PREVENTION

Since the use of live measles vaccine, methods to prevent measles have changed dramatically. Prevention today is ideally carried out long before an anticipated exposure to measles by the administration of live vaccine during the early part of the second year of life. However, there are rare occasions when passive immunization against measles with immune globulin must be used.

Included in the group of persons for whom passive immunization is recommended are those who are at high risk for developing severe or fatal measles, are susceptible, and/or have been exposed to the infection. This includes children with malignant disease, particularly if they are receiving chemotherapy, radiotherapy, or both, and children with significant deficits in cell-mediated immunity, including patients with AIDS. Babies younger than 1 year (including newborns whose mothers have measles) are also at increased risk after an exposure to measles. Because measles has been reported even after vaccination in HIV-infected children, it has been recommended that they also be passively immunized with immune globulin after a recognized exposure.[117,118,128,136] To be effective, passive immunization must be given within 6 days after an exposure; administration after 6 days would not be expected to influence the course of the disease.

For a healthy infant who has been exposed to measles, the modifying dose of immune globulin is 0.5 mL/kg intramuscularly (IM). An infant passively immunized in this fashion should be given live measles vaccine at age 15 months.[128] Immunocompromised children should receive a similar dose of immune globulin, with a maximum of 15 mL.

Active immunization against measles was developed in the early 1960s. Live and killed measles vaccines were licensed for use in the United States in 1963. Killed vaccine was withdrawn from the market in 1967, after the recognition of atypical measles in recipients of this vaccine. The first marketed live measles vaccine was the Edmonston B strain. This vaccine was associated with a fairly high incidence of moderately severe side reactions, such as rash and fever, and it was therefore often administered along with a dose of immune globulin. Subsequently, more attenuated vaccines were developed from the Edmonston strain.[137] Because the incidence of vaccine reactions is low with these vaccines, immune globulin is no longer given along with measles vaccine.

In 1976 it was recommended that all healthy children be given live measles vaccine at 15 months. At present it is recommended that children be immunized between the ages of 12 and 15 months (usually given as measles-mumps-rubella [MMR] vaccine or measles-mumps-rubella-varicella [MMRV] vaccine).[128] A second dose is recommended later in childhood.[114,128] Properly administered measles vaccine has been associated with persistence of immunity to measles for many years.[137,138,139,140] In one study, although measles HI antibodies were no longer detectable in some subjects, antibodies were demonstrated by neutralization, and revaccination was associated with a classic booster antibody response.[138] The estimated rate of secondary immune failure was calculated as less than 0.2%. In the general population, 95% of properly immunized children can be expected to respond serologically to measles vaccine. MMRV has been licensed for use in healthy children (not adults) in the United States and in many other countries. After two doses this vaccine provides an adequate immune response to all four viral antigens with a single injection (see Chapter 316).[141]

Vaccination is not usually recommended for infants younger than 12 months because the induction of immunity may be suppressed by residual transplacentally acquired antibodies. In situations in which the incidence of natural measles before the age of 1 year is high, live measles vaccine may be given at 6 to 9 months of age but should be routinely followed by additional doses.[46] Measles antibody titers are lower in women vaccinated as children than in women who have had natural measles, and the offspring of vaccinated women often lose transplacentally acquired measles antibodies before they are 1 year of age.[142,143] Therefore vaccination can be routinely given as early as 12 months of age because most women in their childbearing years today were vaccinated as children. For individuals who were passively immunized after an exposure to measles, vaccination should not be performed for 6 months after a dose of 0.5 mL/kg.[128] Transient fever and rash develop about 1 week after vaccination in 5% to 15% of children. In a 1986 study of 1162 twins who were given either MMR or placebo, there were side effects (fever, irritability, drowsiness, conjunctivitis) in 0.5% to 4%.[144] Symptoms of CNS dysfunction after measles vaccine are exceedingly rare.[145] Because measles may be severe in adults, immunization of adults who were not vaccinated previously, who have no history of measles, and who were born after 1956 is recommended by the CDC.[114] A 1986 Chicago study of hospital employees, however, indicated that only 1 of 266 (0.03%) was susceptible to measles; about one-third were born after 1957.[146]

A number of reasons for apparent primary vaccine failures of measles vaccine have been proposed.[25] These include improper storage of vaccine at temperatures exceeding 4°C, failure to use the proper diluent for the lyophilized vaccine, exposure of the vaccine to light or heat, and vaccination in the presence of low levels of passive antibody. The latter may occur if infants are immunized at 12 months of age or younger, if children are vaccinated 1 or 2 months after receiving an injection of immune globulin, if the more attenuated vaccines are given with immune globulin, or if live measles vaccine is administered soon after killed measles vaccine. No deleterious effects have been associated with measles revaccination. Although it is probably unusual, sustained transmission of measles has been reported in secondary schools, even when 95% of the students were immune and greater than 99% were immunized.[147,148]

Live measles vaccine is contraindicated in persons with deficits in cell-mediated immunity and in pregnant women. Fatal measles in children with AIDS has been reported.[136,149] Although the potential risks of measles vaccine in these children are unknown, they are less than the disease itself. It is currently recommended that children with known asymptomatic HIV infection receive measles vaccine after the age of 12 months.[116,128] The use of measles vaccine should also be considered for children with known HIV infection who manifest symptoms if their CD4 T-cell levels are relatively well preserved, especially if they live in locations where there may be transmission of measles, such as certain inner city areas.[128] One case of fatal measles pneumonia resulting from vaccine virus in an HIV-infected vaccinated young adult has been described after a second dose of vaccine.[117,150] Children who have been treated for malignant disease may be given measles vaccine 3 months after they complete their course of therapy.[128] High-risk children, such as those described, may be given monovalent measles vaccine or MMR, but they should not be given MMRV vaccine, which contains a significantly higher dose of the varicella component. No safety data for MMRV in high-risk children are available.[151]

Serious hypersensitivity reactions to measles vaccine in persons allergic to egg protein have been reported. At one time it was recommended that persons with a history of anaphylactic reactions after the ingestion of eggs should be vaccinated only with extreme caution.[152] More recent studies, however, have indicated that it is safe to immunize such children.[153]

Susceptible persons who are exposed to measles, with the exception of young infants, pregnant women, and immunocompromised persons, may be given live measles vaccine to prevent disease, as an alternative to immune globulin. If the vaccine is given shortly after exposure, clinical cases of measles may be prevented because clinical manifestations associated with measles vaccine occur in about 7 days, compared with an incubation period of 10 days for clinical measles.[20]

An experimental measles vaccine, a derivative of the original Edmonston B vaccine strain termed *Edmonston-Zagreb vaccine*, administered at a dose 10 to 100 times higher than usual, proved to be immunogenic in 4- to 6-month-old infants.[154] Despite its short-term safety, however, the rate of mortality from causes other than measles in these vaccinees in Senegal was significantly higher than that in children who received standard vaccine.[155] Therefore this vaccine is no longer in use.

The possibility that an abnormal immune reaction to vaccine-type MV in MMR vaccine might cause autism in young children was raised in 1998 by Wakefield and coworkers.[156] This idea was never accepted by the vast majority of the scientific community, and 12 of the original 13 authors of the paper eventually withdrew their names from the article in retraction of its content, in part because of conflict of interest by Wakefield.[157] After extensive review, numerous national committees, including the Institute of Medicine, concluded that there is no evidence to support this hypothesis.[158–160] A recent large retrospective cohort

study of 95,727 children, including those with an elevated baseline risk for autism, found no association between receipt of the MMR vaccine and an increased risk of autism.[161] Numerous other scientific studies have also failed to identify an association between MMR vaccine and subsequent development of autism.[162–167] In the United Kingdom, where there has been extensive adverse publicity about MMR, the incidence of measles has increased as a result of suboptimal vaccination rates.[168]

THERAPY

Patients with measles should be given supportive therapy, such as antipyretics and fluids as indicated. Bacterial superinfection should be promptly treated with appropriate antimicrobials, but prophylactic antibiotics to prevent superinfection are of no known value and are therefore not recommended.

Vitamin A, 200,000 IU administered orally to children once daily for 2 days, has been reported to decrease the severity of measles, especially in those with vitamin A deficiency.[169–171] Children aged 6 months to 1 year should receive 100,000 IU for 2 days. Children younger than 6 months should receive 50,000 IU for 2 days. Children with clinical signs and symptoms of vitamin A deficiency should receive an age-specific dose (a third dose) 2 to 4 weeks later. Side effects include transient vomiting and headache.[172] WHO recommends vitamin A for all children with acute measles, regardless of their country of residence.[128] Administration of vitamin A has been reported to reduce seroconversion in vaccinees and should therefore be avoided at or after immunization.[173] The efficacy of ribavirin administered intravenously or by aerosol for treatment of severe measles is unproven.[125,136,174]

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161 Zoonotic Paramyxoviruses: Nipah, Hendra, and Menangle Viruses

Anna R. Thorner and Raphael Dolin

SHORT VIEW SUMMARY

Definition
- Hendra and Nipah viruses are highly pathogenic zoonotic paramyxoviruses that emerged during the 1990s in Australia and Southeast Asia, respectively, and cause meningoencephalitis in humans.

Epidemiology
- In 1994, in Queensland, Australia, Hendra virus caused two outbreaks of fatal illness in horses and their human caretakers. Additional outbreaks involving humans have occurred subsequently.
- Nipah virus was first recognized when it caused an outbreak of severe encephalitis in pig farmers in Malaysia and abattoir workers in Singapore in 1998 and 1999. Multiple outbreaks have been detected since then in Bangladesh and India, including cases that have involved human-to-human transmission. Drinking raw or fermented date palm sap has been identified as a risk factor in these outbreaks. An outbreak associated with exposure to sick horses occurred in the Philippines in 2014.
- Menangle virus, another paramyxovirus, caused decreased farrowing rates and stillbirths in pigs, and illness in two humans in Australia in 1997.
- The *Pteropus* genus of fruit bat, also known as the flying fox, is the reservoir of all three viruses.

Microbiology
- Nipah and Hendra viruses are members of the *Henipavirus* genus within the Paramyxoviridae family. They are single-stranded, negative-sense RNA viruses.

Clinical Manifestations
- Nipah virus causes a severe and often fatal meningoencephalitis. Clinical manifestations of Hendra virus infection range from a self-limited influenza-like syndrome to a fatal respiratory illness or encephalitis. Menangle virus caused an influenza-like illness in two people.

Diagnosis
- Diagnostic tests that can be used to detect Nipah and Hendra viruses include culture, electron microscopy, immunohistochemistry, serology, and real-time reverse-transcriptase polymerase chain reaction.

Therapy
- Because no specific antiviral therapies have been evaluated for the treatment of Nipah virus or Hendra virus infection, treatment involves only supportive care, such as intravenous hydration and mechanical ventilation, when indicated.
- A recombinant human monoclonal antibody, m102.4, directed against the *Henipavirus* glycoprotein protects animals from disease after inoculation with Hendra virus or Nipah virus.

Prevention
- Several vaccines have been developed against Nipah virus and Hendra virus, but none is currently available for use in humans.
- A Hendra virus subunit vaccine was shown to be highly effective at preventing Hendra virus infection in horses after a lethal challenge; it became available for use in horses in Australia in 2012.

Hendra and Nipah viruses are highly pathogenic zoonotic paramyxoviruses that emerged during the 1990s in Australia and Southeast Asia, respectively. In 1994, in Queensland, Australia, Hendra virus caused two outbreaks of fatal illness in horses and their human caretakers.[1,2] Additional outbreaks involving humans occurred in Australia in 2004, 2008, and 2009.[3–5] Nipah virus was first recognized when it caused an outbreak of severe encephalitis in pig farmers in Malaysia and abattoir workers in Singapore in 1998 and 1999.[6,7] Multiple outbreaks have occurred subsequently in Bangladesh and India,[8–18] and one outbreak has occurred in the Philippines.[19] In 1997 in Australia, another zoonotic paramyxovirus, Menangle virus, caused decreased farrowing rates and stillbirths in pigs, and an influenza-like illness in two people who had occupational exposure to infected pigs.[20,21] The *Pteropus* species of fruit bat, also known as the flying fox, is the reservoir of all three viruses.[9,22–24] Because Nipah and Hendra viruses are Biosafety Level 4 agents, research with these pathogens has been difficult to perform.[25,26] However, the development of pseudovirus and reverse genetics techniques has increased opportunities for their investigation.[25–27]

VIROLOGY

Classification

Nipah and Hendra viruses are species within the *Henipavirus* genus of the Paramyxoviridae family, which also includes the *Rubulavirus* genus in which Menangle virus is a species.[28–31] *Rubulavirus* also includes mumps; Newcastle disease; human parainfluenza types 2, 4A, and 4B; and Menangle viruses. Other genera in the Paramyoxviridae family are *Respirovirus* (human parainfluenza virus types 1 and 3), *Morbillivirus* (measles virus), and *Avulavirus*. Nipah and Hendra viruses are most similar to the viruses of the *Respirovirus* and *Morbillivirus* genera, but there is significantly less sequence homology between Nipah or Hendra virus and the members of either of these genera.[29] Nipah and Hendra viruses share a high degree of sequence homology and have similar genomic organization.[32]

Structure and Molecular Biology

Nipah and Hendra Viruses

Nipah and Hendra viruses have a single-stranded, nonsegmented, negative-sense RNA genome that is fully encapsidated by protein.[33] Nipah virus particles range in diameter from 120 to 500 nm,[29] and Hendra virus particles range from 40 to 600 nm.[34] The paramyxovirus envelope contains two transmembrane glycoproteins: an attachment protein or cell receptor-binding glycoprotein (G) and a fusion protein (F).[29] Thin-section electron microscopy (EM) of infected cells reveals filamentous nucleocapsids contained within cytoplasmic inclusions and incorporated into virions budding from the plasma membrane. Hendra virus has a double-fringed appearance caused by projections on the surface of the viral envelope, whereas Nipah virus has only a single layer of surface projections (Fig. 161.1).[29,35]

Henipaviruses cause the formation of syncytia in infected Vero cells.[35] An unusual feature of Nipah virus infection is that nucleocapsid aggregates form at the periphery of infected syncytial cells late in infection. In contrast, Hendra virus nucleocapsid aggregates form

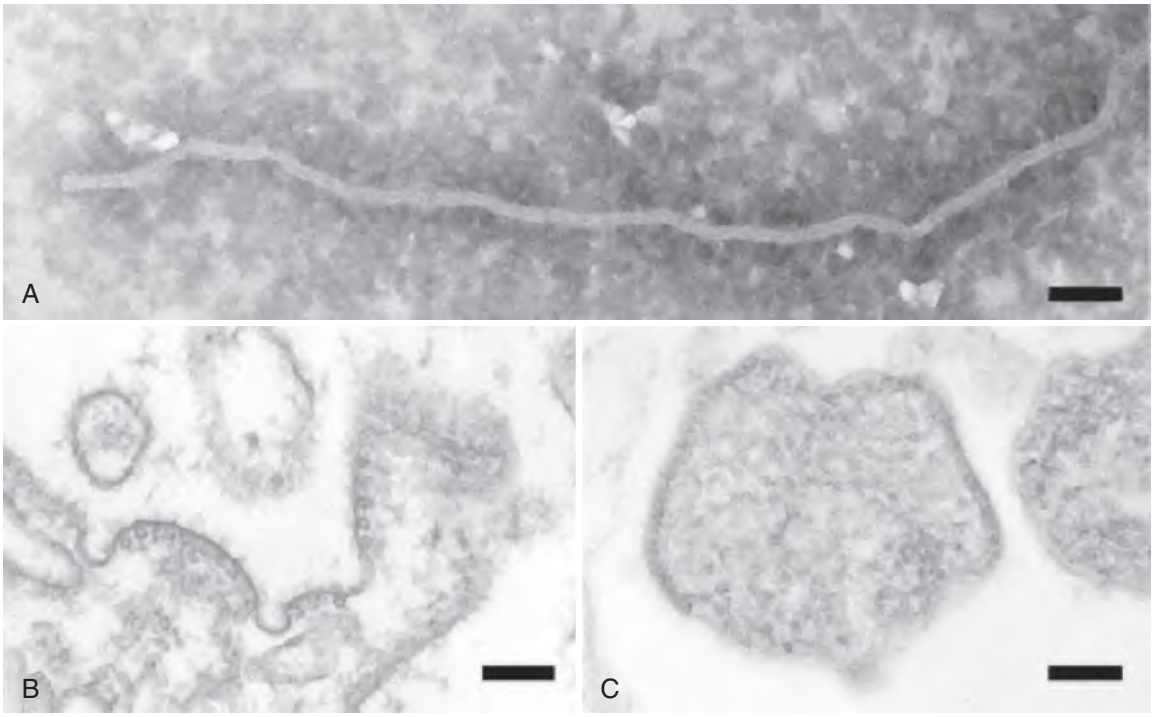

FIG. 161.1 Ultrastructural characteristics of Nipah virus isolate in cell culture as seen with negative stain (A) and thin-section (B and C) electron microscopy. (A) A single nucleocapsid with the typical herringbone appearance characteristic of the family Paramyxoviridae. (B) Viral nucleocapsids, as seen in cross and longitudinal sections, aligned along the plasma membrane of Nipah virus–infected Vero E6 cells. (C) Extracellular Nipah virus particle showing a curvilinear tangle of nucleocapsids enclosed within the viral envelope. Scale bars = 100 nm. *(From Chua KB, Bellini WJ, Rota PA, et al. Nipah virus: a recently emergent deadly paramyxovirus.* Science. *2000;288:1432–1435.)*

randomly throughout the cytoplasm. Both Nipah and Hendra viruses have herringbone nucleocapsid structures.[29,34] Both viruses, but particularly Nipah virus, cause tubule-like structures to be present in the cytoplasm of infected cells.[35] These structures are unique to Hendra and Nipah viruses and Sendai virus, a paramyxovirus that infects animals such as mice. The attachment proteins (G) of Hendra and Nipah viruses lack hemagglutinin and neuraminidase activity, which most other paramyxovirus attachment proteins possess.[33,36,37] The attachment proteins of Nipah and Hendra viruses use ephrin-B2 and ephrin-B3 as receptors, which are present in neurons and arterial endothelial cells.[38–41]

The genomes of the henipaviruses have six transcription units that encode six major structural proteins.[33] The transcription units include the nucleocapsid (N), phosphoprotein (P), matrix protein (M), fusion protein (F), glycoprotein or attachment protein (G), and large protein or RNA polymerase (L). This genome arrangement is most similar to those of the *Respirovirus* and *Morbillivirus* genera. The mRNA of the P genes of Nipah and Hendra viruses are cotranscriptionally edited by the insertion of G residues at editing sites, which leads to the translation of multiple gene products.[32,33]

Hendra and Nipah viruses share 68% to 92% amino-acid homology in the protein-coding regions and 40% to 67% nucleotide identity in the untranslated regions.[42–45] Both viruses are approximately 18.2 kb in length. The length of the genomes of the henipaviruses is in multiples of six nucleotides, a property that is important for efficient replication.[26,48]

A Nipah virus strain isolated during an outbreak in Bangladesh was six nucleotides shorter than the strain isolated in Malaysia and shared 92% sequence homology with it.[49] Although isolates from patients in Malaysia were nearly identical to one another, isolates from patients in Bangladesh differed by 0.9% in the nucleoprotein open reading frame.[16,44] In addition, genetically distinct sequences of Nipah virus were detected from patients in two adjacent districts of Bangladesh over a short period of time, indicating that multiple lineages circulated concurrently in a localized region.[50]

Menangle Virus

Like the other paramyxoviruses, Menangle virus consists of an enveloped, nonsegmented, negative-sense RNA genome that is tightly associated with nucleocapsid proteins.[31] EM reveals that the morphology of Menangle virus is similar to that of other members of the Paramyxoviridae family.[21] The viral particles appear spherical or pleomorphic and range from 30 to 100 nm in diameter. Virus isolated from the lungs, brains, and hearts of affected piglets caused cytopathic effects, including vacuolation and syncytia formation, when grown in baby hamster kidney (BHK21) cells. Menangle virus, like Nipah and Hendra viruses, contains herringbone nucleocapsids. It has an envelope with a single fringe of surface projections 17 ± 4 nm in length. Its genome encodes all six of the major structural proteins described previously for Hendra and Nipah viruses. Sequencing of the nucleoprotein (NP), P, M, F, and hemagglutinin-neuraminidase (HN) genes revealed that Menangle virus is a member of the *Rubulavirus* genus.[31] Ultrastructural analysis also supports this classification.[21] Unlike the other rubulaviruses, Menangle virus lacks hemagglutinin and neuraminidase activity.[31] The HN protein shares only 16% to 20% sequence homology with other *Rubulavirus* HN proteins. Menangle virus also lacks the hexapeptide NRKSCS, which is conserved in the other rubulavirus and the respirovirus HN proteins and is thought to be necessary for neuraminidase activity; only the last two amino acids in the sequence motif are conserved in the Menangle virus HN protein.

EMERGENCE OF HENIPAVIRUSES

Phylogenetic analyses show that Nipah and Hendra viruses are old viruses,[34,51] which suggests that their emergence in the 1990s was due to ecologic factors rather than virus mutations.[52] Ecologic change that drew flying foxes closer to horses, pigs, and humans was probably the largest contributor to the emergence of Hendra and Nipah viruses.[53,54] Deforestation has caused flying foxes to move into suburban and urban areas to use the trees in these regions for roosting. Climate change is likely to be causing an expansion of the geographic areas that are suitable for the bat host species of henipaviruses.[55]

NIPAH VIRUS

Epidemiology

An outbreak of severe encephalitis occurred in pig farmers in the Perak state of Malaysia, including the village of Nipah, in September 1998.[6,7,56]

By December, the outbreak had spread to pig farmers in the Negeri Sembilan state. In March 1999, 11 abattoir workers in Singapore who came into contact with pigs that were imported from Malaysia developed an encephalitis syndrome with associated pneumonia.[57] The last cases occurred in May 1999 in the Selangor state of Malaysia.[58] In total there were 276 cases of acute Nipah virus infection, 106 of which were fatal (case-fatality rate of 38%).[29,57,59] Of the cases in Malaysia, 70% occurred in individuals who worked directly with pigs,[58] and a case-control study showed that exposure to sick pigs was a risk factor for Nipah virus infection.[57] The pig farm that was the site of the initial outbreak in Malaysia was also an orchard, which meant that flying foxes were in close proximity to pigs.[52] The outbreak ended after several interventions were implemented, including the deployment of personal protective equipment to individuals in contact with sick pigs, restriction on movement of livestock, and culling of nearly 1 million pigs.[16]

Nipah virus was isolated from the respiratory secretions and urine of 8 of 20 patients with acute infection in Malaysia.[60] Despite evidence of Nipah virus shedding from infected patients during the outbreaks in Malaysia and Singapore, serologic studies of exposed health care workers did not show evidence of nosocomial transmission.[61,62] However, there was a single case report of a nurse who cared for patients with Nipah virus encephalitis who developed the characteristic lesions of Nipah virus encephalitis on magnetic resonance images despite having no signs or symptoms of infection.[63]

Subsequent outbreaks of Nipah virus encephalitis have occurred in Bangladesh nearly every year since 2001.[9–18,50,64,65] Outbreaks also occurred in regions of India neighboring Bangladesh in 2001 and 2007.[8,64] All of the outbreaks in this region occurred between December and May.[65] Several epidemiologic features distinguished the outbreaks in Bangladesh and India from those that occurred in Malaysia and Singapore, including the absence of an intermediate host, evidence of person-to-person spread, and higher case-fatality rates in Bangladesh and India. Some of the outbreaks in Bangladesh and India involved multiple cases of person-to-person transmission and clustering of cases within households.[8,9,14,17,50,64–68] During the outbreak in India in 2001, 45 of 60 patients (75%) had a history of exposure to individuals infected with Nipah virus.[8] Many of the affected individuals were health care workers, suggesting that nosocomial transmission played an important role in the outbreak. In an outbreak of 23 cases in Kerala State, India in 2018, all but the index case acquired the infection in the hospital; the index case transmitted Nipah virus directly to 19 contacts, whereas 3 cases were secondary cases, acquiring the infection from earlier cases.[66] Nipah virus has also been detected on environmental surfaces in hospitals where patients with Nipah virus infection were receiving care.[12,69] However, two studies did not demonstrate evidence of nosocomial spread during outbreaks in Bangladesh, although in one of them, individuals who lived with or cared for patients with Nipah virus were more likely to acquire Nipah virus infection.[9,70] In a case-control study in Bangladesh, subjects with Nipah virus were 7.3 times more likely to have had contact with a person with Nipah virus than controls (95% confidence interval [CI], 4.0–13.4).[71]

For at least two patients in Bangladesh, the only known exposure was contact with a deceased individual who had had Nipah virus infection.[50] The increased incidence of person-to-person spread in Bangladesh and India compared with Malaysia and Singapore may have been due to the higher rates of respiratory involvement in patients in Bangladesh and India, which could have facilitated transmission.[11,66] In ferrets that were exposed to a strain of Nipah virus from either Malaysia or Bangladesh, significantly higher levels of viral RNA were recovered from the oral secretions of ferrets infected with the Bangladesh strain, suggesting that higher levels of oral shedding could be an important factor for human-to-human transmission.[72]

Other risk factors associated with the outbreaks in Bangladesh or India, or both, included consuming raw date palm sap[13,71,73,74] or fermented date palm sap,[75] climbing trees,[10] exposure to sick cows,[9] exposure to pigs or goats,[65,67] and colder temperatures.[76] Flying foxes feed from pots that are used to collect date palm sap, which probably leads to contamination of the sap that is later consumed by people.[13] Although most patients affected by Nipah virus were adults, in two outbreaks in Bangladesh the median age of affected individuals was 12 years.[10,11] The lower median age and male predominance in one of these outbreaks were probably due to a behavior that increased the risk of infection, such as climbing trees.[10]

In 2014, an outbreak of severe neurologic and influenza-like illness caused by Nipah virus or a related virus occurred in humans and horses in two villages in the Philippines and included cases of horse-to-human, human-to-human, and foodborne transmission.[19] There were a total of 17 cases in humans, including 11 cases of encephalitis, 5 cases of influenza-like illness, and 1 case of meningitis; 9 of 11 patients (82%) with encephalitis died, whereas no patients with influenza-like illness or meningitis died. Of the 17 patients, 7 (41%) had been involved with horse slaughtering and horse meat consumption, and 3 (18%) had only consumed horse meat and had no history of slaughtering or meat preparation. Five patients (29%) had been exposed to other human cases, but not to horses. Of these, 2 were health care workers who did not visit the affected villages, had no contact with sick horses, and did not consume horse meat.

The case-fatality rate of Nipah virus infection was substantially higher in the outbreaks in Bangladesh and India than in Malaysia and Singapore. In a case series that included 92 patients from four of the outbreaks in Bangladesh, the case-fatality rate was 74% compared with 38% in the outbreak in Malaysia and Singapore.[11,29,57,59] During an outbreak in Bangladesh in 2005 that involved 12 individuals, the case-fatality rate was 92%.[13] In the outbreak in Kerala State, India in 2018 that involved 23 patients, the case-fatality rate was 91%.[66] It has been proposed that the increased mortality observed in the outbreaks in Bangladesh and India may have been due to increased virulence of the Nipah virus strains involved, although other factors, such as inadequate facilities for the care of critically ill patients and malnutrition, could have played a role.[11]

Researchers cultured a paramyxovirus from cerebrospinal fluid (CSF) that caused syncytia formation in Vero cells from two of the first three patients who died from Nipah virus.[56] The virus stained with anti–Hendra virus antibodies by indirect immunofluorescence, and an enzyme-linked immunosorbent assay (ELISA) for anti-Hendra immunoglobulin M (IgM) antibodies was positive in the CSF of all three patients. Given the epidemiologic and clinical differences between Hendra virus and the outbreak of encephalitis in Malaysia, it was proposed that a paramyxovirus that was related to but distinct from Hendra virus had caused the outbreak.[56] It was named Nipah virus after the village in which the first patient from whom the virus was isolated resided.[56,58]

Reservoirs and Intermediate Hosts

After the initial outbreak of Nipah virus in Malaysia, bats were screened for the presence of anti–Nipah virus antibodies because they were already known to be the reservoir of Hendra virus. Island flying foxes *(Pteropus hypomelanus)* and Malayan flying foxes *(Pteropus vampyrus)* were found to have neutralizing antibodies to Nipah virus.[24] Subsequently, viruses that caused Hendra virus–like cytopathic effect in Vero cells and stained strongly for Nipah and Hendra virus antibodies were identified from two urine samples from *P. hypomelanus* and from fruit that had been partially eaten by a fruit bat.[77] *Pteropus giganteus* is the reservoir of Nipah virus in Bangladesh[9] and India.[78] Motion sensor infrared cameras have documented flying foxes visiting date palm trees in communities where sap was collected for human consumption.[73,79]

Anti–Nipah virus antibodies have been detected in *Pteropus* species of bats in other countries, such as Cambodia, Thailand, Indonesia, and Madagascar.[80–83] In addition, anti–Nipah virus antibodies, Nipah virus RNA, or both, have been found rarely in non-*Pteropus* species of bats in several countries, including Malaysia, Thailand, and Ghana.[24,81,84] Specimens collected from western and southern Africa revealed a diverse array of paramyxoviruses in African bats, including 19 novel species of *Henipavirus*-like viruses distinct from the Nipah and Hendra viruses found in Southeast Asia and Australia.[85] In a study in Cameroon, Nipah virus cross-neutralizing antibodies were detected in 48% of bat specimens and 3% to 4% of human specimens; seropositive human samples were found almost exclusively in individuals who reported butchering bats for bushmeat.[86] This may represent an opportunity for cross-species infection of newly emerging henipaviruses from bats to humans in such regions.

Other animals may serve as reservoirs of related paramyxoviruses. In 2012, three people who had been working in an abandoned mine in China developed severe pneumonia of unknown etiology and died.[87] Anal swab specimens were collected from rats, bats, and musk shrews in the mine. A novel henipa-like virus, Mojiang paramyxovirus, was detected from anal swab specimens from the rats, but not from the bats or musk shrews, with polymerase chain reaction (PCR)–based sequencing techniques. A tissue specimen from a rat was also positive. The positive anal swab samples were cultured in various cell lines for virus isolation, but no cytopathic effect or viral replication was detected.

The only animals that are known to have served as intermediate hosts of Nipah virus are pigs during the initial outbreak in Malaysia and Singapore[6,7] and horses during the outbreak of Nipah virus or a related virus in the Philippines.[19] Nipah virus infection has been demonstrated serologically in cats, dogs, goats, and cattle[58,88,89]; among these species, only dogs have been shown to have clinical disease.[7]

Clinical Manifestations

The incubation period of Nipah virus in Malaysia ranged from several days to 2 months, although more than 90% of patients had an incubation period of 2 weeks or less.[90] In four of the outbreaks in Bangladesh, the incubation period ranged from 6 to 11 days, with a median of 9 days.[11] Although subclinical infection can occur, the ratio of symptomatic to subclinical infection was approximately 3:1 during the outbreak in Malaysia.[91]

In the outbreaks in Malaysia and Singapore, patients typically presented with fever, headache, dizziness, and vomiting.[58,90,92,93] More than 50% of patients had a decreased level of consciousness and brainstem dysfunction, including such signs as myoclonus, areflexia, hypotonia, hypertension, and tachycardia. Cerebellar signs were also common.[93] Some severely ill patients also had multisystem organ dysfunction, including sepsis, gastrointestinal bleeding, and renal failure.[90] Respiratory findings were uncommon in the outbreaks in Malaysia and Singapore, with only 14% of patients in Malaysia having a nonproductive cough[90] and 3 of 11 patients in Singapore having evidence of pneumonia.[57] In contrast, in a study of four of the outbreaks in Bangladesh, 62 of 90 patients (69%) had respiratory difficulty and at least 5 patients had acute respiratory distress syndrome.[11]

In the most severely affected patients in the outbreak in Malaysia, electroencephalogram (EEG) revealed bilateral temporal periodic complexes of sharp and slow waves occurring every 1 or 2 seconds.[58] The typical magnetic resonance imaging (MRI) findings were multiple 2- to 7-mm lesions best visualized in the T2-weighted images, without associated cerebral edema or mass effect; they were disseminated throughout the brain but were most commonly present in the subcortical and deep white matter of the cerebral hemispheres (Fig. 161.2A).[90,94] In a study of eight patients with Nipah virus encephalitis in Malaysia, all patients had multiple small bilateral foci of T2 prolongation in the subcortical and deep white matter, and in the periventricular areas and the corpus callosum.[95] Five patients also had cortical involvement, three had brainstem lesions, and one had a thalamic lesion. In five of the patients, diffusion-weighted images showed increased signal. Four patients had leptomeningeal enhancement, and four had enhancement of the parenchymal lesions. In a follow-up study 1 month after the outbreak in Malaysia, 5 of 12 patients had widespread small foci of high signal intensity on the T1-weighted images, especially in the cerebral cortex.[96] The diffusion-weighted images showed decreased prominence or disappearance over time. At the 6-month follow-up, there was no radiographic evidence of progression or relapse. In three patients who underwent MRI for acute Nipah virus encephalitis in Bangladesh, confluent high-signal lesions in both the gray and white matter were seen.[97] Relapsed or late-onset encephalitis, or both, occurred in some patients after the outbreaks in Malaysia and Bangladesh.[90,94,98,99]

A study performed 24 months after the outbreak in Malaysia, which included 160 patients who survived Nipah virus infection, found that 12 patients (7.5%) developed a relapse of encephalitis.[98] Of 89 patients who initially had nonencephalitic disease or asymptomatic infection, 10 patients (11%) had late-onset encephalitis, with a mean time to neurologic findings of 8.4 months. Four of the 22 patients (18%) with relapsed or late-onset encephalitis died. Brain MRI images in patients with relapsed disease usually showed patchy confluent cortical gray matter lesions (see Fig. 161.2B).[90,94,98]

In a study of long-term neurologic and functional outcomes that included 22 patients from Bangladesh who survived Nipah virus illness, 17 of whom had encephalitis and 5 of whom had febrile illness, all but 1 patient had disabling fatigue that lasted for a median of 5 months

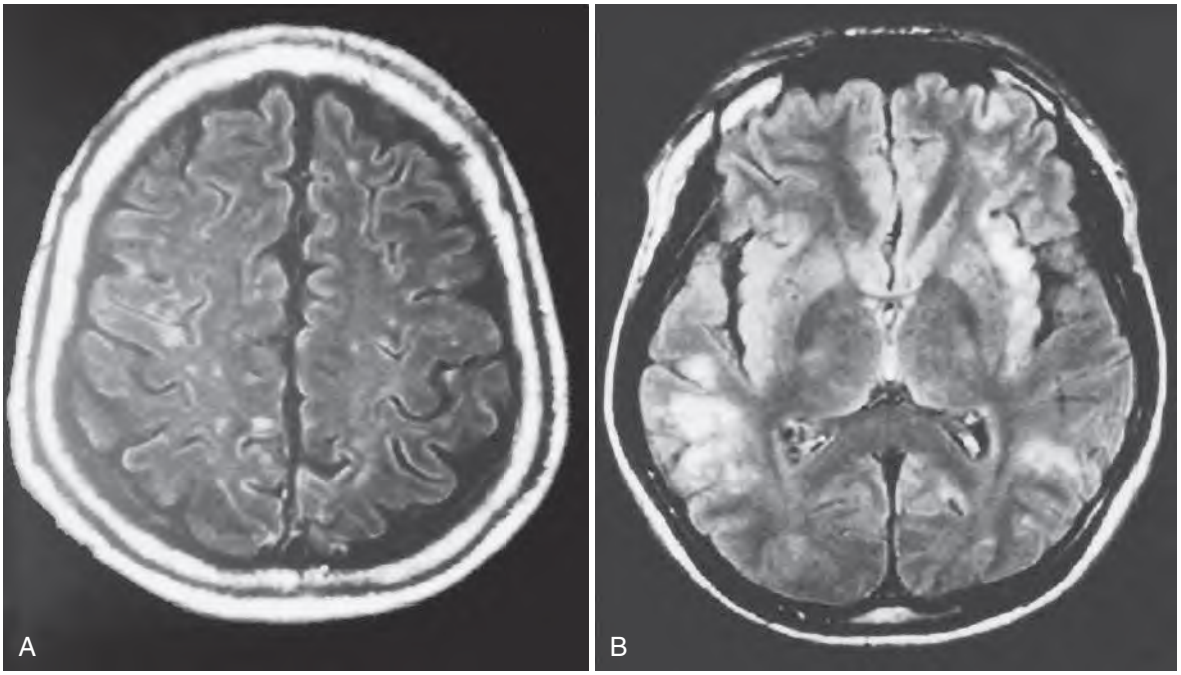

FIG. 161.2 Axial magnetic resonance imaging findings in patients with acute (A) and relapsed (B) Nipah virus encephalitis with use of fluid-attenuated inversion recovery. (A) Multiple discrete hyperintense lesions in the white and gray matter of a patient with acute Nipah virus encephalitis. (B) Confluent lesions primarily involving the cortical gray matter in a patient with relapsed Nipah virus encephalitis. *(From Goh KJ, Tan CT, Chew NK, et al. Clinical features of Nipah virus encephalitis among pig farmers in Malaysia.* N Engl J Med. *2000;342:1229–1235.)*

(range, 8 days to 8 months).[99] Seven of 17 patients (41%) with encephalitis, but none with febrile illness, had persistent neurologic deficits, including encephalopathy, ocular motor palsies, cervical dystonia, focal weakness, and facial paralysis. Four patients had late-onset neurologic abnormalities months after the acute illness.

Laboratory Abnormalities

Leukopenia (11%), thrombocytopenia (30%), and elevated levels of alanine aminotransferase (33%) and aspartate aminotransferase (42%) were the most common laboratory abnormalities in the outbreak in Malaysia.[90] The CSF was abnormal (elevated white blood cell [WBC] count or protein level, or both) in 75% of 92 patients evaluated; the mean CSF WBC count was 41 cells/μL (range, 0 to 842 cells/μL), and the mean protein level was 0.69 g/L (range, 0.12 to 2.15 g/L).[90] CSF glucose was normal in all patients. Unlike in the outbreak in Malaysia, all 18 patients evaluated during an outbreak in India and 4 of 6 patients evaluated during outbreaks in Bangladesh had a normal CSF WBC count (≤5 cells/μL).[8,11] Among patients with acute Nipah virus infection in Malaysia, virus-specific antibodies were present in the serum in more than 70% of samples but in less than one-third of CSF samples.[58] Isolation of Nipah virus from the CSF was strongly associated with mortality.[100] Stored CSF samples from 84 patients with Nipah virus encephalitis (27 fatal and 57 nonfatal) were cultured for Nipah virus. The virus could be cultured from the CSF in 17 fatal cases and 1 nonfatal case.[100]

Diagnostic Tests

Methods that can be used for the diagnosis of Nipah virus infection include culture, serology, EM, immunohistochemistry, reverse-transcriptase polymerase chain reaction (RT-PCR), and serum neutralization tests.[101–104] Real-time RT-PCR is more sensitive than conventional RT-PCR for the diagnosis of Nipah virus infection.[105] Nipah virus can be detected from urine and respiratory secretions by culture and PCR.[60] Serology is most useful for epidemiologic studies.

Pathology

Nipah virus causes a multiorgan vasculitis with a predilection for the central nervous system.[106,107] At autopsy, patients with Nipah virus infection from the outbreak in Malaysia exhibited widespread endothelial involvement characterized by vasculitis, thrombosis, ischemia, and parenchymal necrosis. This was most marked in the central nervous system, although the lungs, heart, and kidneys were also involved. Syncytial giant cell formation was present in affected vessels. Viral inclusions were detectable with both light microscopy and EM. Immunohistochemistry revealed the presence of Nipah virus antigens in the endothelial and smooth muscle cells of blood vessels, and in neurons and other affected cells.

In experimental infections in African green monkeys, a Nipah virus strain from Bangladesh was uniformly fatal, whereas a strain from Malaysia was fatal in only half of infected monkeys.[108] Histopathology of lungs and spleens from monkeys infected with the Bangladeshi strain showed more severe findings than from the monkeys infected with the Malaysian strain.

Therapy

The mainstay of therapy for Nipah virus infection is supportive care, including monitoring in the intensive care unit for patients with severe illness. In an open-label study of oral or intravenous ribavirin in patients with Nipah virus encephalitis in Malaysia, 45 of 140 patients (32%) in the ribavirin group died, compared with 29 of 54 (54%) in the control group.[109] Although these observational data suggest that ribavirin may be beneficial, it is not possible to draw conclusions about efficacy.

On the basis of its in vitro activity against Nipah virus, the antimalarial agent chloroquine was assessed, individually and in combination with ribavirin, for the treatment of Nipah virus infection in a golden hamster model.[110] Ribavirin delayed death in infected hamsters by approximately 5 days. Chloroquine did not protect hamsters when administered either alone or in combination with ribavirin. In another study, ribavirin delayed but did not prevent death in hamsters after a lethal challenge with Nipah virus.[111] A monoclonal antibody, m102.4, directed against the henipavirus G glycoprotein has been shown to have in vitro activity against Nipah virus and to protect ferrets and African green monkeys from a lethal challenge with Nipah virus, but it is not available for clinical use.[112–114] Delivery of antifusion lipopeptides via the respiratory route before and after a lethal challenge of Nipah virus in hamsters and nonhuman primates may reduce the mortality rate of Nipah virus infection.[115]

Prevention

Because many of the cases of Nipah virus infection in Bangladesh have been caused by people drinking raw date palm sap that has been contaminated with Nipah virus by flying foxes, it is important to avoid drinking it. It has been suggested that diverting a larger proportion of the supply of date palm sap to be used for making molasses will help reduce Nipah virus infections because the sap is heated to temperatures at which Nipah virus cannot survive during the manufacturing process.[16] Another preventive measure includes using barrier techniques to prevent access of flying foxes to date palm trees from which sap is being collected; this approach was shown to be effective in a randomized trial.[79] Infection control measures should include standard precautions.[16] Experts have noted that a higher level of infection control may not be feasible in Bangladesh, but that counseling family members to wash their hands with soap after caring for patients with Nipah virus infection can reduce the risk of human-to-human transmission.[12,16]

No vaccine is available for the prevention of Nipah virus, although several vaccines have shown promising results in animal models.[26,116–123] As an example, a Hendra virus G glycoprotein subunit vaccine afforded African green monkeys complete protection against subsequent Nipah virus infection, with no evidence of clinical disease, virus replication, or pathology observed.[118] The status of research and development of vaccines for Nipah virus was reviewed by the World Health Organization in a July 2015 report published in 2016.[124] Despite encouraging studies in animal models, clinical trials with candidate Nipah virus vaccines have not yet been carried out.

HENDRA VIRUS

Epidemiology

In September 1994, an outbreak of an acute respiratory illness occurred in thoroughbred horses in Hendra, a suburb of Brisbane in Queensland, Australia.[1,125] Affected horses had fever, facial swelling, severe respiratory distress, ataxia, and copious frothy nasal discharge, which was sometimes blood-tinged. The index case was a pregnant mare at pasture, which died after a 2-day illness. During the next 2 weeks, 13 more horses at the same stable died or were euthanized. There were four nonfatal cases, two of which had mild neurologic sequelae. Three more horses were found to have seroconverted without having had signs of clinical illness. Within 1 week of the death of the equine index case, a horse trainer and a stablehand became ill with a severe influenza-like illness. The trainer died after developing respiratory and renal failure, whereas the stablehand recovered. Infection with Hendra virus was demonstrated in both human patients with viral culture, immunoelectron microscopy, serology, and PCR using primers derived from other paramyxoviruses.[1,34,125]

In October 1995, a second outbreak of Hendra virus was retrospectively discovered after the death of a thoroughbred stud owner who had developed a relapse of encephalitis 13 months after a mild and self-limited episode of meningitis.[2] It was subsequently determined that two horses had died in August 1994 on his farm in Mackay, in central Queensland, Australia, approximately 1000 km from the original outbreak. The first horse was a pregnant thoroughbred that had developed severe respiratory distress, ataxia, and swelling of the cheeks and supraorbital fossa during a 24-hour period. The second horse had licked the face of the dead mare through a fence. The second horse, a 2-year-old colt, died 11 days later, after a 24-hour clinical course of aimless pacing, muscle trembling, and hemorrhagic nasal discharge. The etiologic agent was originally called equine morbillivirus, but the name was later changed to Hendra virus, after the suburb where the first outbreak had been identified.[52]

In 2004, a veterinarian in Cairns, Queensland, developed a fever, dry cough, sore throat, cervical lymphadenopathy, myalgias, and malaise 1 week after performing a necropsy on a horse that was later found to have Hendra virus.[3] She was diagnosed with Hendra virus by convalescent

serologies but recovered fully. An outbreak in five horses in Brisbane, Queensland, in July 2008 resulted in Hendra virus infection in two veterinary workers, one of whom died.[4] In August 2009, a veterinarian developed fatal encephalitis after close contact with the respiratory secretions of an infected horse.[5] In total, 7 cases have occurred in humans, including 4 deaths, and more than 60 cases have occurred in horses. Sporadic cases continue to occur in horses. In addition to Queensland, several cases have occurred in horses in New South Wales.

Reservoirs and Intermediate Hosts

A serologic survey of wildlife species that were present at the site of the outbreaks was performed.[22] A total of 168 animals from more than 16 species were tested, including rodents, marsupials, birds, amphibians, and insects, yet none were seropositive for Hendra virus. Nomadic birds and flying foxes (bats of the *Pteropus* genus) were then targeted as likely reservoirs, given their presence in the regions of both outbreaks and their ability to travel long distances. Anti–Hendra virus antibodies were found in several types of flying fox throughout Queensland. Hendra virus was subsequently isolated from the reproductive tract of a pregnant gray-headed flying fox that had become entangled on a wire fence and from tissue from aborted flying fox fetuses.[23] The bat isolates were identical to the isolate that infected the horses that died. Flying foxes are thought to have subclinical infection.[52,126,127] Pteropid bat species in geographic regions other than Australia, such as Papua New Guinea and Madagascar, have been found to have antibodies against henipaviruses.[83,128] In a survey of 2840 flying foxes from three of the four Australian mainland species (*Pteropus alecto, Pteropus poliocephalus,* and *Pteropus scapulatus*) captured from the wild in Queensland and New South Wales, specimens of a range of types (urine; serum; urogenital, nasal, oral, and rectal swabs) were collected from anesthetized bats and tested for Hendra virus RNA with a quantitative RT-PCR assay.[129] Hendra virus RNA was detected in *P. alecto* specimens, but not in *P. poliocephalus* or *P. scapulatus* specimens. Forty-two of 1410 *P. alecto* animals had Hendra virus RNA detected in at least one specimen and yielded a total of 78 positive samples, at an overall detection rate of 1.76% among all specimens tested from this species (78/4436). Urine was the most common specimen type to be positive (26 of 29 urine specimens).

Horses are the only animals known to have served as intermediate hosts.[5] Rarely, dogs have been found to have had natural infection after exposure to infected horses.[130–132] In an experimental model, beagles have been infected after oronasal exposure, but generally remain asymptomatic.[133]

Clinical Manifestations

Clinical manifestations of Hendra virus infection range from a self-limited influenza-like syndrome to a fatal respiratory illness or encephalitis. The incubation period is approximately 5 to 21 days.[1,3,5,37,125] One patient had an influenza-like illness characterized by fever, myalgias, headache, lethargy, and vertigo.[1] He was unwell for 6 weeks and then recovered. Another individual had a dry cough, sore throat, cervical lymphadenopathy, myalgias, fatigue, and fever that lasted for 4 days.[3] The illness lasted for a total of 8 days. Another patient presented with a similar syndrome but rapidly developed respiratory distress necessitating mechanical ventilation.[1,34] He also had acidosis, dehydration, an arterial thrombosis in his right lower extremity, and cardiac irritability. He died 6 days after the onset of illness from asystolic cardiac arrest. Two patients presented with an influenza-like illness, with subsequent progression to encephalitis; one of these patients recovered and the other one died.[5] Another patient presented with encephalitis immediately after completing a 5-day course of postexposure prophylaxis with intravenous ribavirin and hydroxychloroquine.[5] This patient had an incubation period of 21 days, compared with an incubation period of 5 to 16 days in the other patients.

Relapsed disease manifesting as encephalitis occurred in one patient 14 months after a horse that he had cared for died from Hendra virus.[2] The patient was a 35-year-old man who had aseptic meningitis in August 1994, after caring for two sick horses and assisting with their necropsies. He initially developed a sore throat, headache, drowsiness, vomiting, and neck stiffness, and he was found to have 560 WBCs/μL in the CSF, with a polymorphonuclear predominance, and negative bacterial and viral cultures. He recovered fully, but 13 months later he developed irritability, low back pain, and a seizure. During the following week he had a low-grade fever and recurrent seizures. By day 7 of hospitalization, he had a right hemiplegia, brainstem signs, and a decreased level of consciousness and required intubation. He remained febrile and unconscious and was found through the EEG to be having seizure activity despite control of clinically apparent seizures. He died 25 days after admission.

Laboratory Abnormalities

One of the patients who died from Hendra virus infection had thrombocytopenia and elevated levels of creatine kinase, lactate dehydrogenase, aspartate aminotransferase, alanine aminotransferase, and glutamyltransferase.[34] He also had signs of dehydration and acidosis. In contrast, a patient who survived had no laboratory abnormalities.[37]

Diagnostic Tests

Diagnostic tests that can be used to detect Hendra virus include culture, EM, immunohistochemistry, serology, and RT-PCR.[5,134] For patients with possible Hendra virus infection, real-time RT-PCR is the diagnostic method of choice.[5,135,136] However, this method may not detect all variants because many different strains are circulating in the bat population in Australia.[5,137,138] Sample types that have yielded positive RT-PCR results include nasopharyngeal aspirates or swabs, urine, blood, and CSF.[139] The use of more than one technique improves the diagnostic yield.[5]

Hendra virus forms syncytia when grown in Vero cells.[35] Primary bat cells also support the growth of Hendra virus.[5,140] The typical EM findings of the herringbone nucleocapsid and the double-fringed appearance of the surface projections of the viral envelope aid in its identification.[34,35] Immunohistochemistry also helps confirm the diagnosis.[5] Serology is useful for epidemiologic and surveillance studies, but not for the diagnosis of acute infection.[5]

Pathology

The autopsy of the horse trainer who died of acute Hendra virus infection revealed interstitial pneumonia characterized by lung congestion, edema, and hemorrhage.[34,125] Histologic examination revealed focal necrotizing alveolitis with giant cells, syncytia formation, and viral inclusions. He also had mild chronic myocarditis and regions of inflammation with necrosis in the kidney, and a pulmonary embolism.[125] Kidney tissue inoculated in cell culture caused formation of syncytia, whereas lung, liver, and spleen tissue did not.[34,125]

The autopsy of the patient who died from relapsed encephalitis revealed a leptomeningitis with a cellular infiltrate that contained lymphocytes and plasma cells.[2] There were discrete foci of necrosis in the neocortex, basal ganglia, brainstem, and cerebellum, with sparing of the subcortical white matter. Rare multinucleated endothelial cells were detected in the brain, liver, spleen, and lungs. Immunohistochemistry of brain tissue was positive for Hendra virus. EM revealed aggregates of nucleocapsids in cell remnants. Hendra virus could not be cultured from the brain.

Therapy

Because no specific antiviral therapies have been proven effective for the treatment of Hendra virus infection, treatment involves only supportive care, such as intravenous hydration and mechanical ventilation, when indicated. Agents that have been evaluated or are being evaluated for the treatment of Hendra virus infection include ribavirin; chloroquine; LJ001, a broad-spectrum antiviral small molecule targeting entry of enveloped viruses[141]; the HIV-1–specific fusion inhibitor enfuvirtide; and a Hendra virus–specific monoclonal antibody.[5] Although ribavirin and chloroquine have activity in vitro, neither one has been effective in animal models of infection.[110,111,142,143] The most promising experimental agent is a recombinant human monoclonal antibody directed against the henipavirus G glycoprotein, m102.4, which protects African green monkeys from disease after inoculation with Hendra virus.[144] This agent was given to two individuals in 2010 and one in 2013 who had a "high-risk" exposure to Hendra virus. None developed Hendra infection, but the effect, if any, of the monoclonal antibody could not be determined.[145] It has also been given to several additional patients who remained well

and experienced no adverse effects.[146] A phase I clinical trial assessing the safety of m102.4 in healthy subjects is underway in Australia.[147]

Prevention

Measures to limit the spread of Hendra virus from infected horses to humans include the use of infection control precautions to prevent contamination of mucosal surfaces and nonintact skin.[5] An important strategy to reduce transmission from flying foxes to horses involves minimizing the opportunity for horses to contact potentially infected body fluids from flying foxes; this can be accomplished by preventing horses from grazing beneath trees in which flying foxes are roosting or feeding, locating yards and stables away from such trees, and placing feed and water stations away from trees and ideally under artificial cover.[5]

Several vaccines have been evaluated in animal models, but none is available for use in humans; vaccines that have shown promising results include a recombinant Hendra virus G glycoprotein–based subunit vaccine and a recombinant adeno-associated virus vector vaccine.[119,148,149] A Hendra virus subunit vaccine was shown to be highly effective at preventing Hendra virus infection in horses after a lethal challenge; it became available for use in horses in Australia in 2012.[150] The efficacy of the vaccine in naturally occurring infections in horses has not been formally tested. The increase in detection of cases of Hendra virus infection in horses in 2013 and 2014 has raised questions about the efficacy of the vaccine.[152] Another likely contributor is the limited acceptance of the vaccine by horse owners.[153]

MENANGLE VIRUS

Epidemiology

From April to September 1997, the farrowing rate at a commercial piggery in Menangle, a suburb of Sydney in New South Wales, Australia, decreased from 82% to 60%.[21] The number of live piglets declined, and the rate of mummified and stillborn piglets increased. Occasional abortions also occurred. The stillborn piglets had abnormalities that included severe degeneration of the brain and spinal cord, arthrogryposis, brachygnathia, and, rarely, fibrinous body cavity effusions and pulmonary hypoplasia. Of pigs at the affected piggery, 96% had neutralizing antibodies against the virus, whereas serum and plasma samples collected from pigs at the piggery before May 1997 were negative.[21,154]

A large colony of fruit bats was found to roost within 200 m of the affected piggery from October to April.[21] Given the proximity of the fruit bats to the piggery and the previous Hendra virus outbreak, fruit bats were immediate targets of the investigation. Forty-two (34%) of 125 serum samples from fruit bats in New South Wales and Queensland, Australia, were positive for neutralizing antibodies against Menangle virus. Antibodies were found in several species of fruit bat.[155] Other species in the area, including rodents, birds, cattle, sheep, cats, and a dog, were all seronegative.[21]

A total of 251 humans who had had potential exposures to infected pigs were tested serologically.[21] Only two of these individuals were found to have neutralizing antibodies, one of whom worked at the affected piggery and one of whom worked at a piggery that had received a shipment of pigs from the affected piggery. Both of these individuals had an influenza-like illness during the weeks after exposure to likely infectious material. They were also tested for a broad range of viruses, bacteria, and parasites that could have caused this illness, but none of the results suggested an alternative diagnosis.[20]

Pathogenesis

Respiratory spread has been proposed as the likely mode of transmission among pigs, but it is less clear how the virus spreads from pigs to humans.[20] Both of the humans who were infected had exposure to body fluids of infected pigs. The first individual helped to birth pigs, and he reported that splashes of amniotic fluid and blood often occurred. He also reported having frequent minor wounds of his hands and forearms. The second patient performed necropsies on pigs without wearing gloves or protective eyewear.

In a study of experimental infection and transmission in which pigs were infected with Menangle virus via intranasal inoculation, the incubation period was approximately 2 to 3 days in duration and was followed by viral shedding from nasal and oral secretions, feces, and urine, lasting for less than 1 week.[156] Cessation of shedding correlated with the development of neutralizing antibodies in sera. Secondary lymphoid organs and intestine were identified as major sites of viral replication and dissemination, as assessed with real-time RT-PCR and immunolabeling for viral antigen. Viremia occurred but was of short duration and low titer.

Clinical Manifestations and Diagnostic Tests

The first individual with Menangle virus reported that in early June 1997 he had the sudden onset of malaise and chills, followed by severe headache and myalgias.[20] He remained in bed for 10 days. Four days into the illness, he developed a spotty erythematous rash. His physician noted that in addition to the rash, he also had abdominal tenderness and lymphadenopathy. He returned to work after 2 weeks but continued to have fatigue. He lost 10 kg during the illness. An evaluation 2 months after the illness revealed mild right lower abdominal tenderness and an enlarged spleen on ultrasound. The liver size was at the upper limit of normal. Urinalysis findings, complete blood count (CBC), blood chemistries, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) level were all normal.

The second patient also became ill in early June 1997 with fever, chills, rigors, sweats, malaise, back pain, severe frontal headache, and photophobia.[20] The headache lasted for 4 or 5 days. On the fourth day of illness, he noticed a spotty erythematous truncal rash, which lasted for 7 days. His acute illness lasted for approximately 10 days, during which time he lost 3 kg. Two months after the illness, he underwent a clinical evaluation. Urinalysis findings, CBC, ESR, and CRP level were normal. Blood chemistries were normal, except for mildly elevated hepatic enzymes. However, he was found to have antibodies against hepatitis C, which could have explained these findings. Abdominal ultrasound examination revealed mild hepatomegaly and a spleen size at the upper limit of normal. Both patients had antibodies against Menangle virus, with titers of 1:128 and 1:512, respectively. As noted earlier, a real-time RT-PCR assay for Menangle virus RNA has been used in a study of experimental infection in pigs.[156]

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The complete reference list is available online at Expert Consult.

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162 Vesicular Stomatitis Virus and Related Vesiculoviruses (Chandipura Virus)

Steven M. Fine

SHORT VIEW SUMMARY

Definition
- Vesicular stomatitis virus (VSV) is a vesiculovirus that causes outbreaks of stomatitis, mainly in livestock but sometimes also in humans.

Epidemiology
- A large percentage of people who live in endemic areas are infected and usually have had mild or subclinical disease.
- Seasonal outbreaks among horses, cattle, and other livestock have the potential to cause large economic losses, particularly in the southwestern United States.
- Endemic disease occurs in areas of Central and South America.
- The mode of transmission is not known, although the virus is believed to be introduced to a herd by an insect vector, and it can then spread from animal to animal by direct contact.
- Related viruses, such as Chandipura virus, have caused outbreaks of encephalitis in India, mainly in children. Chandipura virus has been shown to be spread via mosquitoes and sand flies.

Microbiology
- VSV is a single-stranded, negative-strand RNA virus that is a member of the Rhabdoviridae family, genus *Vesiculovirus.*

Diagnosis
- Samples from lesions can be tested by enzyme-linked immunosorbent assay, complement fixation, or isolation of virus in tissue culture, as well as by polymerase chain reaction assays.
- The main purpose of diagnosis is to quickly distinguish the disease from the more dangerous foot-and-mouth disease in livestock.

Therapy
- Therapy in humans is generally not required.
- There is no known antiviral treatment, so therapy consists of supportive measures.

Prevention
- Quarantine of animals from infected ranches is commonly instituted.
- Protective clothing and gloves should be worn when handling infected animals.
- There are currently no licensed vaccines. Experimental vaccines tested during outbreaks showed immunogenicity but not efficacy.

Vesicular stomatitis virus (VSV) most prominently causes a vesicular disease in domestic animals that resembles foot-and-mouth disease. Outbreaks within domestic animal herds decrease production and result in restrictions on the transport and sale of animals and animal products, which results in significant economic losses. VSV infects a high percentage of people who live in endemic areas. VSV-associated disease in humans is generally mild, although significant morbidity can occur. In addition, because the VSV-G (glyco)protein can bind to numerous cell types, VSV has earned a major role in molecular biology research involving the transduction of genetic material into cells as a vaccine vector and as an antitumor agent.

VIROLOGY

Classification and Structure

Vesicular stomatitis virus is enveloped and contains a single strand of negative-sense RNA that encodes five structural proteins: the glycoprotein (G), membrane (or matrix) protein (M), nucleoprotein (N), and two internal proteins (L and P).[1,2] It belongs to the family Rhabdoviridae, genus *Vesiculovirus*,[3] and it assumes the bullet morphology characteristic of the Rhabdoviridae. Approximately 1200 identical copies of the G protein cover its surface in an ordered, densely packed array of spikes, which present only one antigenic determinant accessible to neutralizing antibodies.[4,5] Of the 9 confirmed and 22 tentative species of vesiculoviruses discovered thus far (Table 162.1), 6 cause animal or human disease: VS-New Jersey (VS-NJ), VS-Indiana (VS-I), VS-Alagoas, Chandipura, Isfahan, and Piry.[6–9]

MOLECULAR BIOLOGY

The VSV-G protein binds to the surface of most cell types. Thus molecular biologists often replace the envelope proteins in other viral vectors with VSV-G to expand the host range of the vector. Viruses produced in cell lines that express the VSV-G protein are thus *pseudotyped* with VSV-G on their surfaces. They can infect a large variety of cells and are therefore tremendously useful for gene transduction.[10,11] VSV is also used as an expression vector in candidate vaccines and can be reengineered to abrogate neurotoxicity and circumvent humoral immunity.[12–14] Live-attenuated VSV vectors are being tested for use in vaccines for human immunodeficiency virus,[15,16] Ebola disease,[17,18] malaria,[19] tuberculosis,[20] and avian influenza.[21]

EPIDEMIOLOGY

Epizootic

In North America VSV disease caused by VS-NJ or VS-I appears in sporadic, epizootic outbreaks in domesticated horses and cattle, mainly in the central and southwestern United States, Canada, and Mexico. Outbreaks of VS-I occurred in 1942, 1956, 1964, 1965, 1997, and 1998[22] and of VS-NJ in 1944, 1949, 1957, 1963, 1982, 1985, 1995, 1997, 2006, 2012, and 2014–15.[23–25] Outbreaks typically begin in late spring, spread to adjacent or remote herds, and abate after heavy frost. The vector is not known, although insects are suspected. VSV was isolated from a mosquito during an epizootic outbreak in New Mexico[26] and from biting midges and black flies that may be responsible for long-distance transport of the virus.[27–29] The 1995 epizootic episode in the southwestern

TABLE 162.1 Vesiculoviruses

SPECIES	LOCATION OF ISOLATION
VS-Indiana	United States
VS-New Jersey	United States
VS-Alagoas	Brazil[6]
VS-Carajas	Brazil[8]
Maraba	Brazil[8]
Piry	Brazil
Cocal	Trinidad[22]
Chandipura	India[74]
Isfahan	Iran[7]

Modified from Travassos da Rosa AP, Tesh RB, Travassos da Rosa JF, et al. Carajas and Maraba viruses, two new vesiculoviruses isolated from phlebotomine sand flies in Brazil. Am J Trop Med Hyg. *1984;33:999–1006.*

United States began in May, in horses in New Mexico, and by October had spread to 367 premises in Arizona, Colorado, New Mexico, Utah, and Wyoming. Seventy-eight percent of cases were in horses and 22% in cattle; one case was in a llama. Production losses, quarantines, and restrictions on livestock shows, auctions, and rodeos cost an estimated $50 to $100 million.[23] The 2015 outbreak of VS-NJ spread to 823 premises in eight states.[30]

Enzootic

In parts of Central and South America and in the United States on Ossabaw Island, Georgia, outbreaks of disease from enzootic VS-NJ predictably appear near the beginning of the dry season (November) and last through March. Farms located near forests and those with poultry experience higher rates of attack.[31] One year in Costa Rica, 9% to 11% of cattle on affected farms developed disease, which constituted 2.6% of cattle overall.[31,32] Lactation and a high acute VSV antibody titer increase the risk of disease for a given animal, but other diseases do not predispose to VSV disease.[31] The reservoirs and vectors for enzootic disease are not known, but phlebotomine sand flies harbor virus in enzootic areas.[6–8,33–35] They can transmit VSV to animals and transovarially to a new generation of sand flies, in which it can then replicate.[34,36,37] Mosquitoes can also harbor VSV and can transmit infection to animals in a laboratory setting.[37] In addition, VSV-infected black flies feeding on uninfected mice can horizontally infect other black flies feeding on the same mouse.[29] The high prevalence rate of VS-NJ antibodies in cows in enzootic areas of Costa Rica (82% for VS-NJ and 17.7% for VS-I)[32] indicates a high lifetime probability of infection, and many of these infections are probably subclinical. Wild animals in enzootic areas also have VS-I and VS-NJ antibodies, with VS-I mainly in arboreal and semiarboreal species and VS-NJ in bats, Carnivora, some rodents, and white-tailed deer.[38,39] Animals with high titers of neutralizing antibodies can become reinfected with other strains.[29]

Animal Disease

Vesicular stomatitis virus infection causes an acute vesicular disease in horses, cattle, swine, goats, llamas, and some wild animals.[23,40] Excess salivation, with fever and blisters or vesicles in and around the mouth, nose, hooves, or teats, appears after a 2- to 8-day incubation period. Vesicles may burst, and the epithelium may slough, leaving large contiguous areas exposed and irritated. Secondary bacterial infection that leads to mastitis may complicate the course, and lameness from foot lesions can develop. A debilitating, nonvesicular manifestation with systemic symptoms, such as fever and weight loss, sometimes occurs. Most animals recover after 2 to 3 weeks,[31,41] but viral sequences may persist.[42]

CLINICAL MANIFESTATIONS

Humans usually contract VSV during close contact with infected animals.[43,44] Most human VSV infections go unrecognized, which indicates either mild or subclinical illness; however, infection is not always benign. Of eight animal handlers who contracted VS-I during a 1965 epizootic episode in cattle, seven reported an illness that included fever, malaise, myalgias, emesis, and pharyngitis, and two had oral vesicular lesions develop in 24 to 48 hours. Although most had mild illness that quickly resolved, one otherwise healthy man had pharyngeal and buccal lesions, lymphadenopathy, and a 20-lb weight loss over 3 weeks.[41] In another case, 30 hours after self-inoculation with VS-I a laboratory worker developed fever, chills, retro-orbital pain, myalgias, nausea, emesis, and diarrhea, which resolved in 3 days.[45]

Vesicular stomatitis virus is neurotropic in baby mice,[46] and two cases of VSV meningoencephalitis have been reported in children. In one case a 3-year-old boy from Panama infected with VS-I had fever, chills, emesis, and generalized tonic-clonic seizures and remained neurologically impaired at discharge.[9]

A related vesiculovirus, Chandipura virus (CHPV), appears to infect humans more readily than VSV and has recently been implicated in outbreaks of encephalitis in parts of India. In 2003 an outbreak of encephalitis occurred in children in Andra Paresh, India, with a high fatality rate (183 of 329 cases, or 55%). CHPV was detected in 28 of 51 cases (51%) with either presence of viral RNA, immunoglobulin M antibodies, or both.[47] In 2004 an outbreak of encephalitis in children was noted in Eastern India and involved 26 cases, with a fatality rate of 78.3%. Laboratory studies in 9 of 13 patients[48] revealed CHPV RNA in serum specimens. CHPV has been isolated from sand flies,[49] which were believed to be the likely vector of spread in the first outbreak but were not detected in a small number of sand fly samples in the second outbreak. Mosquitoes have also been shown to be a vector. CHPV continues to be implicated in outbreaks of encephalitis in rural India,[50] and in 2016 an outbreak of CHPV-associated encephalitis was reported in the Bihar state and affected four children.[51–53]

In an area of Iran enzootic for the Isfahan vesiculovirus, all residents older than 5 years were seropositive in one study,[7] and in a VS-Alagoas–endemic area of Colombia, 62% to 83% of people were seropositive,[6] indicating previous infection. This relatively high rate of seropositivity, which increases with age, also occurs with other serotypes in their respective enzootic areas.[33,38,39,54]

DIAGNOSIS

VSV causes lesions that look like those of the more dangerous foot-and-mouth disease; therefore VSV outbreaks demand urgent diagnosis. Current diagnostic methods include complement fixation, serum neutralization, enzyme-linked immunosorbent assay, or viral isolation in tissue culture.[23] Recently developed assays, with reverse transcription (RT) and polymerase chain reaction (PCR), ease the collection of samples, can be used to identify viral RNA from lesions previously treated with toxic substances, and are being adapted for general use.[55]

Diagnosis of CHPV infection can be made by detection of virus through highly sensitive and specific RT-PCR. CHPV may also be detected in a variety of sand fly and mosquito tissue culture cell lines, and in vertebrate cell lines.[52]

HOST RESPONSE

Studies in mice showed that the presence of B cells and antibody responses was associated with recovery and the development of resistance to VSV; however, $CD4^+$ T cells also contribute to long-term survival, and secretion of interleukin-12 may be beneficial.[56–58] Antibody-mediated neutralization blocks virus-to-cell binding and requires 200 to 500 VSV-G protein–specific immunoglobulin G molecules per virus particle.[2,4] Neutralizing antibodies bind to the same G-protein epitope and protect against infection with the same strain.[5,59] However, in endemic areas, up to 10% of cattle with high titers of neutralizing antibodies become infected each year with clinical symptoms, presumably from mutant strains.[31]

THERAPY

Human infections with VSV are usually mild and generally do not necessitate treatment. No specific treatments exist, and antiviral agents have not been evaluated in vivo. Interferon (IFN)-α, IFN-β, and IFN-γ inhibit VSV growth in vitro[60–62] and protect newborn mice from lethal VSV infection.[63] Prostaglandins A_1 and A_2,[64,65] ribavirin,[66,67] and some experimental compounds[68–70] inhibit VSV in vitro. Doxycycline has been recently reported to inhibit VSV replication in vitro.[71] In animals

secondary bacterial infections of the mouth, teats, and hooves should be treated appropriately, and a mild antiseptic mouthwash may relieve pain from blisters.[72] Nutritional support may help animals that stop eating.

PREVENTION AND VACCINATION

Experimental vaccination with a recombinant vaccinia vector that expresses the VSV-G protein stimulated neutralizing antibody production and protected mice against lethal VSV disease after intravenous challenge. In cattle protection was incomplete, but it correlated with high antibody titer.[73] There are no licensed VSV vaccines for livestock available in the United States. Previously, vaccines used on a restricted basis during outbreaks showed immunogenicity but not long-term effectiveness.

Vaccines against CHPV are also under development.[52]

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The complete reference list is available online at Expert Consult.

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163 Rabies (Rhabdoviruses)

Brett Williams, Charles E. Rupprecht, and Thomas P. Bleck

SHORT VIEW SUMMARY

Definition
- Rabies is a zoonotic encephalitis caused by different species of neurotropic viruses in the *Lyssavirus* genus, Rhabdoviridae family.

Epidemiology
- Rabies is one of the oldest human diseases, with the highest case-fatality rate.
- Approximately 3.3 billion people live in regions where rabies is enzootic.
- An estimated 25,000 to 159,000 people die from rabies each year.
- Although all age groups are susceptible, rabies is most common in children younger than 15 years.
- Bites by rabid dogs cause 99% of human deaths globally.
- In the United States bats account for most human rabies cases.
- Rabies virus transmission may also occur through tissue or organ transplantation.

Microbiology
- Lyssaviruses are bullet-shaped, single-stranded RNA viruses of the order Mononegavirales, family Rhabdoviridae, and genus *Lyssavirus.*
- Rabies virus is the type species of the *Lyssavirus* genus.

Diagnosis
- Development of an acute neurologic syndrome after the bite of a rabid animal is recognized most often in developing countries.
- Encephalitic rabies occurs in the majority of patients, with fever, hydrophobia, aerophobia, agitation, autonomic overactivity with hypersalivation, coma, and paralysis.
- Paralytic rabies is characterized by ascending flaccid quadriparesis and coma.
- Antemortem diagnosis can be made by direct immunofluorescent staining of skin biopsy specimens, reverse-transcriptase polymerase chain reaction of saliva or skin biopsy specimens, or detection of antivirus antibodies in serum or cerebrospinal fluid.

Therapy
- There is no proven antiviral therapy.
- Most patient with rabies virus infection die within a few days to 2 weeks after the onset of illness.

Prevention
- Annually, more than 12 million people receive rabies postexposure prophylaxis.
- After exposure to a rabid animal, prevention consists primarily of prompt wound cleansing, infiltration of rabies immune globulin, and rabies vaccine according to the Advisory Committee on Immunization Practices and World Health Organization guidelines.
- Bat rabies causes the majority of human cases in the United States, and postexposure prophylaxis depends on the type of exposure and availability of the animal for rabies diagnosis.
- Preexposure prophylaxis should be targeted to high-risk groups, including veterinarians, laboratory workers, and certain travelers and children living in remote vampire bat–enzootic areas.

Rabies is a viral disease that produces an almost uniformly fatal encephalitis in humans and other mammals. This zoonosis has been present throughout recorded history and classical literature and likely predates the evolution of humans. Rabies remains one of the most common viral causes of mortality in the developing world. Exposure to the virus has profound medical and economic implications throughout the world, with as many as 12 million people annually receiving postexposure prophylaxis (PEP) to prevent rabies.[1] Although current technology can produce safe biologics for prevention, deviations from recommended regimens can lead to prophylaxis failure and fatal illness.

Rabies, Latin for "madness," derives from *rabere*, to rave, and is related to the Sanskrit word for violence, *rabhas.* The Greek term for rabies, *lyssa*, also means madness, and provides the genus name *(Lyssavirus)*. The Babylon Eshnuna code contains one of the first suggested mentions of rabies in the 23rd century BCE.[2] Democritus provided a clear description of animal rabies in about 500 BCE. Wound cauterization was the preferred treatment in the CE 1st century and was recommended for the management of rabid animal bites until the late 19th century.[2] This treatment remained the only real therapy until Pasteur introduced a vaccine based on an attenuated rabies virus in 1885.

Rabies in the Western Hemisphere likely predated Columbus but remained rare because of low canine population density.[3] Bats spread the disease among livestock and humans in the Americas during the early 16th century.[4] Rabies epizootics began in the northern and eastern United States in the 18th century, reflecting in part the introduction of rabid dogs and importation of foxes for hunting.[5] Globally, bites by rabid domestic dogs cause 99% of human deaths.[1]

Rabies was not diagnosed clinically until 1903, when Adelchi Negri described the cytoplasmic inclusions that bear his name.[6] These inclusions were the only pathologic marker before the development of the fluorescent antibody test in 1958.[7]

VIROLOGY

Classification

The Rhabdoviridae are rod-shaped, negative-sense, nonsegmented, single-stranded RNA viruses. Four major genera infect animals *(Lyssavirus, Vesiculovirus, Ephemerovirus, Novirhabdovirus)*. The genus *Lyssavirus* comprises multiple serotypes; rabies (serotype 1) is the type species of the genus,[5] vesicular stomatitis virus that of the *Vesiculovirus* genus, bovine ephemeral fever that of the *Ephemerovirus* genus, and infectious hematopoietic necrosis virus of the *Novirhabdovirus* genus. Rabies is enzootic, and sometimes epizootic, in a variety of mammals. Australian bat lyssavirus (ABLV) is genetically distinct from rabies virus but causes encephalitis in Australian flying foxes and insectivorous bats. Three human deaths in Australia have been reported due to encephalitis from ABLV infection acquired from animals.[8] The other members of the genus rarely cause human disease (Table 163.1). Current rabies biologics have excellent cross reactivity with members in phylogroup 1 but less so with other more disparate members of the genus. New putative *Lyssavirus* spp. are still emerging in bats around the world.[9,10]

Vesiculoviruses share many of the virologic characteristics of the lyssaviruses. They infect a large number of animal and insect species; humans are occasionally infected via contact with animals, typically via respiratory secretions.[11] Seven vesiculoviruses are known to

TABLE 163.1 Members of the *Lyssavirus* Genus

VIRUS	PHYLOGROUP	RESERVOIRS
Rabies	1	Bats and carnivores, found worldwide except for Antarctica, New Zealand, Sweden, Norway, Spain, Taiwan, Japan, and some other islands
Lagos bat	2	Probably enzootic in fruit bats; no reported human cases
Mokola	2	Probably an insectivore or rodent species limited to parts of Africa; a few domestic animal and two human cases reported
Duvenhage	1	Insectivorous bats; cases identified in South Africa, Zimbabwe, and Senegal
European bat lyssavirus 1 (EBLV1)	1	European insectivorous bats *(Eptesicus serotinus)*
European bat lyssavirus 2 (EBLV2)	1	European insectivorous bats (e.g., *Myotis dasycneme, Myotis daubentonii*)
Australian bat lyssavirus	1	Flying foxes (e.g., *Pteropus* spp.) and insectivorous bats (e.g., *Saccolaimus flaviventris*); three human deaths reported
Irkut	1	Siberian insectivorous bats (e.g., *Murina leurogaster*)
Aravan	1	Central Asian insectivorous bats (e.g., *Myotis blaythi*)
Khujand	1	Central Asian insectivorous bats (e.g., *Myotis mystacinus*)
West Caucasian Bat	3	Insectivorous bats (e.g., *Miniopterus schreibersi*), Caucasian Region
Shimoni	2	Bats (e.g., *Hipposideros commersoni*), Kenya
Ikoma	2	Thought to be African bats
Gannoruwa	1	Fruit bats (e.g., *Pteropus* spp.) in Sri Lanka
Lleida	3	Common bent-winged bat (e.g., *Miniopterus* spp.) in Spain

occasionally infect humans.[12] Another negative-sense single-stranded RNA virus, Borna disease virus, produces central nervous system (CNS) infection in birds and primates, and some authors have suggested that it is related to human psychiatric disorders.[13] However, in contrast to the rhabdoviruses, Borna disease virus replicates in the nucleus rather than in the cytoplasm. It is not a rhabdovirus and is classified within its own family.

EPIDEMIOLOGY

Human Rabies

Rabies is distributed worldwide except for Antarctica, New Zealand, Sweden, Norway, Japan, and some other islands. In 2007, the last year for which global data are available, at least 103 nations reported the presence of rabies, and 42 reported its absence.[14] In the United States an average of three human cases were reported annually during the past decade (Fig. 163.1).

World Health Organization (WHO) estimates that approximately 59,000 humans die of rabies annually. These deaths probably represent an underestimate of the worldwide incidence of the disease, which may cause as many as 159,000 deaths annually.[15,16] A disproportionate number of those affected are children younger than 15 years.[17] An estimated 12 million persons receive PEP annually, with some people still administered unsafe vaccines that carry a risk of neurologic complications.[1,18]

The epidemiology of human rabies reflects that of local animal rabies.[19] In developing areas where canine rabies remains common, most human cases result from dog bites. In regions where dogs are immunized, most human cases follow exposure to rabid wildlife.[20] In the United States 67 of the 22,478 (0.3%) dogs and 244 of 23,101 (1.1%) cats tested in 2015 were positive for rabies.[21] During 2015 (Table 163.2) such national rabies surveillance identified the four major animal reservoirs as bats, raccoons, skunks, and foxes, which accounted for 91% of the 5508 total cases of animal rabies reported.[21] Small rodents, such as chipmunks, squirrels, gerbils, rabbits, rats, and mice, are not a risk group for viral transmission.[20] A leading source associated with cases in the United States is the silver-haired bat *(Lasionycteris noctivagans)*. However, imported cases of canine rabies continue to be reported, and in 2013 a case of human rabies occurred in a Guatemalan immigrant detained at the Texas border, which was confirmed as a canine rabies virus variant.[22] The patient had a dog in Guatemala that died of unknown causes in 2011, but there were no reported bites.

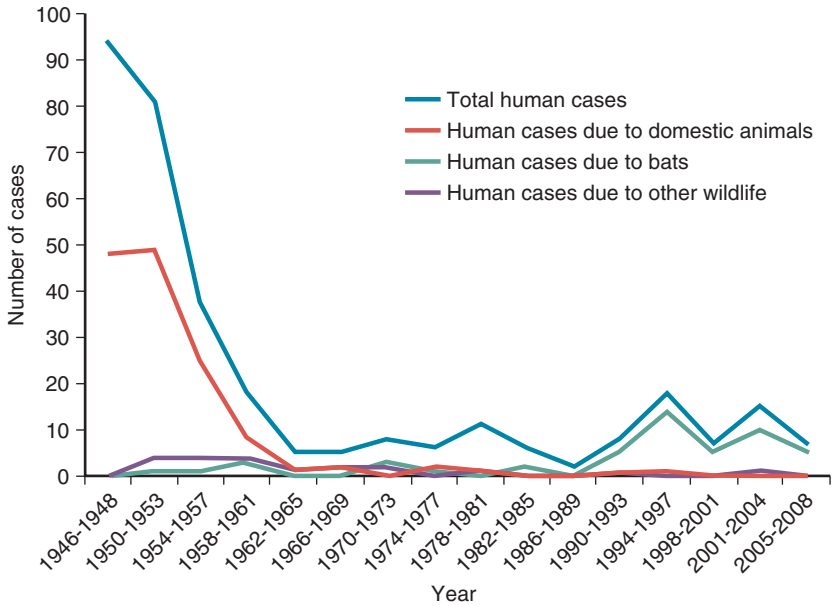

FIG. 163.1 Reported cases of human rabies in the United States by year, 1945–2008. *(From McNabb SJN, Jajosky RA, Hall-Baker PA, et al. Summary of notifiable diseases: United States, 2006.* MMWR Morb Mortal Wkly Rep. *2008;55:1–94.)*

TABLE 163.2 Cases of Rabies in Humans in the United States and Puerto Rico, January 2003–October 2018, by Circumstances of Exposure and Rabies Virus Variant

DATE OF ONSET	DATE OF DEATH	REPORTING STATE	AGE (YR)	SEX	EXPOSURE[A]	RABIES VIRUS VARIANT[B]
Feb. 10, 2003	Mar. 10, 2003	VA	25	M	Unknown	Raccoon, eastern United States
May 28, 2003	Jun. 5, 2003	PR	64	M	Bite, Puerto Rico	Dog-mongoose, Puerto Rico
Aug. 23, 2003	Sept. 14, 2003	CA	66	M	Bite	Bat, Ln
Feb. 9, 2004	Feb. 15, 2004	FL	41	M	Bite, Haiti	Dog, Haiti
Apr. 27, 2004	May 3, 2004	AR	20	M	Bite (organ donor)	Bat, Tb
May 25, 2004	May 31, 2004	OK	53	M	Liver transplant	Bat, Tb
May 27, 2004	Jun. 21, 2004	TX	18	M	Kidney transplant	Bat, Tb
May 29, 2004	Jun. 9, 2004	TX	50	F	Kidney transplant	Bat, Tb
Jun. 2, 2004	Jun. 10, 2004	TX	55	F	Arterial transplant	Bat, Tb
Oct. 12, 2004	Survived	WI	15	F	Bite	Bat, unknown
Oct. 19, 2004	Oct. 26, 2004	CA	22	M	Unknown, El Salvador	Dog, El Salvador
Sept. 27, 2005	Sept. 27, 2005	MS	10	M	Contact	Bat, unknown
May 4, 2006	May 12, 2006	TX	16	M	Contact	Bat, Tb
Sep. 30, 2006	Nov. 2, 2006	IN	10	F	Bite	Bat, Ln
Nov. 15, 2006	Dec. 14, 2006	CA	11	M	Bite, Philippines	Dog, Philippines
Sept. 19, 2007	Oct. 20, 2007	MN	46	M	Bite	Bat, unknown
Mar. 16, 2008	Mar. 18, 2008	CA	16	M	Bite, Mexico	Fox, Tb related
Nov. 19, 2008	Nov. 30, 2008	MO	55	M	Bite	Bat, Ln
Feb. 25, 2009	Survived	TX	17	F	Contact	Bat, unknown
Oct. 5, 2009	Oct. 20, 2009	IN	43	M	Unknown	Bat, Ps
Oct. 20, 2009	Nov. 11, 2009	MI	55	M	Contact	Bat, Ln
Oct. 23, 2009	Nov. 20, 2009	VA	42	M	Contact, India	Dog, India
Aug. 2, 2010	Aug. 21, 2010	LA	19	M	Bite, Mexico	Bat, Dr
Dec. 24, 2010	Jan. 10, 2011	WI	70	M	Unknown	Bat, Ps
Apr. 30, 2011	Survived	CA	8	F	Unknown	Unknown
Jun. 30, 2011	Jul. 20, 2011	NJ	73	F	Bite, Haiti	Dog, Haiti
Aug. 14, 2011	Aug. 31, 2011	NY	25	M	Contact, Afghanistan	Dog, Afghanistan
Aug. 21, 2011	Sept. 1, 2011	NC	20	M	Unknown (organ donor)[C]	Raccoon, eastern United States
Sep. 1, 2011	Oct. 14, 2011	MA	40	M	Contact, Brazil	Dog, Brazil
Dec. 3, 2011	Dec. 19, 2011	SC	46	F	Unknown	Bat, Tb
Dec. 22, 2011	Jan. 23, 2012	MA	63	M	Contact	Bat, My sp.
Jul. 6, 2012	Jul. 31, 2012	CA	34	M	Bite	Bat, Tb
Jan. 31, 2013	Feb. 27, 2013	MD	49	M	Kidney transplant	Raccoon, eastern United States
May 16, 2013	Jun. 11, 2013	TX	28	M	Unknown, Guatemala	Dog, Guatemala
Sept. 12, 2014	Sept. 26, 2014	MO	52	M	Unknown	Bat, Ps
Jul. 30, 2015	Aug. 24, 2015	MA	65	M	Bite, Philippines	Dog, Philippines
Sept. 17, 2015	Oct. 3, 2015	WY	77	F	Contact	Bat, Ln
Nov. 25, 2015	Dec. 1, 2015	PR	54	M	Bite	Dog-mongoose, Caribbean
May 5, 2017	May 21, 2017	VA	65	F	Bite	Dog, India
Oct. 6, 2017	Oct. 21, 2017	FL	56	F	Bite	Bat, Tb
Dec. 28, 2017	Jan. 14, 2018	FL	6	M	Bite	Bat, Tb
Jul. 15, 2018	Aug. 23, 2018	DE	69	F	Unknown	Raccoon, eastern United States

[A]Data for exposure history are reported when plausible information was reported directly by the patient (if lucid or credible) or when a reliable account of an incident consistent with rabies virus exposure (e.g., dog bite) was reported by an independent witness (usually a family member). Exposure histories are categorized as bite, contact (e.g., waking to find bat on exposed skin) but no known bite was acknowledged, or unknown (i.e., no known contact with an animal was elicited during case investigation).

[B]Variants of the rabies virus associated with terrestrial animals in the United States and Puerto Rico are identified with the names of the reservoir animal (e.g., dog or raccoon), followed by the name of the most definitive geographic entity (usually the country) from which the variant has been identified. Variants of the rabies virus associated with bats are identified with the names of the species of bats in which they have been found to be circulating. Because information regarding the location of the exposure and the identity of the exposing animal is almost always retrospective, and much information is frequently unavailable, the location of the exposure and the identity of the animal responsible for the infection are often limited to deduction.

[C]Infection was not identified until 2013, when an organ recipient developed rabies.

Dr, Desmodus rotundus; *Ln,* Lasionycteris noctivagans; *My sp., Myotis* species; *Ps,* Perimyotis subflavus; *Tb,* Tadarida brasiliensis.

From Ma X, Monroe BP, Cleaton JM, et al. Rabies surveillance in the United States during 2017. J Am Vet Med Assoc. *2018;253:1555–1568.*

Transplant-Associated Rabies

In 2004 rabies developed in four recipients of kidneys, an arterial segment, and the liver from a man in Texas who died of encephalitis of unknown cause.[23] Immunohistochemical and direct fluorescent antibody (DFA) staining demonstrated rabies virus in multiple tissues from all recipients, and Negri bodies were also seen in brain tissue of all recipients. Investigations conducted after the rabies diagnosis revealed that the infected donor had reported being bitten by a bat. A series of transplant-associated rabies cases in Germany was reported from an infected donor.[24] All six transplant patients received PEP with rabies immune globulin (RIG) and vaccine. There were three deaths among recipients of lung, kidney, and pancreas transplants. Two of the survivors had received corneal transplants, and one liver transplant recipient had received rabies immunization as a child and had detectable rabies virus antibodies on a pretransplantation blood sample. Transmission of rabies virus had been previously documented in corneal transplantation recipients.[25,26] One recipient received standard PEP plus interferon and did not develop rabies.[26] During 2013 four patients in the United States received organ or tissue donation from a man who had been infected with a raccoon rabies virus variant. One of the recipients subsequently died of rabies. The other recipients, all of whom received PEP, have not developed symptoms of rabies.[27] Two kidney transplant recipients in China died of rabies after receiving allografts from a boy who died of encephalitis in 2015; two recipients of corneal transplants from the boy remain well after receiving PEP.[28] The virus in the Chinese cases was found to be a recently described viral variant (Chinese lineage II).[29] These cases, in addition to transplant-associated West Nile encephalitis and lymphocytic choriomeningitis, have led guidelines to recommend rejection of potential organ donors with encephalitis of unknown cause.[30,31]

Animal Rabies

In the developing world rabies is predominantly a problem of domestic animals. As all developed nations have eliminated rabies from dogs, wildlife is the major affected group. Wildlife accounted for approximately 92% of the reported cases of rabies in the United States in 2015.[21] The prevalence of wildlife rabies waxes and wanes dependent upon surveillance bias and has been decreasing in most animal species except in bat populations (Fig. 163.2).[21] Bats are increasingly the source of human rabies in the United States.[32] The epizoology of bat rabies is changing from typical reservoirs (e.g., the common big brown bat) to include previously rarely affected species (e.g., the silver-haired bat).[33] Most of the human rabies cases known to have been contracted from bats in the United States since 1980 involve the silver-haired/eastern tricolored bat rabies virus variants.[34] Most cases of rabid skunks during 2015 were reported in south-central and eastern states.[21] An outbreak of raccoon rabies in the United States began in 1977 near the Virginia–West Virginia border, and in the ensuing 2 decades the territory expanded to involve most of the eastern states. More than 20,000 cases of raccoon rabies have been reported to date, with several thousand secondary cases in dogs and other animals.[35] Raccoon rabies remains enzootic in 19 states and the District of Columbia, but in 2015 cases were declining.[21]

Rabies reports had also been increasing among bats, but appears to have plateaued.[21,36] Although there was concern that in some wildlife species (e.g., spotted hyenas) rabies virus infection may not lead to clinically apparent disease, such speculation has not been supported.[37] In at least one bat species, *(Eptesicus serotinus)*, salivary samples may contain viral RNA without detectable RNA in concurrent brain samples, but the relevance of amplicon detection in lieu of viral isolation remains questionable.[38] Barring alternative explanations, the potential implications of this finding for routine screening of wildlife after potentially infectious human contact remain to be determined.

Previously unidentified viral variants from potential cross-species transmission are still emerging around the world.[39,40] Molecular epidemiologic evidence reinforces the importance of avoiding contact with downed bats or other ill wildlife.[41,42]

Composition

Lyssaviruses are bullet-shaped, with an average length of 180 nm and an average diameter of 75 nm.[43] The complete virion includes a helical nucleocapsid with 30 and 35 coils between 4.2 and 4.6 nm in length.[44] This is enclosed in a lipoprotein envelope 7.5 to 10 nm thick, from which glycoprotein (G protein) spikes project 10 nm.[45] These spikes cover the surface of the virus except at the blunt end (Fig. 163.3).

The *Lyssavirus* genome encodes five genes: *N, P (NS), M* (or M_2), *G,* and *L* (Table 163.3).[46] Phosphorylation of the nucleoprotein is necessary for efficient replication of the viral RNA,[47,48] and the nucleoprotein is potentially immunogenic.[49] It is needed to switch from the transcription of gene products to the production of full-length positive-strand RNA.[50] Use of monoclonal antibodies directed against the N protein is used routinely for antigenic typing of virus variants. Molecular sequence analysis of the *N* and, increasingly, *G, P,* and *L* genes, adds resolution and identifies virus lineages among virus variants. The P protein may control the L protein, an RNA-dependent RNA polymerase.[51,52] The M, or matrix, protein is located between the nucleocapsid and the lipoprotein envelope,[53] and, in concert with the G protein, is responsible for the

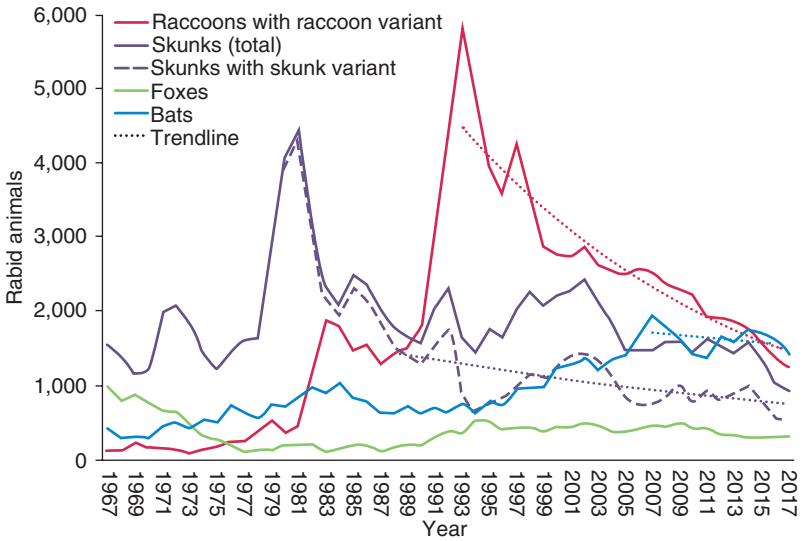

FIG. 163.2 Cases of rabies among wildlife in the United States by year and species, 1967–2017. *(From Ma X, Monroe BP, Cleaton JM, et al. Rabies surveillance in the United States during 2017.* J Am Vet Med Assoc. *2018;253:1555–1568.)*

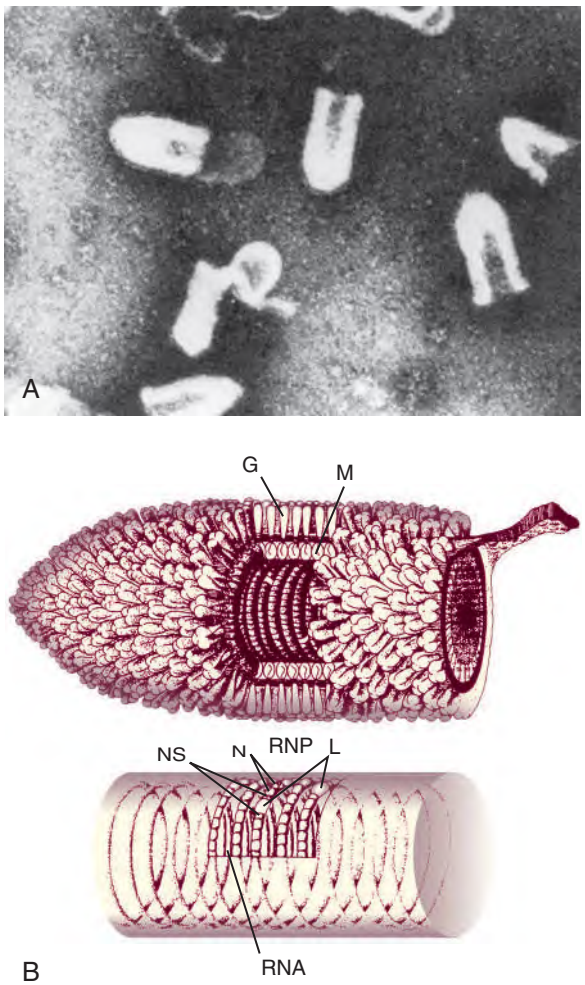

FIG. 163.3 Rabies virus morphology. (A) Electron micrograph of rabies virus. Original magnification, ×55,000. (B) Schematic illustration of rabies virus *(top)*, showing surface glycoprotein *(G)* projections extending from lipid envelope surrounding ribonucleoprotein *(RNP)* and matrix protein *(M)* lining envelope. Helical RNP *(bottom)* comprises single-stranded *RNA* genome, plus nucleoprotein *(N),* phosphoprotein *(NS),* and transcriptase *(L). (From Dietzschold B, Rupprecht CE, Fu ZF, Koprowski H. Rhabdoviruses. In: Fields BN, Knipe DM, Howley PM, et al, eds.* Fields Virology. *3rd ed. Philadelphia: Lippincott Raven; 1996:1137–1159.)*

TABLE 163.3 Rhabdovirus Genes and Products

GENE	SIZE (kDa)	FUNCTION
N (nucleoprotein)	50	Encapsidates the viral RNA and major constituent of the nucleocapsid
P (phosphoprotein)	40	Originally thought to encode a nonstructural (NS) protein but now known to produce a structural protein that is phosphorylated by kinases in the host cell and that joins with L
M (matrix)	26	Responsible for the assembly and budding of bullet-shaped particles, in concert with the G protein
G (glycoprotein)	65	Attachment to host cell receptors
L (large polymerase)	160–190	RNA-dependent RNA polymerase; required for transcription of the negatively stranded viral RNA; appears to form a complex with NS

Modified from Rupprecht CE, Smith JS, Fekadu M, Childs JE. The ascension of wildlife rabies: a cause for public health concern or intervention? Emerg Infect Dis. *1995;1:107–114.*

assembly and budding of bullet-shaped virions.[54] The N and M proteins determine a balance between transcription and replication.[55,56]

The G protein is involved in cellular reception and is the antigen that induces virus-neutralizing antibodies. Variability in this protein is responsible for serotypic differences among lyssaviruses,[57] and mutations at one position, 333 (with substitution of glutamine or isoleucine for arginine), can disrupt virulence.[58] This arginine residue appears to be essential for fusion of the viral envelope with neurons.[59] Molecular modifications of the G protein can increase its antigenicity.[60]

PATHOGENESIS

Natural rabies infection appears to require a period of local viral replication, perhaps to increase the inoculum, before nervous system infection occurs. Timely administration of RIG and active immunization can prevent spread of the virus into the nervous system, thereby preventing disease. The virus does not tolerate a pH below 3 or above 11 and is inactivated by ultraviolet light, sunlight, desiccation, and various chemicals, such as formalin, phenol, ether, trypsin, β-propiolactone, and many detergents.

Once the virus has entered peripheral nerves, current therapeutic techniques probably do not readily prevent subsequent replication and spread, and the virus quickly moves centrally. After the virus enters through a break in the skin, across a mucosal surface, or through the respiratory tract, it may replicate in muscle cells and, in so doing, infects the muscle spindle. In muscle the virus binds to the nicotinic acetylcholine receptor.[61] Some studies suggest that the neuromuscular junction is a major site of neuronal invasion,[62] and blocking acetylcholine receptors inhibits viral attachment.[63] Partial sequence homology exists between rabies virus G protein and several snake neurotoxins that bind to this receptor.[64] However, rabies virus can enter neurons that do not express acetylcholine receptors,[65] probably by G protein binding to nerve growth factor P75NTR or neural cell adhesion molecule (CD56).[66,67] Once bound to the receptor, virus is internalized with receptor-mediated endocytosis via a clathrin coated pit.[68] Rabies virus, after internalization into endosomes, ascends via endosomic trafficking along microtubules.[68] Endosomes acidify upon arrival at the soma, releasing viral particles into the cytoplasm.[68] Herpes simplex virus and tetanus toxin also make use of the microtubular transport systems.[69] Replication occurs in neurons but not usually in glia, either peripheral or central. The viral envelope forms from host membranes into which the G and M proteins are inserted.[70] The envelope includes small amounts of host proteins.[52]

Virus may be present in dorsal root ganglia within 60 to 72 hours of inoculation and before its arrival in spinal cord neurons, which confirms its transport within sensory neurons. After reaching the spinal cord the virus spreads throughout the CNS, following established patterns of synaptic connectivity.[71] Virtually every neuron is infected.[72]

After CNS infection, virus spreads to the rest of the body via peripheral nerves.[73] The high concentration of virus in saliva results from viral shedding from sensory nerve endings in the oral mucosa[70] and also reflects substantial replication in the salivary glands.

The mechanisms by which rabies virus damages the CNS are obscure because pathologic evidence of neuronal necrosis is frequently minimal or absent.[74] Animal models suggest that rabies virus may interfere with neurotransmission[75] and with endogenous opioid systems,[76] and the almost 30-fold increase in local nitric oxide production[77] suggests an excitotoxic mechanism. Analysis of CSF metabolites from rabies patients, including two survivors, suggests that production of quinolinate, an *N*-methyl-D-aspartate receptor agonist, is overproduced, potentially leading to excitotoxicity.[78] Cerebrospinal fluid (CSF) quinolinate levels fell with time in survivors but rose with time in those who died.[78] Rabies virus P protein also appears to increase production of reactive oxygen species by interaction with mitochondrial complex I.[79] This oxidative stress and resultant excitotoxicity leads to axonal swellings, degeneration, and impaired axonal growth but few morphologic changes or neuronal death.[80] Rabies virus antigen concentrates in the red nucleus and midbrain raphe, areas where lesions are known to cause aggression; this, along with interference with endogenous opioid receptors, may be the basis for the characteristic behavioral changes.[76,81,82]

An inverse relationship is found between the concentration of G protein produced and the pathogenicity of different viral variants, and

a monotonic relationship is found between pathogenicity and the induction of neuronal apoptosis.[83]

Pathology

The brain in encephalitic rabies (see subsequent discussion) usually appears unremarkable grossly,[84] except for the vascular congestion. The microscopic pathology of rabies is typically encephalitis with Negri bodies (Fig. 163.4). However, not all autopsy specimens show the perivascular lymphocytic cuffing and necrosis that characterize encephalitis, and some cases look histologically like meningitis.[85] Negri bodies are concentrated in hippocampal pyramidal cells and less frequently in cortical neurons and cerebellar Purkinje cells.[84] They are round or oval, usually eosinophilic, cytoplasmic inclusions between 1 and 7 µm across, and they contain viral nucleocapsids.[86] The acidophilic lyssa body is ultrastructurally identical to the Negri body.[87] Negri bodies and lyssa bodies are detected in only a relatively small percentage of the cells that are infected (as determined with immunohistochemistry).[88]

Paralytic rabies affects primarily the spinal cord, with severe inflammation and necrosis.[89] The brainstem is involved to a lesser extent. A few patients have cortical Negri bodies. Segmental demyelination occurs in the peripheral nerves and resembles acute inflammatory polyneuropathy (Guillain-Barré syndrome).

Systemic pathology is most remarkable for the presence of myocarditis.[90] This cardiac disorder resembles the myocarditis that occurs in hypercatecholaminergic states, such as pheochromocytoma, subarachnoid hemorrhage, and tetanus.[69] Negri bodies are found in the hearts of some patients, which suggests a viral role in this condition.[91] Atrial ganglioneuritis suggests that the virus reaches the myocardium via spread from the nervous system.[92]

Immune Responses

The immune response to natural rabies virus infection is generally insufficient to prevent disease. Rabies can produce immunosuppression,[93] potentially by replicating in and inducing apoptosis in T lymphocytes.[94,95] Unvaccinated patients who develop a measurable antibody response develop it late in the course.[96] Rabies virus may evade immune responses by using cell-to-cell transfection, disseminating through dendritic cells carrying infectious viral nucleic acid.[97] Persistence in macrophages may explain some cases of very long incubation periods.[98] The rabies *P* gene appears to prevent cellular immune response to infected muscle cells by suppressing expression of the interferon-β gene.[99] Patients with development of a cellular immune response tend to have the encephalitic form rather than the paralytic form, and they die faster than those who do not have such a response.[100] Differences in the host immune response appear more likely to explain whether encephalitic or paralytic rabies develops than do differences in the strains of virus that cause the natural infection.[101] Some investigators believe that interleukin-1 production in the CNS may explain the immunosuppressive effect of the virus.[102]

CLINICAL MANIFESTATIONS

Human Rabies

Several variables affect the risk of rabies and the rate of clinical disease development after exposure to a rabid animal.[103] The viral inoculum is important, reflected by the relationship between the extent of exposure to the saliva and the rapidity of progression. A bite with prominent salivary contamination (e.g., through exposed skin) is more likely to produce rabies than a bite through thick clothing, which removes saliva from the animal's teeth. Multiple bites are more likely to transmit the disease than a single bite. The location of the bite also influences the risk of rabies: Bites on the face are more likely to result in disease than those on the extremities. Salivary contamination of a preexisting wound can transfer virus, as can exposure of mucous membranes or the respiratory tract to aerosolized virus.[104]

Incubation Period

The reported incubation period for rabies varies from a few days to more than 19 years, although 75% of patients become ill in the first 90 days after exposure. Moreover, very long incubation periods in hyperendemic regions are quite difficult to document when one considers more likely, subtle routes, such as unrecognized nonbite exposures to puppies or kittens that may have occurred more recently. The solid-organ transplant patients described previously generally became ill within 45 days of transplantation, although one patient became ill 18 months after kidney transplantation.[27] The shorter period of incubation in some patients is theorized to be related to their immunosuppressed state.[23]

Prodromal Symptoms

The initial symptoms of rabies resemble those of other systemic viral infections, including fever, headache, malaise, and disorders of the upper respiratory and gastrointestinal (GI) tracts (Table 163.4).[105] Initial neurologic symptoms may include subtle changes in personality and cognition and paresthesias or pain near the exposure site. Rabies is rarely considered early in the differential diagnosis. In one series physicians considered rabies in only 3 of 21 patients on the first visit, despite an exposure history in many.[105] The prodrome typically lasts about 4 days, but up to 10 days may elapse before more specific symptoms and

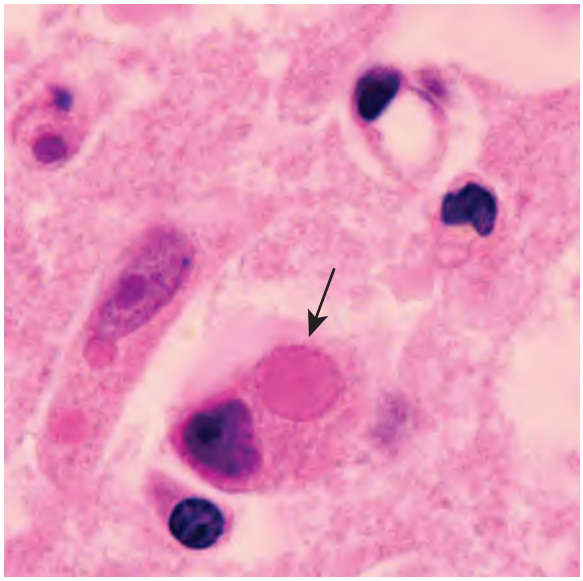

FIG. 163.4 Negri body *(arrow).* Original magnification, ×400. *(Courtesy Maria-Beatriz Lopes, MD, Division of Neuropathology, University of Virginia.)*

TABLE 163.4 Durations of Different Stages of Rabies

Stage	DURATION (% OF CASES)	ASSOCIATED FINDINGS
Incubation period	<30 days (25%) 30–90 days (50%) 90 days to 1 year (20%) >1 year (5%)	None
Prodrome and early symptoms	2–10 days	Paresthesias or pain at the wound site; fever; malaise; anorexia; nausea and vomiting
Acute neurologic disease; furious rabies (80% of cases)	2–7 days	Hallucinations; bizarre behavior; anxiety; agitation; biting; hydrophobia; autonomic dysfunction; syndrome of inappropriate antidiuretic hormone (SIADH)
Paralytic rabies (20% of cases)	2–7 days	Ascending flaccid paralysis
Coma, death[a]	0–14 days	—

[a]Rare recoveries have been reported.

Data modified from Fishbein DB. Rabies in humans. In: Baer GM, ed. The Natural History of Rabies. *2nd ed. Boca Raton, FL: CRC Press; 1991:519–549.*

signs supervene.[105] Myoedema (mounding of part of the muscle struck with a reflex hammer, which then disappears in a few seconds) is present during the prodrome and persists throughout the disease.[106]

Human rabies virus infections are divided into two forms: encephalitic ("furious") and paralytic ("dumb"). The encephalitic form presents with the hydrophobia, delirium, and agitation that form the common picture of rabies. About a fifth of patients present with the paralytic form and have little clinical evidence of cerebral involvement until late in the course. The spinal cord and brainstem bear the brunt of the illness in the paralytic form. The pathogenetic distinction between the two types of rabies is unclear; it does not appear to be based on virologic or antigenic differences.[107] In either form the symptomatic course usually runs 2 to 14 days before coma supervenes. In one series of 32 patients the median duration of illness was 19 days.[108] On occasion, atypical features have been described that do not fit into these classic forms of rabies.[109] Atypical cases can present with sensory or motor deficits, choreiform movements of the bitten limb during the prodromal phase, focal brainstem signs, cranial nerve palsies, myoclonus, and seizures.[109,110]

Not all infections may lead to death or even clinical illness as suggested by a study of Peruvians in the Amazon with a high incidence of bat bites[111]; 6 of 63 residents without history of rabies vaccination were seropositive for rabies virus–neutralizing antibodies, suggesting prior subclinical infection.

Encephalitic (Furious) Rabies

Hydrophobia is the symptom most identified with *encephalitic rabies*. Sir William Gowers[112] provides a seminal depiction of hydrophobia and its sequelae, in which he described:

> "…some discomfort about the throat, an occasional sense of choking, or a little difficulty in swallowing liquids… The attempt to drink occasions some spasm in the pharynx, which increases in the course of a few hours, and spreads to the muscles of respiration, causing a short, quick inspiration, a 'catch in the breath…' This increases in severity to a strong inspiratory effort, in which the extraordinary muscles of respiration, sterno-mastoid, scaleni, etc., and even the facial muscles, take part; the shoulders are raised, and the angles of the mouth drawn outwards. As the intensity of the spasm increases, so does the readiness with which it is excited. It may be caused by the mere contact of water with the lips, and a state of cutaneous hyperæsthesia develops, so that various impressions, such as a draught of air, which normally excite a respiratory effort, bring on the spasm. The mere movement of air caused by raising the bedclothes may be sufficient. The patient is often unable to swallow the saliva, which is usually abundant and viscid, so that it hangs about the mouth and is expelled with difficulty… Vomiting is common… The attacks of spasm are very distressing to the patient; the mental state which they occasion increases the readiness with which they are produced; and in some cases the mere sight of water or the sound of dropping water will cause an attack. It may even be excited by visual impressions which cause a similar sensation, as the reflection from a looking glass, or even a strong light. The sufferer's horror and dread of these excitants becomes intense. Thus the disturbance in the act of swallowing liquids, which constitutes… the first symptom and keynote of the disease, spreads, on the one hand, to mental disturbance, and on the other to extensive muscular spasm. In each of these directions further symptoms develop. The spasm, at first confined to the muscles of deglutition and respiration, spreads to the other muscles of the body, and the paroxysms, at first respiratory, afterwards become general, and assume a convulsive character, although still excited by the same causes. The convulsions may consist of general muscular rigidity, sometimes tetanoid in character, with actual opisthotonus… Actual delusions occasionally supervene, and there may even be wild delirium. The mental derangement is most intense during the paroxysms of spasms, and the frenzied patient may spit his saliva at those about him, and often attempts to bite them with his teeth, making occasional strange sounds in his throat which have been thought to resemble the barking of a dog."

Hydrophobia represents an exaggerated irritant reflex of the respiratory tract, possibly arising from the nucleus ambiguus.[113] Other findings include episodic hyperactivity, seizures, and aerophobia. Hyperventilation is frequently present. Along with coma, evidence of pituitary dysfunction often develops, especially disordered water balance (either inappropriate antidiuresis or diabetes insipidus). Hyperventilation gives way to forms of periodic and ataxic respiration,[113] and eventually apnea supervenes. Cardiac arrhythmias are common, predominantly supraventricular tachycardias, and bradycardias, and they reflect either brainstem dysfunction or myocarditis.[114] Autonomic dysfunction is observed, including pupillary dilation, anisocoria, piloerection, markedly increased salivation and sweating, and, rarely, priapism[115] or spontaneous ejaculation.[116] Two cases are reported of rabies-associated cerebral artery vasospasm that was treatable with drugs directed at the nitric oxide synthase pathway.[117] This vasospasm may relate to a deficiency of tetrahydrobiopterin and might be ameliorated by its replacement.[118]

With exceptions in some rare reports, patients entering coma generally die within 1 to 2 weeks, or earlier, despite maximal supportive care. Patients with encephalitic rabies who receive maximal intensive care support may survive for a longer-than-expected period and appear to pass through the paralytic phase before death.[107]

Paralytic (Dumb) Rabies

Patients with *paralytic rabies*, unlike those with the furious form, do not have hydrophobia, aerophobia, hyperactivity, or seizures. Their initial findings suggest an ascending paralysis, including hypophonia, resembling acute inflammatory polyneuropathy (Guillain-Barré syndrome), or a symmetrical quadriparesis. Paralysis is usually more severe in the bitten limb. Meningeal signs (headache, neck stiffness) may be prominent despite a normal sensorium. As the disease progresses the patient becomes confused and then declines into coma.

Nonneurologic Findings

In addition to the cardiac arrhythmias already mentioned, the systemic complications of rabies are similar to those of other critically ill patients. The virus disseminates to many organs,[119,120] but proof of its role in other organ dysfunction is lacking. GI disturbances include bleeding, vomiting, diarrhea, and ileus.[121] Death is usually from cerebral edema or myocarditis, with cardiac arrhythmia or congestive heart failure as mechanisms.[122]

Animal Rabies

A complete description of the effects of rabies on behavior in all of the species that can be infected is beyond the scope of this text. Prior WHO studies have established a crude ranking of rabies virus susceptibility, which is summarized in Table 163.5.[123] However, overt susceptibility is related in part to viral dose, route, and variant and host attributes. Descriptions of the behavioral changes of rabid animals are available elsewhere.[111,124]

DIAGNOSIS

The diagnosis of rabies poses little difficulty in a nonimmunized patient with hydrophobia after a bite by a known or suspected rabid animal. The presentation in areas where domestic animals are immunized is seldom this straightforward. During the incubation period, no diagnostic studies in the patient are useful; recognition of an exposure to a potentially rabid animal should prompt PEP before illness. When symptoms begin, standard laboratory testing does not reliably distinguish rabies from other encephalitides. The CSF may be normal; however, a mild lymphocytic pleocytosis and a modest increase in protein are often described.[125,126]

Because of limited sensitivity of any one methodology, antemortem diagnosis of rabies requires several specimens, for instance, skin biopsy, saliva, serum, or CSF, and multiple testing methods, including virus-specific immunofluorescent staining, antibody detection in serum or CSF, and use of reverse-transcriptase polymerase chain reaction (RT-PCR)

TABLE 163.5 Generalized Susceptibility of Various Animal Species to Rabies

VERY HIGH	HIGH	MODERATE	LOW
Wolves	Hamsters	Dogs	Opossums
Foxes	Skunks	Primates	
Coyotes	Raccoons		
Kangaroo rats	Domestic cats		
Cotton rats	Rabbits		
Jackals	Bats		
Voles	Cattle		

Data modified from World Health Organization. Sixth Report of the Expert Committee on Rabies: Technical Report Series 523. *Geneva: World Health Organization; 1973.*

in saliva or skin biopsy.[127] DFA staining of biopsy or necropsy (animal or human) tissue remains one standard for the real-time diagnosis of rabies. In humans the procedure of choice is DFA analysis of a full-thickness skin biopsy (5–6 mm in diameter containing approximately 10 hair follicles) obtained from the nape of the neck above the hairline.[128] The virus tends to localize in cutaneous nerves at the base of hair follicles. During the first week of symptoms, about 50% of samples reveal rabies virus, with an increasing percentage thereafter.[129] RT-PCR on biopsy specimens and saliva is another diagnostic procedure of choice in suspected human rabies.[130,131] RT-PCR of skin biopsy specimens has a high (98%) sensitivity and (100%) specificity, and saliva samples can be similarly sensitive if three successive samples are tested.[127] RT-PCR and sequencing allows more specific determination of the geographic and host species origin of a particular rabies virus.[132,133] It may in some cases be successfully performed on decomposed brain material,[134] although the use of fresh CNS tissue is much preferred.[131] Current recommendations for diagnostic testing in animals and humans, with instructions for sample collection and submission, are available at www.cdc.gov/rabies or by calling the rabies laboratory of the Centers for Disease Control and Prevention (CDC) at 404-639-1050. In the United States the state health department should be consulted whenever the diagnosis of rabies is suspected. The state rabies consultation contact phone numbers are also available at the CDC website.

The rapid fluorescent focus inhibition test (RFFIT) is the reference standard serologic test for neutralizing antibodies to the rabies virus G protein.[135] A few untreated patients have detectable antibody by day 6 of clinical illness, 50% by day 8, and usually 100% by day 15. Antibodies in CSF appear later but are diagnostic of infection, even in patients who have received PEP.[136] Immunofluorescent antibody tests that detect rabies virus–specific IgM and IgG antibodies, predominantly to the virus ribonucleoprotein, represent sensitive serologic methods that can be used in serum and CSF.[108]

Computed tomographic scan results of the brain are usually normal early in the course,[26] unless hypoxia has supervened. Later, evidence of cerebral swelling may supervene if the patient receives prolonged critical care support (Fig. 163.5). Magnetic resonance (MR) images show areas of increased T_2 signal in the frontal lobes, hippocampi, hypothalamus, brainstem, and sometimes other areas.[137,138] Fig. 163.6 shows a T_2 image, with involvement of the thalamus and hypothalamus highlighted. Late in the course, gadolinium enhancement may occur in the most profoundly involved areas, indicating breakdown of the blood-brain barrier. In paralytic rabies MR imaging of the spinal cord and nerve roots may be useful.[139] No imaging finding is pathognomonic for rabies.

FIG. 163.5 **Noncontrast computed tomographic scan showing areas of both severe cerebral edema *(arrows)* and more widespread swelling.**

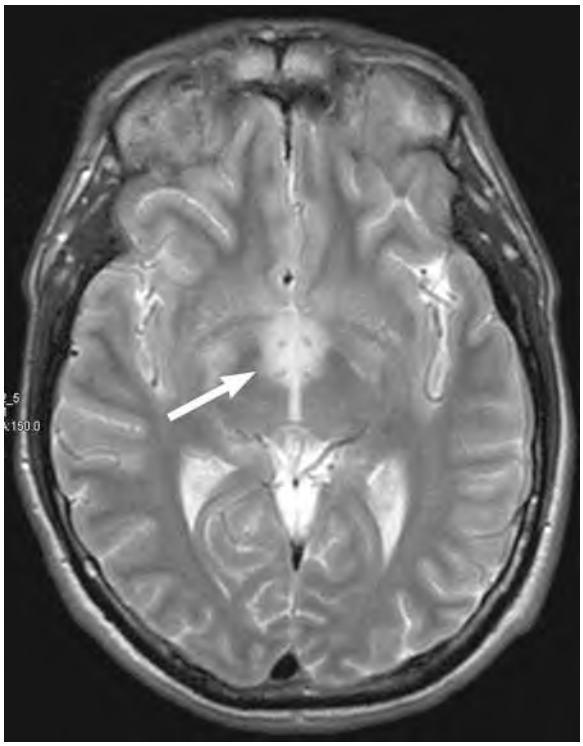

FIG. 163.6 **T2-weighted magnetic resonance image showing increased signal in diencephalon *(arrow).***

Differential Diagnosis

With encephalitic rabies, the major differential consideration is another viral encephalitis.[140] However, rabies patients are significantly more likely to develop aerophobia, hydrophobia, dysphagia, and paresthesias or localized pain and weakness than nonrabies cases.[136] Because the CSF and electroencephalographic (EEG) findings in rabies may mimic those of herpes simplex encephalitis, some patients receive empirical therapy with acyclovir while awaiting a more secure diagnosis (e.g., with PCR). Tetanus is occasionally confused with rabies because opisthotonic posturing may be seen in either.[69] However, the other symptoms of rabies, such as hydrophobia, are not seen in tetanus, and CSF and EEG results are normal in tetanus. Rabies may be misdiagnosed as cerebral malaria and vice versa: At least one atypical case of the latter presented with phobic spasms.[141] Strychnine poisoning should be considered and can be excluded with laboratory testing.

Paralytic rabies may resemble acute inflammatory polyneuropathy, transverse myelitis, or poliomyelitis. Electromyographic studies may be useful in distinguishing rabies from polyneuropathy. In transverse myelitis a finding of pain at the level of the lesion may be helpful, as may the finding of a high T_2 signal lesion. A sensory level is characteristic of transverse myelitis, whereas in rabies, sensory function is typically normal.[115] Fever usually precedes weakness in poliomyelitis, and the resolution of fever with the onset of neurologic findings favors this diagnosis. A history of poliomyelitis immunization should be sought.

The sometimes prolonged incubation period of rabies recalls the slow infections of the CNS caused by conventional viruses (e.g., progressive multifocal leukoencephalopathy).[142] However, rabies requires neither a defect in host immunity nor a mutation in the virus to produce disease, which distinguishes it from other neurotropic viruses. Spongiform changes in brain tissue in rabies[143] may resemble those seen in the prion diseases.[144]

Although CNS reactions to the rabies vaccines available in developed countries are exceptionally rare, patients who receive older vaccine forms that contain myelin determinants occasionally have development of acute disseminated encephalomyelitis (ADEM; also called postvaccinial encephalomyelitis; see "Prevention"). ADEM is a syndrome with many precipitants other than rabies vaccine. It resembles encephalitis, or occasionally it presents as a mass lesion that resembles a brain abscess. It typically begins 10 to 14 days after vaccine exposure, which would constitute an unusually brief incubation period for rabies. Spinal fluid serology, viral isolation, or RT-PCR can differentiate rabies from ADEM after immunization.[145] ADEM produces high T_2 signal lesions visible with MR imaging.[146] However, differences in the distribution of the MR lesions in rabies and ADEM may aid in the differential diagnosis.[147]

Patients potentially exposed to suspect animals may have a psychological reaction termed *rabies hysteria*.[148] They may refuse to drink water; in contrast, the patient with rabies attempts, at least initially, to drink but is halted by pharyngeal spasms.

PREVENTION

Preexposure Prophylaxis

Although management of animal rabies is central to prevention of human disease, very few nations have truly eliminated it, and those that have been successful, in lieu of enzootic wildlife rabies, usually maintain quarantine procedures lest the disease reappear.[149] Therefore prophylactic procedures for domestic animals and humans remain essential. Prophylaxis for cats and dogs in many countries is required by law; in the United States the use of 1-year or 3-year vaccines is common, although only the 3-year vaccines are recommended.[150] Vaccination should be performed or supervised by a veterinarian; improper administration can lead to lack of immunity.[151] Measurement of animal seroconversion rates may be considered for exportation but is unnecessary to ensure protection,[152] and immunization of horses or valuable livestock is recommended in areas of increasing rabies prevalence or in petting zoo settings.

Rather than culling, vaccination of wildlife is an effective public health measure.[153] The use of vaccines effective after oral bait delivery allows immunization of wild animals.[154] One intensive 4-year campaign in Belgium eliminated rabies from the fox population.[155] This approach may also be effective in free-ranging dogs.[156] Veterinary vaccines cost about $0.50 or less per dose in the United States. In contrast, Semple-type (grown in sheep brain cultures) human vaccines cost about $5 per course, Vero cell vaccine in France about $160 per course, and human diploid cell rabies vaccine (HDCV) or purified chick embryo vaccine (PCEC) in the United States more than $500 per course.[157]

Preexposure prophylaxis should be targeted to people at high risk of rabies virus exposure, such as veterinarians, laboratory workers who handle rabies virus, cavers, and certain international travelers to countries with high rabies prevalence and poor access to medical care and PEP. For example, in some remote parts of Amazonia, routine exposure to vampire bat predation may warrant consideration of preexposure vaccination, particularly for pediatric populations at risk.[158] Even where PEP is available, it is important to note that patients often do not receive optimal PEP under real-world conditions, and shortages of RIG are common.[159] Current recommendations for international travelers are available at the CDC website (www.cdc.gov/travel/). A series of three intramuscular (IM) or intradermal injections (days 0, 7, and 21 or 28) is sufficient; antibody response determination is not required in healthy hosts. Preexposure vaccination does not eliminate the need for medical attention after a suspect exposure but eliminates the need for RIG and decreases the doses of vaccine required (two postexposure injections at days 0 and 3). Serology and booster doses every 2 to 3 years, if the titer is inadequate, are recommended for individuals frequently at risk of exposure. An adequate antibody response is generally considered to be complete neutralization at the 1:5 serum dilution level (0.1 IU/mL) with the RFFIT, which is in contrast to the 0.5 IU/mL antibody value suggested by WHO.

Postexposure Prophylaxis

Although the incidence of human rabies is low in the United States, animal rabies remains common, and approximately 16,000 to 39,000 people with contact to potentially rabid animals receive rabies PEP annually.[160] The estimated annual expenditure for such rabies prevention is approximately $584 million, with PEP accounting for almost half of this expenditure.[161] Despite the relative high costs, the recent cost-effectiveness analysis of the Advisory Committee on Immunization Practices (ACIP) showed that it is always cost saving to administer PEP if a patient is bitten by a animal that has tested positive for rabies or if a patient is bitten by a reservoir species, even if the animal is not available for testing.[160]

The cornerstone of rabies PEP is proper wound care, which potentially reduces the risk of rabies by 90%.[162] Thorough washing with a 20% soap solution is as effective as the formerly recommended quaternary ammonium compounds.[163] Irrigation with a virucidal agent, such as povidone-iodine, is also used.[164] After wound care the clinician must decide whether to institute specific PEP. Prompt consultation with public health officials is advised because this decision is based in part on the current prevalence of rabies in the animal species involved in the exposure.[165] In countries of low prevalence a healthy dog or cat that has bitten or otherwise transferred saliva to a human is observed for 10 days. If the animal's behavior remains normal, the patient need not receive PEP beyond proper wound care. If the animal's behavior changes, it should undergo immediate pathologic examination for evidence of rabies virus infection. If infection is confirmed, there is adequate time to institute PEP. Wild mammal exposure, especially if the animal exhibits uncharacteristic behavior, warrants PEP in most circumstances. If the animal is available for pathologic examination, and if pathologic examination of the brain does not indicate the presence of rabies virus, PEP may be discontinued (Fig. 163.7). Discovery of a bat in a room with an infant or a sleeping individual or mentally disabled person, who cannot report the occurrence of a bite reliably and on whom no bite is found, raises the issue of PEP. If the bat is captured and tests positive for rabies, PEP is indicated; if the bat is not available, the decision about PEP must be individualized. In a recent 17-year population survey of 14,453 households with bedroom bat exposure without a recognized bite, the number of individuals needed to be treated to prevent a single case of bat rabies ranged from 314,000 to 2.7 million persons at a cost of CAD$228 million to CAD$2 billion.[166] PEP appears to be safe in pregnant women and should not be withheld when an indication exists.[167]

Modern PEP should always include administration of both passive antibody (RIG) and vaccine for both bite and nonbite exposures in persons with no previous vaccination for rabies. Rabies immune globulin is available in human (HRIG) and equine forms (pooled antirabies antiserum [ARS] of equine origin and purified antirabies serum of equine origin [ERIG]). These immune globulins are purified from the sera of hyperimmunized donors. Two HRIG preparations are available in the United States: Imogam Rabies-HT (Sanofi Pasteur; Swiftwater, PA) and HyperRab S/D (Grifoils; Barcelona, Spain). HRIG is given only once in a dose of 20 IU/kg and is applicable to all age groups, including children. The most recent WHO and ACIP recommendations call for the entire dose to be infiltrated into the wound if anatomically feasible.[160] Any remaining volume should be administered IM at an anatomic site distant from that used for the active vaccine. The recommended dose of ERIG is 40 IU/kg. Failure to infiltrate wounds with RIG or surgical closure of wounds before RIG infiltration has been associated with the development of rabies in patients despite otherwise proper PEP.[168] If RIG is not administered when active vaccination is begun, it can be administered through day 7 of the PEP series.[160] Human monoclonal antibodies to rabies have been successfully administered as PEP during clinical trials and have demonstrated favorable side effect and pharmacokinetic profiles, suggesting that RIG may be replaced in the future by a more readily available and safer product.[169,170]

Many different forms of rabies vaccine have been produced since Pasteur's original success in 1882. In some developing nations, such as Ethiopia and Bolivia, nervous tissue–type vaccine (NTV) is still used, but it carries a risk of central and peripheral neurologic complications in the range of 1 per 200 to 1600 vaccinees.[105] Production of vaccine in sheep CNS, a common method of Semple-type vaccine production, also carries the theoretical risk of transmitting the scrapie prion.[171] Suckling mouse brain vaccine is effective and safer, with a neurologic complication rate of approximately 1:8000, but all NTVs should be discontinued in favor of much safer alternatives.

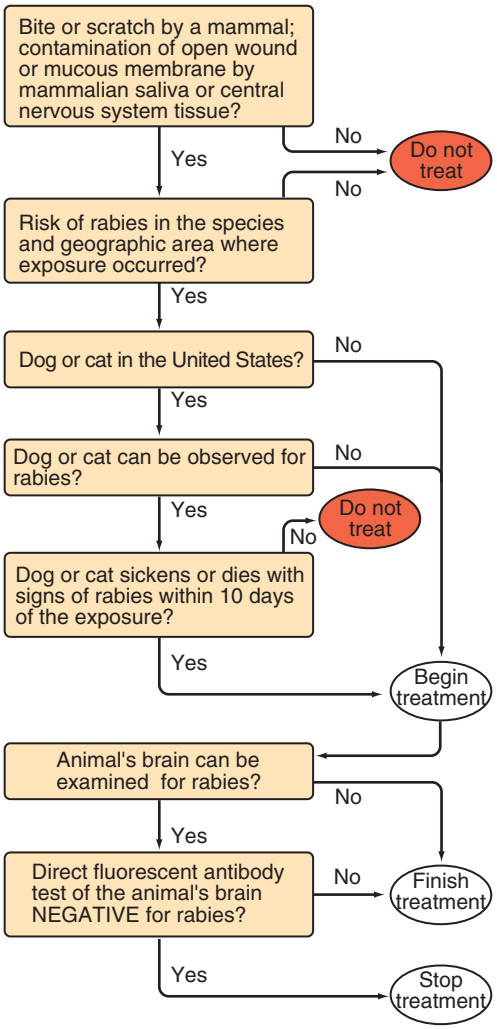

FIG. 163.7 Algorithm for human rabies postexposure prophylaxis. In highly suspect animals, prophylaxis should be started immediately and discontinued if fluorescent antibody test results of the animal brain are negative. In some cases with low risk, treatment may be delayed for up to 48 hours, pending the result of fluorescent antibody testing.

The currently available vaccines licensed for human use in the United States include HDCV (Imovax; Sanofi Pasteur) and PCECV (RabAvert; Novartis; Basel, Switzerland). Both are inactivated virus vaccines that are remarkably safe and immunogenic. Local reactions (pain, swelling, or induration) are common, but systemic symptoms (fever, headache, malaise, nausea, abdominal pain, or adenopathy) occur in only a minority of patients. Serious reactions have been exceedingly uncommon, with suggested Guillain-Barré syndrome reported rarely.[172] To report a vaccine reaction, call the appropriate number for each manufacturer. Corticosteroids should be given only to patients with a life-threatening vaccine reaction because they interfere with the development of immunity. Patients with immunocompromise may not respond adequately to vaccination, and antibody titers should be measured 2 to 4 weeks after immunization.[166]

In other countries other PEP vaccines (e.g., vaccines grown in duck embryos or Vero cell cultures)[173] and regimens are often used. Consultation with the rabies officer of the state health department may be helpful for the management of patients in whom PEP has been initiated with a vaccine not approved for use in the United States.

Cross-protection is provided by rabies vaccines to some other lyssaviruses, such as ABLV.[174] Protocols for potential exposure to ABLV encourage thorough cleaning of the wound, use of the same vaccine doses and schedules as recommended for rabies virus, and testing the bat, if possible, for ABLV. Protocols differ in the specific recommendations regarding the influence of both bat testing and type of exposure on PEP.[175]

The schedule for PEP vaccine administration in an unvaccinated individual changed in 2010 to a four-dose schedule (1.0 mL IM), starting as soon as possible after exposure (day 0) and repeated on days 3, 7, and 14.[176] However, immunocompromised individuals should continue to receive the five-dose schedule.[160,176] Patients who have been previously vaccinated receive 1.0 mL IM on days 0 and 3 only, without RIG.[160]

For adults the vaccination should always be administered in the deltoid area rather than the gluteal area. Vaccine injections into the gluteal result in lower neutralizing antibody titers.[177] In small children vaccination may be given in the lateral thigh. Other schedules are available for use; physicians not familiar with their use should consult local public health authorities and review the most recent WHO and ACIP recommendations.[1,160] The vaccine must not be given in the same region as the immune globulin. Because of the high cost by volume for the IM route of vaccination, intradermal vaccine (uses at least 60% less than IM vaccine) administration is recommended by the WHO as an alternative in developing countries.[1] A single case of transient false-positive enzyme-linked immunosorbent assay results for human immunodeficiency virus after HDCV immunization was reported in 1994.[178] Subsequent screening of samples from people recently immunized against rabies virus revealed no similar cases,[179] but in view of similar phenomena with other vaccines, physicians should be aware of this possibility.

Personnel who care for patients with rabies should practice standard infection control precautions, including use of gloves, gowns, masks, and eye protection, when there is risk of aerosols.[180] PEP should be offered to health care workers or family members if mucous membrane or nonintact skin comes in contact with potentially infectious body fluids such as saliva.

TREATMENT

There is no proven therapy for rabies.[181] An experimental protocol called the Milwaukee protocol (www.mcw.edu/Pediatrics/InfectiousDiseases/PatientCare/Rabies.htm) has been used in patients with rabies. The original patient was a 15-year-old girl from Wisconsin who had a bat bite 1 month before symptom onset.[182] The patient was admitted with fever, obtundation, ataxia, myoclonus, and dysarthria. The patient was placed into electrographic burst suppression with ketamine and midazolam. Antiviral therapy commenced with ribavirin and amantadine. Neither rabies vaccine nor RIG was administered because rabies was not suspected at onset, and during antemortem testing, the patient had an immune response in both the serum and the CSF. The patient was discharged to home on the 76th day after admission with persistent choreoathetosis and ballismus. Twenty-seven months after exposure the patient was taking college courses and attending to her own activities of daily living, with only mild neurologic deficits.[183] To date, she remains well. Since the description of the Milwaukee protocol, there have been 29 reported cases of rabies in the United States, United Kingdom, and Canada; two of those patients survived, and one received components of the Milwaukee protocol.[184,185,186] Eleven other patients received major components of the Milwaukee protocol (therapeutic coma, ketamine) but did not survive.[186] The routine use of this treatment protocol is not recommended, pending further data.

Despite excellent intensive care, the case-fatality rate approaches 100%, and most patients die within a few days to weeks of illness onset. All three documented surviving patients had evidence of rabies virus–specific antibodies in serum and CSF at clinical presentation, but no viral antigens or RNA were identified. This finding may represent a favorable prognostic factor.

Favipravir, a purine analog with activity against a broad range of RNA viruses, was effective in a mouse model as PEP and may be effective in a mouse model of CNS infection, but efficacy in humans is yet to be documented[187,188] (see also Chapter 164). During 2017 the drug was used in a Virginia resident who acquired the infection in India, but the patient succumbed (www.richmond.com/life/health/virginia-health-officials-investigating-rare-case-of-human-rabies-in/article_0aa5bfc3-3875-570b-8d4e-eadbab253b5d.html). A better understanding of basic viral pathobiology, the rational design of antiviral drugs, and development of relevant animal models are necessary for progress in therapeutic interventions.

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The complete reference list is available online at Expert Consult.

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164 Marburg and Ebola Virus Hemorrhagic Fevers

Thomas W. Geisbert

SHORT VIEW SUMMARY

Definition

- Marburg hemorrhagic fever and Ebola hemorrhagic fever (HF) are severe and often fatal diseases characterized by fever, headache, malaise, myalgia, coagulation disorders, and multiorgan failure.

Epidemiology

- Human outbreaks occur sporadically in regions of Central Africa.
- An unprecedented epidemic of Ebola hemorrhagic fever involving more than 28,000 cases and 11,000 deaths occurred in West Africa in 2013–16.
- Recent evidence suggests that bats may play a role as a reservoir host.
- The manner in which filovirus outbreaks are initiated is unknown; however, it is thought that the initial cases occur as a result of contact with an infected animal.
- Nosocomial transmission has occurred frequently during outbreaks of filovirus HF in endemic areas.

Diagnosis

- Clinical symptoms are nonspecific, but a constellation of symptoms, including fever, headache, malaise, myalgia, sore throat, vomiting, diarrhea, and, in particular, the appearance of a maculopapular rash, may indicate infection with a filovirus.
- Antigen-capture enzyme-linked immunosorbent assay and polymerase chain reaction are the most frequently used assays to diagnose filovirus infection.

Treatment

- There are no approved postexposure treatments for filovirus infections.
- Treating patients infected with marburgviruses or ebolaviruses consists primarily of intensive supportive care, which is directed toward maintaining effective blood volume and electrolyte balance.
- Several experimental treatments have shown promise in nonhuman primate models of filovirus infection, including small interfering RNAs, antisense oligonucleotides, RNA polymerase inhibitors, and pools of monoclonal antibodies; of these interventions, ZMapp, a pool of three monoclonal antibodies, showed promise in humans when used in phase II/III trials during the 2013–16 Ebola epidemic.

Prevention

- There are no approved vaccines against marburgviruses or ebolaviruses, although a vesicular stomatitis virus–based vaccine expressing the *Zaire ebolavirus* glycoprotein showed remarkable success in a ring-vaccination cluster randomized trial in Guinea during the 2013–16 Ebola epidemic.
- Barrier nursing procedures include the donning of protective clothing, masks, and eye shields.
- Infected patients and close contacts should be isolated.
- Avoid contact with bush meat and sick animals, particularly nonhuman primates, in endemic regions.

Viral hemorrhagic fever (VHF) is a syndrome characterized by fever, malaise, myalgia, and blood coagulation disorders that can progress to multiorgan failure, shock, and death in many cases. VHF is caused by members of four different families of RNA viruses. Among the VHFs members, the genera *Marburgvirus* and *Ebolavirus* in the family Filoviridae are the most feared because of their dramatic clinical presentation and unusually high case-fatality rates of up to 90%, and because their natural history remains a mystery. In addition to concerns of natural outbreaks and epidemics in regions of Central and Western Africa, ebolaviruses and marburgviruses are known to have been the subjects of former biological weapons programs and have the potential for deliberate misuse.[1,2] Currently, there are no filovirus vaccines or treatments approved for human use. For these reasons, ebolaviruses and marburgviruses have recently been included as only 2 of 11 human pathogens and only 2 of 4 viruses on the United States Department of Health and Human Services Tier 1 list of Category A select agents.[3] In addition to causing significant disease in humans, filoviruses have decimated populations of great apes in the Congo basin, further impacting an already endangered species.

VIRUS CHARACTERIZATION

The family Filoviridae is divided into two genera: *Marburgvirus* and *Ebolavirus*. The *Marburgvirus* genus contains a single species, *Marburg Marburgvirus,* which contains two genetically distinct members: Marburg virus (MARV) and Ravn virus (RAVV). The *Ebolavirus* genus consists of five distinct species: *Bundibugyo ebolavirus* (BDBV), *Reston ebolavirus* (RESTV), *Sudan ebolavirus* (SUDV), *Tai Forest ebolavirus* (TAFV) (more commonly known as *Ivory Coast ebolavirus*); and *Zaire ebolavirus* (EBOV).[4] Nucleotide and amino-acid differences between the genera *Marburgvirus* and *Ebolavirus* are each approximately 55%, and there is no serologic cross-reactivity between these viruses. In comparison, ebolavirus species show 37% to 41% differences in nucleotide and amino-acid sequences, and there is varying degrees of cross-reactivity among the ebolavirus species.

Filoviruses are enveloped, nonsegmented, negative-stranded RNA viruses. Filovirus particles take on a variety of forms, from circular or "6"-shaped to prototypical straight filaments, for which the virus family is named (Fig. 164.1). Although the length of the virions is variable, with marburgvirus particles averaging close to 800 nm and ebolavirus virions measuring about 1 μM, the diameter of all filovirus particles uniformly measures about 80 nm.[5] Filovirus particles contain an approximately 19-kilobase noninfectious genome that encodes seven structural proteins, with a gene order of 3′ leader, nucleoprotein (NP), virion protein (VP) 35 (VP35), VP40, glycoprotein (GP), VP30, VP24, RNA-dependent RNA polymerase L protein, and 5′ trailer. Four of these proteins are associated with the viral genomic RNA in the ribonucleoprotein complex: NP, VP30, VP35, and the L protein. Some proteins of the ribonucleoprotein complex have additional functions.

For example, VP35 has been shown to act as an interferon antagonist.[6] VP40 serves as the matrix protein and mediates particle formation and, in the case of marburgviruses, has also been shown to interfere with host innate immune responses.[7] VP24 is another structural protein associated with the membrane and for ebolaviruses also interferes with interferon signaling.[8]

The GP is the surface glycoprotein that forms the spikes on the virion and is the effector for receptor binding and membrane fusion. An important distinction of ebolaviruses from marburgviruses is that although the marburgvirus GP is encoded in a single open reading frame (ORF), the ebolavirus GP is encoded in two ORFs.[9,10] The single marburgvirus ORF translates into the structural surface GP. In contrast, the two ebolavirus ORFs are linked together by slippage of the L polymerase at an editing site (a string of seven consecutive template uracil residues) to insert an eighth uracil. This process results in the production of a messenger RNA (mRNA) transcript that permits read-through translation of full-length GP. However, only about 20% of the mRNA transcripts are edited and translate into structural surface GP. The remaining 80% of unedited mRNA transcripts result in the production of a truncated soluble GP (sGP) that is secreted in large quantities from infected cells. Although the function of sGP has not been fully elucidated, it has been postulated that sGP subverts the host immune response by both passively absorbing antibodies directed at the full-length structural GP[11,12] and by triggering the proliferation of B cells that preferentially bind sGP.[13]

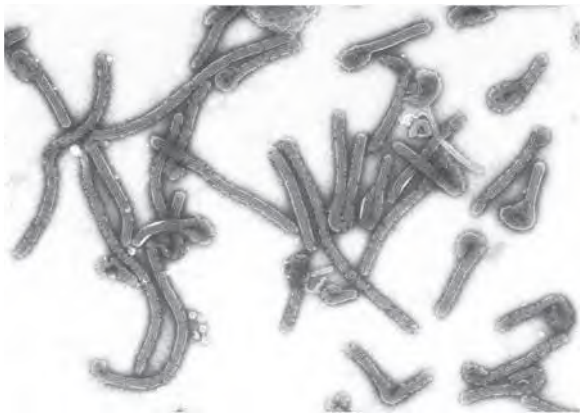


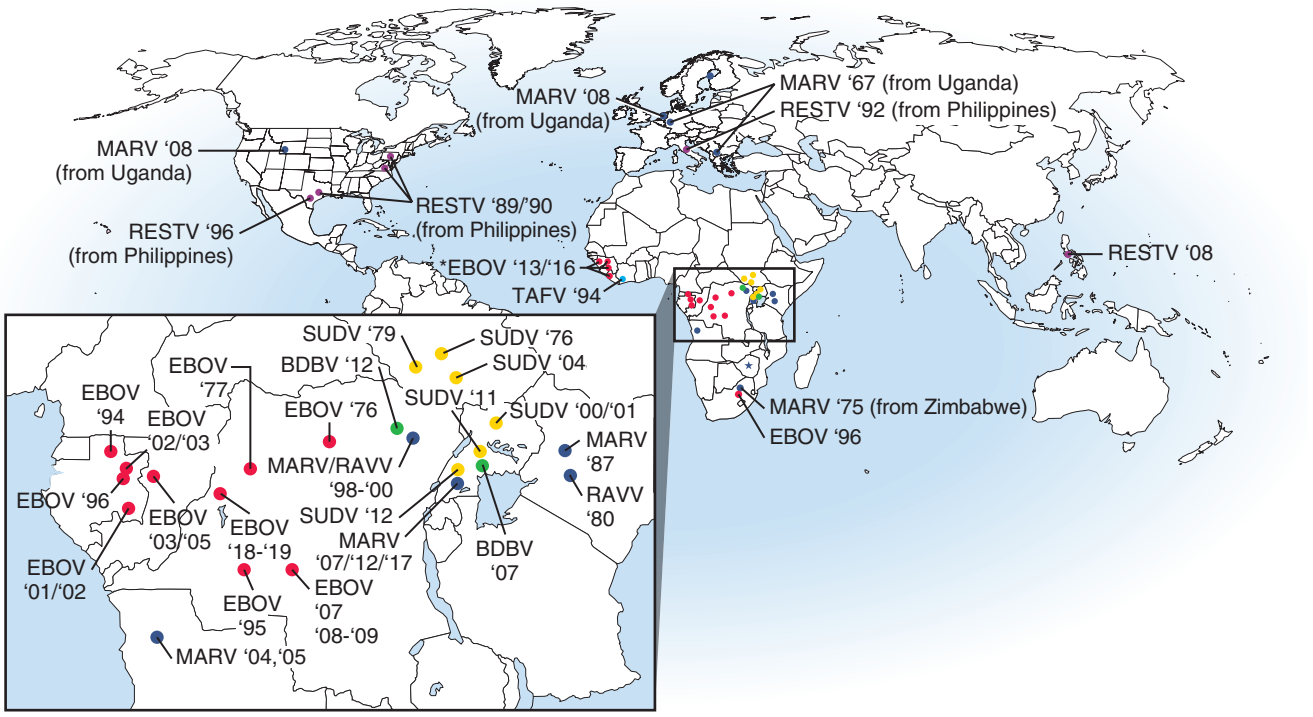
FIG. 164.1 Electron micrograph of *Sudan ebolavirus* virions. Negatively contrasted filovirus particles obtained from culture fluids from infected Vero cells (original magnification ×12,000).

EPIDEMIOLOGY

Marburg Hemorrhagic Fever

The first documented outbreak of VHF caused by a filovirus occurred in 1967, when there were three concurrent episodes of lethal marburgvirus infection in Marburg and Frankfurt, Germany and in Belgrade (in the former Yugoslavia) among laboratory workers exposed to blood and tissue products of African green monkeys imported from Uganda (Fig. 164.2).[14] Secondary transmission to medical staff and family members was also documented. In total, 31 patients became infected, and seven of these patients died. During the next 2 decades, marburgvirus was associated with sporadic, isolated, usually fatal cases among residents and travelers in southeast Africa.

In 1998–2000 there was a prolonged outbreak involving 154 cases of Marburg hemorrhagic fever (HF) in Durba, Democratic Republic of the Congo (DRC), which was associated with individuals working in an underground gold mine.[15] Case-fatality rates from this outbreak are unclear but may be up to 83%. This outbreak was unique and complicated by the fact that it had multiple introductions of marburgviruses of different phylogenetic lineages and included variants that are thought to be more pathogenic (Angola)[16,17] than others. The largest and most lethal marburgvirus outbreak to date occurred in 2004–05 in northern Angola.[16] This outbreak involved 252 cases, with a case-fatality rate of 90%. The epidemic was driven largely by nosocomial transmission;



*In regard to the 2013-16 West African outbreak of EBOV, several cases in the US and Europe occurred that were associated with direct contact with medically evacuated patients or imported cases.

FIG. 164.2 Locations of filovirus infections and outbreaks. *BDBV, Bundibugyo ebolavirus; EBOV, Zaire ebolavirus; MARV,* Marburg virus; *RAVV,* Ravn virus; *RESTV, Reston ebolavirus; SUDV, Sudan ebolavirus; TAFV, Tai Forest ebolavirus.*

however, community-acquired infection was documented toward the end of the outbreak. Between 2007 and 2017 several small episodes of Marburg HF were reported in Uganda, with two cases being exported to the United States[18] and the Netherlands,[19] respectively.

Ebola Hemorrhagic Fever

Ebolavirus was first recognized during near-simultaneous explosive outbreaks in 1976 in small communities in the former Zaire (now the DRC) and Sudan (see Fig. 164.2).[20,21] There was significant secondary transmission through the reuse of unsterilized needles and syringes and nosocomial contacts. These independent outbreaks involved the serologically distinct species EBOV and SUDV. The EBOV outbreak consisted of 318 cases and 280 deaths (88% mortality), whereas the SUDV outbreak involved 284 cases with 151 deaths (53% mortality). Since 1976 EBOV has appeared sporadically in Central Africa, causing several small- to middle-size outbreaks between 1976 and 1979. In 1995 there was a large epidemic of Ebola HF involving 315 cases of EBOV, with an 81% case-fatality rate, in Kikwit, a community in the former Zaire.[22] Meanwhile, between 1994 and 1997 there were smaller outbreaks caused by EBOV in Gabon. Since 2000 there have been near-yearly occurrences of EBOV in Gabon, DRC, or the Republic of Congo. These EBOV outbreaks have also involved a catastrophic decline in populations of great apes.[23,24] Before 2013 the largest EBOV outbreak on record involved 425 cases, with a 53% case-fatality rate.[25] This outbreak occurred in 2000–01 in Sudan and was caused by SUDV. Smaller outbreaks of SUDV have occurred in Sudan in 2004 and in Uganda in 2011 and 2012. In December 2013 an unprecedented outbreak caused by EBOV began in the West African countries of Guinea, Liberia, and Sierra Leone. The epidemic continued unabated for more than 2 years, finally ending in January 2016 with 28,616 cases and 11,301 deaths.[26] In May of 2018 an EBOV outbreak was reported in the DRC, and as of June 20, 2018, there have been 61 cases and 28 deaths reported.[27] World Health Organization reported on June 22, 2018 that they believe that outbreak had been largely contained.[28]

In 1989–90 a third species of the genus *Ebolavirus,* RESTV, appeared in Reston, Virginia in association with an outbreak of VHF among cynomolgus macaques imported to the United States from the Philippine Islands.[29] Hundreds of monkeys were infected (with high mortality) in this outbreak, but no human cases occurred. Four animal caretakers seroconverted to REBOV with no overt disease. Epizootics in cynomolgus monkeys recurred at other facilities in Europe and the United States through 1992 and again in 1996. Subsequently, RESTV has been found in the Philippines on several occasions, with surprising reports documenting infections in domestic pigs.[30]

A fourth species of the genus *Ebolavirus,* TAFV, was identified in Côte d'Ivoire in 1994.[31] The virus was isolated from an ethnologist who had worked in the Tai Forest reserve and became infected after a necropsy on a chimpanzee. The individual became ill with symptoms consistent with filovirus infection and survived infection. The chimpanzee originated from a troop that lost several members to an illness that was subsequently identified as being caused by TAFV.

The latest and fifth species of the genus *Ebolavirus,* BDBV, was discovered in Uganda late in 2007 during an outbreak that involved 56 confirmed cases and an approximate 40% case-fatality rate.[32] A more recent outbreak of BDBV occurred late in 2012 in the DRC and involved 52 probable cases and a 48% case-fatality rate.[33]

Natural History

Human and nonhuman primates are susceptible to filovirus infection and are considered to be end hosts rather than potential reservoirs. Surveys to identify animal reservoirs and arthropod vectors have been aggressively undertaken in endemic areas, particularly after most large filovirus outbreaks. Until recently these efforts have been unsuccessful. Ecologic studies in 2003–06 in Gabon and the Republic of Congo demonstrated the initial evidence for the presence of EBOV in three different species of fruit bats.[34] These studies showed the presence of viral RNA and antibodies, although the investigators were unable to isolate infectious EBOV. Subsequent studies in 2007 detecting MARV RNA and isolating infectious MARV from cave-dwelling fruit bats in Uganda further support the view that bats may serve as a reservoir for filoviruses.[35] More recently, antibodies against RESTV were detected in fruit bats in the Philippines.[36] Although current data suggest a role for bats in maintaining filoviruses in nature, it remains unclear whether bats serve as the primary reservoir or whether other species are involved.

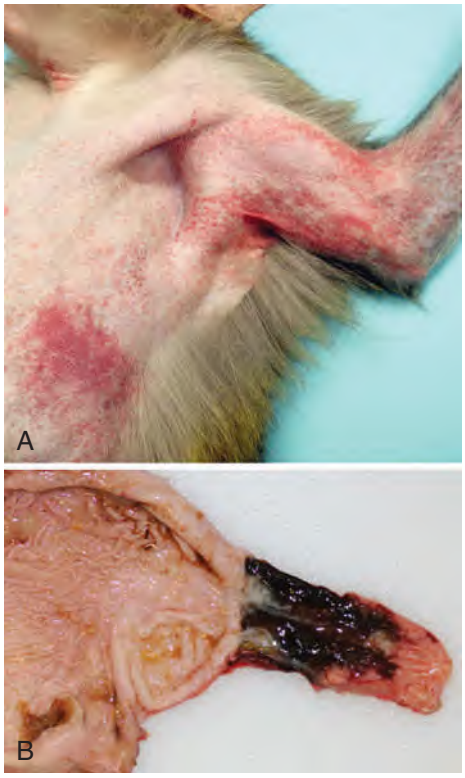


FIG. 164.3 Representative gross necropsy lesions from nonhuman primates experimentally infected with filoviruses. (A) Typical petechial rash of the left arm and chest of a rhesus macaque 11 days after infection with Marburg virus. (B) Marked congestion of the duodenum at the gastroduodenal junction of a rhesus monkey 9 days after infection with *Zaire ebolavirus*.

Clinical Manifestations and Diagnosis

Clinical and laboratory features of marburgvirus and ebolavirus infection are nonspecific and include an incubation period of 2 to 21 days (mean, 4–10) with a sudden onset of fever, malaise and/or myalgia, and may include a variety of other nonspecific symptoms (reviewed in references 4 and 37). The presence of an erythematous, maculopapular rash may be observed (Fig. 164.3). A constellation of other coagulation disorders may occur, including bleeding from venipuncture sites and the gastrointestinal tract (see Fig. 164.3). Clinical pathology findings include leukopenia and lymphocytopenia with increased levels of neutrophils, thrombocytopenia, and increased serum levels of the liver-associated enzymes aspartate aminotransferase and alanine aminotransferase. Prolonged blood coagulation times and increased circulating levels of D-dimers are also associated with filovirus infections.[38,39]

Confirmation of filovirus infection requires detection of virus in blood or other tissues or the demonstration of filovirus-specific antibody. Assays most frequently used to diagnose filovirus infections include immunofluorescent antibody tests, the enzyme-linked immunosorbent assays for filovirus antigen and specific immunoglobulin M (IgM) and IgG antibodies, and reverse-transcriptase polymerase chain reaction.[25,40–43] Rapid tests to detect antigen were optimized for point-of-care diagnosis during the 2013–16 EBOV epidemic.[44] Other assays that have been used to confirm filovirus infection included immunohistochemistry of skin and other tissues, virus culture, and electron microscopy.[40,45,46]

PATHOGENESIS

Filoviruses are thought to enter the host through mucosal surfaces, small abrasions and/or tears in the skin, or by parenteral introduction.

Both marburgviruses and ebolaviruses have a broad cell tropism, infecting a wide variety of cell types. Ultrastructural examination of tissues from fatal human cases and from experimentally infected nonhuman primates show that monocytes, macrophages, dendritic cells, endothelial cells, fibroblasts, hepatocytes, adrenal cortical cells, and several types of epithelial cells all lend support to replication of these viruses.[47,48,49–52,53] Systematic studies in nonhuman primates experimentally infected with MARV or EBOV suggest that monocytes, macrophages, and dendritic cells are the early and preferred replication sites.[51,52] Filovirus infection of mononuclear phagocytes appears to trigger a cascade of events involving the production and release of the procoagulant protein tissue factor,[38] as well as a variety of proinflammatory cytokines/chemokines and oxygen free radicals.[54,55] It is thought that the triggering of this chain of events is equal, or in fact more critical, to the development of the observed pathology than is any structural damage induced directly by the actual process of viral replication in host cells and/or tissues.

During filovirus infection, lymphoid depletion and necrosis are frequently observed in spleen, thymus, and lymph nodes of patients with fatal disease and in nonhuman primates that are experimentally infected (Fig. 164.4). Although lymphoid tissues are primary sites of filovirus infection, there is usually little inflammatory cellular response in these or other infected tissues. Despite the large die-off and loss of lymphocytes during the disease course, the lymphocytes themselves do not support the production of progeny virus. Large numbers of lymphocytes undergo apoptosis in man[56] and in experimentally infected nonhuman primates,[57] in part explaining the progressive lymphopenia and lymphoid depletion at death. Other morphologic lesions include focal necrosis in a number of organs, particularly liver, where Councilman bodies are a prominent finding (see Fig. 164.4).

Coagulation disorders are a hallmark feature of filovirus infection, and results from many studies have shown biochemical and histologic evidence of disseminated intravascular coagulation in both humans and experimentally infected nonhuman primates.[a] The mechanism(s) responsible for triggering the coagulation disorders that typify filovirus infection are not completely understood. Results from several studies strongly suggest that expression or release of tissue factor from monocytes and macrophages infected with filoviruses plays a pivotal role in inducing the development of coagulation irregularities reported in filovirus HF.[38] However, coagulopathy noted during filovirus HF could be caused by several factors, especially during the later stages of disease.

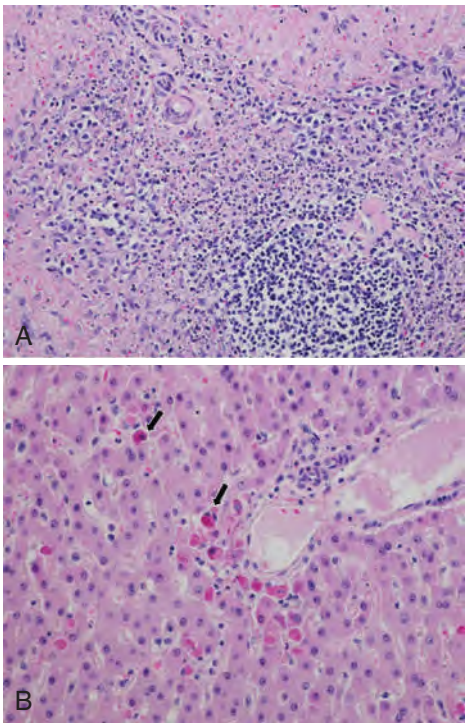


FIG. 164.4 Histopathology of nonhuman primates experimentally infected with filoviruses. (A) Necrosis and apoptosis of lymphocytes with concomitant lymphoid depletion in spleen of a rhesus monkey 9 days after infection with *Zaire ebolavirus* (hematoxylin and eosin stain; original magnification, 20×). (B) Councilman-like bodies *(arrows)* in the liver of a rhesus monkey 9 days after infection with *Zaire ebolavirus* (hematoxylin and eosin stain; original magnification, 20×) *(Photographs courtesy Karla Fenton, University of Texas Medical Department–Galveston, TX.)*

COUNTERMEASURES

Prevention

In the past there has been little commercial interest for developing vaccines against filoviruses, primarily because of the geographic location of epidemic areas and the small global market. However, the classification of filoviruses as important biological defense pathogens, bolstered by the increased press coverage of the recent EBOV epidemic in West Africa, has dramatically changed perspectives regarding the need for vaccines against marburgviruses and ebolaviruses. Effective and fast-acting filovirus vaccines would be valuable for at-risk medical personnel, first responders, military staff and researchers, and also for targeted vaccination in the most affected populations (e.g., primarily health care workers and family members of confirmed or probable cases).

Although there are no approved vaccines or postexposure treatment modalities available for preventing or managing filovirus infections, there are at least nine different vaccine systems that have shown promise in completely protecting nonhuman primates against either Ebola or Marburg HF, with five of these vaccines protecting animals against both ebolaviruses and marburgviruses (reviewed in references 60–62). Several of these vaccines require multiple injections to confer protective efficacy. However, in the setting of pathogens such as marburgviruses and ebolaviruses, which are indigenous to Africa and are also potential agents of bioterrorism, a single-injection vaccine is preferable. In the case of preventing natural infections, multiple-dose vaccines are both too costly and not practical (logistics and compliance) in developing countries. In the case of a deliberate release of these agents, there would be little time for deployment of a vaccine that requires multiple injections over an extended period of time. Thus, for most practical applications, a vaccine against the filoviruses necessitates a single immunization or, at the most, two injections within a very short time frame. Of the prospective filovirus vaccines, only two systems—one based on replication-defective adenoviruses (Ad5 or ChAd3) and the other based on the recombinant vesicular stomatitis virus (rVSV)—have been shown to provide complete protection to cynomolgus macaques when administered as a single-injection vaccine.[63–66]

Substantial progress was made in the advancement of candidate preventive vaccines during the 2013–16 EBOV epidemic. Several candidate EBOV vaccines were used in phase I and/or II studies during this time. These included rVSV-EBOV,[67–71] Ad5-EBOV,[72–74] rAd5-EBOV in combination with rVSV-EBOV,[75] ChAd3-EBOV either with or without modified vaccinia Ankara (MVA)-EBOV,[76–79] Ad26-EBOV combined with MVA-EBOV,[80] and a DNA vaccine that encoded multiple filovirus GPs.[81,82] Overall, these EBOV vaccines generated good immunogenicity against the EBOV GP, with no serious adverse events reported. Although at least 16 phase I and/or II trials were conducted during the 2013–16 EBOV epidemic, there were few opportunities to conduct phase III trials, and the results of only a single phase III trial were reported. Final results of the phase III rVSV-EBOV vaccine, which was used in a ring-vaccination, open-label, cluster-randomized trial in Guinea, were published in 2017.[83] This study demonstrated statistically significant protection, with no cases of EBOV among individuals from day 10 after vaccination in both randomized and nonrandomized clusters. The remarkable success of this phase III trial of the rVSV-EBOV vaccine should pave the way for future licensure.

Although promising results have been obtained in regard to EBOV vaccines in humans, many challenges remain. Among the most significant obstacles are the identification of a seemingly more pathogenic variant of MARV (variant Angola) in 2004–05 and the identification of a new

[a]References 21, 38, 39, 49, 58, 59.

species of EBOV, BDBV, in 2007. Until recently, filovirus vaccine development has primarily focused on two EBOV species, SUDV and EBOV, and one MARV variant (variant Musoke). Future efforts focusing on the filoviruses other than EBOV are needed. Preclinical studies in nonhuman primates suggest that multivalent filovirus vaccines are possible.[60–62]

Treatment

Before the 2013–16 EBOV epidemic, treating patients infected with MARVs or EBOVs in endemic areas consisted primarily of intensive supportive care directed toward maintaining effective blood volume and electrolyte balance. Several interventional therapies, including interferons, heparin, convalescent serum, and equine anti-EBOV IgG were used to treat natural and laboratory-acquired filovirus infections with little to no success.[59,84–86]

During the 2013–16 EBOV epidemic a number of different postexposure treatment approaches were used (reviewed in references 87 and 88). These primarily consisted of the interventions that showed the greatest promise as therapeutics in preclinical nonhuman primate models. These treatments included the anti-EBOV monoclonal antibody pool known as ZMapp (Mapp Biopharmaceutical; San Diego, CA), small interfering RNAs, and the small molecules favipiravir and GS-5734. Most of these interventions showed the ability to completely protect nonhuman primates against EBOV even when treatment was not initiated until the onset of illness or later. Clinical trials conducted during the West African EBOV epidemic using some of these drugs were unable to enroll enough patients to achieve statistically significant data; however, ZMapp showed promising results.[89] It was clear from all of the trials that patients with very high viral loads at the initiation of antiviral treatment were unlikely to survive, which is not surprising given that the case-fatality rate for EBOV is greater than 90% when the viral load exceeds 10^7 genome copies per mL of blood. Although most EBOV patients were treated in resource-poor settings in West Africa, some patients were treated in Europe or the United States. In these cases advanced supportive care, including real-time electrolyte balance, ventilation, and dialysis, appeared to be critically important successful treatment.[87,88,90,91] Specifically, only 2 of the 12 EBOV patients (17%) treated in the United States died. One patient who died shortly after arriving in the United States was at a very advanced stage of disease when treatment was initiated. There were also reports of 15 EBOV cases treated in Europe during the epidemic, of whom three died (20%). By comparison, mortality for EBOV reported in treatment centers in West Africa ranged from 37% to 4%.[92–96] It is evident from these cases that advanced supportive care clearly improved patient survival. It is also important to note that many EBOV patients who were medically evacuated to the United States or Europe also received various experimental antiviral therapies. Thus the combination of experimental antiviral interventions and advanced supportive care likely contributed to the improved outcome.

As with the development of preventive vaccines as a result of the 2013–16 EBOV epidemic, progress on the development of treatments for EBOV has outpaced the advancement of treatments for other filoviruses. However, the recent development of human monoclonal antibody MR-191, which completely protected nonhuman primates against both MARV and RAVV when treatment was delayed until after the onset of illness,[97] offers hope for other filoviruses. In addition, the recent investment in the development of panhuman monoclonal antibodies with antiviral activity against all species of EBOVs is encouraging.[98–100]

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165 Influenza Viruses, Including Avian Influenza and Swine Influenza

John J. Treanor

SHORT VIEW SUMMARY

Definition
- Influenza viruses are enveloped, negative-sense, single-stranded RNA viruses whose genome is segmented. They cause epidemic acute respiratory disease characterized by fever, cough, and systemic symptoms. Three types (A, B, and C) are recognized, with many subtypes within the type A viruses.

Epidemiology
- Influenza viruses are transmitted by the respiratory route and cause large epidemics, which generally occur during the winter in temperate climates. In addition to infecting humans, influenza A viruses infect a wide variety of animals, particularly migratory waterfowl. New influenza A virus subtypes sporadically emerge in humans to cause widespread disease, or pandemics.

Microbiology
- Influenza viruses are readily isolated in eggs or mammalian cell culture at 33°C. They undergo constant antigenic evolution, referred to as antigenic drift or shift, which allows them to reinfect individuals who have had previous infections.

Diagnosis
- In the context of recognized epidemics, influenza is usually diagnosed clinically on the basis of characteristic symptoms of fever and cough. Rapid detection of virus in respiratory secretions also can be accomplished through antigen detection or molecular techniques such as polymerase chain reaction assay.

Therapy
- Antiviral therapy with oseltamivir, zanamivir, or peramivir is available and may shorten the duration of illness and reduce the rate of complications. Therapy is most effective when used early in the course of illness (Table 165.1).

Prevention
- Influenza vaccines are effective in the prevention of influenza illness, although improved vaccines are needed.
- Inactivated and live-attenuated vaccines are available in trivalent and quadrivalent formulations (Table 165.2).
- The objectives of vaccination include protection of the individual and protection of the population through herd immunity. Antiviral drugs can also be used prophylactically in selected circumstances.

Influenza is an acute, febrile illness caused by infection with influenza type A or B virus that occurs in outbreaks of varying severity almost every winter in temperate climates, and year-round in tropical climates. The most common clinical manifestations are fever, malaise, and cough. Two unique features of influenza are the epidemic nature of the disease and the mortality that results in part from its pulmonary complications.

HISTORY

Influenza virus has been causing recurrent epidemics of febrile respiratory disease every 1 to 3 years for at least the past 400 years. Although the disease is not associated with a characteristic manifestation such as rash, the high attack rate, the explosive nature of the epidemic, and the frequency of cough allow the identification of past epidemics. For example, Hirsch tabulated 299 outbreaks occurring at an average interval of 2.4 years between 1173 and 1875.[1] The greatest pandemic in recorded history occurred in 1918–1919 when, during three "waves" of influenza, 21 million deaths were recorded worldwide, among them 549,000 in the United States.[2]

The modern understanding of influenza was ushered in with the isolation by Smith and associates of influenza A virus in ferrets in 1933.[3] Influenza B virus was isolated by Francis in 1939[4] and influenza C virus by Taylor in 1950.[5] The discovery by Burnet in 1936 that influenza virus could be grown in embryonated hens' eggs allowed extensive study of the properties of the virus and the development of inactivated vaccines.[6] Animal cell culture systems for the growth of influenza viruses were developed in the 1950s.[7] The phenomenon of hemagglutination, which was discovered by Hirst in 1941, led to simple and inexpensive methods for the measurement of virus and specific antibody.[8]

Evidence of the protective efficacy of inactivated vaccines was developed in the 1940s.[9] The use of live vaccines for influenza was first suggested shortly after the virus was discovered,[10] but the first live vaccine was not licensed in the United States until 2003, approximately 70 years later. Finally, antiviral agents in two classes have been approved for prevention and treatment of influenza. These include the so-called M2 inhibitors, amantadine in the mid-1960s, rimantadine in 1993, and the neuraminidase inhibitors (NIs) zanamivir, oseltamivir, and most recently peramivir.

THE VIRUSES

Classification

Influenza viruses belong to the family Orthomyxoviridae and are classified into three distinct types: influenza A, influenza B, and influenza C virus. There are significant differences in genetic organization, structure, host range, epidemiology, and clinical characteristics among the three influenza virus types (Table 165.3). However, all three viruses share certain features, including the presence of a host-cell–derived envelope, envelope glycoproteins of critical importance in virus entry and egress from cells, and a segmented genome of negative-sense (i.e., opposite of message sense), single-stranded RNA. The standard nomenclature for influenza viruses includes the influenza type, place of initial isolation, strain designation, and year of isolation. For example, the influenza A virus isolated by Francis from a patient in Puerto Rico in 1934 is given the strain designation A/Puerto Rico/8/34, sometimes referred to as PR8 virus. Influenza A viruses are further divided into subtypes based on the hemagglutinin (H, or HA) and neuraminidase (N, or NA) antigens (e.g., H1N1 or H3N2).

TABLE 165.1 Antiviral Chemotherapy and Chemoprophylaxis for Influenza

INDICATION	DRUG	ROUTE	DOSAGE
Influenza A and B: treatment	Oseltamivir	Oral	Adults: 75 mg bid ×5 days Children aged 1–12 yr: 30–75 mg bid, depending on weight,[a] ×5 days
	Zanamivir	Inhaled orally	Adults and children aged ≥7 yr: 10 mg bid ×5 days
	Peramivir	Intravenous	Adult: 600 mg IV once Children 2–12 yr: 12 mg/kg once
	Baloxavir	Oral	Adults: 80 mg PO once Children ≥12 yr and <80 kg: 40 mg PO once
Influenza A: treatment	Amantadine[b]	Oral	Adults: 100 mg qd or bid ×5–7 days Children aged 1–9 yr: 5 mg/kg/day (maximum, 150 mg/day) ×5–7 days
	Rimantadine[b]	Oral	100 mg qd or bid ×5–7 days in adults
Influenza A and B: prophylaxis	Oseltamivir	Oral	Adults: 75 mg/day Children aged ≥1 yr: 30–75 mg/day, depending on weight[a]
	Zanamivir	Inhaled orally	Adults and children aged ≥5 yr: 10 mg/day
	Peramivir	Intravenous	Not FDA approved for prophylaxis
Influenza A: prophylaxis	Amantadine[b] or rimantadine[b]	Oral	Adults: 200 mg/day Children aged 1–9 yr: 5 mg/kg/day (maximum, 150 mg/day)

[a]For detailed dosage recommendations in children aged <1 yr, see www.cdc.gov/flu/professionals/antivirals/summary-clinicians.htm.
[b]Amantadine and rimantadine are not considered for use because of widespread resistance in influenza A/H3N2 and A/H1N1 viruses currently circulating. They may be considered if sensitivities become reestablished.
FDA, US Food and Drug Administration.

TABLE 165.2 Influenza Vaccines Available in the United States, 2017–2018 Influenza Season

VACCINE (MANUFACTURER)	DOSE	AGES
IIV Quadrivalent, Standard Dose (SD-IIV4)		
Afluria Quadrivalent (Seqirus)	15 µg	≥5 yr
Fluarix Quadrivalent (GSK)	15 µg	≥3 yr
FluLaval Quadrivalent (ID Biomedical)	15 µg	≥6 mo
Fluzone Quadrivalent (Sanofi)	15 µg	≥6 mo
IIV Quadrivalent, Standard Dose, Cell Culture-based (ccIIV-4)		
Flucelvax Quadrivalent (Seqiris)	15 µg	≥4 yr
Recombinant Influenza Vaccine, Quadrivalent (RIV-4)		
Flublok Quadrivalent (Protein Sciences/Sanofi)	45 µg	≥18 yr
IIV Quadrivalent, Standard Dose, Intradermal (ID IIV-4)		
Fluzone Intradermal Quadrivalent (Sanofi)	9 µg	18–64 yr
IIV Trivalent, Standard Dose (SD-IIV-3)		
Afluria (Seqirus)	15 µg	≥5 yr
Fluvirin (Seqirus)	15 µg	≥4 yr
IIV Trivalent, High Dose (HD-IIV-3)		
Fluzone High Dose (Sanofi)	60 µg	≥65 yr
Adjuvanted IIV Trivalent, Standard Dose (aIIV-3)		
Fluad (Seqirus)	15 µg	≥65 yr
Live-Attenuated Influenza Vaccine, Quadrivalent (LAIV-4)		
FluMist Quadrivalent (MedImmune)	10^7 FFU	2–49 yr

For adults and older children, the recommended site of vaccination is the deltoid muscle. The preferred site for infants and young children is the anterolateral aspect of the thigh. Specific guidance regarding site and needle length for intramuscular administration may be found in the Advisory Committee on Immunization Practices General Recommendations on Immunization.
[a]Immunization providers should check US Food and Drug Administration–approved prescribing information for the most complete and updated information, including (but not limited to) indications, contraindications, and precautions. Package inserts for US-licensed vaccines are available at http://www.fda.gov/BiologicsBloodVaccines/Vaccines/ApprovedProducts/ucm093833.htm.
FFU, Fluorescent focus units; *ID,* intradermal; *IIV,* Inactivated influenza vaccine; *IIV-3,* inactivated influenza vaccine, trivalent; *IIV-4,* inactivated influenza vaccine, quadrivalent; *IM,* intramuscular; *IN,* intranasal; *LAIV,* live-attenuated influenza vaccine; *RIV,* recombinant influenza vaccine.
Modified from Centers for Disease Control and Prevention. Summary recommendations: prevention and control of influenza with vaccines: recommendations of the Advisory Committee on Immunization Practices (ACIP)—United States, 2017–18. http://www.cdc.gov/flu/professionals/acip/summary-recommendations.htm#table1.

TABLE 165.3 Differences Among Influenza A, B, and C Viruses

	INFLUENZA A	INFLUENZA B	INFLUENZA C
Genetics	8 gene segments	8 gene segments	7 gene segments
Structure	10 viral proteins M2 unique	11 viral proteins NB unique	9 viral proteins HEF unique
Natural host range	Humans, swine, equine, birds, marine mammals[a]	Humans only	Humans and swine
Epidemiology	Antigenic shift and drift	Antigenic drift only; two main lineages cocirculate	Antigenic drift only; multiple variants
Clinical manifestations	May cause large pandemics with significant mortality in young persons	Severe disease generally confined to older adults or persons at high risk; pandemics not seen	Mild disease without seasonality

[a]Influenza A viruses have also been isolated from mink, dogs, and cats.

Virology

Influenza viruses are enveloped viruses that may exist in spherical or filamentous forms of 80 to 120 nm (Fig. 165.1), with surface projections consisting of HA and NA spikes. A schematic diagram of an influenza A virus is shown in Fig. 165.2.

The HA is the viral attachment protein, and the receptor binding site is located in the globular head of the molecule. Each rod-shaped HA spike measures approximately 4 nm in diameter by 14 nm in length. They can be removed from the intact virion by sodium dodecyl sulfate, by bromelain, or by chymotrypsin. Each spike is a trimer composed of three HA polypeptides, each with a molecular weight of 75,000 to 80,000, resulting in a trimer with a molecular weight of approximately 224,640. The HA is synthesized as a monomer (HA_0), which is cleaved by host-cell proteases into HA_1 and HA_2 components that remain linked together.

In birds, the ability of the HA to be cleaved by proteases plays an important role in pathogenesis. Proteases capable of cleaving the HA of avirulent viruses, such as tryptase Clara,[11] are restricted in distribution to cells of the respiratory and gastrointestinal mucosa, thereby limiting replication to these areas. However, addition of several basic amino acids to the cleavage site[12] renders the HA capable of being cleaved by ubiquitous cellular furin-like proteases[13] and allows these viruses to escape the confines of the mucosa and replicate systemically in chickens.[14] However, the potential role of HA cleavability in pathogenesis in humans is unknown.

FIG. 165.1 **Electron micrograph of influenza A/USSR/77 H1N1 (×189,000).**

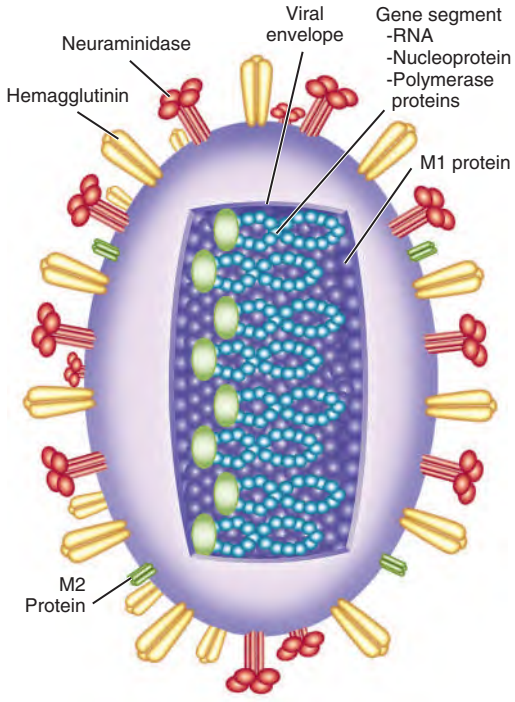

FIG. 165.2 **Schematic model of an influenza A virus.**

The viral NA is an enzyme that catalyzes the removal of terminal sialic acids (*N*-acetyl neuraminic acid) from sialic acid–containing glycoproteins. The NA spike is shaped like a mushroom rather than a rod and has a molecular weight of 240,000. The intact NA consists of a tetramer of NA polypeptides, each with a molecular weight of 58,000. The enzyme active site is located in the mushroom-shaped head.

At least 16 highly divergent, antigenically distinct HAs have been described in influenza A viruses (H1 to H16), in addition to at least nine distinct NAs (N1 to N9). In addition, two distinct HA subtypes have been detected in bats,[15,16] and two possible neuraminidase subtypes, although without actual neuraminidase activity.[17] Based on sequence comparisons, the HA subtypes of influenza A viruses can be grouped into two subgroups: group 1, containing H1 and H5 among others, and group 2, containing H3 and H7. A third integral membrane protein, the M2 protein, is also present in small amounts on the viral envelope.

Interior to the envelope is the matrix, or M1, protein. This protein provides structure to the virion and is important for virus assembly. Within the virion are eight physically discrete nucleocapsid segments (Table 165.4). Each nucleocapsid is composed of a single segment of genomic RNA intimately associated with the viral nucleoprotein (NP), with the three polymerase proteins PB1, PB2, and PA bound to one end. Two nonstructural viral proteins, NS1 and NS2 (also referred to as the nuclear export protein, or NEP), are also found within infected cells. Small amounts of NEP are present within virions.

Although influenza B viruses have a similar structure, they do not exhibit the same type of antigenic and genetic variation in the HA and NA, and therefore do not have subtypes. However, since 2001, two antigenically distinct lineages of influenza B viruses, termed the "Victoria" lineage and the "Yamagata" lineage, have cocirculated in humans.[18]

Influenza viruses enter the cell by attachment of the viral hemagglutinin to sialic acid–containing receptors on the cell membrane, followed by internalization of the virus into an acidic endosome. In the acidic environment of the endosome, the HA undergoes a conformational change that liberates a fusion peptide and results in fusion of the viral envelope with the endosomal membrane. At the same time, the third envelope protein, the M2 protein, acts as an ion channel allowing H^+ ions to enter the virion from the endosome. This in turn allows the viral gene segments to leave the virion and enter the cytoplasm, a process known as uncoating.

Viral gene segments are transported to the nucleus, where the viral polymerase complex, composed of the proteins PB1, PB2, and PA, directs the synthesis of the plus-sense messenger RNA and synthesis of negative-sense copies that will serve as progeny genomic RNA. The polymerase proteins also play a role in disruption of host cell protein synthesis. Because replication takes place in the nucleus, some mRNAs are spliced, giving rise in the case of influenza A viruses to the M2 protein from the M gene segment, and the NS2 or NEP (nuclear export protein) from the NS gene segment. Alternative start codons also give rise to a number of additional proteins from the polymerase genes of influenza A viruses including PB1-F2[19] and PA-X,[20] which may play roles in pathogenesis.[21]

TABLE 165.4 Genes and Protein Products of Influenza A Virus

RNA SEGMENT	GENE PRODUCT	NAME	PROPOSED FUNCTIONS
1	PB2	Basic polymerase 2	Cap-binding of host mRNA molecules
2	PB1 PB1-F2	Basic polymerase 1	RNA-dependent RNA polymerase Proapoptotic, immune evasion, proinflammatory
3	PA PA-X	Acidic polymerase	Endonuclease activity Multiple functions, inhibits host innate response
4	HA	Hemagglutinin	Viral attachment to cell receptor, membrane fusion
5	NP	Nucleoprotein	Encapsidates RNA, forms ribonucleoproteins
6	NA	Neuraminidase	Releases virus from cell, prevents aggregation of virions
7	M1 M2	Matrix protein 1 Matrix protein 2	 Ion channel, required for uncoating
8	NS1 NEP	Nonstructural protein 1 Nuclear export protein	Antagonizes interferon Transport of newly assembled ribonucleoproteins from nucleus to cytoplasm

Negative-sense daughter virion RNAs are encased in nucleoprotein, associated with one copy of the polymerase complex, and transported to the cytoplasm for assembly at the cell surface. Envelope proteins are glycosylated and transported to the cell surface. Virions bud from selected lipid rafts at the cell surface, acquiring an envelope derived from the cell membrane and decorated with HA, NA, and small amounts of M2 protein. Finally, the NA removes sialic acid from receptors on the cell surface or on the viral envelope, allowing the progeny viruses to leave the infected cell.

Because the genome of influenza virus is segmented, segments can be exchanged between viruses infecting the same cell, a process referred to as reassortment. Genetic reassortment plays an important role in the generation of pandemic influenza A viruses, and has also been taken advantage of for the construction of attenuated live influenza vaccines.

EPIDEMIOLOGY AND DISEASE IMPACT

Disease Impact

Influenza epidemics are regularly associated with excess morbidity and mortality, usually expressed in the form of excess rates of pneumonia and influenza-associated hospitalizations and deaths during epidemics. In order to estimate the mortality burden of influenza, observed pneumonia- and influenza-related deaths during periods of influenza epidemic activity are compared with an expected seasonal baseline derived from a time-series regression model, and the excess mortality attributable to influenza is calculated. A historical tabulation of levels of excess pneumonia and influenza deaths attributable to influenza epidemics[22] is shown in Table 165.5, along with the estimated percentage of isolates that were typed as influenza A (H3N2), A (H1N1), or B in each year. In general, the level of excess mortality is highest in years when influenza A (H3N2) viruses predominate, but influenza B and to a lesser extent H1N1 viruses also can be associated with excess mortality. Because not all influenza-related deaths manifest as pneumonia, the pneumonia and influenza mortality statistics probably underestimate the true impact of influenza on the population.[23] Table 165.5 also lists the all-cause excess mortality, defined as deaths due to any cause, above a similarly derived baseline, that occur during periods of influenza epidemic activity. As many as 51,000 deaths annually in the United States can be attributed to influenza.[24] After correction for underreporting by assessing the fraction of cases with diagnostic testing, it has been estimated that there were between 155,000 and 624,000 hospitalizations, 18,000 to 95,000 intensive care unit (ICU) admissions, and 5000 to 27,000 deaths per year in the United States attributable to influenza.[25] Surveillance data have suggested that pneumonia and influenza hospitalizations and inpatient deaths in the United States may have declined from 1996 to 2011, coincident with expanded recommendations for influenza vaccination and the introduction of conjugate pneumococcal vaccines.[26]

Mortality is only the most severe manifestation of influenza impact, and similar techniques can be used to estimate excess morbidity due to influenza epidemics.[27] Data from the Tecumseh Community Health Study have been used to estimate that influenza is responsible for 13.8 to 16.0 million excess respiratory illnesses per year in the United States among individuals younger than 20 years, and for 4.1 to 4.5 million excess illnesses in older individuals.[27] Much of the impact of influenza is related to the malaise and consequent disability that it produces, even in young, healthy individuals. It has been estimated that a typical case of influenza, on average, is associated with 5 to 6 days of restricted activity, 3 to 4 days of bed disability, and about 3 days lost from work or school.[28] Direct medical costs of illness account for only about 20% of the total expenses of a case of influenza, with a major proportion (30%–50%) of the economic impact due to loss of productivity.[29] In one study, influenza in schoolchildren resulted in 37 missed school

TABLE 165.5 Estimated Excess Pneumonia- and Influenza-Related Deaths and Excess Mortality of All Causes During Influenza Epidemics

YEAR	PERCENT OF ISOLATES THAT WERE OF THE FOLLOWING (SUB)TYPE			PNEUMONIA- AND INFLUENZA-RELATED EXCESS DEATHS (RANGE)	ALL-CAUSE EXCESS DEATHS (RANGE)
	H3N2	H1N1	B		
1972/73	90	0	10	7900 (5500–10,300)	18,300 (1200–35,000)
1973/74	20	0	80	0	0
1974/75	100	0	0	6500 (4100–8900)	15,100 (0–32,100)
1975/76	70	0	30	11,800 (9200–14,400)	24,600 (3400–45,900)
1976/77	5	0	95	0	0
1977/78	60	26	14	8300 (6000–10,500)	46,200 (19,800–72,700)
1978/79	0	98	2	0	0
1979/80	2	1	97	5100 (3500–6700)	17,300 (600–34,100)
1980/81	77	23	0	11,700 (9,100–14,200)	47,200 (27,800–66,600)
1981/82	1	24	75	2100 (600–3700)	0
1982/83	79	10	11	4700 (2800–6700)	9600 (0–19,200)
1983/84	5	50	45	3500 (1600–5400)	8200 (0–17,600)
1984/85	97	0	3	8100 (6600–9600)	36,200 (17,700–54,700)
1985/86	24	0	76	6700 (4900–8500)	34,000 (6800–61,200)
1986/87	—	—	—	1800 (1100–2500)	16,800 (1900–31,700)
1987/88	0	80	20	7400 (5600–9100)	33,400 (12,900–53,800)
1988/89	45	45	10	5100 (3600–6600)	10,500 (800–20,200)
1989/90	90	1	9	10,100 (8500–11,700)	43,600 (27,600–59,600)
1990/91	4	3	93	4200 (2400–6100)	23,000 (0–46,000)
1991/92	19	81	0	6600 (5600–7700)	41,700 (19,600–63,700)

Modified from Simonsen L, Clarke MJ, Williamson DW, et al. The impact of influenza epidemics on mortality: introducing a severity index. Am J Public Health. *1997;87:1944–1950.*

days by children and 20 days of missed work by parents, per 100 children.[30] Influenza is also associated with decreased job performance in working adults[31] and reduced levels of independent functioning in older adults.[32]

Influenza is usually associated with a U-shaped epidemic curve. Attack rates are generally highest in the young, whereas mortality is generally highest among older adults (Fig. 165.3).[33] In some studies, up to three-fourths of all influenza-related deaths occur in those older than 65.[25] Excess morbidity and mortality are also high in those with certain high-risk medical conditions, including adults and children with cardiovascular and pulmonary conditions such as asthma, or those requiring regular medical care because of chronic metabolic disease, renal dysfunction, hemoglobinopathies, or immunodeficiency, and in individuals with neurologic conditions that compromise the handling of respiratory secretions.[34] A summary of these conditions is shown in Table 165.6. Influenza-related death rates in nursing home residents with comorbid conditions are as high as 2.8% per year.[35]

The increased risk of influenza during pregnancy was dramatically demonstrated during the 2009 pandemic.[36] Previous studies had identified an increased risk of hospitalization associated with influenza epidemics during pregnancy, especially in the second and third trimesters and in the immediate postpartum period.[37] During the 2009 H1N1 pandemic, women in each stage of pregnancy, or in the immediate postpartum period, were significantly overrepresented among those admitted to hospitals and ICUs.[36,38] A meta-analysis has suggested that the overall risk of influenza-related hospitalization was more than twice as great during pregnancy.[39] The mechanism(s) by which pregnancy enhances the risk of influenza are not clear but might include the increased cardiovascular demands of pregnancy, and hormonally mediated changes to the innate and adaptive immune response.[40]

Obesity also emerged as a risk factor for influenza morbidity and mortality during the 2009 pandemic that had not been recognized in previous seasonal epidemics or pandemics.[41,42] Although compromise to respiratory mechanics as a direct result of extreme obesity undoubtedly plays a role, there is also evidence to support a detrimental role of adipose tissue in the inflammatory response that might also enhance the influenza disease process.

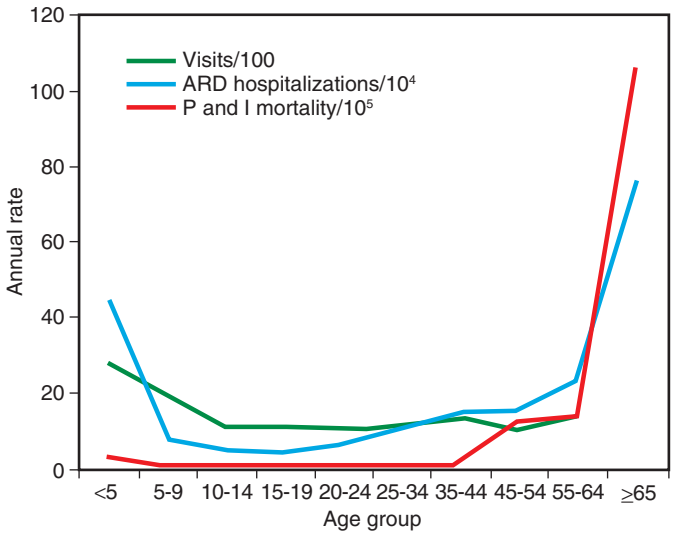

FIG. 165.3 Typical epidemic curve in the interpandemic era. Shown are the rates of medically attended illness (*green line,* rate per 100), hospitalizations for acute respiratory disease (ARD, *blue line,* rate per 10,000), and pneumonia *(P)*- and influenza *(I)*-related mortality (*red line,* rate per 100,000) by age for several seasons of influenza in Houston, Texas. Attack rates and hospitalizations occur at both extremes of age, but mortality occurs largely in those older than 65 years. *(Data from Glezen WP, Keitel WA, Taber LH, et al. Age distribution of patients with medically attended illnesses caused by sequential variants of influenza A/H1N1: comparison to age-specific infection rates, 1978–1989.* Am J Epidemiol. *1991;133:296–304.)*

Influenza is recognized as an important health problem in young children. Rates of influenza-related hospitalizations are particularly high in healthy children younger than 2 years, in whom rates approach those of older children with high-risk conditions.[43–45] In addition, a high rate of secondary complications, particularly otitis media and pneumonia, occur in children with influenza infection.[46] Rates of outpatient clinic visits for laboratory-documented influenza have been observed at 50 to 95 per 1000 person-years, and of emergency room visits at 6 to 27 per 1000 person-years in children younger than 5.[47] Although rare, influenza-related deaths occur each year in previously healthy children.[34] Notably, many of these deaths occur in children who were not recognized to have high-risk conditions before their illness.

Epidemic Influenza

An epidemic is an outbreak of influenza confined to one location, such as a city, town, or country. A graphic description of a typical and well-characterized epidemic due to influenza A (H3N2) in 1976 is shown in Fig. 165.4. The epidemic began abruptly, reached a sharp peak in 2 to 3 weeks, and lasted 5 to 6 weeks.[48] The first indicator of influenza activity was reports of increased numbers of children with febrile respiratory illness, followed by the occurrence of influenza-like illnesses among adults. These reports were subsequently followed by increased hospital admissions for patients with pneumonia, exacerbation of chronic obstructive pulmonary disease, croup, and congestive heart failure, and finally by increased school and industrial absenteeism. However, individual seasonal epidemics do not always follow this pattern.

During epidemics, attack rates in unvaccinated populations are estimated to be 10% to 20%, but rates as high as 40% to 50% have been reported.[49] The factors that lead to termination of an outbreak in any given location are unclear, but often the outbreak ends before the theoretical supply of susceptible individuals is exhausted.

In temperate climates in either hemisphere, epidemics occur almost exclusively in the winter months (generally November to April in the Northern Hemisphere and May to September in the Southern Hemisphere). Seasonal periodicity is also observed in tropical climates, with increased activity during periods of low absolute humidity, although influenza can occur throughout the year and seasonal fluctuations are not as marked.[50] The reasons for these seasonal changes are not entirely clear but might be the result of more favorable environmental conditions for virus survival.[51] Using a statistical model called convergent cross mapping, temperature and absolute humidity were shown to be important

TABLE 165.6 Groups at Higher Risk for Influenza Complications

Children and Adolescents at Higher Risk for Influenza Complications
Children younger than 4 years
Children with chronic pulmonary (including asthma), cardiovascular (except hypertension), renal, hepatic, hematologic, or metabolic (including diabetes mellitus) disorders
Children who are immunosuppressed, including children infected with human immunodeficiency virus and those taking immunosuppressive medications
Children with a condition that can compromise respiratory function or handling of respiratory secretions that can increase the risk for hypertension (e.g., cognitive dysfunction, spinal cord injuries, seizure disorders, or other neuromuscular disorders)
Children who are receiving long-term aspirin therapy and who therefore might be at risk for developing Reye syndrome
Children who are residents of chronic-care facilities
Those who will be pregnant during the influenza season
Adults at Higher Risk for Influenza-Related Complications
Persons aged 65 years or older
Women who will be pregnant during the influenza season
Persons with chronic pulmonary (including asthma), cardiovascular, renal, hepatic, hematologic, or metabolic disorders (including diabetes mellitus)
Persons who have immunosuppression (including immunosuppression due to medication or human immunodeficiency virus infection)
Persons with any condition that can compromise respiratory function or the handling of respiratory secretions or increase the risk of aspiration.
Persons with obesity, defined as a body mass index greater than 40
Residents of nursing homes and other chronic-care facilities

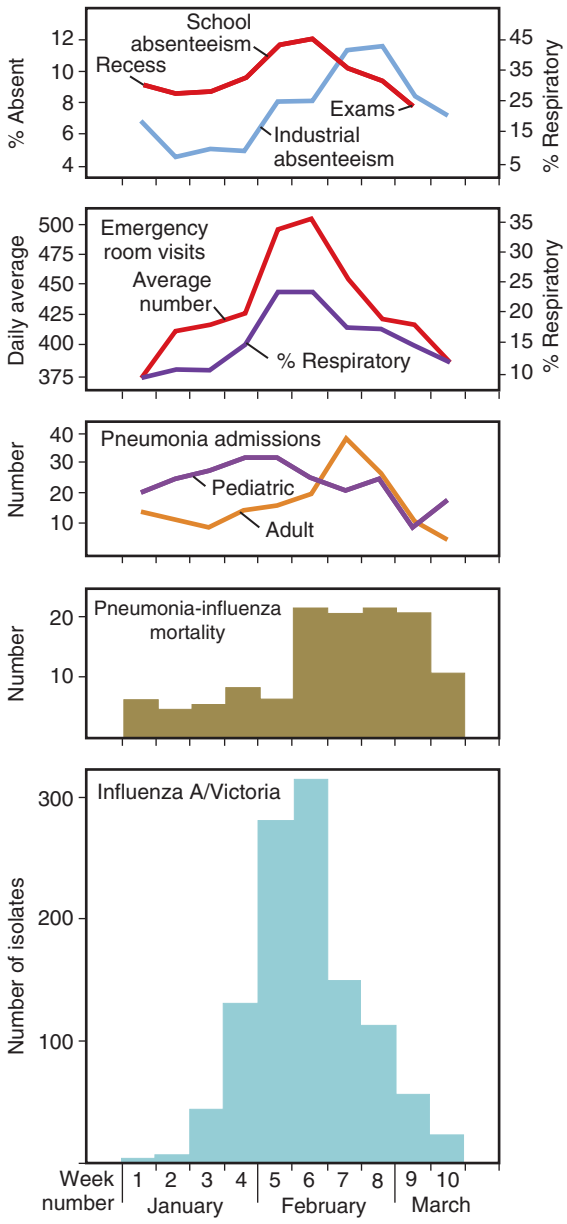

FIG. 165.4 Correlation of the nonvirologic indexes of epidemiologic influenza with the number of isolates of A/Victoria virus according to week, Houston, 1976. Industrial absenteeism is determined by the percentage with respiratory complaints. *(From Glezen WP, Couch RB. Interpandemic influenza in the Houston area, 1974–1976.* N Engl J Med. *1978;298:587–593.)*

drivers of seasonal epidemicity.[52] Studies in a model of transmission of influenza in guinea pigs have also supported a role for conditions of cold temperature and dry humidity in facilitating transmission.[53] Seasonality may also be associated with behavioral changes that may increase transmission, such as indoor crowding or school attendance. Possibly for this reason, the effects of weather variability may be greater in young children.[54] Modeling studies based on medical claims data have suggested that winter holidays delay seasonal epidemic peaks and shift disease visits toward adults.[55]

In general, a single strain of influenza virus will be the predominant cause of cases during an epidemic. However, this is not always the case, and in some seasons two different lineages or strains within a single subtype or two different influenza A subtypes (H1N1 and H3N2) or concomitant outbreaks of influenza A and B have occurred. In some years, the end of the influenza epidemic season is characterized by cases due to a new strain. These limited outbreaks, which have been referred to as a "herald wave," sometimes predict the predominant strain in the next influenza season.[56]

Transmission

Influenza viruses are transmitted from person to person via the respiratory route. Three potential modes of transmission have been suggested.[57] Coughing and sneezing could generate small-particle aerosols (<10 μm mass diameter) that can remain suspended in air for many hours and could transmit infection to individuals at a substantial distance. Larger particles or droplets will typically fall to the ground within 3 meters of the infected person and would be expected to infect individuals in direct contact. Finally, viral particles could land on surfaces, where influenza viruses remain infectious and could infect others through indirect contact. There is substantial evidence for all three modes of transmission in experimental studies and epidemiologic observations, but the relative roles of each mode of transmission are uncertain and remain controversial, with obvious implications for infection control practices and for potential interventions to mitigate pandemics.

Small-particle aerosols are generated by infected humans, and influenza genome can be detected in these small particles by polymerase chain reaction (PCR) techniques.[58,59] It has not been proven that these aerosols contain significant amounts of infectious virus, but experimental studies in humans have shown that very small amounts (approximately five infectious particles) may be sufficient to infect humans by the aerosol route.[60,61] Aerosol transmission has also been demonstrated in animal models in which infected and exposed ferrets or guinea pigs are separated by several meters, with transmission occurring in the direction of airflow.[53,62]

Airborne transmission has also been implicated in multiple observations of outbreaks in which an airborne route of transmission appears to be the most plausible explanation for the characteristics of the outbreak. The most often cited such outbreak occurred in a commercial airliner that was delayed for approximately 4½ hours with a poorly functioning ventilation system. The risk of transmission of influenza A from the index case to other passengers was related to the amount of time passengers spent on the aircraft, and not on their seating proximity to the index case. Because most of the passengers did not have direct contact with the index case, airborne transmission appears to be likely.[63] In a well-investigated hospital outbreak, nosocomial cases occurred significantly less frequently in a hospital ward where the air was treated with ultraviolet (UV) light than in an otherwise similar ward without UV light treatment.[64] In an outbreak in a long-term care facility, there appeared to be an association between the risk of nosocomial influenza and the air-handling systems in several wards.[65]

Additional observations consistent with airborne transmission were made during an early study of zanamivir prophylaxis of influenza in families. In this study,[66] subjects who received short-term prophylaxis with inhaled zanamivir were protected compared with placebo recipients, but recipients of zanamivir administered by nasal spray were not. In addition, the combination of nasal and inhaled zanamivir was no better than inhaled zanamivir alone.

In most of these outbreaks, there were alternative explanations for the observations that could at least partially explain the epidemic behavior without requiring aerosol transmission,[67] and the real role of aerosol transmission remains controversial. If aerosol transmission plays a dominant role in influenza, then health care workers would need to wear filtering face masks, and patients would require negative pressure isolation, to prevent nosocomial transmission of influenza. This has prompted several studies that have attempted to evaluate the role of face masks in infection prevention in hospitals. In one large randomized trial, nursing staff who were randomly assigned to wear N95 respirators had the same rate of influenza as staff assigned to wear simple surgical masks while caring for patients with influenza.[68] This study suggests that airborne transmission does not play a major role at least in nosocomial influenza, although it has been pointed out that cases in the N95 group could have been acquired outside the hospital, and that compliance with use of these masks is frequently poor. In contrast, hand hygiene and simple surgical masks were reported to be modestly effective in the prevention of influenza transmission in households,[69] suggesting that in this setting, droplet spread was the predominant modality.

Pandemic Influenza

Pandemics are typically associated with the emergence of an antigenically variant influenza virus toward which the population has little or no prior immunity. The World Health Organization (WHO) has defined four levels of influenza pandemic activity (interpandemic, alert, pandemic, and transition), and currently defines pandemic influenza as the period of global spread of human influenza caused by a new influenza subtype.[70]

The epidemiologic behavior of pandemics differs in many ways from that of epidemic, seasonal influenza. In addition to higher attack rates overall with substantially greater disease impact, pandemics are typically associated with a different age distribution of cases, with greater impact in younger persons and relative sparing of the elderly. The reasons for this are unclear, although in some pandemics older segments of the population may have been exposed to antigenically related viruses in childhood, sometimes referred to as "antigenic recycling." Pandemics also often occur outside of the usual window of seasonality, possibly because their inherently greater transmissibility allows them to spread even under conditions that would not be favorable for transmission of seasonal influenza viruses. Many pandemics are characterized by multiple waves of activity during the pandemic period, such as the H1N1 pandemic, with an initial wave in the spring followed by a more severe wave of cases in the early fall in much of the United States. The intervals between pandemics are quite variable and unpredictable, but it is likely that pandemics of influenza will continue to occur in the future.

ANTIGENIC VARIATION

One of the unique and most remarkable features of influenza virus is the frequency with which changes in antigenicity occur, collectively referred to as antigenic variation. Alteration of the antigen structure of the virus leads to infection with variants to which little or no resistance is present in the population at risk. The phenomenon of antigenic variation helps explain why influenza continues to be a major epidemic disease of humans. Antigenic variation involves principally the two external glycoproteins of the virus, HA and NA, and is referred to as antigenic drift or antigenic shift, depending on whether the variation is small or great.

Antigenic Drift

Antigenic drift refers to relatively minor antigenic changes that occur frequently within the HA and/or NA of the virus. Drift has been studied most intensively for the HA and is the result of gradual accumulation of amino-acid changes in one or more of the five identified major antigenic sites on the HA molecule.[71] Because antibody generated by exposure to previous strains does not neutralize the antigenic variant as effectively, immunologic selection takes place and the variant supplants previous strains as the predominant virus in the epidemic. Antigenic variants can be produced in cell culture in the presence of limiting amounts of antibody, and these variants have similar single amino-acid sequences in the HA similar to those of drift variants.[72]

Although not studied as intensively, antigenic drift also occurs by amino-acid substitutions in the antibody epitopes in the NA.[73] A phenomenon analogous to antigenic drift has also been described for some epitopes recognized by cellular immune effectors.[74] Fitness costs to the virus, and the diversity of HLA types in the population, have been speculated to limit the extent of such variability.[75]

Comparison of the HA gene sequences of influenza viruses isolated in successive years reveals differences in the patterns of HA evolution among influenza A, B, and C viruses. In general, a single lineage, or relatively few lineages, of influenza A virus circulate in humans, and the accumulation of point mutations in the HA is linear, with each strain replacing the previously circulating one.[76] In contrast, multiple lineages of influenza C virus cocirculate, as shown by sequence comparisons of the HEF gene. The evolution of influenza B viruses is somewhere between these two examples, with relatively few lineages of the HA gene (but more than one) cocirculating.[77] Currently, two antigenically distinct lineages of influenza B viruses, referred to as the "Yamagata" lineage and the "Victoria" lineage, are cocirculating worldwide.

Although there is generally a very tight correlation between genetic changes and antigenic distance[78] the antigenic significance of any individual mutation can be more difficult to assess.

Antigenic Shift

At unpredictable intervals, influenza A viruses with more radical changes in the antigenicity of the HA and/or NA have emerged to cause widespread disease, or pandemics. These major antigenic changes are referred to as antigenic shifts, and result in viruses toward which the population has little or no prior immunity and is therefore highly susceptible. Fig. 165.5 ties together the concepts of antigenic shift and antigenic drift in relation to population immunity.[79] When a new virus, here called HxNx, to which immunity is lacking, is introduced into a population, pandemic influenza results. After one or more waves of pandemic influenza, the proportion of immune individuals in the population increases. Subsequent epidemics of seasonal epidemics due to strains of influenza A/HxNx that exhibit antigenic drift occur, with selection of new variants. After an unpredictable period of circulation of antigenic variants of the HxNx virus, a new virus, HyNy, will emerge, and the process repeats itself.

The pattern of replacement of HA and NA subtypes during the most recent century of pandemics is shown in Fig. 165.6. In addition, serologic studies have suggested that H3 subtype viruses circulated before 1918.[80] Virologic and PCR studies have shown that the "Spanish" pandemic of 1918 (mentioned previously) was caused by an H1N1 virus, which in turn was supplanted in the "Asian" pandemic of 1957 by H2N2 viruses. In 1968, the "Hong Kong" pandemic was caused by viruses of the H3N2 subtype. In 1977, viruses of the H1N1 subtype were reintroduced through an unknown mechanism. These viruses are genetically identical to the H1N1 viruses that were circulating in 1950. From 1977 to 2009, these viruses cocirculated with those of the H3N2 subtype. In 2009, a new variant of H1N1 viruses, referred to as pH1N1, emerged from pigs and replaced the previous H1N1 viruses.

The origin of new pandemic strains has been the subject of intense interest and study, for obvious reasons. The most plausible explanation for their origin takes into account three features of this phenomenon: that the virus has a segmented genome, that pandemics occur only with influenza A viruses, and that influenza A viruses, but not other influenza viruses, maintain a large reservoir of genetic diversity, primarily in birds.

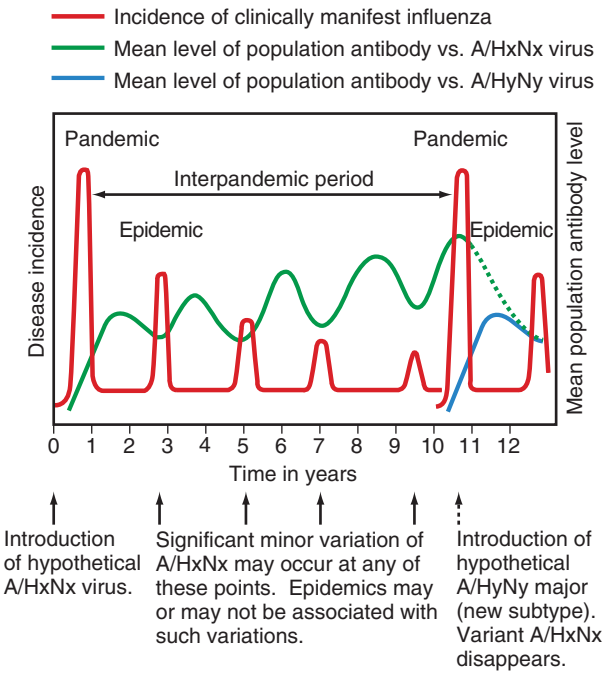

FIG. 165.5 Schema of the occurrence of influenza pandemics and epidemics in relation to the level of immunity in the population. A/HxNx and A/HyNy represent influenza viruses with completely different hemagglutinins and neuraminidases. *(Modified from Kilbourne ED. The epidemiology of influenza. In: Kilbourne ED, ed.* The Influenza Viruses and Influenza. *New York: Academic Press; 1975:483.)*

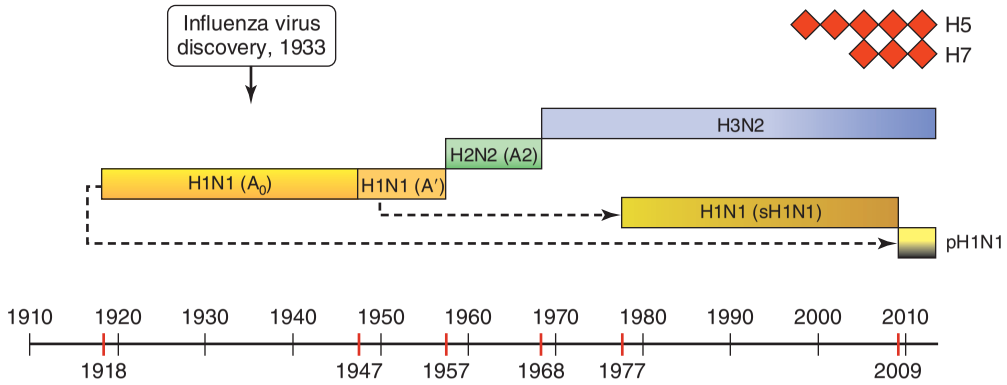

FIG. 165.6 Recent pandemics of influenza. The duration of circulation of viruses of various subtypes is shown by the boxes. Because the nature of influenza epidemics prior to 1918 is known only by serologic means, those boxes are *shaded tan*. Below the time line are the ages of individuals in 2004 who were alive during the various epidemic periods of earlier influenza subtypes. For example, individuals currently living who are between the ages of 47 and 86 probably experienced their first influenza A infection as an H1N1 virus. Individuals who are 36 years of age or younger have never been infected with H2N2 viruses.

Emergence of Pandemic Viruses From Birds

Extensive surveillance studies have identified influenza A viruses of 16 HA subtypes and all 9 NA subtypes in migratory waterfowl. In these birds influenza A causes mild illness or may be shed asymptomatically at high levels and for long duration in the feces. These birds may transmit influenza to other animals, including domestic poultry, horses, swine, and marine mammals, which may in turn transmit these viruses to humans. Comparisons of sequence data from animal and human influenza viruses isolates has suggested that the 1918 virus was introduced into humans from such an animal population. In contrast, the 1957 and 1968 pandemic influenza viruses were reassortant viruses that derived some genes from previously circulating human viruses, while deriving the HA and sometimes NA genes from an avian influenza virus.[81]

Because of the likely role of avian influenza viruses in the generation of emerging pandemics, there has been intense interest in recent outbreaks involving transmission of avian influenza viruses to humans, with resulting disease. Most of these transmission events have been quite limited, with small numbers of persons affected, relatively mild disease, and little or no evidence of person-to-person transmission. In most cases, virus has been transmitted to humans from infected domestic poultry, but cases have also occurred in association with marine mammals and possibly wild birds. These infections have involved primarily viruses of the H7, H5, H9, and H10 subtypes, with H7 and H5 viruses being most common.

Human Infections With H5 Viruses

H5N1 viruses were first recognized in humans in 1997[82] and have continued to cause substantial numbers of human cases since that time. The status of H5N1 infection is updated monthly by WHO. From 2003 until 2018, a total of 860 laboratory confirmed human cases of H5N1 infection, of which 454 (53%) cases were fatal, and 19 cases of H5N6 infection had been reported to WHO. Patients have ranged in age from 3 months to 75 years, with the median age being 20 years. Half of all cases have been in people younger than 20 years, and 90% of cases have been in people younger than 40 years. The median duration from onset of illness to hospitalization has been 4 days (range, 0–18 days). The case-fatality rates have been the highest for those in the 10- to 19-year-old age group, lowest for people 50 or older, and in between for children aged <10 years.[83]

Most cases have had close contact with ill poultry in the week before the onset of illness. Activities such as plucking and preparing diseased birds; playing with birds, especially asymptomatically infected ducks; and handling fighting cocks are risk factors for infection.[84] Other apparent modes of acquisition have included eating undercooked poultry or drinking raw duck blood, or exposure to contaminated water.[85] Person-to-person transmission has been rare, although family clusters of infection involving two or more family members have been documented.[86,87] Sequence comparisons of viruses from human cases and those of concurrent avian cases have established that at least some of the cases in the cluster represented person-to-person transmission.

Descriptions of the signs and symptoms of H5N1 infection are mostly from hospitalized patients. Most patients have presented with nonspecific complaints of a fever, cough, and shortness of breath. In many of the patients, there is a progression of symptoms leading to respiratory failure necessitating ventilation and other supportive measures. Atypical symptoms such as nausea, vomiting, encephalopathy, and bleeding gums and nose have been reported.[88] Watery diarrhea may be present before the onset of respiratory symptoms. The majority of patients have an abnormal chest radiograph with diffuse and multifocal or patchy infiltration, but pleural effusions are rare.

Laboratory abnormalities include significant lymphopenia and leucopenia, mild-to-moderate thrombocytopenia, and elevated transaminases. These abnormalities are poor prognostic signs.[89] Most patients have had negative bacteriologic cultures of sputum and blood. Pathologic changes include diffuse alveolar damage in the lungs, reactive hemophagocytosis in the marrow, and lymphoid depletion with atypical lymphocytosis in the spleen and lymphoid tissues.[90] Centrilobular hepatic necrosis and acute tubular necrosis have been reported.

Highly pathogenic avian H5N1 viruses have undergone significant genetic diversification and dissemination throughout Asia, the Middle East, and Europe since their initial detection, especially after a large outbreak in birds at Qinghai lake in Western China in 2005.[91] These viruses are currently classified into 10 clades (clades 0–9), of which viruses in clade 0, 1, 2, and 7 have been responsible for disease in humans.[92] Clade 2 viruses have been further classified into subclades, which are antigenically distinct. Current human infections have been mainly due to clade 2 viruses, including subclades 2.1, 2.2, 2.3, and 2.4.

Human Infections With H7 Viruses

Sporadic human infections with H7 viruses have been detected for many years, and early infections were typically relatively mild and limited. A characteristic of these infections has been the presence of conjunctivitis.[93,94] Human cases have typically been associated with outbreaks in birds, although one case of human H7 infection was reported in a laboratory worker who was sneezed on by a seal that was infected with an H7N7 influenza virus.[95] A large cluster of H7N7 infections of humans occurred in 2003 in association with an outbreak of highly pathogenic avian influenza in commercial poultry farms in the Netherlands.[96,97] Although almost all cases in this outbreak were mild or subclinical, there was one confirmed fatal case in a 57-year-old otherwise healthy veterinarian, who developed pneumonia.[97] Subsequent serologic surveys have suggested that there were substantial numbers of asymptomatic infections in this outbreak.[98] Since that time, several additional, small outbreaks of human infections with H7N7 and H7N3 viruses have been reported.[99–101]

The spectrum of disease associated with H7 viruses was radically altered when major outbreaks of severe human disease due to viruses

of the H7N9 subtype began to occur, first noted in Shanghai and with subsequent cases throughout a large area of eastern China. These outbreaks occurred annually between 2013 and 2017, during the winter with noted cocirculation of seasonal human H3N2 viruses. Coinfection with both H3N2 and H7N9 viruses has been reported,[102] but does not appear to have spread reassorted viruses.

Infection was associated with severe respiratory illness resulting in acute respiratory distress syndrome (ARDS) and ICU admissions, with a case-fatality rate of 27% among recognized cases.[103] Lymphocytopenia was seen in almost all of the patients, and the majority had extensive consolidation on chest radiographs, with ground-glass opacities. In contrast to previous outbreaks of avian influenza in humans, the mean age of the affected patients (61 years) was substantially higher, and 42% of those affected were 65 or older. Older age and the presence of chronic medical conditions have been demonstrated to be risk factors for severe illness, somewhat similar to the findings in seasonal influenza.[103,104] H7N9 viruses frequently have mutations (H279Y) in the NA gene, suggesting resistance to oseltamivir.

Exposure to infected birds in live bird markets has been identified as a major risk factor for infection.[103,104] However, control of outbreaks of H7N9 in domesticated poultry has been more difficult than H5 outbreaks because the initial H7 viruses did not exhibit high pathogenicity in birds, making infected flocks much more difficult to recognize. It has been speculated that the older age distribution of human cases may reflect social differences by age in patronizing these markets.[105] However, an alternative explanation may be related to the age-related previous exposures to influenza viruses. Individuals born after 1968, whose primary influenza exposure was due to H3N2 viruses, might be relatively resistant to H7 viruses compared with older individuals whose primary exposure was to an H1N1 virus.[106]

In 2016–2017 there was a fifth wave of human cases, associated with a high-pathology variant of the H7 virus.[107] The HA of the fifth wave virus was antigenically distant from the viruses isolated in the first four waves, and sera from humans immunized against earlier H7N9 viruses did not recognize this new variant. However, only three human cases of H7N9 infection were detected in 2017–2018, bringing the total number of cases to 1567. Because of the potential for person-to-person transmission and intrinsic virulence, H7 viruses are currently considered the most threatening pandemic risk as assessed by the Centers for Disease Control and Prevention (CDC) Influenza Risk Assessment Tool (IRAT).[108]

Human Infections With H9 Viruses

To date, human infections with H9 viruses have been rare. Influenza H9N2 virus was isolated from two children in Hong Kong with mild febrile pharyngitis in 1999.[109] Subsequently, H9N2 infection has been detected from five individuals with typical influenza in China[110] and from a child with relatively severe influenza in Hong Kong. Although these isolated incidents have been uncommon, H9 viruses remain a high priority for human surveillance because other threatening avian viruses such as H5 and H7 are often reassortants with H9 viruses.

Factors Controlling Host Range

Fortunately, avian influenza A viruses appear to be relatively restricted in their ability to replicate in humans.[111] The precise molecular mechanisms responsible for the host-range preferences of avian influenza A viruses are not completely known, but several factors probably play a role.[112] The divergent evolution of the genes of these viruses in avian hosts could have resulted in less efficient interactions between undefined viral and mammalian host cell components. The relative attenuation of avian-human influenza reassortants for man[113] supports a role for non-HA genes in this restriction. In addition, the HAs of avian and mammalian influenza viruses display a different host-cell receptor specificity, with avian viruses preferring receptors containing sialic acid–galactose linkages of the α2-3 variety, and mammalian viruses tending toward α2-6 linkages.

Extensive sequence analysis has suggested at least two mechanisms by which avian viruses can circumvent these barriers to interspecies transmission. These studies have shown significant sequence similarity between the HA, NA, and PB1 gene segments of the pandemic H2N2 virus and avian viruses,[81] and between the H3 and PB1 gene segments of the pandemic H3N2 virus and avian viruses,[81,114] suggesting that in some circumstances, new pandemic viruses arise by reassortment between avian viruses, which provide novel surface glycoproteins, and human viruses, which provide genes allowing efficient replication in humans. Reassortment would be facilitated by the presence of a third species that is susceptible to infection with both avian and human viruses, such as the pig, which contains both types of receptors.[115] However, there are likely to be constraints on what types of reassortants are viable; in particular, it has been suggested that the hemagglutinins of recent human influenza A viruses are not compatible with the matrix genes of current avian viruses,[116] and phenomena of this type may limit the possibilities for generation of pandemic viruses by reassortment.

A second mechanism would involve adaptation of avian viruses to the human host by evolution in swine, and this is supported by sequence analysis showing that the 1918 pandemic was most likely the result of direct introduction of an avian or swine influenza A virus into humans.[117]

The finding of limited transmission of H5N1 in humans is consistent with experiments in the ferret model, in which aerosol transmission over distances can be shown with seasonal H3N2 viruses, but not with human isolates of H5N1 viruses. A series of studies have been reported in which various manipulations were attempted to determine whether H5N1 viruses were capable of adapting to ferrets and eventually acquiring the ability to transmit from ferret to ferret.[118,119] These studies showed that several mutations are required for H5 viruses to gain the ability to transmit from ferret to ferret, including changes in the polymerase, changes in the receptor binding specificity of the HA, and changes that alter the stability of the HA under acidic conditions. Sequence analysis has suggested that some of these changes may already be present in naturally occurring H5 viruses,[120] and it will be important to continue monitoring isolates for sequences suggestive of successful human adaptation.

Emergence of Pandemic Viruses From Swine

Domestic swine have also been recognized as a potential source of pandemic influenza viruses in man. Genomic data have suggested that influenza A (H1N1) viruses were introduced into swine populations at around the same time that H1N1 viruses emerged in man in 1918.[121] Since that time these H1N1 viruses, or classic swine viruses, continued to be maintained in domestic swine, where they caused minor illnesses and underwent relatively little antigenic evolution. During this time, swine were also occasionally infected with influenza A viruses from humans and from birds, and have always been considered to represent a potential "mixing vessel" in which reassortment between human and avian influenza viruses could occur. This concept was strengthened by the recognition that the swine respiratory tract contains abundant receptors of both the α2-3 and α2-6 types favored by avian and human viruses, respectively.[115]

In 2009, a novel virus in which gene segments derived from four different sources (the so-called "quadruple reassortant") emerged from pigs to cause widespread disease in humans with sustained person-to-person transmission. This virus, the pandemic H1N1 virus, was derived from the classic swine triple reassortant virus (HA, NP, and NS from classic swine; PA and PB2 from avian influenza virus; PB1 from human virus) but with a substitution of the NA and M gene segments from a Eurasian swine lineage. The HA of the pH1N1 virus was closely related to the HA of human viruses circulating in the early 20th century and was not recognized well by antibody to recent seasonal H1N1 viruses. This virus went on to cause a pandemic of influenza with a major impact in children and adolescents, and replaced previous H1N1 viruses as the new seasonal H1N1. Subsequent studies in guinea pigs have identified the Eurasian swine M gene segment as playing a critical role in transmission phenotype of this virus.[122]

In the late 1990s, H3N2 viruses emerged in swine as a result of transmission of H3N2 viruses from humans to pigs, and of genetic reassortment between human and swine influenza viruses. Three different genotypes of H3N2 viruses have been observed in swine: nonreassortant human H3N2 viruses; reassortants bearing the HA, NA, and PB1 genes from human H3N2 viruses and the remaining gene segments from classical swine influenza viruses; and the "triple reassortant" viruses

that derived their HA, NA, and PB1 genes from human H3N2 viruses, their M, NP, and M gene segments from classical swine viruses, and their PA and PB2 gene segments from AI viruses.[123,124]

More recently, a number of cases of human infection with quadruple reassortant H3N2 viruses were reported. These viruses are genetically identical to pH1N1 viruses, except that the HA and NA genes are derived from circulating swine H3N2 triple reassortant viruses.[125] Important to note, the M gene segment is derived from the Eurasian swine lineage, perhaps increasing the likelihood of sustained transmission of these viruses in humans. Influenza viruses that circulate in swine are referred to as "variant" viruses when isolated in humans, designated H3N2v virus.

During the period from July to September 2012, a total of 306 cases of human infection with H3N2v influenza viruses were reported.[126] H3N2v has been associated with typical influenza illness, and there were 16 H3N2v-associated hospitalizations and one death. Almost all cases have documented histories of swine exposure, and the majority of cases were associated with attendance at state fairs.[127] However, there are some cases that have suggested the presence of limited person-to-person transmission. The H3N2v viruses isolated from humans are phylogenetically most closely related to human influenza viruses from the mid 1990s, particularly the A/Wuhan/95 and A/Sydney/99 viruses.[125,128] Clinical cases of H3N2v have occurred primarily in children 12 years of age and younger—that is, individuals born after these viruses last circulated in humans. Studies based on serosusceptibility have also suggested that children younger than 10 years would be largely susceptible to infection.[128,129]

CLINICAL FINDINGS

Uncomplicated Influenza

Typical uncomplicated influenza often begins with an abrupt onset of symptoms after an incubation period of 1 to 2 days. Many patients can pinpoint the hour of onset. Initially, systemic symptoms predominate, including feverishness, chilliness or frank shaking chills, headaches, myalgia, malaise, and anorexia. In more severe cases, prostration is observed. Usually, myalgia or headache is the most troublesome symptom, and the severity is related to the height of the fever. Myalgia may involve the extremities or the long muscles of the back. In children, calf muscle myalgia may be particularly prominent. Severe pain in the eye muscles can be elicited by gazing laterally, and arthralgia but not frank arthritis is commonly observed. Other ocular symptoms include tearing and burning. The systemic symptoms usually persist for 3 days, the typical duration of fever. Respiratory symptoms, particularly a dry cough, severe pharyngeal pain, and nasal obstruction and discharge, are usually also present at the onset of illness but are overshadowed by the systemic symptoms. The predominance of systemic symptoms is a major feature distinguishing influenza from other viral upper respiratory infections. Hoarseness and a dry or sore throat may also be present, but these symptoms tend to appear as systemic symptoms diminish, and thus they become more prominent as the disease progresses, persisting 3 to 4 days after the fever subsides. Cough is the most frequent and troublesome of these symptoms and may be accompanied by substernal discomfort or burning. Older adults may simply present with high fever, lassitude, and confusion without the characteristic respiratory complaints, which may not occur at all. In addition, there is a wide range of symptomatology in healthy adults, ranging from classic influenza to mild illness or asymptomatic infection.

Fever is the most important physical finding. The temperature usually rises rapidly to a peak of 100°F to 104°F, and occasionally to 106°F, within 12 hours of onset, concurrent with the development of systemic symptoms. Fever is usually continuous but may be intermittent, especially if antipyretics are administered. On the second and third days of illness, the temperature elevation is usually 0.5°F to 1.0°F lower than on the first day, and as the fever subsides, the systemic symptoms diminish. Typically, the duration of fever is 3 days, but it may last 4 to 8 days.

Early in the course of illness, the patient appears toxic, the face is flushed, and the skin is hot and moist. The eyes are watery and reddened. A clear nasal discharge is common, but nasal obstruction is uncommon. The mucous membranes of the nose and throat are hyperemic, but exudate is not observed. Small, tender cervical lymph nodes are often present. Transient scattered rhonchi or localized areas of rales are found in less than 20% of cases. A convalescent period of 1, 2, or more weeks to full recovery then ensues. Cough, lassitude, and malaise are the most frequent symptoms during this period.

At the extremes of age, there are prominent differences in influenza. Influenza attack rates are higher in children than in adults. Maximal temperatures tend to be higher among children, and cervical adenopathy is more frequent among children than among adults. Croup associated with influenza virus infection occurs only among children.[130,131] Among older adults, fever remains a very frequent finding, although the height of the febrile response may be lower than among children and young adults. Pulmonary complications are far more frequent in older adults than in any other age group.

Complications of Influenza

Pulmonary Complications

Two manifestations of pneumonia associated with influenza are well recognized: primary influenza viral pneumonia and secondary bacterial infection. In addition, less distinct and milder pulmonic syndromes often occur during an outbreak of influenza that may represent tracheobronchitis, localized viral pneumonia, or possibly mixed viral and bacterial pneumonia. Comparative features of these clinical syndromes are shown in Table 165.7.

Primary Influenza Viral Pneumonia

The syndrome of primary influenza viral pneumonia was first well documented in the 1957–1958 outbreak. However, many of the deaths of young healthy adults in the 1918–1919 outbreak may have been a result of this syndrome. In outbreaks since 1918, primary influenza viral pneumonia has occurred predominantly among persons with cardiovascular disease, especially rheumatic heart disease with mitral stenosis, and to a lesser extent in others with chronic cardiovascular

TABLE 165.7 Comparative Features of Pulmonary Complications of Influenza

	PRIMARY VIRAL PNEUMONIA	SECONDARY BACTERIAL PNEUMONIA	MIXED VIRAL AND BACTERIAL PNEUMONIA
Setting	Cardiovascular disease; pregnancy; young adult (pH1N1)	Adults and children	Any associated with A or B
Clinical history	Relentless progression from classic 3-day influenza	Improvement, then worsening after 3-day influenza	Features of both primary and secondary pneumonia
Physical examination	Bilateral findings, no consolidation	Consolidation	Consolidation
Sputum bacteriology	Normal flora	*Pneumococcus, Staphylococcus, Haemophilus influenzae*	*Pneumococcus, Staphylococcus, H. influenzae*
Chest radiography	Bilateral findings	Consolidation	Consolidation
Detection of influenza virus	Yes	Not always	Yes
Response to antibiotics	No	Yes	Often
Mortality	High	Variable	Variable

and pulmonary disorders. The illness begins with a typical onset of influenza, followed by a rapid progression of fever, cough, dyspnea, and cyanosis. Physical examination and chest radiographs reveal bilateral findings consistent with the adult respiratory disease syndrome but no consolidation. Blood gas studies show marked hypoxia, Gram stain of the sputum fails to reveal significant bacteria, and bacterial culture yields sparse growth of normal flora, whereas viral cultures yield high titers of influenza A virus. Such patients do not respond to antibiotics, and mortality is high. At autopsy, findings consist of tracheitis, bronchitis, diffuse hemorrhagic pneumonia, hyaline membranes lining alveolar ducts and alveoli, and a paucity of inflammatory cells within the alveoli (see Figs. 165.9 and 165.10).

Secondary Bacterial Pneumonia

Secondary bacterial pneumonia often produces a syndrome that is clinically indistinguishable from that occurring in the absence of influenza.[132,133] The patient has a classic influenza illness followed by a period of improvement lasting usually 4 to 14 days. Recrudescence of fever is associated with symptoms and signs of bacterial pneumonia such as cough, sputum production, and an area of consolidation detected at physical examination and on a chest radiograph. The two pathogens that are currently most commonly associated with influenza are *Streptococcus pneumoniae* and *Staphylococcus aureus,* which is otherwise an uncommon cause of community-acquired pneumonia. Community-acquired methicillin-resistant *S. aureus* (MRSA) has been seen in children following influenza,[134] and a study has suggested that the addition of a second active antibiotic may be important in children with MRSA complicating infection.[135]

During an outbreak of influenza, many patients do not clearly fit into either of the aforementioned categories. The disease is not relentlessly progressive, and yet the fever pattern may not be biphasic. These patients may have primary viral, secondary bacterial, or mixed viral and bacterial infection of the lung. In addition, milder forms of influenza viral pneumonia involving only one lobe or segment have been described that do not invariably lead to death, and that are more likely to be confused with pneumonia caused by *Mycoplasma pneumoniae* than to pneumonia produced by bacterial infection. Some studies have suggested that elevated levels of procalcitonin or C-reactive protein can be helpful in distinguishing secondary bacterial from primary viral pneumonia.[136,137]

Pulmonary Complications in Immunosuppressed Patients

Influenza has been noted to cause severe disease with an increased incidence of pneumonia in immunosuppressed children with cancer compared with age-matched individuals without immunosuppression.[138] Severe disease associated with pneumonia and death has been reported, particularly in bone marrow transplant recipients and leukemic patients.[139,140] Relatively more immunosuppressed individuals early after transplantation appear to be at greater risk. Influenza virus shedding can be quite prolonged in immunosuppressed children,[141] particularly those with human immunodeficiency virus (HIV) and low $CD4^+$ counts.[142] Because of the prolonged, unchecked replication of influenza viruses in these individuals, resistance to antiviral drugs eventually occurs in many treated patients.[143,144]

Other Pulmonary Complications

In addition to pneumonia, other pulmonary complications of influenza have been recognized. Bronchiolitis may also occur as a result of influenza A or B virus infection, but respiratory syncytial virus and parainfluenza virus type 3 are more important causes of bronchiolitis. Significant numbers of cases of croup occur in influenza A and B outbreaks.[130] Croup associated with influenza A virus appears to be more severe but less frequent than that associated with parainfluenza virus types 1 or 3 or respiratory syncytial virus infections (see Chapters 156 and 158). Acute exacerbation of chronic bronchitis is a common complication of influenza and may result in a permanent loss of pulmonary function.[145] Exacerbations of asthma and worsening pulmonary function in children with cystic fibrosis may also occur.[146]

Nonpulmonary Complications

Myositis

Myositis and myoglobinuria with tender leg muscles and elevated serum creatine phosphokinase (CPK) levels have been reported, mostly in children, but they can also occur in adults.[147] Symptoms may be sufficiently severe to interfere with walking.

Cardiac Complications

Both myocarditis and pericarditis have been rarely associated with influenza A or B virus infection,[148,149] although not observed at autopsy among those who died of primary influenza viral pneumonia.[150] Myocardial infarction may also be triggered by influenza infection,[151] possibly as an effect of platelet aggregation.[152] Studies have shown a substantially increased risk of myocardial infarction in the 7 days after hospitalization for influenza.[153]

Toxic Shock Syndrome

In recent outbreaks of influenza A or B, a toxic shock–like syndrome has occurred in previously healthy children or adults, presumably because viral infection changed colonization and replication characteristics of the toxin-producing staphylococci.[154–156]

Central Nervous Complications

Guillain-Barré syndrome (GBS) has been reported to occur after influenza A infection, as it has after numerous other infections, but no definite etiologic relationship has been established. In addition, cases of transverse myelitis and encephalitis have occurred rarely.[157] Influenza has also been associated with acute encephalitis and encephalopathy,[158] which have a wide variety of manifestations.[159] Most cases occur in children, but associated morbidity is higher in adults.[160] The specific pathogenesis of influenza-associated encephalitis is unclear but may be related to the cytokine response.

Reye Syndrome

Reye syndrome is associated with many viral infections, prominently including influenza and varicella in children. The classic manifestation is a change in mental status occurring several days after a typical respiratory illness. Manifestations range from lethargy to delirium, obtundation, seizures, and respiratory arrest. Lumbar puncture reveals normal protein values and normal cell counts, confirming the presence of encephalopathy rather than encephalitis or meningoencephalitis. The most frequent laboratory abnormality is elevation of the blood ammonia value, which occurs in almost all patients. Reye syndrome is almost exclusively seen in children who have been given aspirin for treatment of febrile illnesses due to influenza and other viruses, and it is important to use other antipyretics such as nonsteroidal antiinflammatory drugs in this situation. Children who require continuous aspirin therapy are an important target group for influenza vaccination to reduce the risks of Reye syndrome.

PATHOGENESIS

Cellular Pathogenesis

Influenza virus infection is acquired by means of a mechanism involving the transfer of virus-containing respiratory secretions from an infected to a susceptible person. Once virus is deposited on the respiratory tract epithelium, it can attach to and penetrate columnar epithelial cells if not prevented from doing so by specific secretory antibody (immunoglobulin A [IgA]), by nonspecific mucoproteins to which virus may attach, or by the mechanical action of the mucociliary apparatus. After adsorption, virus replication begins, leading to cell death through several mechanisms. There is a dramatic shutoff of host-cell protein synthesis that occurs at several levels. Newly synthesized cellular mRNAs are degraded (probably because cleavage by the virus cap endonuclease renders these transcripts susceptible to hydrolysis by cellular nuclease), whereas translation of already-synthesized cytoplasmic mRNAs is blocked at both initiation and elongation. Finally, expression of the influenza virus PA protein has been shown to induce generalized degradation of coexpressed proteins.[161] Ultimately, the loss of critical cellular proteins very likely contributes to cell death.

In addition to effects leading to cell necrosis, infection of cells with influenza A and B viruses causes cell death by apoptosis.[162] Bronchiolar