pus, hypothalamus, deep and subcortical white matter, and deep and cortical gray matter with varying degrees of severity depending on the stage of disease. Gadolinium enhancement is clearly shown only in later stages (Figs. 1 and 2). Although a seemingly similar involvement of thalamus and midbrain on magnetic resonance images can also be seen in Japanese encephalitis, more prominent hypersignal T2 changes and foci of hemorrhages are observed in this arboviral encephalitis than in rabies [9].

8 DIFFERENTIAL DIAGNOSIS

Differential diagnosis includes encephalitis caused by arboviruses such as Japanese, eastern equine, and West Nile viruses and enterovirus 71 and Nipah virus infections [9]. Diffuse flaccid paralysis was found in 10% of patients with West Nile virus encephalitis, with no discernible ascending pattern of progression [27]. Asymmetrical weakness in an unimmunized patient in an epidemic setting suggests paralytic poliomyelitis or atypical forms of Japanese encephalitis [9]. The Trinidad outbreak of paralytic rabies was initially thought to be poliomyelitis.

Acute hepatic porphyria with neuropsychiatric disturbances can be confused with rabies. Phobic and inspiratory spasms are seen only in rabies. Other conditions mimicking rabies include intoxication by a variety of substances such as atropine-like compounds and cannabis, alcohol withdrawal (delirium tremens), and acute serotonin syndrome from taking serotonin reuptake inhibitors [1]. Tetanus resembles rabies only in the form of reflex spasms [1]. All tetanus patients have a clear sensorium. Rabies patients do not have persistent rigidity or sustained contraction of axial musculature such as the jaw, neck, back, and abdomen as in tetanus. Spasms in rabies predominatly involve accessory respira-

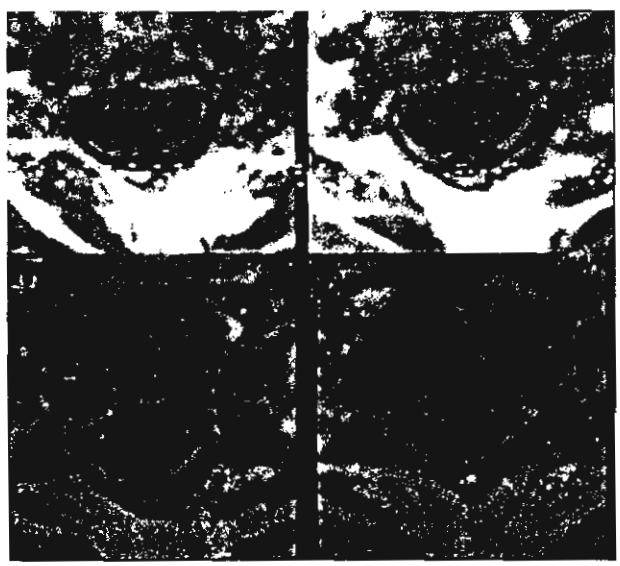

Figure 2 Axial T1-weighted MRI with gadolinium. Enhanced lesions in the gray matter of cervical cord and nerve roots in a paralytic rabies patient. (Courtesy of Dr. Jiraporn Laothamatas, Ramathibodi Hospital, Bangkok, Thailand.)

tory muscles and the diaphragm, whereas in tetanus spasms occur in axial muscles. Opisthotonos is present extremely rarely if ever in rabies.

In some parts of the world where nervous tissue rabies vaccine is still widely used, allergic encephalomyelitis must be included in the differential diagnosis. These vaccine-induced "accidents" develop in as many as 1 in 400 Semple vaccine–treated patients and less often in subjects who received mouse brain vaccine [3,4]. Neither phobic spasms, paresthesias at bite sites, nor fluctuating consciousness are present in these postvaccination reactions.

Acute motor axonal neuropathy (AMAN), an axonal form of GBS, shares many clinical features with paralytic rabies [28,29]. AMAN following *Campylobacter jejuni* infection may have preceding diarrhea that may be mistakenly diagnosed as a prodromal symptom of rabies. Areflexic quadriparesis and bilateral facial weakness without sensory deficits are observed in both conditions. Urinary incontinence is a common early symptom in paralytic rabies but rare in GBS. Inspiratory spasms with abnormal behavior may appear late in the clinical course and may be masked by generalized paralysis and superimposed metabolic disturbances that may occur in both conditions. Acute inflammatory demyelinating GBS (AIDP) or acute motor sensory axonal neuropathy (AMSAN) and GBS-like syndrome following nervous tissue rabies vaccine may exhibit some degree of sensory deficit. These are usually absent in paralytic rabies. Furthermore, the presence of local prodromal symptoms, even without a history of bite exposure and early autonomic dysfunctions (especially hypersalivation, abnormal pupils, and piloerection) suggest paralytic rabies.

9 ESTABLISHING THE DIAGNOSIS

An early antemortem diagnosis of rabies is extremely important. Delays in diagnosis result in much anxiety, potential spread of contamination, and an unnecessary expensive postexposure prophylaxis.

The diagnosis of furious rabies can be made with confidence when three classic or major cardinal signs are present together: fluctuating consciousness, phobic spasms, and autonomic dysfunction. However, in areas where bats are the principal vector of rabies to humans, these clinical expressions may be variable [9,10]. Phobic spasms were found in only half (10 of 20) of the cases. However, either phobic spasms alone or the presence of three or more of the following—agitation, confusion, seizures (16 of 20) or dysphagia (7 of 20), hypersalivation (10 of 20), limb pain, paresthesias (9 of 20), limb weakness, and paralysis or ataxia (3 of 20)—were significantly associated with antemortem diagnosis [18]. Local prodromal symptoms alone have to be interpreted with great caution because they may be modified by the patient's fear of rabies. A definite history of a bite, although commonly found in crv cases, is not helpful in cases associated with brv. Of the six brv cases reported between 1998 and 2000 in North America, only one had a definite history of a bat bite.

Serological testing in the serum and CSF may produce variable results [1,10,30,31]. Only 20% (6 of 31) of nonvaccinated Thai rabies patients had CSF rabies antibody (by rapid fluorescent focus inhibition test) within 1–26 days after the onset of the disease. All antibody-positive serum samples were obtained within 9 days after onset (3/6 within the first 3 days). None of the 27 Thai nonvaccinated crv-rabies patients were CSF antibody–positive to rabies virus [1,30]. Results obtained from the analysis of 102 samples from 39 cases in the United States and 16 in France since 1960 showed that serum antibody

usually developed if the patients survived more than 8 days (6 of 43 between days 1 and 8 versus 34 of 59 from day 9). Antibody in the CSF appeared later (0 of 19 between days 1 and 8 versus 10 of 28 on day 9) [10,31].

Rabies virus may be isolated in mouse neuroblastoma cells from saliva specimens [10]. This cell culture isolation is sensitive and specific, and results are known within 4–5 days. However, all samples to be tested must be maintained frozen after collection with no preservatives. The success rate also depends on the status of rabies antibody (13 of 15 were positive in antibody-negative patients, compared to 0 of 17 in antibody-positive patients). The intermittence of rabies virus shedding in the saliva also confuses these findings. One must also understand that false negative results may be obtained from decomposed brains.

Rabies viral antigen may be detected by fluorescent antibody technique performed on frozen sections of nuchal skin samples [8–10,31]. An examination of at least 20 sections is required to detect the rabies nucleocapsid inclusions around the base of hair follicles. The result is unrelated to the presence or absence of antibody. Earlier studies showed that the proportion of positive results tends to increase as the disease progresses [1]. However, in another 26 rabies patients, antigen could be detected in as many as five of six (82%) within 4 days after onset. This number dropped to six of 10 (60%) between days 5 and 8 and to seven of 10 (70%) from day 9 [31]. Detection of rabies viral antigen in corneal and salivary impression smears may yield false positive or negative results [32].

Brain biopsy with antigen detection yielded the highest sensitivity in two series [32]. False negative results may occur when brain biopsy is performed during the first few days of the clinical illness. This may be due to a relative lack of viral antigen in the frontotemporal region and can be overcome by RT-PCR. Our experience with the nested PCR in 500 dog and five human brain samples showed 100% sensitivity without positive or false negative results [30,33].

In addition to CNS tissue, saliva, CSF, tears, skin biopsy samples, and urine may be sources for detection of rabies virus RNA by RT-PCR or nucleic acid sequence based amplification (NASBA) [8,9,33–35. Serial samples should be tested, because not all are positive, owing to intermittent shedding of virus. Sensitivity was not affected by antibody status of the patients. The success rate also depends on primer selection and surveillance of genetic variation among different reservoirs in a certain geographical location

Where postmortem diagnosis from a complete brain autopsy is not possible, brain tissue can be obtained by Trucut needle aspiration through a transorbital approach [9]. It should be noted that only a minimal amount of brain tissue can be obtained by this technique and antigen detection by fluorescent antibody test may give a false negative result [personal experience].

10 MANAGEMENT

There is no specific treatment for rabies once clinical signs develop. Treatment is purely symptomatic, aiming to lessen the degree of agitation and to comfort the patient and family as much as possible. Preexposure vaccination is recommended for attending nurses and physicians who routinely care for patients with rabies. Only those who had true exposure despite precautions should receive postexposure treatment. Treatment with interferon and antiviral drugs such as ribavirin, adenine arabinoside, acyclovir, and inosine pronobex, intrathecal or systemic administration of rabies polyclonal antibody, and immunosuppre-

sive therapy such as high-dose steroids or antithymocyte globulin did not alter the course of the disease in humans [9,30].

11 PREVENTION

Successful postexposure prophylaxis (PEP) relies on prompt start of treatment whenever the biting animal cannot be killed and its brain examined immediately by fluorescence microscopy [5,8] (see Table 2). Whether or not a dog in an endemic area has attacked without provocation should not be considered in the treatment decision. Treatment may be discontinued if the dog or cat, but no other mammal, remains healthy throughout an observation period of 10 days. Treatment must be initiated if the dog or cat develops abnormal signs during this observation period. Treatment should be started in an exposed person regardless of the time interval that elapsed since exposure but is usually not administered after a time interval longer than one year. Pregnancy is not a contraindication for PEP.

Postexposure prophylaxis consists of local wound care (thorough cleansing with soap and water, followed by application of 70% ethanol or a solution of iodine), use of modern tissue culture rabies vaccine, and wound infiltration with human or purified rabies immunoglobulin (RIG) in the case of single or multiple transdermal bites or scratches or licks over mucous membranes [8] (Table 2). RIG serves to neutralize some, if not all, of the virus inoculum at the bite site and closes the time gap until neutralizing antibody elicited

Table 2 Guide for Postexposure Treatment

Category	Type of contact with a suspect or confirmed domestic or wild[a] animal, or animal unavailable for observation	Recommended treatment
I	Touching or hand feeding of animals; licks on intact skin	None, if reliable case history is available.
II	Nibbling of uncovered skin; minor scratches or abrasions without bleeding; licks on broken skin	Administer vaccine immediately.[b] Stop treatment if animal remains healthy throughout an observation period[c] of 10 days or if animal is euthanized and found to be negative for rabies by appropriate laboratory techniques.
III	Single or multiple transdermal bites or scratches; contamination of mucous membrane with saliva (i.e., licks)	Administer rabies immunoglobulin and vaccine immediately.[b] Stop treatment if animal remains healthy throughout an observation period[c] of 10 days or if animal is killed humanely and found to be negative for rabies by appropriate laboratory techniques.

[a] Exposure to rodents, rabbits, and hares seldom, if ever, requires specific anti-rabies treatment.

[b] If an apparently healthy dog or cat in or from a low-risk area is placed under observation, it may be justified to delay specific treatment.

[c] This observation period applies only to dogs and cats. Except in the case of threatened or endangered species, other domestic and wild animals suspected of being rabid should be euthanized and their tissues examined using appropriate laboratory techniques.

by active immunization appears. The wound should not be sutured. As much as possible of the RIG [human (HRIG) 20 IU/kg; equine (ERIG) 40 IU/kg] should be injected in and around the wounds. We discourage the practice of multiple punctures during this process because this, like wound suturing, may cause additional injuries to nerves [11]. If the entire volume cannot be administered at the wound, the remainder can be administered at a distant site, such as the deltoid opposite the vaccine dose or the anterior thigh. In the case of multiple severe wounds, where RIG is insufficient in volume for infiltration of all wounds, dilution with saline solution to an adequate volume is recommended. RIG can be administered with a delay of up to 7 days after the initiation of vaccine treatment. A skin test prior to ERIG administration is required. ERIG is approximately 80% less expensive than HRIG in Thailand. Unfortunately, a shortage of ERIG supply may occur in the near future [36]. All tissue culture rabies vaccines such as human diploid cell (HDCV), purified Vero cell (PVRV), and purified chick embryo cell (PCECV) rabies vaccines are equally safe and effective. These vaccines can be given by the intramuscular (IM) route (at deltoid or anterolateral thigh muscles in children) on days 0, 3, 7, 14, and 28 or 30. The economical Thai Red Cross intradermal (ID) multisite regimen (2–2–2–0–1–1) consists of 0.1 mL of any potent tissue culture vaccine injected at two different sites on days 0, 3, and 7 and at one site on days 30 and 90. The Oxford intradermal multisite regimen (8–0–4–0–1–1) consists of 0.1 mL of any potent tissue culture vaccine injected intradermally at eight sites on day 0 (both sides of deltoid, lateral thigh, suprascapular region, and lower quadrant of the abdomen), at four sites on day 7, and at one site on days 28 and 90 [8]. Both intradermal regimens have been approved by WHO.

Persons who have been previously vaccinated with either pre- or postexposure regimens using a tissue culture or avian origin vaccine should receive two boosters on days 0 and 3 after a potential reexposure. No RIG is needed [8].

To date, there is no approved PEP for HIV-infected individuals. These patients have shown a low or even absence of antibody response after rabies vaccination [8].

Preexposure vaccination is recommended for subjects who are at a continuing risk of exposure (e.g., laboratory technicians and veterinarians.) [8]. The recommended preexposure immunization schedule consists of three intramuscular doses or three intradermal (0.1 mL) injections on days 0, 7, and 28 at the deltoid area. Also, neutralizing antibody titers should be checked every 6 months. If the value is less than 0.5 IU/mL, a booster dose of vaccine should be given using either the intramuscular or intradermal route.

For an individual who is on antimalarial chemoprophylaxis or is immunocompromised, IM injections are preferable.

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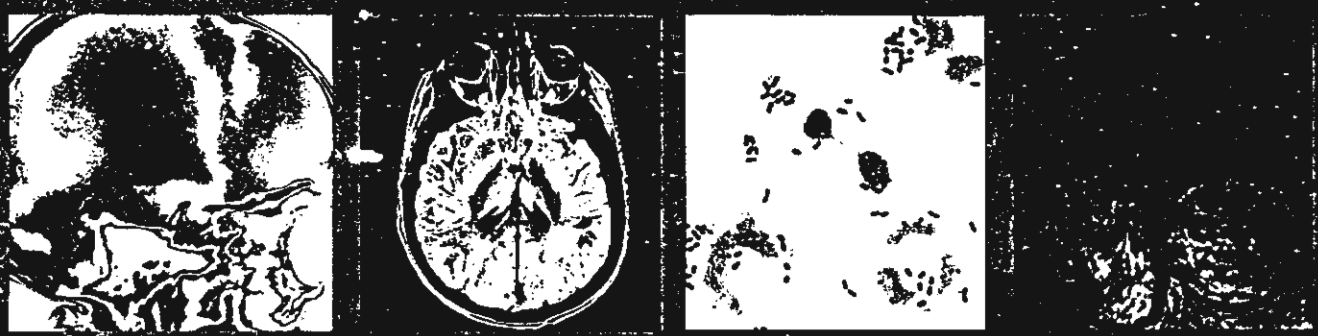

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Principles of NEUROLOGIC Infectious Diseases

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CHAPTER 11

Rabies

Thiravat Hemachudha and Charles E. Rupprecht

Rabies is an acute progressive encephalitis with the highest case to fatality ratio of any infectious disease. The causative agents are neurotropic RNA viruses in the family Rhabdoviridae, genus *Lyssavirus*, of seven putative genotypes. Rabies has been recognized since antiquity and is the most significant viral zoonosis today. At present, rabies kills more people than yellow fever, dengue fever, and Japanese encephalitis.[1] Despite millions of human exposures and thousands of human deaths, primarily in developing countries in Asia, Africa, and Latin America, the World Health Organization (WHO) ranks rabies low on its list of important infectious diseases. In contrast, rabies virus infections in humans in the United States and other developed countries are uncommon, accounting for no more than a few cases each year. Mammalian reservoirs include species

in the Carnivora and Chiroptera, but rabid dogs still pose the greatest hazard worldwide.

Clinical symptomatology, once believed to be unique, may be more variable, particularly in bat-related cases. This causes diagnostic confusion. Differences in cellular tropism either at the inoculation site or in the nervous system, as well as differences in routes of spread, viral variants, and participation of host factors, may account for this diversity. Unreliable epidemiologic data and the complexity of clinical manifestations are compounded by limitations in diagnostic laboratories. With rapid movement of people and animals, cases can appear in regions where rabies has been eliminated or never recorded. Strict adherence to WHO guidelines for rabies prophylaxis has proven effective, and deviations should not be allowed. Physicians should diagnose and differentiate rabies from other neurologic conditions and understand how to provide proper postexposure prophylaxis (PEP).

▶ HISTORY

Rabies is one of the oldest recognized infectious diseases. Few other maladies possess the mythical linkage with animals, exposures, and ensuing dramatic illness. Although the annual disease burden is less than direct human contagions such as smallpox, influenza, and acquired immunodeficienct syndrome (AIDS), the striking clinical manifestations and inevitably fatal progression of rabies have secured its place to the present as the most important viral zoonosis.[2] Ancient civilizations were aware of the disease.[3–5] Passages described in the pre-Mosaic Eshnunna Code of Mesopotamia from the twenty-third century B.C. harken to the dangers of bites from the "mad dog," as well as the ensuing legal penalties to the dog's owner. Familiarity with rabies is apparent from Greek, Roman, Hebrew, and Chinese literary, religious, and medical writings. Careful observers surmised that a poison or a "virus" was present in saliva. Inquisitive scholars rightly noted clinical aspects of infection, including that the patient seemed tortured by thirst and the concomitant repulsion toward water. Early suggestions for treatment included excision of tissue and cauterization of the wound, as well as dunking victims into water, and all manner of ingested potions and apothecary concoctions. Throughout the ages, various talismans were believed to be curatives, as was the need for divine intervention. Although superstitions and supposed cures would persist to the present day in several cultures (including the suggestion for salves of hot chili peppers or animal venoms applied directly to the wounds), many examples from the classical periods to the Renaissance contained otherwise remarkable moments of clarity and pragmatism. For example, the Italian physician Fracastoro, in a treatise of 1546 entitled, *The Incurable Wound*, vividly described a human case: ". . . its incubation is so stealthy, slow and gradual that the infection is very rarely manifest before the 20th day, in most cases after the 30th, and in many cases not until four or six months have elapsed . . . cases recorded in which it became manifest a year after the bite . . . the patient can neither stand nor lie down; like a madman he flings himself hither and thither, tears his flesh with his hands, and feels intolerable thirst . . . the most distressing symptom, for he so shrinks from water and all liquids that he would rather die than drink or be brought near to water; it is then that they bite at other persons, foam at the mouth, their eyes look twister, and finally they are exhausted and painfully breathe their last."[6]

Despite its widespread occurrence and recognition in the Old World for apparently thousands of years, the history of rabies in the New World is complicated by a paucity of written records prior to European colonization. Likely, it was present well before Columbus' arrival in the fifteenth century. For example, as cited by Koprowski in an allusion to vampire bats, the bishop Petrus Martyr-Anglerius wrote: " . . . in places bats not much smaller than turtle doves used to fly at them in the early evening with brutal fury and with their venomous bites brought those injured to madness, . . . bats . . . come in from the marshes on the river and attack our men with deadly bite." Several hundred years would elapse before the major role of bats in rabies was better appreciated.[7] Elsewhere in North America, a rabid wolf was reported by the Reverend Lucio Marmolejo in Mexico during 1709, and some 200 years after initial Spanish invasion, canine rabies was described in 1753 from the Virginia Colony and later among foxes.[3,8] Human migration had left its mark, and the risk posed by translocation of animals as viral portals continues to this day.

For centuries, the bite of a rabid animal was thought to be the likely source of rabies infection, but it was Zinke (1804) who demonstrated experimental disease transmission using dog saliva.[9] In 1879, Galtier experimented with rabies in rabbits as a model and transmission from rabbit to rabbit.[5] Clinical descriptions, paired with animal inoculation, formed the basis for rabies diagnosis until the advent of light microscopy and observation of microscopic lesions. Negri (1903) described the cytoplasmic inclusions that bear his name (Negri bodies) in neurons of rabid animals.[10] While the value of Negri bodies was appreciated and debated throughout the twentieth century, their nature and composition awaited development of electron microscopy in the 1960s (when virions actually were visualized). Laboratory-based diagnostics did not improve significantly until development of the immunofluroscent technique.[11]

Pasteur's collected work on rabies is probably the best known historical achievement in the field.[12] Build-

ing on the knowledge that preceded them, Pasteur and his colleagues in their cramped Parisian quarters adapted the virus to animals and succeeded in altering its properties. This remarkable French team applied basic scientific concepts and approaches toward the first protective protocol against rabies. Desiccated spinal cords from infected rabbits formed the raw material for the vaccine. Progressive inoculations of material, from oldest to freshest, amounted to increased doses of infectious viral inocula. Despite severe reservations over preparedness for human experimentation, on July 6, 1885, a young boy, Joseph Meister, who was bitten at multiple sites by a rabid dog 3 days earlier, received the first of multiple inoculations of vaccine and, to everyone's relief, became the first registered survivor.

After considerable medical debate, Pasteur's method became the accepted approach throughout the world in the early twentieth century as other vaccine evolutions began.[13] Gradual improvements to the technique included the addition of chemical fixation and adaptation to duck embryos and suckling mice to improve vaccine safety.[14–17] Continual modifications were needed because improperly inactivated virus could cause rabies, and brain tissue induced neuroparalytic accidents.[18] Also, vaccine alone was not totally effective in cases of severe bites, such as those inflicted on the face and head by rabid wolves. Postexposure prophylaxis (PEP) against rabies through the simultaneous administration of antirabies serum and vaccine was suggested as early as the late 1800s.[19] The combined approach found few adherents until the 1940s, when international interest was revived. After a WHO-sponsored trial in 1954, analysis of the combined use of serum and vaccine was found to be more effective than vaccine alone.[20,21] Today, the combination approach is the recommended standard for human rabies prophylaxis.

Additional adaptation of the virus to primary and, later, continuous cell lines removed the need for animal vaccine production.[22,23] In the 1960s and 1970s, a rabies virus grown in human diploid cells was used as a source of purification and concentration for production of a safe and efficacious vaccine[24] and eliminated many of the problems connected with neuronal tissue and poor potency from other cultures. The human diploid cell vaccine (HDCV) is used widely throughout the world, although for economic reasons several developing countries still use nervous tissue vaccines. After demonstration of the primary utility of HDCV, other cell-culture vaccines were developed, including those produced in Vero cells and avian embryos.[25] Newer generations of vaccines throughout the twenty-first century have an impressive legacy to exceed and will carry the considerable burden of maximum purity, potency, efficacy, and safety while still being affordable in those regions afflicted with enzootic dog rabies.

▶ EPIDEMIOLOGY

A better understanding of the epidemiology of this complex zoonosis requires an appreciation of the agent, its hosts, and the environmental facets that will shape its distribution over time.[26] At one point only a single agent was believed to cause rabies. However, in the 1960s and 1970s, various serotypes were suggested between rabies virus and what were then termed *rabies-related viruses* before use of the designation *Lyssavirus*.[27] During the 1970s and 1980s, placement within the taxonomic group was defined by serologic cross-reactivity of viral antigens (e.g., by complement fixation, immunofluorescence, neutralization tests, etc.) based on antigenic sites on the nucleoprotein (N) and glycoprotein (G) using monoclonal antibodies (MAbs).[28,29] More recently, sequence data became available, with a trend toward phylogenetic classification.[30–35] Application of MAb typing and gene sequencing to the study of lyssaviruses provided substantive evidence for considerable antigenic and genetic variation. Major virus species currently or putatively assigned to the *Lyssavirus* genus.[36] all isolated from mammals, include rabies virus, Lagos bat virus (LBV), Mokola virus (MOK), Duvenhage virus (DUV). European bat virus type 1 (EBV-1), European bat virus type 2 (EBV-2), and Australian bat virus (ABV). Several viruses, such as Obodhiang and Kotonkan, found only in Africa and originally isolated from mosquitoes or midges,[37] and Rochambeau virus,[38] isolated from mosquitoes in French Guiana, have been suggested by some for consideration in the genus, primarily based on serologic data alone, but are unlikely candidates. Other more likely *Lyssavirus* prospects, such as those isolated from bats, with shared antigenic and genetic properties, and known to produce encephalitis, await ultimate taxonomic assignment.[39]

Modern molecular methods have been particularly useful in determining the extent of natural variation among lyssaviruses isolated from wildlife reservoirs within restricted geographic areas or from separate continents over time.[40] In particular, distinctions between viruses isolated from bats and terrestrial carnivores became obvious.[41] Notwithstanding genetic variation and the general tendency or relatively rapid evolution of RNA viruses, striking global patterns have emerged that are highly suggestive of a rather conservative strategy presented by any particular variant.[35] Intragenotypic viral clusters are distinguishable, suggestive of historical, geographic, or host-species relationships. Combined with historical temporal and spatial disease surveillance data, antigenic characterization with MAbs and nucleotide sequence analysis can help to assign isolates to different animal rabies reservoirs. In addition, antigenic and molecular characterization is useful in investigating unusual or unexpected mortality from rabies, especially in domestic animals or humans with no obvious expo-

sure history. The antigenic patterns or nucleotide sequences obtained can be compared with variants from known animal reservoirs. For example, analysis of recent human rabies cases from the United States implicate specific viral variants associated with insectivorous bats in the etiology of infection.[42]

Rabies is enzootic on all continents except Antarctica. Rabies virus is the prototype species of the *Lyssavirus* genus and can infect warm-blooded animals (including birds) under experimental conditions, but only mammals are significant natural hosts. Host range is broad, stretching from an exotic alphabet of species from armadillos to zebras, but most of these are epidemiologic dead ends, even if an episode is as dramatic as an infected pachyderm.[43] Major species responsible for transmission include dogs, foxes, jackals, coyotes, wolves, cats, raccoons, skunks, mongoose and their relatives, and bats.[44] Several countries, such as Japan and many islands, are reportedly "rabies free" either because of their isolation or due to considerable animal control efforts, but bats can reach even distant islands.[45]

The lyssaviruses DUV, LBV, and MOK are restricted to Africa. Although bats and small mammals such as shrews have been suggested as potential reservoirs,[46] relatively little is known about these African agents compared with rabies virus. Compared with other lyssaviruses, MOK appears to be the most disparate member recognized to date, with divergent amino acid changes in the antigenic sites mapped to the G protein, partially explaining the absence of cross-protection with vaccine.[47,48] In Europe, phylogenetic analysis of EBV suggests at least two genetically distinguishable lineages, possibly related to spatiotemporal introduction from different geographic locations, such as North Africa.[49] Bats, such as *Eptesicus serotinus, Myotis dasycneme,* and *M. daubentonii,* appear to be principal reservoirs for EBV. Australia was believed to be free of the disease until 1996,[50] when indigenous lyssaviruses were discovered among bats. These viruses appear to use either frugivorous Megachiroptera or insectivorous Microchiroptera as primary reservoirs and have caused at least two human deaths. The ultimate importance of the nonrabies lyssaviruses remains a matter of speculation. All mammals are likely susceptible to some degree, but experimental results suggest a rough hierarchy for species susceptibility. However, most experimental animal studies occurred before antigenic or genetic differences between lyssaviruses were appreciated. Wild canids are very susceptible to infection. Cats, important as vectors but not as reservoirs, and some wildlife species, such as raccoons, appear moderately susceptible to infection. The opossum (*Didelphis virginiana*) appears especially resistant.[51] Despite their varying ability to infect different cell cultures, no lyssaviruses have been isolated from cold-blooded vertebrates. Laboratory rodents, such as mice, have been used extensively for rabies diagnosis, vaccine testing, and pathogenesis studies, but laboratory rodents are epidemiologically insignificant as *Lyssavirus* vectors or reservoirs[52] compared with species among the Carnivora and Chiroptera.

An accurate calculation of the true incidence of human rabies from a global perspective is difficult due to the lack of reporting and accurate diagnosis in most developing countries but has been estimated at between 0.1 to 29 cases per 1 million inhabitants.[53] In developing countries, although most age ranges may be represented, most are young (<20 years), and more than 50 percent are male.[54] Clearly, human rabies may be prevented by avoiding exposure to rabid animals or by the application of prophylaxis after exposure occurs. Practical animal control efforts should be based on local rabies epidemiology. Most developed countries have eliminated canine rabies, and human fatalities are at or near zero, either imported or related to wildlife cases. Nevertheless, when exposures do occur, especially en masse, most tend to be associated with an infected domestic animal, even in the United States.[55]

In effect, *Lyssavirus* epidemiology is influenced in part by host species distribution, abundance, demographics, behavioral ecology, dispersal, and interactions with humans. Due to its severe consequences when ignored, rabies is a reportable disease in several, but not all, countries. Epidemiologic information on rabies usually originates from results of the examination of brain material submitted to public health or veterinary diagnostic laboratories when contact with wildlife or a domestic animal is suspected. The spatiotemporal distribution and relative intensity of rabies infection in various mammalian species thus often are described based on the passive monitoring of suspicious contact cases and frequently on a clinical basis only.

Rabies virus does not persist in the environment but rather is perpetuated in a variety of reservoirs. The dog is the principal host and major vector of rabies throughout the world.[56] International reporting of both human and animal rabies cases, suggested in the tens of thousands and tens of millions, respectively, grossly underestimates the magnitude of the problem. Predominant wild reservoirs include foxes in Arctic areas (*Alopex lagopus*), central and western Europe (*Vulpes vulpes*), and scattered foci elsewhere throughout the United States (e.g., *Urocyon cinereoargenteus*); the raccoon dog (*Nyctereutes procyanoides*) in Eurasia; jackals (*Canis* species) and other wild canids in Asia and Africa; skunks (*Mephitis mephitis, Spilogale putorius*) in North America; procyonids, such as the raccoon (*Procyon lotor*), in the eastern United States; and viverrids (e.g., the yellow mongoose, *Cynictis penicillata*) in Asia and Africa. The Indian mongoose (*Herpestes auropunctatus*) was introduced into several Caribbean islands during the nineteenth century and persists as a major agricultural and public health threat. Rabies detection in

rodents and lagomorphs is uniformly rare. Bat rabies (due to true rabies virus, as opposed to ABV, DUV, EBV, LBV, etc.) predominates as a New World phenomenon, described primarily among the insectivorous species of the United States and Canada (some 40 species) and the three hematophagus vampire species (principally *Desmodus rotundus*) ranging from northern Mexico to Argentina. Renewed investigations suggest that other bat species also may be important throughout Latin America.

In contrast to parts of Europe and North America, where wildlife rabies predominates, in Asia, Africa, and much of Latin America, dogs continue to be the principal causative vectors to humans. Vaccination of a critical percentage of dogs, on the order of 40 to 70 percent, should be adequate to interrupt canine rabies transmission.[57] Realizing the importance of dogs as the primary reservoirs, the Pan American Health Organization began a program in 1983 to eliminate urban rabies from the principal cities of Latin America by the year 2000, and results to date have been very encouraging.[58] From the 1980s to the 1990s, the annual number of human rabies cases decreased from some 350 to less than 114; rabies-specific mortality declined from 1.3 to less than 0.2 deaths per 1 million exposures, and the proportion of 414 cities free of rabies increased from 75 percent to more than 80 percent. These results suggest that widespread vaccination of canine populations can reach sufficient levels for the herd immunity needed to prevent rabies epizootics and that elimination of canine rabies, at least in major urban areas, may be an achievable goal. Rabies transmission by hematophagous bats, unique to the Americas, is an emerging public health problem, in addition to being a historically important disease of livestock with widespread economic implications.

Properly applied, regional epidemiologic surveillance on animal rabies can reduce human morbidity (from inappropriate treatment) and mortality ascribed to lyssaviruses significantly by identifying typical versus unlikely reservoirs, developing treatment algorithms, assessing occupational groups at risk, and targeting veterinary efforts in animal control. Efforts in the United States illustrate the benefits of a systematic surveillance approach that defines rabies as single- or multiple-species assemblages.[59] For example, during 2001, 7437 cases (an increase of ~1 percent over 2000) were recorded. In contrast to widespread canine rabies of the 1940s, more than 90 percent of current cases are from wildlife. Most of the cases (37 percent) resulted from the continued spread of raccoon rabies due to progression of an outbreak initiated in the late 1970s when animals from an infected location in the southeastern United States were transported to the Virginias. Rabies cases increase after primary introduction, whereas the infection spreads successively within local populations.[60] Other major wildlife contributors included bats (17 percent), skunks (31 percent), and foxes (6 percent). Cases of coyote rabies from an outbreak in southern Texas continued substantial decline. Dómestic species included cats (4 percent), cattle (1 percent), and dogs (1 percent). Historically, Hawaii remained the only rabies-free U.S. state, never having reported a case of indigenously acquired rabies, due in part to its remote location.

Rabies is not considered a practical candidate for actual global disease eradication at this time because of the numerous wild reservoirs. However, the historical correlation between the reduction of canine rabies and decreased human fatalities has led to the successful application of herd health programs using vaccine-laden baits for wildlife, which ultimately may help to reduce the associated disease burden, if it can be accomplished over large areas with diverse hosts of high population density in a cost-effective manner.[61]

▶ CAUSATIVE AGENTS

Together with its taxonomic allies, rabies virus with its distinct bullet shape is in the family Rhabdoviridae and in the genus *Lyssavirus*. Both terms are of Greek derivation, meaning "rod" and "rage or frenzy," respectively.[62,63] At present, the genus *Lyssavirus* contains seven putative genotypes. Rabies virus is representative of genotype 1, whereas the other six genotypes are composed of: LBV (genotype 2), MOK (genotype 3), DUV (genotype 4), EBV-1 and EBV-2 (genotypes 5 and 6), and ABV (genotype 7). All of these, except genotype 2, have been associated with human disease (however, one has to realize that few diagnostic facilities exist in Africa with the laboratory ability to detect and differentiate non-rabies virus infections). Clinical manifestations in most *Lyssavirus* infections share several features of a classic rabies encephalitis.[64] Rabies virus and the other members of the family Rhabdoviridae, as well as several other RNA virus groups (the families Paramyxoviridae, Filoviridae, and Bornaviridae) that contain nonsegmented, negative-sense, single-strand RNA genomes, constitute the order Mononegavirales.

Rabies virus particles measure approximately 180 × 75 nm. Its genome consists of 11,932 or 11,928 nucleotides [based on an analysis of Pasteur virus (PV) and Street Alabama Dufferin (SAD)–B19 strains, respectively] and contains a leader sequence at the 3′ end, followed by five monocistronic genes that encode the nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G), and RNA transcriptase (L).[62,63] At the core of this bullet-shaped virus is the ribonucleoprotein (RNP), which consists of helical RNA and the N, P, and L proteins. The M and G proteins are associated with a lipid bilayer envelope. The G protein forms approximately 400 trimeric spikes covering all but the flat end

of the virion. The events during infection require transcription of these genomes to produce complementary messenger RNA (mRNA) molecules for synthesizing their corresponding proteins and a full-length positive-strand intermediate RNA. This antigenome RNA serves as the template for replication. Transcription and replication are ensured by the RNP complexes of the N, P, and L proteins. The classic neuronal inclusion, or Negri body, is an accumulation of intracellular matrix formed by an excess amount of RNA-protein complex.

▶ PATHOGENESIS

Rabies is the quintessential neurotropic virus infection. It is considered the most dramatic infection of the nervous system owing to its high fatality rate, unpredictable incubation period, and horrific clinical picture. Rabies virus may infect all mammalian species. However, species have different levels of susceptibility and variable transmission potentials.[65] Foxes and other wild canids are extremely susceptible to infection.[63] The pathogenesis of rabies virus infections can be considered according to "bite" or "nonbite" acquisition. However, it is the bite route that usually accounts for human disease. Sequential steps or cascades following peripheral inoculation of rabies virus include an eclipse phase, access to the peripheral nerve (with or without replication in peripheral tissues) with centripetal spread to the central nervous system (CNS), dissemination within and spread from the CNS to extraneural sites (particularly the salivary glands), and neuronal dysfunction and death.

Exposure

Human rabies cases are almost always attributable to a bite from a rabid animal. Animal bites were the cause of 99 percent of 3920 human rabies cases examined at Pasteur Institutes throughout the world in the first half of the twentieth century.[66] Virus does not enter intact skin. Although it is an almost universally fatal disease once signs develop, not all bites from rabid dogs result in death. Mortality after untreated bites by rabid dogs varies from 38 to 57 percent.[65,67] However, exposure to rabid animals of other species, such as wolves, may result in 80 percent or more mortality. Efficient transmission, therefore, depends in part on the degree of severity of the bites, the locations of the wound, the quantity of inoculum in the saliva, and the variant of the virus.

Bites at areas that contain a high density of nicotinic acetylcholine receptors (AchR), such as the head or face, particularly with bleeding, carry the highest risk and usually are associated with a shorter incubation period due to proximity to the CNS. Highest mortality tends to occur in persons bitten on the head and face (up to 80 percent or more), with intermediate mortality in those bitten on the hands or arms (15 to 40 percent) and least in those bitten on the trunk or legs (5 to 10 percent) or through clothing (<5 percent), when no specific prophylaxis was initiated.[68,69] Nevertheless, single bites at any location that are deep enough to reach the muscles should be treated with the same urgency.[70] The risk of rabies acquisition is at least 50 times higher with a bite than with scratches (5 to 80 percent versus 0.1 to 1.0 percent).[71] Most recent bat rabies–associated human deaths did not have a reported exposure source, but these cases are likely due to bat bites in which either the risk was not appreciated or the bites were not recognized by the patient owing partly to the unique ability of these agents to replicate in the nonneural tissues.[42,72,73]

Nonbite transmission includes inhalation from aerosolized virus in caves inhabited by millions of rabid bats and in laboratory accidents with aerosolized virus.[1,2] Transmission is also possible by handling and skinning of infected carcasses and through corneal transplantation. Other exposures, such as aerosols, licks, scratches, or other unusual events that lead to contamination of an open wound or mucous membrane, rarely cause rabies.[1,63] Although there is a potential risk from contact with patients, because secretions commonly contain viable virus,[74–76] there are no such documented cases.[65,77] Since secretions contain viable virus, masks should be worn when caring for these patients. Transplacental transmission has been reported very infrequently and has not been verified recently. The potential mechanism for such an unusual occurrence is not known. In contrast, infants born to mothers with rabies were found to be healthy.[78] Immediate rabies PEP and interferon were initiated at delivery. The importance of oral transmission of rabies infection remains uncertain, although this can occur under experimental conditions in animal models either by direct oral route or by gastric tube administration.[79] Rabies PEP was given to people who consumed nonpasteurized milk from a rabid cow without incident.[80]

Transit to the Central Nervous System (CNS)

Eclipse Phase Versus Direct Nerve Entry

Another characteristic of rabies is an extremely variable incubation period, which may range from less than 7 days to more than 6 years.[81–84] Persistence at the site of exposure may explain the long incubation period. Those who died in less than 1 week usually sustained a direct injury to a nerve or brachial plexus from dog bites.

Following a successful introduction into the wound, rabies virus may go directly into nerves. This

ability was confirmed by inoculation of rabies virus into the anterior chamber of the eye in rats and in a mouse model by inoculation into the masseter muscle or the forelimb.[79,85–87] Alternatively, rabies virus may be localized within the muscle cells, at the neuromuscular junction, or in the skin at the site of exposure and undergo an "eclipse" phase. The eclipse phase during incubation specifically refers to the period after viral entry in the periphery and passage in the axoplasm when we are in the dark as to where virus may be or in what form, hence in eclipse or unable to be visualized. The eclipse phase ends when virus may be detected in the CNS, but in the more common scenario, this occurs with the onset of clinical disease. The role of rabies virus persistence in bone marrow cells to explain this eclipse phase is still intriguing but unproven.[88] The factors that control the length of this silent period are undefined.

In the case of canine rabies virus, the viral G protein (residues 174 to 202) may bind to the alpha subunit of the nicotinic AchR on the muscle and subsequently multiply in the muscle cells.[89–94] In one study in skunks, rabies virus antigen and genome could be demonstrated as long as 2 months after inoculation into muscle.[95] In the case of some bat lyssaviruses, the virus may bind to unknown receptors in the epidermis or dermis.[42,72] This silent phase of localization at exposure sites, in turn, provides an opportunity for host immune clearance and for PEP.[96]

Centripetal Spread to the CNS

After budding from the plasma membrane of muscle cells, virus is taken up into unmyelinated nerve endings at the neuromuscular junctions or at the muscle spindles. Rabies virus is transported to the CNS via retrograde fast axonal transport, which can be blocked by colchicine or vinblastine injection.[97] Studies in rhesus monkeys indicate that motor nerves are preferentially involved.[98] Rabies virus P protein (residues 138 to 172) interacts with dynein light chain 8 (LC 8), a component in actin- and microtubule-based transport, in this retrograde movement within the peripheral nerve and in the CNS.[99–101] The virus replicates again in the dorsal root ganglia and anterior horn cells.[102] At the dorsal root ganglia, viral replication may be recognized and attacked by immune effector cells, resulting in ganglioneuronitis and a clinical prodrome of neuropathic pain at the exposure site.[1,103] This local prodrome is found more frequently in bat-related than in dog-related cases (70 versus 30 percent).[81] Some bat rabies virus variants also may replicate more effectively in the skin than in the muscle.[72] These observations suggest a preferential sensory pathway in bat-related cases.

Travel from the peripheral nerves to the CNS occurs at a constant rate of 8 to 20 mm/day. However, the first development of the local prodrome, even with the absence of any other neurologic deficits, defines the patient's fate. Studies with the fixed-challenge virus standard strain of rabies virus in cocultures of chick spinal cord and muscles showed that the neuromuscular junction is the major site of entry into neurons.[94] Colocalization of virus and endosome tracers within the nerve terminals, which subsequently accumulate in axons and nerve cell bodies, indicates retrograde transport of endocytosed virus from motor-nerve terminals.

Although nicotinic AchR is an important rabies virus receptor for virus spread from the inoculation site to the CNS, it is unlikely to be the only receptor that mediates viral entry into neurons. Nicotinic AchR is not present on all types of neurons susceptible to rabies virus. Rabies virus also may use other central receptors, such as carbohydrates, phospholipids, gangliosides, the neural cell adhesion molecule (NCAM or CD56), and low-affinity nerve growth factor receptor p75 neurotrophin receptor (NTR) to gain entry into cells.[79]

Spread Within the CNS

Once the virus is in the neurons, rapid amplification and dissemination take place. Virus disseminates through plasma membrane budding and direct cell-to-cell transmission or by transsynaptic propagation, which occurs exclusively by retrograde axonal transport.[98,102] It is not known which tract is preferentially involved.[104] The G protein is required for attachment to neuronal receptors, as well as for transsynaptic spread.[62,105]

The hippocampus was once believed to be predominantly involved, suggesting that virus localization to this area may account for the altered behavior and rage reaction. Subsequent studies showed that infection involves the brain stem and thalamus most prominently both in animals and in humans.[106,107] In human rabies, rabies virus antigen is found predominantly in brain stem, thalamus, and spinal cord regardless of the clinical type if the survival period is less than 7 days.[107] Moreover, the hippocampus contains a minimal amount of rabies virus antigen.[106–108] Studies in skunks infected with skunk street rabies virus showed that the areas that contained heavy accumulations were the motor nucleus of the vagus nerve, midbrain raphe, hypoglottis, and red nuclei.[109] These street rabies virus–infected skunks manifested as furious rabies.

Spread from the CNS

An important component of disease is the centrifugal spread of virus back out of the CNS to peripheral sites.[63] Specifically, virus is transmitted to acinar cells of the salivary and submaxillary glands, resulting in salivary excretion of virus. Transport of virus back out of the CNS can lead to infection of head and neck tissues. The observation of virus infection at these sites has resulted in the recognition that biopsies of the nape of the neck

are extremely helpful for diagnostic purposes.[96] Additionally, virus can be found in corneal cells, drawing the association between corneal smears and a rapid diagnosis of rabies infection; although this may not be as sensitive as biopsies of the nape of the neck.[110] Thus corneal transplants can be a source of infection for person-to-person transmission, as reported.[1,63] Virtually all organs can become involved following natural infection, including the heart, kidney, lung, and gastrointestinal tract.[96,111,112]

▶ PATHOPHYSIOLOGY

Lack of Correlation Between Clinical Severity and Pathology

The amount of rabies virus in the CNS does not appear to determine the clinical and functional severity of the disease. Access of the virus to the CNS does not necessarily lead to a rapid development of symptoms and death. High titers of virus in the brain and spinal cord can be found in animals long before clinical signs appear.[113] Rabies virus antigen has been demonstrated extensively in the frontal lobe of one paralytic rabies patient who had quadriplegia and respiratory failure requiring ventilatory support. He was euphoric but still fully conscious at the time of biopsy, which was performed 19 days after onset of the clinical illness.[110] The degree of muscarinic AchR functional modifications in the hippocampus of rabid dogs was not dependent on the amount of virus.[114]

Pathologic findings, although similar to those encountered with other encephalitides, are significantly less extensive. This is in drastic contrast to the striking clinical symptomatology of hydrophobia, frenzied activity, and bizarre behaviors. Perivascular cuffing, neuronophagia, neuronal necrosis, and parenchymal infiltrations are limited.[107,115] Moreover, virus localization may not solely explain limbic symptomatology or the diversity of clinical manifestations (furious and paralytic rabies).

Furious and Paralytic Rabies

Differential Response Versus Differential Infection

In human rabies, cerebral symptoms dominate the clinical picture in furious rabies, and peripheral nerve or anterior horn cell symptoms dominate the clinical presentation in paralytic rabies. There is no correlation between the two distinct clinical patterns and the site of the bite, species of responsible vector, presence or absence of a history of previous immunization, and the incubation period.[65,107,108,116] Almost all human rabies cases in Thailand were associated with canine rabies virus of genotype 1. Similar regional CNS rabies antigen distribution, as well as similar magnetic resonance imaging (MRI) findings, could be found in both furious and paralytic rabies.[107,116] The brain stem and thalamus were predominantly involved. An MRI in a case of nonclassic rabies due to a bat bite revealed similar findings.[117] This suggests that functional changes and clinical manifestations (including mood and behavior, motor weakness, etc.) are due to a differential response of the various CNS regions.[108] Areas that contained a minimal amount of rabies virus antigen, such as the hippocampi, hypothalami, and subcortical white matter, also were abnormal on MRI in both clinical forms.[116]

Specific Rabies Virus Variants in Furious and Paralytic Rabies

Different rabies virus variants associated with particular vectors have been postulated to be responsible for these clinical manifestations. Differences in G protein may affect G protein–receptor interactions, such as nicotinic AchR at the bite site, the NCAM (CD56), and the p75 NTR, as well as glycolipid or ganglioside CNS receptors.[62] Minor variations on the G protein, such as an amino acid substitution of arginine at position 333, also can affect neuroinvasiveness and the use of different neuronal pathways and distribution in the CNS.[62] Furthermore, some bat lyssaviruses may bind to other unidentified receptors in the epidermis or dermis.[72] Patients with some acquired bat-related rabies viruses are reported to have clinical features substantially different from those of dog-related cases.[1] In addition, the rabies viral capsid P protein interacts with the microtubule dynein light chain in retrograde axonal transport.[62] Modifying the dynein light chain binding site can reduce the efficiency of the peripheral spread of certain rabies viruses.[101] Differences in genotypes or in associated structures required for binding and transport may affect rabies virus propagation and spread, thus resulting in variations in clinical manifestations (nonclassic rabies).[1]

Arguing against the concept of specific rabies virus variants in determining clinical manifestations, patients with classic rabies displayed a stereotypic clinical pattern and progression, unlike nonclassic rabies patients.[1] Although rabies acquired after exposure to a particular host, such as a vampire bat, generally is described as the paralytic form, a recent outbreak of rabies in Peru transmitted by vampire bats presented as the encephalitic form.[118,119] A single infected dog in Thailand transmitted the furious form of rabies to one patient and paralytic rabies to another.[110]

Comparison of a 1432-, a 1575-, and an 894-nucleotide region from the rabies virus N, G, and P protein genes of samples obtained from two furious and two paralytic rabies patients associated with canine ra-

bies virus of genotype 1 showed only minor nucleotide differences.[120] Deduced amino acid patterns of N protein were identical among both human and canine samples that belonged to the same geographic location regardless of the clinical forms. All differences in the amino acid of G protein were not in an interactive region with receptors known to be responsible for virus pathogenicity, nor did they lie in an immunodominant G domain. Arginine-333 was present in all samples. None of the amino acid differences of P protein were within the putative interactive site with dynein. These findings support the concept that clinical manifestations are not explained solely by the infecting rabies virus variant.

Peripheral Nerve in Paralytic Rabies

The site of neural involvement responsible for weakness in paralytic rabies is not clearly defined. There have been no reliable electrophysiologic data defining whether the defect is in peripheral nerve or anterior horn cells. Nonetheless, a histopathologic study performed in 11 paralytic rabies patients suggested peripheral nerve demyelination as the prime pathologic change.[121] Such demyelination was absent in patients with encephalitic rabies.[103] In the 17 peripheral nerve specimens studied, there was mild to moderate loss of myelinated nerve fibers in 11 nerves. Segmental demyelination and remyelination were present in 16 teased nerve preparations. Axonal loss of a variable degree was present in only 4 cases, and Wallerian-like degeneration in teased single fibers was noted in 6 nerves. In 9 nerves, the primary abnormality was segmental demyelination and remyelination or myelinated nerve fiber loss either singly or in combination. In none of these cases was Wallerian-like degeneration seen as the only pathologic feature. All spinal nerves studies showed evidence of Wallerian-like degeneration as well as segmental demyelination.[121] Our recent study agrees with a previous pathology report[121] that peripheral nerve is the prime target.[116] There was an intense inflammation and demyelination in spinal nerve roots corresponding to enhancing nerve root lesions on MRI.[116] Inflammatory reactions were much more intense in the spinal cord in furious rabies patients. Only mild inflammation of the spinal nerve roots of all levels was seen.

Immunologic Parameters in Furious and Paralytic Rabies

The particular involvement of the peripheral nerves in paralytic rabies, plus unexplained aggression and extreme excitability in furious rabies (despite similar virus distributions in the CNS and similar MRI patterns in the brain) and the lack of specific virus variants, suggests a participation of host factors. The degree of functional impairment of muscarinic AchR in the brains of rabies virus–infected dogs does not correlate with virus distribution and virus load.[114] Patients with intact T-cell immunity to rabies virus, with a high concentration of serum interleukin 2 (IL-2) receptor and IL-6, die earlier and present with furious rabies, whereas those lacking such responses survive longer and present with paralytic rabies.[108,110,122] Lack of T-cell responses to virus in paralytic rabies is not explained by excessive cortisol production or by a panimmunosuppressive process. Cortisol concentrations, although significantly higher than normal, were similar among patients with furious and others with paralytic rabies. Reaction against myelin basic protein was found in both furious and paralytic rabies patients who died rapidly.[108,110,122] An immune phenomenon also may be responsible for nerve injury, although neutralizing antibody to rabies virus could not be demonstrated in the cerebrospinal fluid (CSF) of paralytic rabies patients.[116] This immune phenomenon can be further supported by a patient with furious rabies who developed paralysis of all limbs soon after intravenous administration of human rabies immune globulin (RIG).[123] An immune attack against virus in the axons has been suggested in one Chinese paralytic rabies patient.[124]

Hypothetical Mechanisms in Furious Rabies

Participation of host response to neuronal infection also may explain aggression, the rage reaction, and autonomic stimulation signs in furious rabies.[1] Rabies virus infection in the brain stem leads to the production of cytokines and proinflammatory molecules, such as interleukins 1 alpha/beta, 6, and 10; tumor necrosis factor alpha (TNF-α), interferons, nitric oxide, and chemokines (Fig. 11-1). These cytokines can modify the hippocampus and other limbic system functions, including electrical cortical activity, the hypothalamic-pituitary-adrenal axis, and serotonin metabolism.[81] In furious rabies, these locally produced cytokines may further activate the p55 TNF-α receptor, resulting in the recruitment of T and B cells.[125] This action may lead to the promotion of immune recognition against rabies virus at "immune privileged" sites and provoke another amplification of the cytokine cascade, intensifying the limbic symptom pattern. Delayed mortality was observed in mice deficient in the p55 TNF-α receptor as a result of an increase in interferon gamma and IL-10 and a reduction in inflammatory cells in the CNS.[125] Furthermore, if Vβ8 T cells are stimulated by rabies virus nucleocapsid antigen,[126] more cytokines are produced, thus exaggerating the functional disturbance in the limbic system even in the absence of virus in such structures. This process possibly explains the relative paucity of limbic dysfunction and the absence of cellular immune activity to rabies virus in patients with the paralytic form.

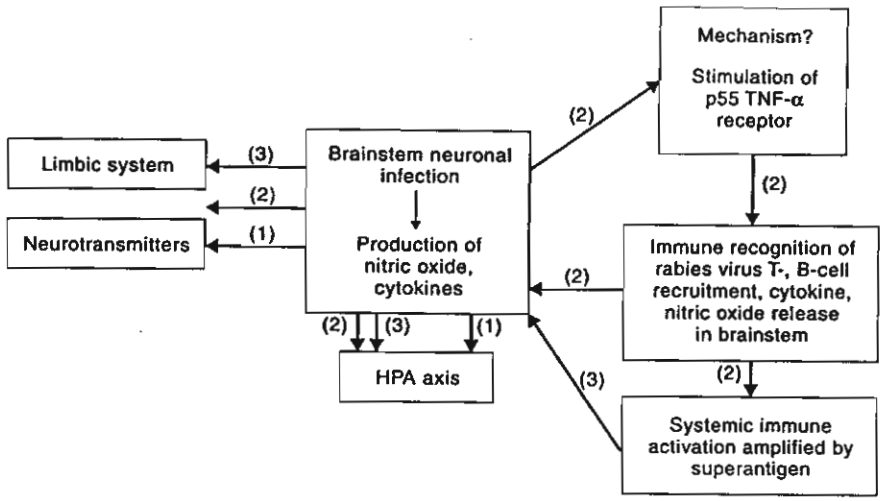


Figure 11-1. Hypothetical mechanisms in encephalitic rabies. Production of proinflammatory molecules results from rabies virus–infected neuronal processes in the brain stem. These substances, in turn, lead to functional modification of the limbic system and stimulation of the hypothalamic-pituitary-adrenal (HPA) axis (arrow 1). In furious rabies, the p55 TNF-α receptors also may be activated. Rabies virus antigen is thus recognized. Subsequently, recruitment of immune cells and intensification of limbic symptoms and HPA stimulation follow (arrow 2). Once Vβ8 T cells are stimulated by rabies virus nucleocapsid antigens (arrow 3), these cytokine cascades are reamplified, exaggerating the disturbance of the limbic and sympathetic nervous systems. *(Reprinted with permission from Human rabies: A disease of complex neuropathogenetic mechanisms and diagnostic challenge. Lancet Neurol 1:101, 2002, Fig. 4; used by permission of Elsevier.)*

Neuronal Death, Vulnerability of Different Neuronal Type, and Neurodestructive Immunity

Programmed cell death during rabies virus infection had been proposed as a principal pathologic mechanism; however, recent studies suggest otherwise.[127] Apoptosis appears to be one of the most important defense mechanisms against rabies virus infection. Apoptosis leads to depolymerization of actin filaments, which would prevent transport of viral nucleocapsid protein and the neuronal spread of virus. The extent of apoptosis correlates with the amount of expression of rabies virus G protein in infected neurons.[128–130] Downregulation of G protein expression in neuronal cells contributes to pathogenesis by preventing apoptosis.[127] However, the process of cell death is also modulated by the types of the neurons infected and the quality of immune response to infecting virus, as well as its virulence.[131] There is a delay of apoptosis in spinal cord motoneurons after rabies virus infection compared with hippocampal cells.[132]

Nonfatal or abortive infection induced by the attenuated strain of Pasteur rabies virus is associated with paralysis.[130] This mechanism of viral clearance is mediated by local recruitment of T cells, as well as the development of apoptosis of infected neurons and surrounding cells. It is hoped that rabies virus clearance can be made possible by other nonlytic mechanisms than this neurodestructive immunity, as shown in the Sindbis virus model.[133]

Depletion of metabolic pools by excessive viral replication, which ultimately leads to downregulation of expression of the late response gene and cell death, is one likely explanation of the virulence of rabies virus.[127]

▶ CLINICAL FEATURES

Rabies continues to be underreported in most developing countries. One of the most important contributors to this is that diagnosis depends on symptomatology alone. Rabies can manifest variably, and once coma supervenes, there is no reliable sign.

Clinical features can be divided into five stages: incubation period, prodrome, acute neurologic phase, coma, and death or recovery.[1,65,134] During the acute neurologic phase, rabies can be distinguished clinically as classic (furious or paralytic forms) and nonclassic forms. Furious and paralytic forms also differ in the survival period (interval between onset of disease and death) and immunologic features (see "Pathophysiology" above).

Incubation Period

The incubation period of rabies is usually 1 to 2 months after exposure. However, patients have been reported who developed the first signs of rabies as early as 5 days after a severe dog bite injury to the brachial plexus with possible direct inoculation of the virus into the nerve.[81] On the other hand, unusually long periods of 2–7 months and 4 and 6 years have been reported.[1] These patients had Australian bat *Lyssavirus* infection and canine rabies variant of genotype 1. The incubation period of more than 1 year is considered exceptionally rare. The WHO recommends that rabies PEP should be given to an exposed person regardless of the time that has elapsed since exposure, but PEP usually is not administered after a time interval of longer than 1 year.[96] Absence of a history of exposure is not uncommon in dog rabies–endemic countries, where exposures frequently occur and tend to be neglected.[1] This is also true in cryptic bat rabies cases, whose exposure may be considered trivial.[135] Most deaths occur because individuals are unaware that they had been exposed and infected with rabies virus.[136]

Prodrome

Prodromal symptoms can be vague and nonspecific, such as fever, muscular aching, flulike symptoms, diarrhea, abdominal pain, etc. Only local symptoms at the bitten region in the form of burning, numbness, tingling, itching, or pruritus are regarded as reliable indicators of rabies.[1] This neuropathic pain is presumed to be due to ganglioneuronitis. As many as a third of patients with dog-related infections (equally common in furious and paralytic rabies) and three-quarters of those with bat-related disease may experience local symptoms.

The appearance of the local symptoms at the bitten region marks the end of the incubation period, and most patients die within the next 2 weeks. This local reaction is intense and progressive, starting at the bite site and spreading gradually to involve the whole limb in a nonradicular pattern or the ipsilateral side of the face. Rarely, these symptoms can occur at locations remote from the bite site. Prodromal symptoms usually last only a few hours or days.

Acute Neurologic Phase

During this phase, objective signs of nervous system dysfunction begin. Furious and paralytic forms of human rabies have been widely recognized, although the latter is not easily diagnosed. These patients display a stereotypic pattern of manifestations (see below).[1,65,81] Increasing awareness of atypical forms of rabies has been emphasized.[81,136] Both paralytic and atypical (or nonclassic) forms of rabies may pose diagnostic problems not only in clinical practice but also in disease surveillance. Underestimation of case numbers is undoubtedly a contributory factor to rabies being ranked low on the priority lists for disease-control programs.[137]

Classic Rabies

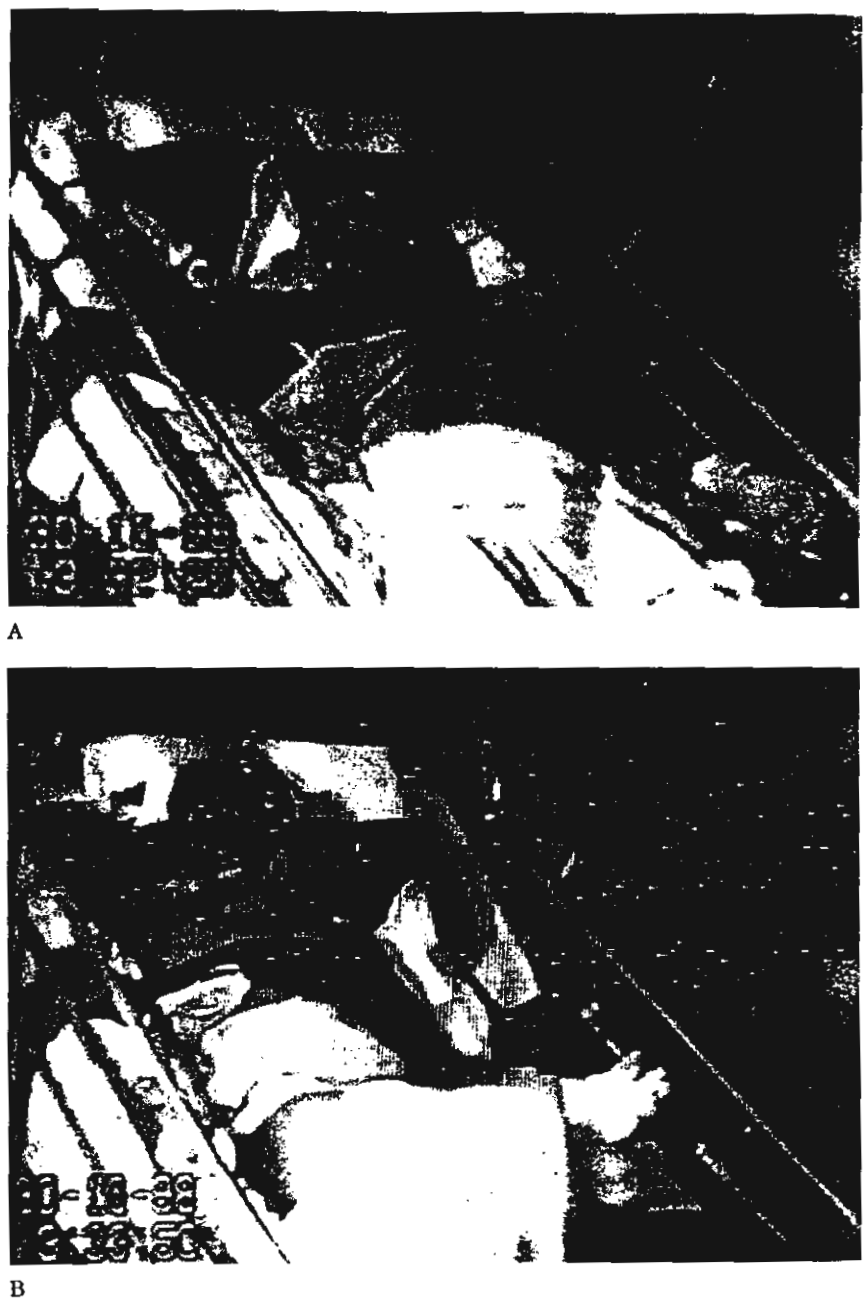
Classic rabies is almost always associated with true rabies virus (genotype 1), particularly the canine rabies virus variant. Mental status abnormalities can be seen in patients with furious rabies, as well as in some with paralytic forms, but to a much greater extent in the former group.

Furious Rabies. Two-thirds of patients with classic rabies have a furious form, and the remainder present with paralysis. Most furious rabies patients die within 7 days (average 5 days) of onset, and the survival period is about 13 days in paralytic cases.[1,65,81]

The earliest feature is hyperactivity, aggravated by internal (fear, thirst, etc.) and external (light, noise, etc.) stimuli. Mentation is preserved, but attention span is shortened. Fever, already apparent during the prodrome, is fairly constant, persisting through the preterminal phase. Cranial nerve deficits are detected rarely. Focal neurologic deficits, such as hemiparesis and hemihypalgesia, are not present. Seizures and hallucinations are rare. There are three major cardinal signs of furious rabies:

Fluctuating consciousness. Mental status alternates between periods of progressively more severe agitation and periods of relative normality and depression (Fig. 11-2). The patient abruptly becomes confused and disoriented without any warning. This bizarre behavior lasts only for minutes and then abates. The patient then becomes lucid and may not recall these events. Confusion becomes severe and may evolve to wild agitation and aggressiveness. Between these episodes of agitation the patient is drowsy but arousable. The period of irritability is gradually succeeded by impaired consciousness and coma. Biting behavior and barking have never been observed (authors' experience). The electroencephalogram (EEG) is normal during the initial stage (Fig. 11-3). It will not be abnormal until the patient exhibits severe aggression, with the appearance of high-amplitude sharp and slow waves interspersed within the slow background (Fig. 11-4).

Phobic spasms. Aero- and hydrophobia are seen in all furious rabies patients at some stage. These spasms can be elicited by blowing or fanning air on the face or chest wall or merely by offering a drink or showing a cup of water. Aerophobia and hydrophobia may not coexist and are not necessarily associated with laryngeal spasms.



A

B

Figure 11-2. Fluctuation of consciousness in furious rabies. This is characterized by alternating periods of relative normality (*A*) and severe agitation (*B*).

However, when these are present, the patient may spit saliva. These phobic spasms (or startle reactions) result from spasms of the accessory neck muscles and diaphragm followed by neck flexion (or rarely by neck extension). During the episode, the patient may have a fearful facial expression. These phobic spasms cannot be explained by a conditioned reflex because many patients have their first hydrophobic attack while bathing with no previous swallowing difficulties. Soft palate and pharyngeal sensations remain intact, but the gag reflex is hyperactive. Phobic spasms cannot be elicited once drowsiness and coma supervene. However, these are replaced by spontaneously occurring inspiratory spasms. Since inspiratory spasms are less intense and less frequent, they can escape notice. Opisthotonos, a characteristic sign of tetanus, is extremely rare.

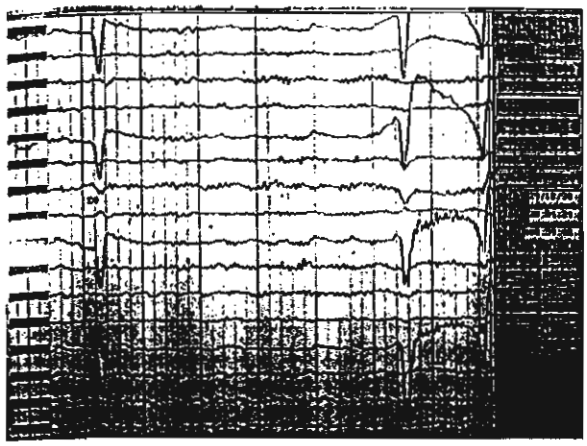


Figure 11-3. Electroencephalogram (EEG) in furious rabies displays a normal finding in the presence of fluctuating consciousness.

Autonomic dysfunction. Hypersalivation is a unique feature. Transient reactions can include fixed, dilated, or constricted pupils; anisocoria; localized (usually on the bitten region) or generalized piloerection; pulmonary edema (only in 3 among more than 170 patients in our experience); excessive sweating; priapism; and spontaneous ejaculations.

PARALYTIC RABIES. This form of rabies resembles the axonal form of Guillain-Barré syndrome (GBS), acute motor axonal neuropathy (AMAN).[124,138] The major cardinal signs appear late and are not prominent. Phobic spasms occur in only half the patients and are less evident because of weakness. Many, but not all, of the patients have weakness starting at the bitten limb. In patients with facial bites, weakness initially may involve facial and oculomotor muscles. Nevertheless, bilateral symmetric weakness of the legs develops in all patients regardless of the bite site. Bilateral weakness in an ascending fashion progressively involves all limbs and the pharyngeal, bulbar, and respiratory muscles. Facial diparesis is common (Fig. 11-5). Loss of deep tendon reflexes is found in all cases. Limb weakness is almost always proximal; however, this also can be seen in GBS. Sensory functions of all modalities are intact except, in some cases, at the bitten region. Persistent fever from the onset of weakness, intact sensory function, percussion myoedema, and bladder dysfunction may differentiate paralytic rabies from GBS.[134] Percussion myoedema is seen from the prodromal to the preterminal stage.[139] This is best elicited by percussion of the chest, deltoid, and thigh regions with a tendon hammer and consists of mounding of a part of the muscle at the percussion site, which then flattens and disappears over a few seconds. Patients with extreme cachectic conditions, hyponatremia, hypothyroidism, and renal failure may have myoedema signs. Myoedema during the late stage in rabies patients may be due in part to severe hyponatremia from the syndrome of inappropriate secretion of antidiuretic hormone.

Nonclassic Rabies

Patients with bat-related rabies have been reported to have clinical features substantially different from those with dog-related rabies.[1,81,113,117] Neuroimaging studies

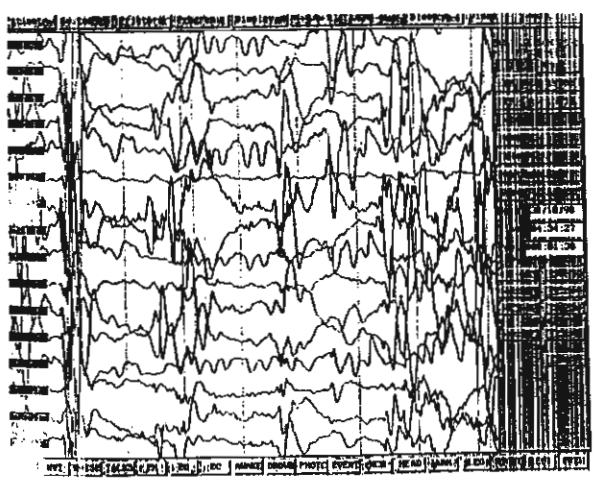


Figure 11-4. Paroxysmal bursts of high-amplitude sharp and slow waves appear only when the patient becomes severely agitated.

Figure 11-5. Facial diparesis in paralytic rabies.

in classic and nonclassic rabies patients appear remarkably similar.[116,117,140] Local neuropathic pain during the prodromal phase is more common in bat-related rabies. Moreover, there are reports of radicular pain, objective motor and sensory deficits, and choreiform movements of the bitten limb. Focal brain stem signs and myoclonus are observed frequently. Other patients have been described as having hemiparesis or hemisensory loss, ataxia, vertigo, or Horner's syndrome. Convulsive and nonconvulsive seizures and hallucinations are common. Phobic spasms were described in only one of six bat-related cases during 1997–2000.[1,74]

These atypical presentations have been observed in at least six patients with dog-related rabies since 1997 at Chulalongkorn University Hospital alone.[113] These included paraparesis, facial and bulbar weakness with preserved arm strength or bilateral arm weakness, repeated spontaneous ejaculations, ocular myoclonus, and hemichorea. One patient had nocturnal agitation but remained calm during the day. Phobic spasms or autonomic hyperactivity and hypersalivation were lacking.

Coma

Once a patient becomes comatose, it is extremely difficult to diagnose rabies. Although some suggest that the oculocephalic reflex (doll's-eye response) disappears early, this also happens in other viral encephalitis patients with brain stem involvement. Only inspiratory spasms can suggest the diagnosis of rabies at this stage. The EEG is nondiagnostic and similar to cases of metabolic encephalopathy (Fig. 11-6). Sinus tachycardia, disproportionate to fever, is usually seen even when hydration is adequate. This is followed by a nodal rhythm and, in some cases, by supraventricular and ventricular arrhythmias. Echocardiography shows reduction of the ejection fraction at the time of, or even before, hypotension. Viral involvement at the conduction pathways and myocardial involvement are the likely responsible mechanisms.[1] Coma precedes circulatory insufficiency, a prime cause of death, in almost all cases. Hematemesis is seen in 30 to 60 percent of patients 6 to 12 hours before death.

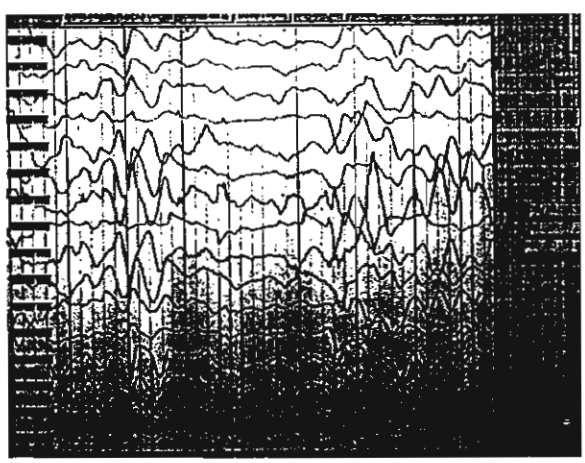


Figure 11-6. EEG during the comatose phase in rabies.

Recovery

Eight rabies survivors have been reported to date.[1,141] Most of them had atypical presentations.[108,113] The first patient (1972), who was exposed to a rabid bat, had unsteady gait, dysarthria, and hemiparesis. The second (1976), exposed to a rabid dog, had quadriparesis and generalized myoclonus at the early stage and later developed cerebellar signs, frontal lobe signs, and bibrachial weakness. The third patient (1977) had aerosol exposure to a highly concentrated fixed rabies virus strain. The five additional survivors include four Mexican children (between 1992 and 1995) and one Indian child (2002). Four patients were bitten by rabid dogs and one by a vampire bat. Each patient promptly received cell culture vaccine but not rabies immune globulin. All had significant sequelae. No spontaneous survivors have been reported to date.

▶ DIAGNOSIS

Antemortem Diagnosis

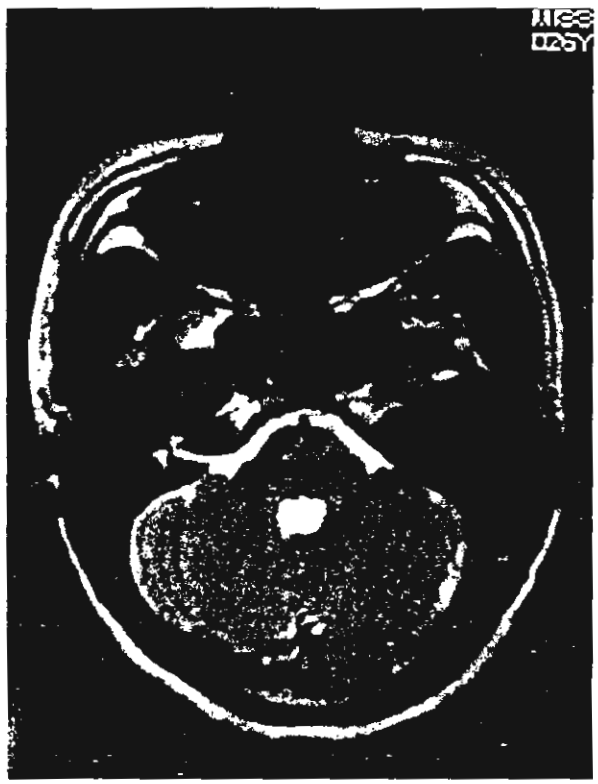
Clinical Features and Routine Laboratory Findings

Diagnosis on clinical grounds alone causes an imprecise assumption of low mortality from human rabies. Lack of a history of exposure is common in bat-related cases and is found in as many as 6 percent of dog-related cases.[113] Several patients with paralysis in Thailand had undergone plasma exchange because of a misdiagnosis of GBS. For a definitive diagnosis, all three cardinal signs of rabies should be present. Such a diagnosis is often not possible. Phobic spasms are evident at some stage. Many patients in developing countries seek medical attention late, and these signs cannot be demonstrated once the patients become comatose. In more developed countries, failure to diagnose rabies may be explained by a lack of medical familiarity even with typical clinical features of the disease. Moreover, clinical presentations may be variable in most cases associated with exposure to rabid bats or other wild animals[74] and even to rabid dogs.[81]

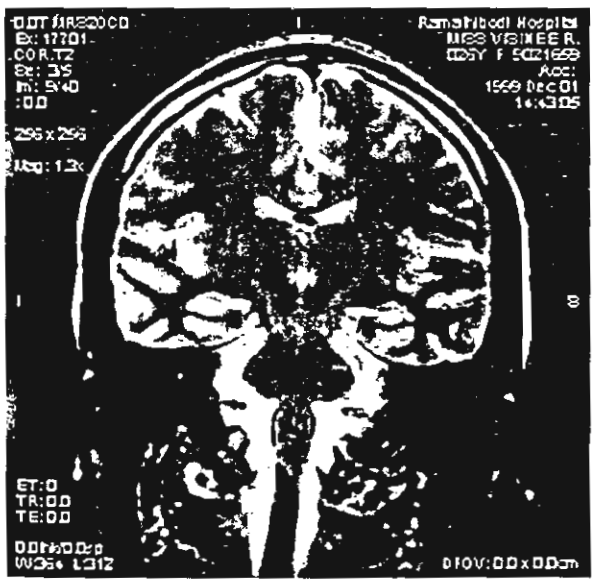
There are no characteristic findings in routine laboratory tests. The CSF examination is normal in most cases or shows only mild pleocytosis with lymphocytic predominance. The EEG shows no specific finding.

Neuroimaging

Computed tomographic (CT) scan of the brain is insufficient to detect abnormal parenchymal changes in



A



B

Figure 11-7. Axial (*A*) and coronal (*B*) T_2-weighted MRI of the brain in a patient with furious rabies demonstrating areas of increased signal intensity in the pons (*A*) and subcortical white matter and hippocampi (*B*). *(Courtesy of Dr. Jiraporn Laothamatas, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand.)*

rabies. The MRI is more precise and can give specific findings suggestive of rabies.[116,117] A nonenhancing mild hyperintensity on T_2-weighted images in the brain stem, hippocampi, hypothalami, subcortical white matter, and deep and cortical gray matter can be demonstrated in noncomatose rabies patients[117] (Fig. 11-7). Enhancement with gadolinium can be seen only when the patient becomes comatose (Fig. 11-8). Human rabies of both forms demonstrate similar MRI abnormalities.

Similar abnormalities in the brain stem on MRI can be seen in Japanese encephalitis (JE); eastern equine encephalitis (EEE); rhombencephalitis from *Listeria monocytogenes*, herpes simplex virus (HSV), and adenovirus infections; acute hemorrhagic leukoencephalitis (AHL); acute disseminated encephalomyelitis (ADEM); and Behçet's disease.

The MRI findings in JE and EEE were similar to rabies in terms of localization but without enhancement. However, a much more prominent nonenhancing increased signal abnormality on T_2-weighted MRI images was found in the former with only a mild degree of cortical gray and white matter involvement.[142,143] Foci of hemorrhages are demonstrated frequently on MRI and CT scan in JE but are unusual in rabies.[116] The abnormality seen in adenovirus rhombencephalitis was composed of a more prominent moderate hyperintensity on T_2-weighted images involving the brain stem and cerebellum with patchy enhancement along the pe-

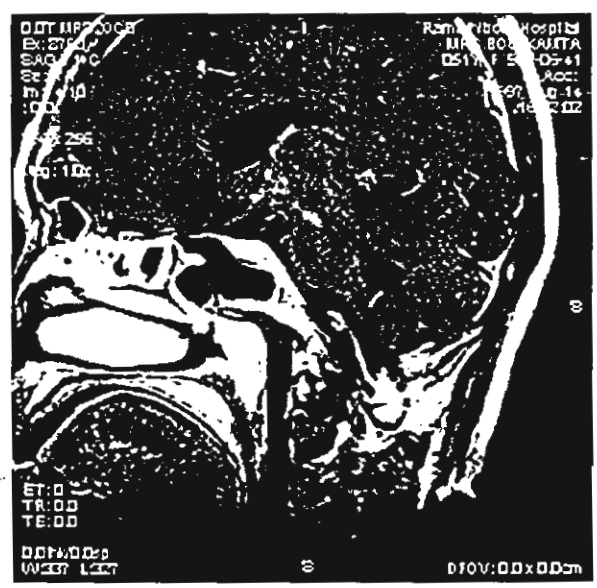


Figure 11-8. Postgadolinium sagittal T_1-weighted MRI of the brain in a comatose patient with paralytic rabies showing mild to moderate enhancement in the hypothalamus, midbrain, dorsal pons, and upper medulla. *(Courtesy of Dr. Jiraporn Laothamatas, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand.)*

riphery of the involved areas.[144] The abnormality seen in AHL and ADEM consisted of bilateral symmetric abnormal hyperintensities on T_2-weighted images that selectively involved the white matter of supra- and infratentorial structures and spinal cord.[145] The MRI in Behçet's disease involved basal ganglia, thalamus, and the central part of the pons with the absence of a predominance of white matter lesions.[146]

Special Laboratory Tests

Serum and CSF can be tested for IgM and IgG rabies virus antibodies (in previously unvaccinated individuals), but these often are not positive when the patient first presents.[1,147] Furthermore, this may produce variable results among diagnostic laboratories.

Rabies virus antigen in the nerve plexus at the hair follicles can be demonstrated by the fluorescent-antibody (FA) technique on frozen sections of the skin from the nape of the neck. However, this may not always be practical because it requires a dedicated cryostat. Earlier studies suggested that the number of positive results tends to increase as the disease progresses, but these findings were not always confirmed.[75] Corneal and salivary impressions for detection of rabies virus antigen may yield conflicting results partly due to differences in technique and difficulty in interpretation.

Isolation of rabies virus in neuroblastoma cells from saliva specimens is sensitive and specific. Results are known within 48 hours; however, all samples tested must be maintained frozen after collection with no preservative. Patients who have serum rabies virus antibody tend to have negative results.[74]

Brain biopsy is not practical but is highly sensitive by the FA test. However, false-negative results can occur due to a relative paucity of virus antigen at the biopsy areas, usually in the frontotemporal region, during the first few days after clinical onset.[74,110] This can be overcome by rabies virus isolation or molecular detection.

Molecular technology can improve clinical diagnosis. Although molecular diagnostic facilities for rabies are limited in developing countries, these do exist in parts of India, the Philippines, Latin America, Sri Lanka, and Thailand. The best specimens include saliva, tear secretions, nuchal skin biopsy specimens, CSF, and urine. Performing reverse-transcription polymerase chain reaction (RT-PCR) or nucleic acid sequence–based amplification (NASBA) on several sequential samples is mandatory in patients suspected of rabies because not all samples are positive.[1,74,75,148,149] Secretion of virus is intermittent in saliva, urine, and even CSF. The CSF appears to be the least sensitive source for rabies virus RNA detection. Any PCR products will need to be sequenced for confirmation because of the occurrence of nonspecific bands. Rapid clinical and, where available, laboratory diagnosis is important to prevent potential exposure to the health care team and to reduce anxiety and treatment costs.

Postmortem Diagnosis

A definitive test for rabies requires an examination of brain tissue. Brain necropsy via the transorbital approach is an alternative whenever a full autopsy cannot be done. Brain examination should be performed in all patients with encephalitis or paralysis who progress to coma and death. The presence of inflammation and Negri bodies is not always indicative of rabies. Moreover, inclusion bodies may not always be present.[2] The direct FA test on brain tissue remains a gold standard in rabies diagnosis. Touch impressions of brain tissue are made on glass slides and fixed in cold acetone and subsequently stained with fluorescein isothiocyanate–labeled polyclonal or monoclonal antibodies against rabies virus antigens. Areas to be examined include brain stem, spinal cord, cerebellum, and hippocampus.[106,107]

Although the FA test on brain impressions is simple, many developing countries have limited facilities to do this. For example, in Thailand, where there are 33 FA diagnostic laboratories, the number of human and animal cases diagnosed by laboratory analysis remains low. Often without maintenance of the cold chain, transfer of the specimens for a FA test in many rural areas cannot be done easily within 24 hours, before the brain begins to decompose (Ministry of Public Health report, Thailand, September 2002). Although brain tissue samples can be stored frozen at the collection site and during transport, this is not always possible in remote areas where there is no or little electricity. Molecular assays may be useful, in such conditions, in confirming a diagnosis and in epidemiologic surveillance. Results may be reliable, even in decomposed brain samples.[150] Rabies virus RNA also can be recovered from brain tissues dried on filter paper and stored at room temperature after 222 days.[151]

▶ DIFFERENTIAL DIAGNOSIS

The differential diagnosis includes encephalitis caused by pathogens that may selectively involve midline structures or those which are associated with behavioral changes and includes arboviruses [JE, EEE, and West Nile (WNV) viruses], enterovirus 71 (EV-71), herpes simplex virus (HSV), and varicella-zoster virus (VZV) infections.[143,152–157] A combination of the clinical symptomatology, the presence of behavioral abnormalities from the onset, the rapidity of disease progression (from onset to coma), the presence or absence of brain stem signs, and MRI abnormalities may differentiate rabies from other pathogens (Fig. 11-9). Most rabies patients

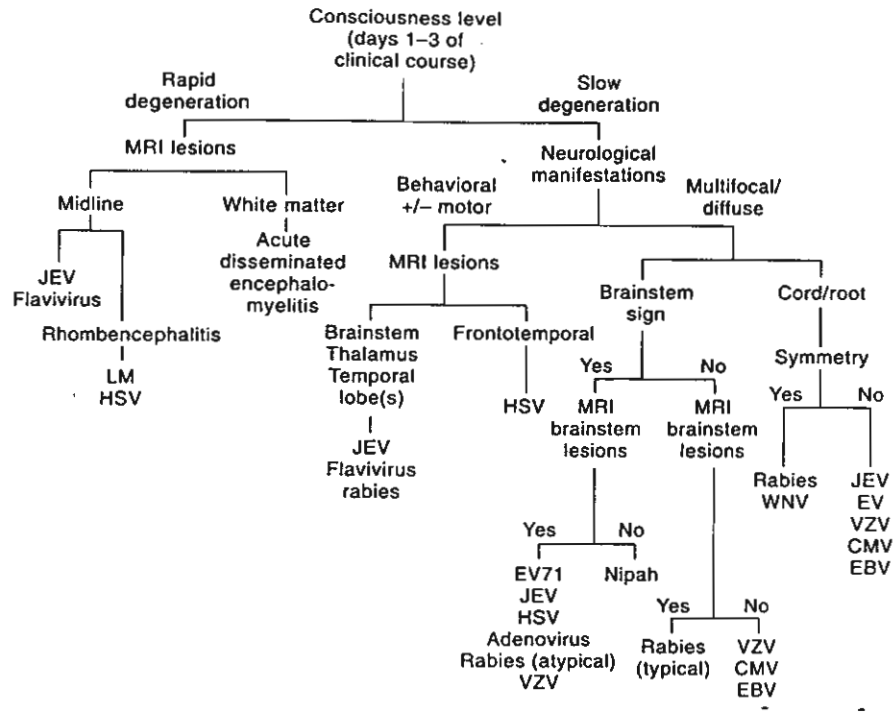


Figure 11-9. Algorithm for the differential diagnosis of rabies. The model considers the rapidity of disease progression, clinical manifestations, the presence or absence of brain stem signs on clinical examination and/or in MRIs, behavioral changes, MRI details, and the pattern of spinal cord/root involvement (see text for details). JEV = Japanese encephalitis virus; LM = *Listeria monocytogenes;* HSV = herpes simplex virus; EV = enterovirus; CMV = cytomegalovirus; EBV = Epstein-Barr virus; VZV = varicella zoster virus. *(Reprinted with permission from Rabies reexamined. Lancet Infect Dis 2:327, 2002, Fig. 11; used by permission of Elsevier.)*

remain alert and able to communicate during the first 3 days after clinical onset.

Patients with rabies and JE and HSV encephalitis may or may not have dramatic behavioral changes. An asymmetric MRI involvement with hyperintense lesions on T_2-weighted images in the frontotemporal region favors HSV encephalitis. Absence of enhancing lesions during the noncomatose phase and less intense signal in T_2-weighted images may separate rabies from other brain stem encephalitides due to EV-71, JE, and adenovirus infections. By comparison, HSV, VZV, and other herpesvirus infections are infrequently associated with brain stem lesions. Myoclonus and other brain stem signs, such as bilateral ptosis and nystagmus (similar to those in patients infected by bat rabies virus), have been found in Nipah virus encephalitis of acute, relapsed, and late-onset forms. However, the MRI usually shows multiple and widespread discrete hyperintense lesions, mainly in white matter, representing vasculitis in the acute form and confluent lesions involving primarily the cortical gray matter in late-onset or relapsed forms of Nipah virus encephalitis.[154,158] Diffuse flaccid paralysis was found in 10 percent of patients with WNV encephalitis. Asymmetric pure motor poliomyelitis-like weakness can be seen in patients with JE and WNV infections. An asymmetric radiculomyelitis may be due to infection with cytomegalovirus (CMV), Epstein-Barr virus, and other herperviruses.

Acute hepatic porphyria with neuropsychiatric disturbances, such as psychosis, seizures, signs of autonomic dysfunctions, and ascending paralysis with bilateral facial weakness, may mimic rabies. Fluctuating consciousness is observed in both conditions, but phobic and inspiratory spasms are seen only in rabies. A family history, ingestion of porphyrinogenic agents, severe abdominal pain, and dark urine color after being exposed to sunlight or added with concentrated nitric acid with elevated urinary delta-aminolevulinic acid and porphobilinogen should establish the diagnosis. Other conditions mimicking rabies are substance abuse, alcohol withdrawal or delirium tremens, and acute serotonin syndrome from taking serotonin reuptake inhibitors. Tetanus resembles rabies only in the form of reflex spasms. Tetanus patients have a clear sensorium. Rabies patients do not have persistent rigidity or sustained contraction of the jaw, neck, back, and abdomen as seen in tetanus. Spasms in rabies predominantly affect accessory respiratory muscles and the diaphragm.

whereas in tetanus spasms occur in axial muscles. Opisthotonos is rarely, if ever, present in rabies.

The axonal form of GBS, AMAN, shares many clinical features with paralytic rabies. Inspiratory spasms with abnormal behavior may appear late in the clinical course and may be masked by generalized paralysis and superimposed metabolic disturbances that can occur in both conditions. Nerve conduction studies cannot differentiate paralytic rabies from GBS.

In some parts of the world where nervous tissue (sheep, monkey, and mouse brain)–derived rabies vaccine is still widely used, neuroparalytic accidents must be included in the differential diagnosis. These developed in as many as 1 in 400 Semple vaccine–treated patients but less often in individuals who received mouse brain vaccine.[159–161] Delayed onset and a picture of chronic progressive encephalitis also have been observed. Phobic spasms, local prodromal symptoms, and fluctuating consciousness are not present in these postvaccination reactions.

▶ MANAGEMENT

Comfort care should be the management goal for the patient with rabies.[162] Previous attempts at treating symptomatic rabies patients have failed.[108,123] Invasive procedures and even respiratory support should be avoided in virtually every laboratory-proven case of rabies. Liberal use of barbiturates and intravenous morphine are best for relief of the terrifying attacks of anxiety, agitation, and respiratory spasms.

▶ PREVENTION

The WHO issued a current guideline for rabies pre- and postexposure prophylaxis (PEP) in humans in November 2002 (*www.who.int/emc.diseases/zoo/RabiesPET.pdf*). Due to reports of rabies prophylaxis failures,[1,163–165] this current guideline emphasizes the need to adhere strictly to its recommendations. Assessment of wounds or bite exposures helps define the risk and the need to use vaccine and RIG. Immediate washing and flushing with soap and water and disinfection with appropriate agents are as important as the use of vaccine and RIG. In less developed countries, where the use of commercial tissue-culture vaccines is not readily affordable, the practice of intradermal (ID) vaccination has been proven effective and economical. Therefore, the use of nerve-tissue vaccines, which carries serious side effects, should be abandoned by the year 2006.

General Considerations in Rabies PEP

Rabies PEP is a medical emergency and, as a general rule, should not be delayed or deferred. There are no contraindications if modern purified rabies biologicals are used. Pregnancy and infancy are never contraindications to rabies PEP. Persons who present for evaluation *even weeks or months after having been bitten* should be dealt with in the same manner as if the contact occurred recently.

Prophylaxis consists of vaccine regimens and routes of administration that have been proven to be safe and effective. Initiation of prophylaxis should *not await* the results of laboratory diagnosis or be delayed by dog observation when rabies is strongly suspected. Wounds should be washed and flushed with soap and water immediately or with water alone and disinfected with ethanol (700 ml/liter) or iodine (tincture or aqueous solution). Prophylaxis may be deferred if the species is unlikely to be infected with rabies and if results of laboratory diagnosis can be obtained in a timely manner, usually within 48 hours. Most dogs tend to shed virus concomitant with or shortly before the development of clinical signs. If the domestic dog or cat is unlikely to be rabid, it may be observed for 10 days. If the animal remains well, no prophylaxis is indicated. For example, the animal may be observed in cases where the bite is provoked and the animal is healthy, up to date on rabies vaccination, well maintained, etc. However, even vaccinated animals may become rabid. If the dog shows any sign of illness during the observation period, the patient should receive full rabies PEP urgently, and the animal's brain should be examined by a competent laboratory for a definitive rabies diagnosis.

Rabies PEP Modalities

Whether to use rabies immune globulin in addition to vaccine, vaccine alone, or none at all depends on the WHO category of exposure. These decisions may vary from country to country because epidemiologic circumstances vary. These broad categories are

Category III. Single or multiple transdermal bites, scratches, or contamination of mucous membrane with saliva (i.e., licks): Use RIG plus vaccine.

Category II. Minor scratches or abrasions without bleeding or licks on broken skin and nibbling of uncovered skin: Use vaccine alone.

Category I. Touching, feeding of animals, or licks on intact skin: No exposure; therefore, no PEP is required if the history is reliable.

Administration of Rabies Immune Globulin

Rabies immune globulin (RIG) must be infiltrated into the depth of the wound and around the wound. As much as anatomically feasible of the RIG should be infiltrated. Any remainder should be injected at an intra-

muscular site distant from that of vaccine inoculation, e.g., into the anterior thigh. The quantity used is 20 IU/kg of human RIG or 40 IU/kg of equine RIG. The total recommended dose should not be exceeded. If the calculated dose is insufficient to infiltrate all wounds, sterile saline may be used to dilute it two- to threefold to permit thorough infiltration. Suturing should be postponed to avoid further wound contamination with virus. If suturing is necessary, it should be ensured that RIG has been applied locally. Antimicrobial agents and tetanus toxoid may be required accordingly.

Rabies PEP: Vaccination

Tissue-culture rabies vaccine can be administered intramuscularly (IM) or ID where licensed or approved by the administrative authority. The efficacy and adverse events are comparable between these two regimens. However, the use of ID vaccination may decrease the cost by as much as 60 to 80 percent. Given that there has been no substantive data comparing one vaccine with the others in terms of vaccine immunogenicity and a change in the route of vaccine administration (e.g., from IM to ID), the routine interchangeability of modern rabies vaccines and routes is not recommended for liability concerns alone. However, when completion of PEP with the same rabies vaccine is not possible, the switch can be done, provided that it is with one of the WHO-recommended cell-culture vaccines.

Intramuscular Regimen

Vaccines should not be injected into the gluteal region. A classic five-dose IM or "Essen" regimen consists of one dose of vaccine on days 0, 3, 7, 14, and 28 in the deltoid region or in small children into the anterolateral area of the thigh muscle. As an alternative, the 2-1-1 regimen may be used. Two doses are given on day 0 in the deltoid muscle and right and left arms. In addition, one dose is given in the deltoid muscle on day 7 and one on day 21.

Intradermal Regimen

Since these regimens require considerably less vaccine than the IM regimens, this method is particularly appropriate where vaccine or funds are in short supply. The ID approach reduces the volume of vaccine required and hence vaccine cost by 60 to 80 percent.

The decision to implement an economical ID PEP scheme rests with government agencies that define rabies prevention and treatment policies in their own countries. When the ID route is used, attention must be given to staff training, conditions and duration of vaccine storage after reconstitution, use of an appropriate 1-ml syringe and short hypodermic needles, etc.

Vaccines suggested for use with the ID regimen are the HDCV Rabivac; the purified vero cell vaccines (PVRV) Verorab, Imovax, Rabies vero, and TRC Verorab; and the purified chick embryo cell vaccine (PCECV) Rabipur. There are two methods of ID administration:

1. Eight-site ID method (8-0-4-0-1-1; 8 sites on day 0, 4 sites on day 7, 1 site on days 30 and 90) for use with HDCV (Rabivac) and PCECV (Rabipur). The volume per ID site for both vaccines is 0.1 ml. Although there has been no supporting scientific evidence, the eight-site regimen may be considered in emergency situations when no RIG is available. The RIG may be administered within 7 days after the first dose of vaccination.
2. Two-site ID method (2-2-2-0-1-1; 2 sites on days 0, 3, and 7; 1 site on days 30 and 90) for use with PVRV (Verorab, Imovax, Rabies vero, TRC Verorab) and PCECV (Rabipur). The volume per ID site is 0.1 ml for PVRV (Verorab, Imovax, Rabies vero, TRC Verorab) and 0.2 ml for PCECV (Rabipur) or, *as an option,* 0.1 ml for PCECV (Rabipur) may be considered for use by national health authorities. This does not apply to any other vaccine brand.

Rabies PEP Prophylaxis in Immunosuppressed Individuals

There is no reliable PEP method to ensure efficacy in immunosuppressed individuals. Therefore, the importance of wound treatment should be further stressed. RIG should be administered deeply into the wound for all exposures. Vaccine always should be administered, and no modification of the recommended number of doses is advisable. An infectious disease specialist with expert knowledge of rabies prevention should be consulted.

Rabies PEP in Previously Vaccinated Persons

Apart from local wound treatment, there is no need for RIG administration. Two boosters, either IM or ID, are required on days 0 and 3. However, full PEP should be given to persons who received pre- or postexposure prophylaxis with vaccine of unproven potency or who have not demonstrated acceptable rabies neutralizing antibody titer.

Preexposure Rabies Vaccination

Three doses of vaccine on days 0, 7, and 21 or 28 are recommended for persons at high risk of exposure to rabies virus (laboratory staff, veterinarians, animal handlers, and wildlife officers). Toddlers and children in highly endemic areas also may be considered for vaccine. A dose is either 1.0 or 0.5 ml (according to the vaccine type) standard IM or 0.1 ml ID (if antimalarial chemoprophylaxis is applied concurrently, IM injections are preferable to ID).

Persons working with rabies virus in diagnostic, research, and vaccine-production laboratories should have serum tested for neutralizing antibody every 6 months and have a booster when the titer falls below 0.5 IU/ml. People in other professions (such as veterinarians, animal handlers, and wildlife officers) who are at frequent risk of exposure to rabies should have serum testing every 2 years and receive a booster when the titer falls below 0.5 IU/ml.

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INFECTIONS OF THE CENTRAL NERVOUS SYSTEM

THIRD EDITION

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16

RABIES

CHARLES E. RUPPRECHT AND THIRAVAT HEMACHUDHA

Rabies, an acute encephalitis caused by a Lyssavirus, is the most significant viral zoonosis recognized today, accounting for millions of human exposures and thousands of human deaths each year, primarily in developing countries in Asia, Africa, and Latin America. In contrast, rabies virus infections in humans in the United States and other developed countries are uncommon, accounting for no more than a few cases each year. Nevertheless, in both global arenas, conversations about rabies may conjure a primal specter that awakens latent memories of horrific historical, literary, and medical images. Historical descriptions of rabies, associated with animal bite, strongly suggest that this entity has existed for millennia. Literary references from many cultures portray a graphic incurable malady. Clinical citations continue to elucidate the neurologic variability of manifest disease, not only in *Homo sapiens* but also in domestic animals, ranging from a pariah dog foaming at the mouth with maniacal behavior to a paralyzed bellowing bull dragging a useless hind limb.

The contrasting ecology of rabies virus infections in animal populations of developing versus developed countries is also striking. Introduction of effective control programs for domestic dogs in developed countries has shifted the attention toward surveillance among wildlife. Unfortunately, the bites of rabid domestic dogs in developing countries still result in tens of thousands of human cases. The presentation of an agitated patient with hydrophobia and frenzied behavior or a child with acute ascending paralysis reinforces the devastating aspects of human disease. Furthermore, the recognition that rabies has the highest case-fatality ratio of any infectious disease should remind public health officials of the renewed need for rabies elimination from urban domestic canine populations, as well as for consideration of disease control among wildlife. Without a better understanding of neuropathology and the absence of a suitable model to serve as a human surrogate, hope for a cure anytime soon remains intangible. Lack of a specific antiviral agent for treatment further demonstrates the necessity for development of improved methods of primary prevention and control. Together with traditional veterinary techniques, recent advances in oral vaccination of animals introduces the possibility of preventing disease in targeted free-ranging populations, such as stray dogs, which may provide for a further reduction of human rabies cases, at the level of the wild reservoir, something of a novelty among zoonoses. Administration of highly effective cell culture vaccines, in combination with local infiltration of rabies immune globulin (RIG), to exposed patients has decreased the incidence of human disease, but the costs of such programs may outweigh health economic benefits because of expensive biologicals and inappropriate delivery. These issues provide a basis for understanding the interaction between rabies virus infections of animals and transmission to humans, especially as it relates to the technological advances that may allow the possibility of inexpensive and highly efficacious vaccines for both populations via integrated holistic public health programs.

This chapter summarizes current knowledge of rabies virus infections of the human central nervous system (CNS). Several excellent books are testament to the commitment of the numerous investigators in the field, both then and now, representative of the multidisciplinary nature that has become the norm for rabies prevention and control (1–3).

HISTORY

Rabies has attracted considerable attention in both veterinary and human circles throughout history (4,5). For example, in the pre-Mosaic Eshunna Code of Mesopotamia, circa 2300 B.C., legal reference was made to fines levied on owners of "mad dogs" whose actions led to the death of bitten persons, with monetary distinctions made between the loss of a slave or the demise of other members of society. Whether this related to rabies per se or to the overt injury inflicted by a vicious dog is uncertain. Other allusions appear in ancient Greek and Roman writings, including those of Democritus, Aristotle, and Galen. At about 100 A.D. Celsus recommended wound cauterization after a bite, and a rather unconventional therapy for those suffering from this "virus" or poisonous slimy liquid, as it was referred, namely throwing the victim into water or a tank of hot oil. Throughout the centuries, various concoctions were proposed as remedies, as was the suggestion for consulting oracles or requesting divine intervention. Indeed, rabies posed such a threat in some portions of medieval Europe that when peasants needed aid, they often turned to St. Hubert, the patron saint of rabies victims. His shrine near Liege, Belgium, drew many of the faithful. Those unable to make pilgrimages to the shrine used iron bars or crosses known as the Keys of St. Hubert, which could either be inserted into the walls of houses to protect believers against the curse of rabies or alternatively to cauterize wounds, a treatment practiced well into the nineteenth century.

In the mid-sixteenth century, the Italian physician, Fracastoro provided detailed clinical descriptions of human rabies

(4,5). Over the next several centuries, other literary reports appeared that elucidated the natural history of human disease and provided a direct association to animal rabies. A gradual recognition of this relationship between rabid animals, and human illness eventually led to animal control programs and resulted in the virtual elimination of disease from wide geographical areas, such as the Scandinavian region and subsequently Great Britain, but not until the early part of the twentieth century.

A major landmark in an understanding of rabies and its rational prevention was a series of experiments performed by Louis Pasteur and his colleagues in the late 1800s. Pasteur demonstrated disease transmission by inoculating homogenized spinal material from a rabid dog into the brain of a previously healthy dog. Thereafter, related observations led to the development of a vaccine, involving more than 90 serial intracerebral passages of rabies virus in rabbits. After drying the spinal cords from affected rabbits, viral infectivity was reduced. This vaccine was tested initially in animals and was believed to be effective. Although administered initially to other patients without notable effect, in 1885 Pasteur gave the vaccine to a child, Joseph Meister, who was bitten by a rabid dog, and remarkably, the boy survived (6). Much introspection from prominent medical experts at the time followed the apparent initial successes of this approach. Increased deployment of a live-virus vaccine over the ensuing years at Pasteur Institutes established around the globe attested to its increasing popularity. These early trials provided a historical context for subsequent vaccine improvements (1–5,7,8).

By the end of the nineteenth century, the scientific literature was replete with clinical case descriptions of human and animal rabies. In 1903, Negri made an important histopathologic observation (9), when he identified cytoplasmic inclusion bodies—now known as "Negri bodies"—in the neuronal cells of animal brains (even though he believed rabies could be due to a protozoan). Though of diagnostic utility when present, approximately 25% to 30% of rabid animals did not develop discernible Negri bodies, necessitating animal inoculation to confirm suspected cases, during which time individuals bitten by animals underwent treatment. Later, in 1912, Babes (10) published the "Traite de la Rage," a compendium of cases, which helped to define both the natural history and the clinical findings of human rabies at the time, and set the stage for a better understanding of the disease.

As the latter half of the twentieth century progressed, additional insights were achieved in the etiology, molecular biology, diagnosis, pathogenesis, prevention, and control of rabies, especially the development of effective vaccines for animals and the prophylactic combination of vaccine and RIG for humans (1–5,7,8,11). Use of vaccines to protect domestic dogs has made human rabies a rarity in developed countries. Nevertheless, the absence of effective control programs for domestic animals in many developing countries still results in tens of thousands of human rabies cases each year. Although it is one of the oldest recognized infectious diseases, the uniformly high fatality rate that is the hallmark of rabies should initiate a renewed advocacy for antiviral introspection and continued medical research toward inexpensive prophylaxis against this devastating agent.

ETIOLOGY

Taxonomy

Taxonomically, rabies virus belongs to the family *Rhabdoviridae*. "Rhabdos" is of Greek derivation, meaning rod. As suggested by the name of the family, these viruses are rodlike or bullet-like in morphology when viewed by electron microscopy (Fig. 16.1). This family of viruses includes pathogens that infect a wide variety of species, including mammals, birds, cold-blooded vertebrates, arthropods, and plants (12). Currently, the Rhabdoviridae affecting vertebrates consist of four genera: *Vesiculovirus*, *Ephemerovirus*, *Novirhabdovirus*, and *Lyssavirus*. Members of these genera can be differentiated according to genetic, antigenic, and biologic characteristics (12,13). Groups that contain members that affect humans include the *Vesiculovirus* and *Lyssavirus* genera. Rabies virus is the prototype species of the *Lyssavirus* genus, which includes several other related members, all with the potential for inducing encephalitis (Table 16.1). Lagos bat virus is the only member to date that has not been implicated in human mortality. The term "Lyssa" is also of Greek derivation, meaning rage or frenzy. From a practical public health perspective, this terminology underscores the point that lyssaviruses cause rabies and are clinically indistinguishable regardless of underlying viral etiology. Within the genera of lyssaviruses, numerous variants may be distinguished by the use of monoclonal antibody typing and genetic sequencing (1–3,11,13–15). Most of this remaining discussion focuses on rabies virus, as the best-known representative of the group.

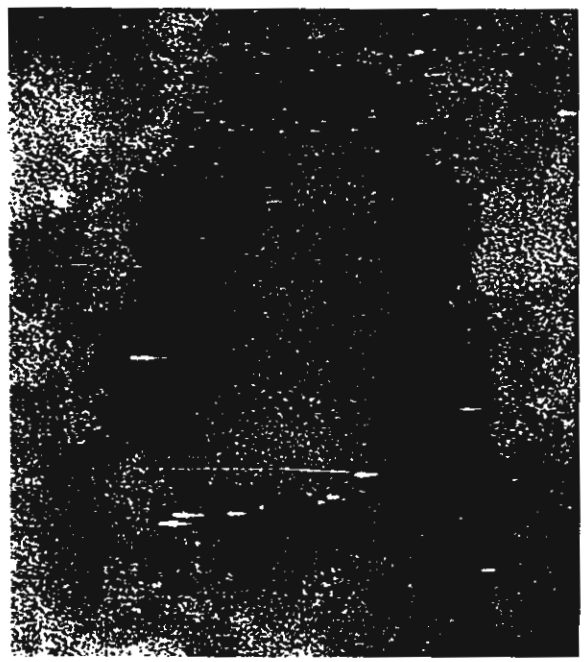

FIG. 16.1. Negatively stained rabies virus particle with typical morphology, as observed by electron microscopy. (Courtesy Sylvia Whitfield, Centers for Disease Control and Prevention.)

TABLE 16.1. MEMBERS OF THE *LYSSAVIRUS* GENUS IN THE RHABDOVIRIDAE FAMILY

Virus	Source Species
Rabies virus[a]	Mammals (dogs, bats, wild carnivores, etc.)
Duvenhage virus[a]	Bats
Lagos bat virus	Bats
Mokola virus[a]	Insectivores (i.e., shrews)?
European bat virus (I, II)[a]	Bats
Australian bat virus[a]	Bats

Subject to change with additional pathogen discovery and taxonomic interpretation.
[a]Associated with human infection.
From Rose JK, Whitt MA. Rhabdoviridae: the viruses and their replication. In: Knipe DM, Howley PM, et al., eds. *Fields virology.* New York: Lippincott Williams & Wilkins, 2001:1221–1244; and de Mattos CA, de Mattos CC, Rupprecht CE. Rhabdoviruses. In: Knipe DM, Howley PM, et al., eds. *Fields virology.* New York: Lippincott Williams & Wilkins, 2001:1245–1277, with permission.

Composition

Rabies virus particles are approximately 75 nm by 180 nm in size and consist of protein-coated, single-stranded, negative-sense nonsegmented RNA, with an estimated molecular weight of approximately 4×10^6 (14,15). The RNA is found within a membrane-bound viral nucleocapsid, or ribonucleoprotein core. The 12-kb RNA genome encodes five multifunctional proteins: an inner nucleoprotein (N), a phosphoprotein (NS or P), a matrix (M) protein, an outer glycoprotein (G), and a large or virion-associated RNA polymerase protein (L). The M and G proteins are associated with a lipid bilayer of the viral envelope. The M protein forms a sheathlike structure around the nucleocapsid core, appears localized on the inner aspect of the envelope, and is believed to play a role in the budding process. The glycosylated G protein projects through the membrane to the outer surface of the envelope, is involved in receptor recognition, and induces virus-specific neutralizing antibodies. The envelope surrounds the nucleocapsid, consisting of a series of helical RNA coils, in a complex with the N, P, and L proteins. The histologic appearance of Negri bodies results from the accumulation of a filamentous matrix composed of viral RNA–protein complexes within the neuronal cytoplasm. Besides identified structural and enzymatic attributes, Lyssavirus proteins function in a variety of complex regulatory, modulating, and stabilizing roles during viral transcription, replication, and assembly.

Viral Stability

Rabies virus does not persist in the environment. The virus is inactivated by heat, such as pasteurization temperatures, as well as by exposure to ultraviolet light, 5% iodine, ether, chloroform, concentrated phenols, 70% ethanol, formalin, β-propiolactone, and most other common lipid solvents, fixatives, and quaternary ammonium compounds (15). Viral susceptibility to surface-active detergents, such as hand soaps, is particularly relevant because local wound care with an agent capable of inactivating rabies virus is essential during first aid. Infectivity may remain stable for long periods if the virus is maintained frozen at approximately -80°C, or colder, but the virus is unstable at extreme ranges of pH levels.

In Vitro Replication

In nature, rabies virus seems predisposed toward primary replication in the neuron, but in the latter twentieth century, it has been adapted in the laboratory to a broad variety of primary cells or continuous cell lines (e.g., baby hamster kidney, neuroblastoma, and Vero). These laboratory strains, or "fixed viruses," have altered characteristics that have served as the basis for vaccine development (7,15). Replication of virus in vitro appears to follow a classic pathway, largely similar to other rhabdoviruses, such as vesicular stomatitis virus, with some unique exceptions (12,14,16). In essence, virions must contact and enter susceptible cells, undergo RNA transcription and replication in the cytoplasm, and reassemble at cell surfaces to exit and begin the process anew. After an initial eclipse phase of several hours, the replication process progresses to completion within approximately 24 hours. Virion assembly is associated with budding from the plasma membrane or occasionally via the endoplasmic reticulum and Golgi apparatus.

In 1958, Kissling accomplished the in vitro propagation of rabies virus (17). Such research prompted progress toward avoiding the necessity to produce a vaccine in animal brains, such as rabbits, sheep, or nonhuman primates. Thus, complications related to allergic encephalomyelitis could be minimized by production of rabies virus vaccine in cell cultures. By the mid-1960s, relatively large quantities of rabies virus could be produced in primary cell cultures (7,18). Thereafter, additional progress occurred with viral adaptation and production in avian embryos, and continuous cell lines, such as WI-38 and Vero cells (19).

PATHOGENESIS AND PATHOLOGY

Pathogenesis

As a zoonosis, human rabies is inextricably linked to infections in animal populations. The potential opportunity for pathogen perpetuation is great, considering that rabies virus is believed capable of infecting all mammals. Older studies, originally summarized collectively by World Health Organization experts, suggested a hierarchy of susceptibility to experimental infection among certain species (Table 16.2). For example, foxes and other wild canids were believed to be extremely susceptible to infection. The apparent lack of immunity among red foxes in enzootic rabies areas, based on the lack of demonstrable antibodies, supported an idea of fairly uniform fatality with infection. In contrast, antibodies were detected regularly in other carnivore species, such as the mongoose, suggesting acquired herd immunity. Nonhuman primates were considered only moderately susceptible to rabies infection, whereas a marsupial, the common opossum, was one of the least susceptible animals (1–3). Many of these animal studies were conducted before the appreciation and laboratory distinction of different viral variants. Thus, overt susceptibility may be predicated upon a variety

TABLE 16.2. RELATIVE MAMMALIAN SUSCEPTIBILITY TO RABIES VIRUS INFECTION

Highest	High	Moderate	Low
Wolves?	Hamsters	Dogs?	Opossums
Foxes	Skunks	Nonhuman primates	Bats?
Coyotes	Raccoons	Humans?	
Rats (kangaroo and cotton)	Domestic cats	Cattle	
Field voles	Rabbits		

From Baer GM, ed. *The natural history of rabies*, 2nd ed. Boca Raton, FL: CRC Press, 1991; Campbell JB, Charlton KM, eds. *Rabies*. Boston: Klumer Academic, 1988; Jackson AC, Wunner WH, eds. *Rabies*. New York: Academic Press, 2002; and Expert Committee reports of the World Health Organization, with permission.

of factors, including the variant of virus, quantity of inoculum, extent of innervation at the site of the infection, individual host species attributes, and so on. Moreover, the relative density of available binding sites upon receptive cells may also be involved. For example, one putative site for rabies virus is the nicotinic acetylcholine receptor, among others (14,20). The role of viral variation in rabies pathogenesis is only slowly being appreciated (1–3).

The pathogenesis of rabies virus infections can also be considered according to the different routes of acquisition. Almost all cases of human rabies occur due to the bite of an infected animal. Rarely, nonbite sources of rabies virus infection may also account for disease. Examples of nonbite transmission include infection by aerosols or by person-to-person transmission, such as following corneal transplantation. After the bite of a rabid animal, virus is inoculated from the saliva into local tissue. As early as 1804, Zinke (21) demonstrated experimentally that saliva from a rabid dog could produce rabies in a healthy subject via wounds as a portal of entry. Besides the CNS, in the salivary glands, acinar cells may support viral replication, but infected rabid animals do not always continuously excrete virus in saliva (22,23).

Even a single rabid animal can successively bite many individuals in a short period One rabid wolf that severely attacked 29 individuals in an Iranian village in 1954 provided some of the first convincing field evidence for the effectiveness of combined vaccine and immune serum administration in postexposure situations, compared to vaccine alone (24). Obviously, it is rather difficult to try to predict relative risk and compare the opportunity for differential viral shedding in affected patients under such extreme circumstances.

After inoculation, virus may be transmitted to the CNS directly by peripheral nerves, without prior local replication (25). Alternatively, local replication of rabies virus may occur at the site of inoculation, with subsequent spread to the CNS (26–28). Taken together, experimental studies provide evidence of either direct transit via the axoplasm or an initial phase of local replication at the inoculation site, before transmission into the CNS.

A series of related experimental observations, including the localization of rabies virus antigen at subneural clefts, commensurate with the distribution of nicotine acetylcholine receptors, the prevention of transmission by compounds interfering with nicotinic acetylcholine receptors, and sequence homology between the G protein of rabies virus and the region with the greatest binding affinity for acetylcholine receptors, have contributed to developing a molecular hypothesis for rabies virus pathogenesis, supporting the concept of receptor-mediated transmission (14,20,29,30). Although the experimental data are convincing that the acetylcholine receptor may be important in rabies pathogenesis, alternative candidates exist as well (14).

Once virus enters local neuronal cells, it is transmitted centripetally (31). The rate of passive retrograde axonal transport of virus is estimated to vary from approximately 8 to 20 mm per day. The manner and form of viral transport is not precisely known, but the cytoplasmic dynein light chain (LC8) has been shown to interact with the rabies virus P protein during retrograde axonal transport. Elimination of the LC8 ligand remarkably diminished pathogenicity after experimental intramuscular inoculation (32). Severing nerve tracts may also affect transmission of virus from peripheral sites of inoculation to the spinal ganglia (33–35). Evidence for infection of dorsal root ganglia includes the demonstration of virions by both culture and electron microscopy, as well as the demonstration of dorsal root edema. At this stage, pain or paresthesia at the wound site may occur, thus presumably related to dorsal root ganglionopathy. From the spinal cord, the virus can ascend extremely rapidly to the brain (31,34,36).

Once virus reaches the brain, its progeny affects many of the lower brain structures with subsequent widespread infection. These include the limbic system, hippocampus, brainstem, and cerebellum (36,37). Subsequent analyses have revealed a more pronounced involvement of the brainstem and thalamus than the hippocampus, in both humans and animals, regardless of clinical manifestations (38,39). Clinical disease in the form of encephalitic (or furious) and paralytic (or dumb) phases may reflect a particular involvement of the limbic and autonomic structures in the former presentation and the spinal cord/peripheral nerves in the latter. A similar pattern of predilection for central midline structures of rabies virus distribution in both clinical presentations has also been confirmed by magnetic resonance imaging (MRI) (40). Further, the degree of functional modification of the muscarinic acetylcholine receptor in the rabies-infected brain has not been correlated with the amount of

virus or the presence of apoptosis of neurons and presence of rabies virus antigen (41,42). Curiously, apoptosis, previously thought to be a principal cause of neuronal death, has been suggested to be a potentially important defense mechanism against rabies virus infection (43). Somewhat surprisingly, given the case-mortality rate, examination of rabies-infected brains usually does not reveal dramatic histologic changes. Taken together, both viral clinical expression and pathogenicity may be more influenced by either physiologic or nonlytic mechanisms attributable to host defense systems and may be due to the intrinsic nature of certain neuronal cell types, such as the interaction between glial cells and neurons (44–46). In general, involvement of the limbic system may result in aberrant sexual activity and loss of behavioral control, whereas brainstem involvement usually results in failure of body temperature control and altered patterns of respiration, leading to respiratory arrest.

Another important component of disease is the centrifugal spread of virus back out of the CNS to peripheral sites. Specifically, virus replication in acinar cells of salivary glands can result in excretion of high virus loads (22,23). Centrifugal transport of virus from the CNS leads to infection of a variety of other organs (1–3,36). Observation of virus infection at locations outside the CNS has resulted in the recognition that tissue biopsies, such as of skin follicles at the nape of the neck, may be extremely helpful for diagnostic purposes. Similarly, virus may be found in corneal cells, drawing the association between corneal impressions and antemortem diagnosis (47) of rabies virus infection (although this may not be as sensitive as compared to biopsies of the nape of the neck, and improper technique may lead to corneal lesions). Thus, corneal transplants can be a source of infection and result in person-to-person transmission (48). End stage, virtually all innervated organs are involved following natural infection, including portions of the heart, kidneys, lungs, and gastrointestinal tract. As opposed to neuronal transport in both centripetal and centrifugal spread, viremia is unimportant in natural infections.

The pathogenesis of rabies virus infections due to "nonbite" exposure requires viral acquisition at alternative sites, such as the mucosa (37). For example, if exposure occurred from an aerosol, as in a laboratory accident, replication of virus may occur in olfactory neuroepithelium, with direct neuronal transmission to the brain by the olfactory tract. Similarly, with corneal infection, direct retrograde spread of virus from the eye to the brain via the optic nerve may occur.

Why does the immune system not clear rabies virus, especially during prolonged incubation periods? It may in some cases, but the relationship between the role of cell-mediated immunity and the prevention of rabies virus propagation is not clear. Current data suggest that cell-mediated immune responses probably play only a minor role in successful clearance of virus, which could explain certain clinical manifestations and may influence apoptosis (43,49–51). In contrast, with the recognized value of the administration of RIG and the generation of humoral antibody responses after vaccine administration, circulating neutralizing antibodies decrease mortality, at least before the advent of clinical signs. Conservatively, abrogation should occur before neuronal acquisition in an ideal sense, even though some preliminary experimental animal intervention studies suggest the effectiveness of certain neutralizing monoclonal antibodies, even after CNS involvement (52).

Replication of rabies virus occurs primarily in perikaryon and dendritic processes of neurons (1–3,53). Long incubation periods may be related to the sequestration of virus either within neurons or outside the nervous system within peripheral muscle fibers, before ascending in nerves (33–35,53). Regardless of the location where virus may reside in the interim, eventual invasion of the CNS via peripheral neuronal pathways is well documented (rather than other nonneuronal routes), although the ultimate explanation as to the precise mechanism for prolonged incubation periods is not understood, unlike the genomic strategies employed by herpes or retroviruses.

Pathology

Gross findings in the brain are usually not remarkable, beyond occasional slight swelling and congestion (54). The typical pathologic observations in rabies are similar to those encountered with other suspected viral encephalitides. Microscopic lesions may be significantly less extensive, especially in light of the otherwise striking clinical symptomatology of hydrophobia, frenzied activity, and bizarre behaviors. Evidence of perivascular cuffing is a nonspecific characteristic of rabies virus infection of the brain, and it may be mild and limited. Sporadic neuronophagia and neuronal necrosis may also present as a component of the acute stages of disease (55). Areas of the CNS commonly involved include the medulla, pons, spinal column, dorsal root ganglia, cerebellum, and hippocampus. A histopathologic study of paralytic rabies patients suggested peripheral nerve demyelination as a prime pathologic change (56). In peripheral nerve specimens studied, there was mild to moderate loss of myelinated fibers in most nerves. Segmental demyelination and remyelination was present in 16 teased nerve preparations. Axonal loss to a variable degree was present in only four, and wallerian-like degeneration in teased single fibers was noted in six nerves. In nine nerves, the primary abnormality was segmental demyelination and remyelination, or myelinated fiber loss, either singly or in combination. In none of these cases was wallerian-like degeneration seen as the only pathologic feature. All spinal nerve studies showed evidence of wallerian-like degeneration, as well as segmental demyelination (56), a finding absent in cases of encephalitic rabies (57).

The striking clinical similarities between paralytic rabies and the Guillain-Barré syndrome, with evidence for peripheral demyelination, may suggest immune-mediated processes as causative factors for the clinical weakness experienced during paralytic rabies.

Upon histopathologic examination of the CNS, a classic finding is the presence of Negri bodies (9) in neurons, as byproducts of viral replication. These inclusions are most commonly found in areas of active viral replication, especially the hippocampus, cerebellum, and brainstem. When correctly identified and differentiated from other intracytoplasmic inclusions, they are considered pathognomonic for rabies virus infection. However, detection in affected brains varies, and these particular inclusions appear only in subsets of affected neurons, compared with more sensitive means of viral antigen detection (58).

Dupont and Earle (55) suggested a relationship between histopathologic findings in the brain with duration of morbidity in rabid humans. Death occurred most rapidly (usually in 1 month) in those patients who had evidence of either encephalitis or congestion only. When Negri bodies were present either alone or in the presence of encephalitis, morbidity extended to approximately 66 and 73 days. These findings imply that Negri bodies appear rather later in the course of disease and again lessen their diagnostic value during early onset, which is not a limitation for more modern means of diagnosis.

EPIDEMIOLOGY

Rabies is distributed among mammals on all naturally inhabited continents (3,11). In general, the essential global epidemiology of human rabies largely parallels rabies infections of domestic animal populations. Additionally, the increasing prevalence of rabies in wildlife populations poses increasing problems for public health. When efforts to control domestic animal rabies, particularly the dog and cat, have succeeded, the incidence of human rabies has fallen dramatically (59). As a recent example (Fig. 16.2), based on data supplied by officials in the National Health Ministry and the Pan American Health Organization, human mortality from canine rabies in Mexico declined from nearly 70 cases per year in 1990 (with more than 10,000 rabid dogs reported) to an unprecedented single human case in 2001 (other human cases were due to wildlife), related to massive canine vaccination campaigns.

Cases of human rabies occur mainly in areas of the world where public health programs designed to control domestic animal rabies have not been adequately implemented. In such situations, based on global surveys by the World Health Organization, the incidence of human rabies may exceed 3 to 4 cases per 100,000 individuals. In contrast, human rabies has declined in other areas of the world to 0.04 per 100,000 individuals, or less. In the United States, the control of rabies in domestic animal populations has led to a significant decrease in the number of infected humans, from more than 30 fatalities in 1946 (when more than 8,000 rabid dogs were reported) to the current level of 1 to 2 cases per year (59,60). Current cases occurring in the United States have been the consequence of individuals having been bitten by rabid dogs outside of the territorial borders of the country or from wildlife (61–64). No human rabies cases have been documented from indigenous domestic animals in more than 25 years.

In areas where domestic animal rabies control programs are in effect (such as the United States, Canada, and Western Europe), indigenous dogs no longer account for cases of human rabies (1–3). In contrast, where domestic animal rabies has not been controlled, dogs may account for more than 90% of cases. In addition, in different areas of the world, various wildlife species can serve as sources for rabies virus infection. These include the fox in Eurasia and Arctic regions, mongoose and jackals in Africa and parts of Asia, the wolf in the Middle East and Asia, vampire bats in Latin America, and other bat species worldwide. Globally, specific rabies virus variants are adapted to different host carnivore or bat species (1–3). These viruses may be compartmentalized and form intraspecific patterns (such as fox rabies and mongoose rabies) or may result in spillover infections in dead-end hosts (e.g., vampire bat rabies virus infections in cattle). Endemic dog-to-dog transmission with specific virus variants may perpetuate in some areas (as in many parts of Asia, Africa, and Latin America). Wildlife may reintroduce rabies into dog populations, in which the disease has been otherwise controlled (63). From a practical perspective, nearly all human cases are due to dead-end spillover infections from affected hosts.

The control of domestic animal rabies in the United States by canine vaccination, leash requirements, and stray animal removal has led to a significant decrease in the number of cases of rabies in humans (59–61,65). The major reduction in human deaths began even before significant improvements in modern prophylaxis. Recognition of the emergence of the disease among wildlife, such as raccoons, skunks, foxes, and coyotes in the United States, not only is a reflection of real epidemiologic phenomena but also relates to the luxury for additional surveillance afforded after active dog rabies control. Besides terrestrial carnivores, another important source of infection, superimposed on the landscape, is bat rabies. Globally, multiple human deaths have resulted from exposure to rabid bats on all inhabited continents (Table 16.3). Recognizing the bias inherent in all passive surveillance systems, bats account for about 10% of the reported

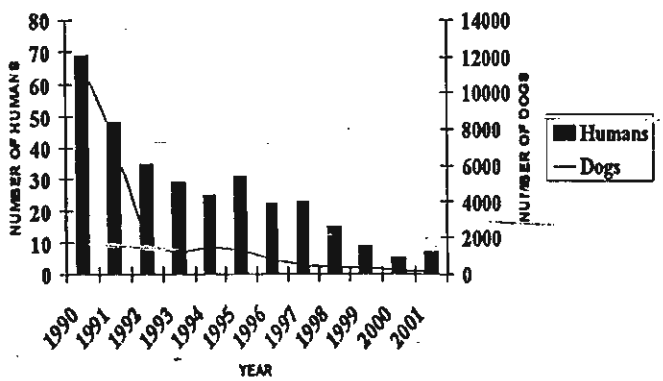

FIG. 16.2. Comparison of human and canine rabies cases in Mexico. (Courtesy the Ministry of Health, Mexico City, and the Pan American Health Organization, Washington, DC.)

TABLE 16.3. HUMAN RABIES FROM EXPOSURE TO BATS, OTHER THAN VAMPIRES

Yr/Region	Location	Nature of Exposure
United States		
1951	Texas	Bite
1956	Texas	Unknown, worked with rabid bats
1958	California	Bite (*L. noctivagans*)
1959	Texas/Mexico	Unknown, frequented bat caves
1970	Wisconsin	Bite
1971	Ohio	Bite (*E. fuscus*)
1973	New Jersey	Bite
1976	Kentucky	Bite
1978	Maryland	Bite
	Oregon	Unknown, bat virus identified
1979	Oklahoma	Unknown, bat virus identified
	Kentucky	Unknown, bat virus identified
1983	Michigan	Unknown, bat virus identified
1984	Pennsylvania	Unknown, bat virus identified
1990	Texas	Bite, bat virus identified
1991	Arkansas	Unknown, bat virus identified
	Georgia	Unknown, bat virus identified
1993	New York	Unknown, bat virus identified
	Texas	Unknown, bat virus identified
1994	California	Unknown, bat virus identified
	Alabama	Unknown, bat virus identified
	West Virginia	Unknown, bat virus identified
	Tennessee	Unknown, bat virus identified
1995	Washington	Unknown, bat virus identified
	California	Unknown, bat virus identified
	Connecticut	Unknown, bat virus identified
	California	Unknown, bat virus identified
1996	Kentucky	Unknown, bat virus identified
	Montana	Unknown, bat virus identified
1997	Montana	Unknown, bat virus identified
	Washington	Unknown, bat virus identified
	Texas	Unknown, bat virus identified
	New Jersey	Unknown, bat virus identified
1998	Virginia	Unknown, bat virus identified
2000	California	Unknown, bat virus identified
	Georgia	Unknown, bat virus identified
	Minnesota	Bite, bat virus identified
	Wisconsin	Unknown, bat virus identified
2002	California	Unknown, bat virus identified
	Tennessee	Unknown, bat virus identified
	Iowa	Unknown, bat virus identified
Canada		
1970	Saskatchewan	Unknown, bat contact
1977	Nova Scotia	Bite
1985	Alberta	Unknown, bat rabies virus identified
2000	Quebec	Unknown, bat rabies virus identified
Latin America		
1999	Mexico	Unknown, bat rabies virus identified
1996	Chile	Unknown, bat rabies virus identified
Asia		
1954	Andraha, India	Bite
Africa		
1970	South Africa (Transvaal)	Bite, Duvenhage virus identified
Eurasia		
1977	Former USSR	Bite, virus not identified
1985	Former USSR	Bite, European bat virus I identified
1985	Finland	Worked with bats, European bat virus II identified
2002	Scotland	Worked with bats, European bat virus II identified
Australia		
1996	Rockhampton	Worked with bats, Australian bat virus identified
1998	Mackay	Bitten by flying fox, Australian bat virus identified

Data from references 1–3, 11, 13, 59–61, 63, 64, 66, 67, 118, and surveillance reports to the World Health Organization.

rabies-infected animals in the United States today (63). Exposure to infected bats accounts for most current human deaths attributed to rabies in this country (64).

The pathologic and clinical findings in animal rabies are similar to the observations encountered with human disease, resulting in either "furious" or "paralytic" manifestations. Animals exhibiting furious behavior can exhibit extreme agitation, altered gait, irritability, nervousness, and aggressive activity toward inanimate objects. With the dumb or paralytic form of disease, there is ascending paralysis with general hindquarter weakness and gait disturbance.

Besides specific viral assemblages and host species contributions to the complex epidemiology of rabies, geographical facets are also apparent. Historically, several areas of the world have been considered "rabies free." To qualify for such a distinction by current World Health Organization standards, no cases of rabies should be identified in the area for at least 2 years, assuming an adequate surveillance system exists. Such countries include many of the islands of the Caribbean, Uruguay, Sweden, Portugal, Iceland, Japan, Oceania, among others. The danger with such an absolute connotation is a certain public health laxity that may evolve, as well as the lack of vigilance needed for maintenance of mitigative measures against the possibility for introduction. The emergence of bat rabies in areas presumed to be rabies free for some time, such as the United Kingdom and Australia, should reinforce the obvious need for continued surveillance and preparedness (66,67).

Human rabies occurrence is not random by age, sex, or season. In areas where the disease is endemic among dogs, human rabies often occurs most often in younger people, particularly in boys younger than 15 years, accounting for nearly 50% of all cases (68). Overall, the male-to-female ratio indicates that the frequency in males may be approximately 60% to 70%. Likely, the higher proportion in males and younger children may relate to a propensity to be outdoors and to be in frequent contact with dogs, with a shorter stature predisposing perhaps to more serious bites and a decreased chance of ready escape to avoid being bitten. As might be expected, the seasons of greatest risk for exposure to rabies virus occur most often during the months of May through September in the United States but will vary elsewhere in the world in part during those times when there is greater outdoor activity between humans and other animals. Likewise, human exposure may vary in regions where bats hibernate or migrate (63).

Current statistics suggest that more than 30,000 human cases occur per year, with most of these cases occurring in India and other parts of Asia (69). The number of actual cases may be far in excess of that actually reported. Overall, however, human cases parallel the gross distribution of animal cases throughout the world. International travelers should be aware of endemic areas of rabies and take proper precautions in these regions to avoid exposure. In urban populations where canine rabies is not under control, the close association between humans and dogs results in a high number of cases. In the United States, even though wild animals are the reservoirs, infected domestic animals usually account for the largest number of human exposures (70).

Other reported historic sources of infection include mucous membrane or wound contamination, inhalation of aerosols (either natural or by laboratory accidents), improperly inactivated vaccine-induced cases, and corneal transplantation with infected tissue (37,48,71–78). Most current human rabies cases associated with bat viruses in the United States have occurred without a history of bite exposure, perhaps in part due to lengthy incubation periods and forgotten episodes, ignorance of the risk associated with bat bite, trivialization of the wounds, or unrecognized bites (61,62,64,79).

CLINICAL SYMPTOMS

Background

Potential contact with a rabid animal does not equate with exposure, and not all humans bitten by a rabid animal will develop a productive viral infection. Infection is a function in part of a variety of factors, such as the virus variant, excretion of virus at the time of the bite, quantity of virus excreted, site and severity of the bite, and determination of whether the bite occurred through an article of clothing. A rather basic understanding of rabies occurrence appreciates the observation that a productive infection usually leads to death and the relationship between the site and severity of exposure, which may be important in the assessment of risk for the bitten subject (Table 16.4). Using the

TABLE 16.4. REPRESENTATIVE MORTALITY IN UNVACCINATED PEOPLE FOLLOWING EXPOSURE TO ANIMALS ASSUMED RABID

Location of Exposure	Extent of Exposure	Mortality (%)
Face	Bites (multiple and severe)	60–100
Other parts of head	Bites (multiple and severe)	50
Face	Bite (single)	30–40
Fingers/hand	Bite (severe)	15–20
Face	Bites (multiple and superficial)	10
Hand	Bites (superficial)	5
Trunk/legs	Bites (through torn clothing)	3
Hands/exposed skin	Bleeding and superficial wounds	2
Skin covered by clothes	Bleeding and superficial wounds	0.5
Recent wounds	Saliva contact	0.1
Wounds >24 hr old	Saliva contact	0.0

From Babes V. *Traite de la Rage*. Paris: Balliese et Fils, 1912:81–119, with permission.

rabid animal bite as a model, we find that mortality appears related to the number, location, and severity of the bites. For example, bites to the face, which are multiple and severe, may be associated with a mortality of about 60% or more in the absence of specific prophylactic intervention, with a similar rate for multiple severe bites to other parts of the head. Historically, wolves in the Old World have been associated with some of the highest rates of fatality per episode. In contrast to head and facial lesions, severe bites to the fingers or hand are associated with lower mortality (about 15%), suggesting a lower rate of productive infection (or perhaps associated with the ability for more adequate cleansing). Superficial exposures to skin or wounds with infectious saliva are associated with even lower mortality rates, ranging at or below approximately 3% (68,80–82). Obviously, merely petting or seeing a rabid animal is not a source of exposure, and neither are fomites. Regardless of the site or severity, any known or likely exposure to a rabid or suspect animal should be considered a possibility for rabies infection, and appropriate medical consultation about the need for prophylaxis measures should be instituted.

Animal bite remains the primary rationale for administering rabies postexposure prophylaxis, because it is the modality most often involved as the cause of human rabies cases worldwide. Nonbite exposures only rarely result in rabies, but nevertheless vaccination is often provided for this rather common scenario. Indirect, nonbite exposures have not been documented to cause rabies. Asymptomatic infection has been suggested, as indicated by the rare detection of low-level antibodies in unvaccinated persons with occupations having potential exposures to rabid animals (83–85).

Events following infection can be divided roughly into five stages: (a) incubation period, (b) prodromal phase, (c) acute neurologic phase, (d) coma, and (e) death (or rarely, recovery).

Incubation Period

The incubation period is extremely variable, from as short as a few days to as long as several years (1–3,86,87). These widely discrepant periods make it one of the most variable of all infectious diseases (59–61,88–96). During the incubation period, the host is free of symptoms. Typically, the interval ranges from 30 to 90 days (Table 16.5). A shorter incubation period may be encountered in those individuals with more severe exposures, such as those cases involving the head and face. The site of the bite varies somewhat between adults and children, with the latter more frequently experiencing bites to the head, face, and neck, explaining a difference in periods by age. Stress and inadequate vaccination after exposure may decrease the incubation period (86,97).

Prodromal Phase

Initial clinical findings of disease are usually vague and nonspecific and include malaise, fever, and myalgia (1,3). Chills with fever may develop in excess of 50% of patients (86). Additional components include nausea, vomiting, abdominal pain, and diarrhea. Patients may report tingling in the bitten extremity and pain at the site of the original bite.

Acute Neurologic Phase

Neurologic findings associated with rabies can be classified into one of two general forms of clinical manifestations: either "furious" rabies or "paralytic" rabies (86). Most patients will suffer from a furious form of rabies, with more than 80% of patients developing agitation, acute behavioral changes, hyperactivity,

TABLE 16.5. CLINICAL PROGRESSION OF RABIES IN HUMANS FOLLOWING EXPOSURE TO RABID ANIMALS

Stage	Duration	Clinical Association
Incubation period	30–90 days: about 50% of cases <30 days: about 25% >90 days to 1 year: about 20% >1 year: about 5%	No clinical findings
Prodromal phase	2–10 days	Paresthesia and/or pain at the site of bite Anorexia, nausea, vomiting Headache
Acute neurologic phase	2–10 days	"Furious rabies" (about 80% of cases) Hallucinations, bizarre behavior, anxiety, agitation, biting Hydrophobia Autonomic dysfunction "Paralytic rabies" (about 20% of cases) Flaccid paralysis Paresis Ascending paralysis
Coma	0–14 days	Syndrome of inappropriate secretion of antidiuretic hormone Diabetes insipidus Multiorgan failure Respiratory/cardiac failure
Death or recovery (very rare)	Variable	Neurologic dysfunction

From Fishbein DB. Rabies in humans. In: Baer GM, ed. *The natural history of rabies.* Boca Raton, FL: CRC Press, 1991:519–551, with permission.

excitability, and hallucinations (86,98). During this furious stage of the disease, patients may become extremely combative, requiring restraint and sedation (Fig. 16.3). A varying percentage of patients have hydrophobia, consisting of episodes that last 1 to 5 minutes and that are associated with an aversion to drinking water (99–101).

Trousseau at the Clinique Médical de l'Hôtel-dieu de Paris, in 1865, provided a rather graphic description of rabies, worth repeating:

> Finally there appears a symptom practically constant in established rabies in man, the horror of water. The site of this liquid often suffices to bring on a general tremor; but it is, above all, when the patient wishes to bring water to his lips that this special horror comes on, those convulsions of the face and of the entire body which make a vivid impression on those who witness an attack. The rabid man completely preserves his reason; he is thirsty; he wishes to drink, he bids his hands to carry to his lips the vessel filled with liquid, but no sooner does it touch him than the unhappy creature withdraws, terrified, sometimes he cries out that he cannot drink; his face shows agony, his eyes are fixed, his features contracted; then his limbs shake and his body quivers. The crisis lasts several seconds, gradually calm seems to return, but the least contact, even a breath of air, suffices to start a new crisis, such as the hypersensitivity of the skin. He cannot wash hands or face or comb his hair without being menaced by convulsions.

In addition, these patients may experience focal or generalized seizures, which have been associated with high mortality.

The alternative form of rabies is known as "paralytic" or "dumb" rabies. For patients with this form of disease, ascending paralysis is the predominant clinical finding (86,101–105). Various forms of paralysis may develop in the afflicted patients, ranging from paralysis of one limb to quadriplegia. Combined motor and sensory involvement may occur, as might the Guillain-Barré syndrome (106,107). Near termination, there is overlap between the furious and the paralytic form of rabies. The duration of either form of disease can last several weeks.

Coma

Patients develop, with varying degrees of rapidity, either coma or extremely rarely recovery. Coma can develop virtually immediately after the onset of clinical symptoms, or it can occur some 14 days after the onset of clinical disease. Death occurs approximately 18 days after onset of illness but may be extended by heroic means. For individuals who receive intensive support, survival following onset of illness averages approximately 25 days (59–61). In general, the furious rabies patient has a shorter survival period than the dumb rabies patient.

Death

Numerous complications contribute to mortality in patients with rabies (86,101) (Table 16.6). Given its predominant neurotropism and replication dynamics, the most important of these relate to involvement of the CNS. Obviously, complications can include multiple organs, because rabies virus can be transmitted peripherally to organs other than the CNS. Neurologic complications are significant and may include inappropriate secretion of antidiuretic hormone, diabetes insipidus, a hypoventilation or hyperventilation syndrome with involvement of the brainstem, alterations of temperature, and inability to control blood pressure (86,101,109). Other organ complications include myocarditis, renal failure, and gastrointestinal involvement (110–112).

To date, only five patients are reported to have recovered from rabies, but none spontaneously (73,113–116). In all cases, patients received either preexposure or postexposure rabies vaccination. Recovery was complete in only one child, but the adults had neurologic sequelae.

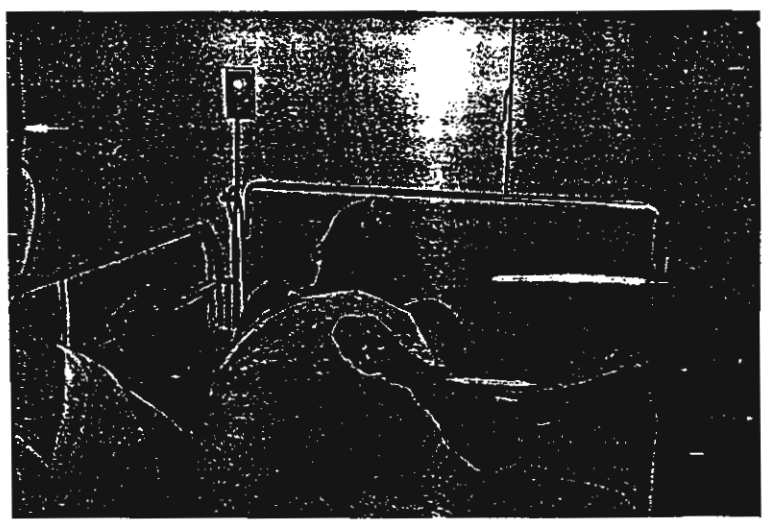

FIG. 16.3. Hospitalized human rabies case in restraints, during the acute neurologic phase. (Centers for Disease Control and Prevention, Public Health Image Library, 1959.)

TABLE 16.6. CLINICAL MANIFESTATIONS AND POSSIBLE COMPLICATIONS OF HUMAN RABIES

Central nervous system
Hyperactive episodes
Hydrophobia, aerophobia
Seizures
Localized neurologic signs
Cerebral edema
Inappropriate secretion of antidiuretic hormone
Diabetes insipidus
Pulmonary system
Hyperventilation
Hypoxemia
Atelectasis
Pneumomediastinum
Apnea/respiratory arrest
Pneumonia
Pneumothorax
Cardiovascular system
Arrhythmia
Congestive heart failure
Hypotension
Arteriovenous thrombosis
Other
Upper gastrointestinal tract bleeding
Ileus
Urinary retention
Hyperthermia
Hypothermia
Renal failure
Priapism

From Fishbein DB. Rabies in humans. In: Baer GM, ed. *The natural history of rabies.* Boca Raton, FL: CRC Press, 1991:519–551, with permission.

DIAGNOSIS

The diagnosis of rabies in the encephalitic patient is based on several parameters, including a history of an association with a known or suspected rabid animal bite or related exposure to the virus, and specific laboratory tests for (a) viral antigen demonstration, (b) serology, (c) viral nucleic acid detection, (d) virus isolation, or (e) histopathology (15,61). These tests may be performed either antemortem or postmortem.

Additional studies may support a diagnosis of rabies in an encephalitic patient, including electroencephalography or radiographic imaging of the CNS, such as axial computed tomography, realizing that such findings may be nonspecific (117,118). MRI may also be helpful (44,119). For example, nonenhancing ill-defined mild hypersignal T2-weighted changes of the brainstem, hippocampus; hypothalamus, deep and subcortical white matter, and deep and cortical gray matter could be demonstrated in noncomatose patients with rabies, either the furious or the paralytic form. Enhancement with gadolinium at the hypothalamus, brainstem nuclei, spinal cord gray matter, and intradural spinal nerve roots could be seen when the patients become comatose. During the prodromal phase, enhancement along the brachial plexus of the bitten arm could also be observed in a patient who had local neuropathic symptoms.

Initial clinical and epidemiologic findings should support a diagnosis of rabies, particularly for individuals living in areas where the disease is hyperendemic, and clinical acumen is maintained. Association of animal bite with the typical neurologic sequence of events provides a strong suggestion of evidence of rabies infection. However, other etiologies may mimic aspects of rabies. Moreover, the disease may be atypical in its presentation and may not be easily recognized by clinicians unfamiliar with its diverse manifestations (61). Rabies should be considered in the differential diagnosis of any unexplained viral encephalitis, regardless of a history of recent animal exposure. Differential diagnosis includes encephalitis caused by arboviruses, such as Japanese, equine encephalitis, and West Nile viruses, and enterovirus-71 and Nipah virus infections (44). For example, diffuse flaccid paralysis was found in about 10% of patients with West Nile virus encephalitis, with no discernible ascending pattern of progression, due to anterior horn cell involvement responsible for the weakness (120,121). Asymmetric, pure motor poliomyelitis-like weakness can be seen in patients with Japanese encephalitis. Myoclonus and other brainstem signs have been found in enterovirus-71 and Nipah virus encephalitis. Patterns of disease progression (acute or more chronic), the rapid or slow degeneration of consciousness levels, specific clinical signs of hydrophobia and hypersalivation, the appearance of brainstem symptoms, and the use of imaging analysis can suggest rabies or rule out other more likely pathogens (11,44). Use of the modern diagnostic virology laboratory is essential if a definitive diagnosis of rabies is to be ascertained, particularly for the antemortem demonstration of viral nucleic acid in infected material via reverse transcriptase polymerase chain reaction and nucleic acid sequence-based amplification (61,122,123). Moreover, the demonstration of rabies virus antigen by a fluorescent antibody technique following either a brain biopsy or a full-thickness biopsy of the nape of the neck, where hair follicles are abundant, may be used routinely for diagnosis of rabies (61,124). The sensitivity of skin biopsy for diagnosis of rabies is in excess of 50% and may be as high as 90%, with a specificity of nearly 100%. Nuchal biopsy should replace corneal smears (61,125) as the diagnostic method of choice, because fluorescent staining of corneal cells may be associated with problems with specificity, as well as unintended corneal lesions.

Related diagnostic approaches include virus isolation from a putatively infected host. Rabies virus can be isolated from the brain or saliva of infected individuals after direct intracerebral inoculation into laboratory animals, such as suckling or weanling mice, or infection of susceptible cultures, such as neuroblastoma cells (15). The frequency of viral isolation may vary according to disease duration and host immune response but can be as high as 60% or more (61). The probability of viral retrieval may be higher in the absence of virus-neutralizing antibodies.

Full postmortem examination of tissues can yield virus isolation, viral antigen, nucleic acids, or histopathologic evidence of infection. The brain is the usual site for isolation of rabies virus, particularly those areas of the brain usually infected by this pathogen. In addition, virus can be isolated from other exit portal tissues at autopsy, such as the salivary gland. Because of the peripheral excretion of rabies virus from saliva, fluids from these patients can pose a theoretical source for nosocomial infection, although there are no recent reports of infected health care workers (126).

Intensive histopathologic examination of the brain may demonstrate the presence of intracytoplasmic inclusions within neurons, or Negri bodies, although the use of immunohisto-

chemistry should improve detection over classic techniques (127,128). The addition of electron microscopy to the evaluation of brain specimens may increase the diagnostic yield of histopathology alone, but it is not the most obvious, effective, or preferred method of investigation.

Serologic approaches to the diagnosis of rabies infection have been conducted on both patient serum and cerebrospinal fluid (CSF) specimens (15,61). A variety of assays exist for determination of serologic status. Of particular interest is the appearance of neutralizing antibodies in the CSF, which is indicative of the diagnosis of rabies virus infection, as is antibody in the serum of naive patients (61,129).

TREATMENT AND DISEASE PREVENTION

Treatment

No specific therapy exists for rabies after disease manifestation, although some recent suggestions have been proposed (86,130). Regardless of sophisticated technology for intensive care of other patients, rabies remains an untreatable infection associated with near-uniform fatality. Current specific management for rabid patients entails palliative care only. Attention to the medical needs of such patients in an intensive care setting may lengthen the morbidity period, but the unrelenting progression of this viral encephalitis does not generate overt clinical optimism.

Prevention

Ideally, prevention of human rabies would occur by the elimination of the virus in the animal community. To date, domestic animal vaccination programs have helped control rabies significantly in the United States and other developed countries during the 1940s to the 1950s (65). Alternatively, wildlife vaccination programs have achieved success in some developed countries. Regarding humans, when exposure does occur, several other components of disease prevention warrant consideration. These include (a) evaluation of the risk for acquiring rabies, (b) local wound cleaning, (c) administration of vaccine, and (d) the administration of RIG (131). Outside the United States, other biologicals and vaccine regimens may be used in rabies prophylaxis, as described by the World Health Organization (www.who.int/vaccines/en/rabies.shtml).

Because of the high mortality rate associated with rabies and the absence of effective antiviral therapy, prevention is paramount. Humans are not the primary source of rabies but are only an uncommon dead-end host as a consequence of contact with infected animals. Thus, interrupting the chain of transmission events is essential. Altering the risk of exposure may be accomplished by decreasing contact with wild or domestic rabid animals or by rapid intervention with prophylactic measures after exposure occurs. Clearly, effective domestic animal rabies control will significantly diminish the number of human rabies cases (65). However, developing countries still experience a large number of cases of rabies in humans, due to inadequate control in poorly supervised dogs. In developed countries, prevention of rabies in wild carnivore populations has been of increasing relevance, but the control of bat rabies will remain a practical problem for which adequate methods do not exist. Novel approaches should continue to exploit opportunities to interrupt the chain of transmission events at multiple portals (Fig. 16.4).

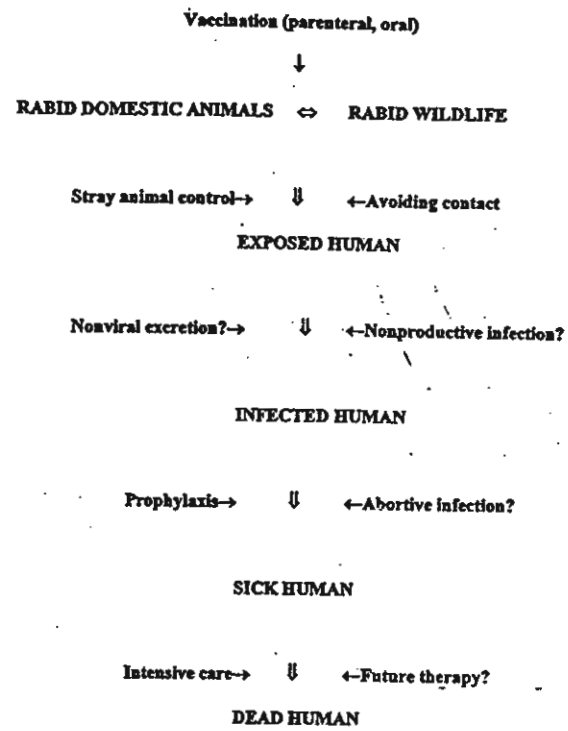

FIG. 16.4. Potential chain of events and approaches to the prevention and control of rabies. (From Hattwick MAW, Gregg MB. The disease in man. In: Baer GM, ed. *The natural history of rabies.* New York: Academic Press, 1975:282–304, with permission.)

Preexposure Prophylaxis

The risk of rabies can be significantly diminished by preexposure prophylaxis in high-risk individuals (131). These include laboratory staff in rabies virus research laboratories and those involved in the production of rabies virus biologicals. Such individuals would likely have continuous exposure to rabies virus. Other categories of individuals at risk with frequent exposure include rabies diagnostic lab workers, veterinarians, animal control staff, cavers, and wildlife workers in rabies epizootic areas. Personnel should have their serologic status verified every 6 months to 2 years, depending on their relative risk of exposure.

Preexposure prophylaxis does not substitute for postexposure requirements. Nevertheless, postexposure prophylaxis can be greatly simplified in the vaccinated individual, because RIG is not needed, and the number of vaccine doses is reduced. A standard preexposure regimen for immunization employed in the United States includes the administration of vaccine intramuscularly over 3 to 4 weeks, on days 0, 7, and 21 or 28 (Table 16.7). Should subsequent exposure to rabies occur, revaccination with 1-mL doses is indicated on two occasions (days 0 and 3). Elsewhere in the world, intradermal vaccination may be practiced with 0.1-mL doses, in an effort to lessen costs.

TABLE 16.7. SCHEDULE OF PROPHYLAXIS RECOMMENDED IN THE UNITED STATES BEFORE EXPOSURE TO RABIES

Type of vaccination and route	Regimens
Primary	
Intramuscular	1 mL of HDCV, PCEC, or RVA in the deltoid area on days 0, 7, and 21 or 28
Preexposure booster[a]	1 mL of HDCV, PCEC, or RVA in the
Intramuscular	deltoid area

[a]The administration of a booster dose of vaccine depends on the degree of continuing risk and serologic monitoring for evidence of rabies virus neutralizing antibodies.
From Centers for Disease Control and Prevention. Human rabies—United States, 1999. *MMWR Recomm Rep* 1999;48(RR-1):1–21, with permission.

Postexposure Prophylaxis

A key element in postexposure prophylaxis is the rapid evaluation of the risk to the individual. This risk varies according to the type and extent of exposure. Unequivocally, bites are the most common source of rabies virus infection from animals to humans. Nonbite exposures rarely result in rabies. Other facets include the species of the biting animal, the prevalence of rabies in the area, and the circumstances of the exposure. For example, if after provocation, a healthy domestic dog or cat bites a child in a major urban area, with no recent occurrence of rabies, observation of this animal for 10 days is adequate for determination of risk of rabies virus infection (65,131). Should abnormal behavior develop in the animal, appropriate intervention by veterinary personnel would include euthanasia, brain removal, and shipment to a qualified local diagnostic laboratory. Under such circumstances, implementation of a postexposure prophylactic regimen is indicated only if the test result is positive. Alternatively, exposure to a wild animal, such as a carnivore or bat that is not available for testing, regardless of altered behavior, warrants implementation of prompt postexposure prophylaxis. Local health department officials are of assistance in determining risks in different areas. In the United States, wildlife commonly affected includes skunks, raccoons, foxes, coyotes, bobcats, and bats (63). In contrast, small mammals such as squirrels, hamsters, chipmunks, rats, mice, and rabbits are not reservoirs. Despite their ubiquity, these animals are infected infrequently, have not been documented to transmit infection to humans, and almost never warrant prophylaxis.

Prompt local wound care with copious flushing is essential to the management of an exposed individual with a documented bite and is probably one of the simplest, economical, and most important means for preventing rabies. Soap or other products used in routine bite wound care can decrease the transmission of experimental rabies virus infection (132–134).

Following local wound care, exposed naive individuals should be given RIG and vaccine (131). Human RIG is prepared by cold ethanol fractionation of the plasma from immunized blood donors. The RIG is given only once, in a per-weight dose (20 IU/kg of body weight). Most, if not all, is infiltrated directly at the wound site, and the rest is administered intramuscularly. Equine serum, obtained following hyperimmunization of horses, can be used as well, if human RIG is not available. The dose of heterologous product under such circumstances is 40 IU/kg of body weight. If the equine RIG is not purified, patients who receive equine products may develop serum sickness or anaphylaxis, compared with minimal adverse effects among individuals receiving material of human origin (131,135).

The historical utility of RIG in rabies prevention has been demonstrated by reduced human mortality after bites by known rabid animals, compared with vaccine alone (7,24). Ordinarily, administration of RIG does not interfere with active antibody generation following vaccination, but it should not be given at the site of vaccine administration or in the same syringe (131). The safety of human RIG has been well documented and has not been associated with the acquisition of any untoward pathogens, such as hepatitis or retroviruses.

Concomitant with the administration of RIG, vaccine should be administered in the deltoid area, as opposed to the gluteal region (136). In the United States, the administration schedule begins as soon as possible after exposure (day 0), and on days 3, 7, 14, and 28 after the initial immunization (Table 16.8). Infants and small children receive the same vaccine dose

TABLE 16.8. SCHEDULE OF PROPHYLAXIS RECOMMENDED IN THE UNITED STATES AFTER EXPOSURE TO RABIES

Vaccination status	Regimen[a]
Not previously vaccinated	Immediate cleansing with soap and water
Rabies immune globulin	20 IU/kg of body weight (if anatomically feasible, all of the dose should be infiltrated into and around the wound or wounds and the rest should be administered intramuscularly); never administer more than the recommended dose; do not use the syringe used for vaccine or inject into the same anatomic site
Vaccine	1 mL of HDCV, PCEC, or RVA intramuscularly in the deltoid area[b] on days 0, 3, 7, 14, and 28
Previously vaccinated[c]	Immediate cleansing with soap and water
Rabies immune globulin	Should not be given
Vaccine	1 mL of HDCV, PCEC, or RVA intramuscularly in the deltoid area[b] on days 0 and 3

[a]The regimens are applicable to all age-groups, including children.
[b]The deltoid area is the preferred site of vaccination for adults and older children. For younger children, the outer aspect of the thigh may be used. Vaccine should not be administered in the gluteal area.
[c]"Previously vaccinated" indicates previous vaccination with HDCV, PCEC, or RVA, or any other type of licensed modern cell culture rabies vaccine, or a documented history of virus neutralizing antibody response.
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