement, has been extremely difficult to justify, in widespread, long-term, government-sponsored programmes, and raises serious economic, ethical, efficacy, and ecological issues. Animal rabies can be controlled by the proper induction of herd immunity. The use of the first effective veterinary vaccines against rabies during the 1920s, and their application throughout Europe and North America at the end of World War II, strikingly lowered the number of cases in domestic animals. Coupled with humane removal of stray animals, the institution of laws on control of animals, early spay and neutering programmes, and the promotion of responsible pet ownership by education, canine rabies can be eliminated (once a heretical idea). The epidemiological luxury provided by dog rabies control allowed the extension of this concept to wildlife during the 1960s, but by the oral, rather than the parenteral, route.[80] Development of oral rabies vaccines and baits throughout the 1970s and 1980s led to the first field trials that targeted the red fox.[81,82] Progress to date in both Europe and North America has shown that rabies in free-ranging terrestrial carnivores can be controlled, with extension to other important species, such as the coyote and raccoon (figure 17). Adherence to rigorous surveillance and diagnostic criteria will be essential in the long-term

Figure 17. A raccoon consuming a bait laden with oral rabies virus vaccine.

definition, assessment, and modelling of programme success. Advances in wildlife vaccination are now being extended to community dogs in less developed countries.[83–85] However, all oral rabies vaccines in current use are self-replicating entities and are not entirely hazard free.[86,87] Careful attention to safety concerns and continued vigilance should keep to a minimum the drawbacks related to rare or perceived health risks.

Bats present different problems. Most bats are endangered or threatened on a global basis, and they carry out important functions of pollination, seed dispersal, and insect predation, especially in tropical areas. Commensal bats should be excluded from human living quarters. Mist-netting of vampire bats and coating them with anticoagulant paste may achieve selective population reduction in certain areas; the bats will fly back to a roost and be groomed by others, resulting in many deaths in a colony. Extension to bats of oral rabies vaccination, or other novel techniques, may occur in the future, particularly if rabies control in terrestrial carnivores is sustainable.[88] Greater attention to prevention and control of animal rabies should lead to greater long-term benefits in public health, rather than a narrow focus on human prophylaxis alone.[89]

Risks to the traveller

Given the ease of international travel, and the long incubation periods involved, rabies can be imported with impunity, even into areas believed to be free of the disease.[5,90] Between 1990 and 2001, at least seven fatal human cases were diagnosed in the USA among travellers exposed to rabies abroad.[26] All of these cases were due to rabies viruses that were associated with infected dogs in the localities visited. Countries included Ghana, Haiti, India, Mexico, Nepal, and the Philippines. These deaths were preventable by avoidance of animal exposure or by seeking prompt postexposure prophylaxis after animal bite. Tourists from more developed countries may not be aware that canine rabies still flourishes in many parts of the world. Cats are also effective vectors, which may be forgotten. The rise in modern ecotourism should carry a requirement for continuing education about likely human health risks, including zoonoses, particularly when medical facilities may not be located nearby. Wildlife should be appreciated at a distance, in line with objective conservation guidelines and practical common sense. Pre-exposure vaccination does simplify postexposure management, although it does not abrogate the need for booster doses of vaccine.

Perpetuation and re-emergence

Invariably, a truly productive rabies infection is a death sentence. This property was cited by some academics as descriptive evidence for categorising rabies viruses as rather "imperfect" parasites. If so, how can rabies have existed for millennia and into yet another century? The traditional epidemiological triad of host, agent, and environment forms an appropriate backdrop. Objective limitations are beset by the need for an observer within a subjective human reference frame, irrespective of actual viral evolution. A partial understanding relates to the domestication of the dog over the past 10 000 years. Human beings gradually accrued many benefits from canine companionship, but their attachment ensured a ubiquitous perpetuation of virus in rather close association with people, especially in densely packed urban areas. Lurking in the forest edge and savannah were many wild social carnivores with their own peculiar viral medley. The ability to fly allowed the Chiroptera to reach urban, suburban, rural, and undeveloped landscapes alike.[91,92] As with the Carnivora, compartmentalisation of bat rabies seems to be the rule, although spill-over infections to other mammals have been documented.[93–95] Are these episodes indicative primarily of false starts and dead-end infections, or opportunities for potential viral evolution and re-emergence? For example, infection with a bat rabies virus was recently found in skunks in the southwestern USA, with the suggestion of sustained transmission, raising intriguing questions about the public-health significance of viral spill-over, adaptation, compartmentalisation, and persistence.[96] With the unpredictability surrounding such emergence, how useful will oral vaccination of wild carnivores be in true disease elimination, without a contingency plan?

Thus, one of the obvious reasons for the staying power of lyssaviruses has been an abundance of diverse hosts. Rabies has been intertwined invariably with the success of certain mammalian assemblages, particularly over the course of civilisation. Lyssavirus domination and adaptive radiation ebbed and flowed with ecological impunity prevailing over genetic bottlenecks and backwaters. Rapid rates of evolution are features shared by RNA viruses, under the imposed constraints of maintained self-identity. Virus persistence in periequatorial regions may be linked to the continued occurrence of many so-called "tropical diseases", due in no small measure to unchecked human overpopulation, poverty, and continual social strife. As a primary zoonosis, rather than a serious human contagion or major veterinary economic concern, apparently rabies does not provide a large enough burden in the minds of decision makers, in comparative terms of mortality, morbidity, or economic hardship, to attract adequate attention, especially if compared with diseases such as smallpox, influenza, plague, polio, foot-and-mouth disease, or even the transmissible spongiform encephalopathies.

Search strategy and selection criteria

We have followed international publications on rabies for longer than 20 years. Data for this review were identified by searches of Medline and Current Contents (especially from the past 5 years), and references supplied from older relevant review articles; numerous pieces were identified through searches of the files of the authors. Consideration for inclusion in the reference list included relevant sources by topic, irrespective of primary language, if an English translation of the title and abstract was provided. Search terms included "rabies", "rabies virus", "rabies vaccine", and "lyssavirus".

Conclusion

Anyone seriously working in the field of rabies has been fascinated by its complexity and frustrated by its mysteries.

Rabies, unlike any other viral zoonosis, offers the possibility of selective elimination, rather than mere control. Moreover, the disease teases with the prospect of a cure, once its mechanisms have been fathomed. All of the major tools to make a significant impact on this fatal disease, and to plough fertile new ground, were present at the end of the 20th century—a new advocacy must be resurrected during this one.

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Conflicts of interest

We have no conflicts of interest.

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