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152 Rubella Virus (German Measles)

Anne A. Gershon

SHORT VIEW SUMMARY

Definition
- Rubella is an infectious illness characterized by fever and maculopapular rash, which may be accompanied by arthritis.
- Many postnatal infections are asymptomatic.
- Postnatal rubella is a benign illness, but congenital rubella can result in a variety of serious medical problems.
- Manifestations of congenital rubella include deafness, cataract or glaucoma, congenital heart disease, autism, and mental retardation.
- Rubella vaccine was developed primarily to prevent congenital rubella from occurring.

Epidemiology
- In temperate climates, disease occurs mainly in the spring (if vaccine is not being used).
- Rubella is somewhat less contagious than measles.
- Widespread vaccination in the Americas led to the elimination of rubella as of 2009.
- Globally, rubella and congenital rubella remain serious medical problems, especially in Africa, some areas of Europe, Southeast Asia, and the Western Pacific.

Microbiology
- Rubella is an RNA virus, a member of the Togaviridae family and the genus *Rubivirus.*

Diagnosis
- Diagnosis can be made serologically (enzyme-linked immunosorbent assay) with acute and convalescent serum samples or by demonstration of rubella immunoglobulin M antibody on a single serum sample.
- A positive test for viral RNA using reverse-transcriptase polymerase chain reaction on throat swabs, cerebrospinal fluid, or amniotic fluid is especially useful for diagnosis of congenital rubella.

Therapy
- None is available.

Prevention
- Live-attenuated rubella vaccine is effective and safe, usually administered along with measles and mumps vaccines (MMR).

Rubella (German measles) is an acute exanthematous viral infection of children and adults. The clinical illness is characterized by rash, fever, and lymphadenopathy and resembles a mild case of measles (rubeola). Although many infections with the agent are subclinical, this virus has the potential to cause fetal infection, with resultant birth defects, and (uncommonly but especially in adults) various forms of arthritis.

Rubella virus was first isolated in 1962 by Parkman and colleagues[1] and by Weller and Neva.[2] Rubella virus is classified in the Togaviridae family[3,4] on the basis of its single-stranded, positive-sense polyadenylated RNA genome; replication strategy; icosahedral capsid; and lipoprotein envelope. Rubella virus is closely related to the alphaviruses, but in contrast to alphaviruses, no vector is required for its transmission, and it is serologically distinct from alphaviruses.[4] Therefore rubella virus alone has been placed in a separate genus, *Rubivirus.* There are 2 clades and 13 genotypes.[5,6] Only one antigenic type, however, is recognized.[6] On electron microscopy, rubella virus is roughly spherical. Its envelope, which has short surface projections, has a diameter of about 60 nm. The envelope surrounds the 20-sided nucleocapsid, which has a diameter of about 30 nm and comprises a helix of protein and RNA. Rubella virus matures by budding from the cell membrane.[7]

Three structural polypeptides associated with rubella virus are termed E1, E2, and C. There are also two nonstructural proteins that are related to replication and transcription. E1 and E2 are transmembrane glycoproteins, and C is the capsid protein that surrounds the RNA of the virion. Hemagglutinin and complement-fixing antigens are composed of varying proportions and mixtures of E1, E2, and C.[8,9] E1 is important in attachment, fusion, hemagglutination, and neutralization.

Rubella virus is relatively unstable. It is inactivated by lipid solvents, trypsin, formalin, ultraviolet light, and extremes of pH and heat, and it is inhibited by amantadine.[10] Cytopathic effects may not be noted in all cell lines in which rubella virus replicates. However, cytopathic effects are readily observed in the rabbit kidney cell line RK-13 and in primary African green monkey cells.[8]

EPIDEMIOLOGY

Rubella was not distinguished clinically from certain other exanthematous infections until the late 19th century. At one time it was termed *third disease,* when measles and scarlet fever were called *first disease* and *second disease,* respectively.[11] Because postnatal rubella is such a mild illness, the disease was considered to be of only minor importance for many years. However, in 1941, when the Australian ophthalmologist Gregg[12] recognized the link between maternal rubella and certain congenital defects, a more complete picture of disease caused by rubella virus began to emerge.

Before widespread vaccine use, the incidence of clinical cases of postnatal rubella in temperate climates was highest in the spring, and it was traditionally recognized to be most common in children 5 to 9 years of age.[13] Rubella is only a moderately contagious illness, in contrast to measles. Therefore in the prevaccine era, only 80% to 90% of adults were immune to rubella, whereas 98% were immune to measles.[14]

Epidemics of rubella of minor proportions occurred in the prevaccine era every 6 to 9 years, and large-scale epidemics occurred at intervals of up to 30 years. The most recent major epidemic in the United States occurred in 1964, during which some 12,500,000 people were infected.[15] Since the licensure of a live-attenuated rubella vaccine in 1969, there have been no large rubella epidemics in countries where the vaccine is widely used. However, limited outbreaks continued to occur in settings such as workplaces, schools, and military camps, where groups of susceptible individuals had close contact with each other.[14,16] In 2001, only 23 cases of postnatal rubella and 3 confirmed cases of congenital rubella syndrome were reported to the US Centers for Disease Control and Prevention (CDC).[17] An increase in susceptibility to rubella was noted among Hispanic young adults for some years, and there were increasing efforts to identify rubella-susceptible women before they became pregnant.[17,18]

In 2004 rubella was declared no longer endemic in the United States.[5,6,19–21] Since 2001, there had been fewer than 25 annual postnatal cases and only rare cases of congenital rubella syndrome. In 2017 the

CDC reported that about 10 annual cases of postnatal rubella occurred in the United States.[22] In 2012, three cases of congenital rubella were reported to the CDC, and all three were likely acquired through mothers who traveled early in their pregnancies in Africa.[23] Rubella was considered eliminated from the United States, as 95% of school-aged children were vaccinated, more than 90% of the population had immunity, there was adequate surveillance to detect outbreaks, and molecular evidence indicated that there were no circulating American genotypes of rubella virus.[24] This was a major advance in public health and occurred at a time similar to that when measles was no longer endemic in the United States, which was also a result of widespread immunization. Isolated cases of rubella may be imported into the United States from another country but with only the possibility of limited spread. Mathematical models indicate that transmission of rubella ceases at 90% immunization levels, which has been achieved in the United States as a whole,[5,25] although levels in individual states may be lower.[26] Measles, by comparison, is more contagious than rubella, and higher immunization rates may be required to prevent measles outbreaks.[27] Global efforts are underway to decrease the global burden of rubella and congenital rubella syndrome.[28] The expansion of vaccination programs worldwide has resulted in significant progress toward the control and ultimate eradication of the disease. A 95% decrease in cases of rubella was reported by the World Health Organization between 2000 and 2014.[29] In April 2015 North and South America became the first regions in the world to be declared free of endemic rubella transmission. As of December 2016, 152 (78%) of 194 countries had introduced rubella vaccination into their immunization schedules, which represented an increase of 53 countries since 2000 and an increase in 22 countries since 2012.[30] The CDC has established the target goal of eradication of rubella and measles to be accomplished by 2020.[31]

TRANSMISSION OF RUBELLA

Rubella virus is spread in droplets that are shed from respiratory secretions of infected individuals. Patients are most contagious while the rash is erupting, but they may shed virus from the throat from 10 days before until 15 days after the onset of the rash. Patients with subclinical cases of illness may also transmit the infection to others.[10]

Infants with congenital rubella shed large quantities of virus from body secretions for many months and therefore may transmit the infection to individuals who care for them. These infants continue to excrete rubella virus despite high titers of neutralizing antibody, a puzzling phenomenon that has yet to be explained.[32] The possibility of immune tolerance due to fetal infection has been raised.[9] Infants of recent immigrants from developing countries who have congenital rubella may infect susceptible individuals if the diagnosis is not made and contacts are not immunized.

People who receive rubella vaccine do not transmit rubella to others, although the virus may be transiently isolated from the pharynx. The quantity of virus shed may be too small to be infectious.[18]

MAINTENANCE OF IMMUNITY TO RUBELLA

After an attack of rubella, lifelong protection against the disease develops in most people. However, the factors responsible for this protection are not precisely understood. Antibody titers to rubella virus develop, but the significance of the decline of antibody titers with time remains unclear. Cell-mediated immunity to rubella virus associated with $CD4^+$ and $CD8^+$ T lymphocytes has also been detected by in vitro assays[8,33] months to years after an attack of rubella. The long-term persistence of humoral and cellular immunity to rubella in a group of cloistered nuns who had no opportunity for reexposure to rubella virus has been documented.[34] The persistence of specific antibody for 14 years after immunization has also been demonstrated.[35,36]

Nevertheless, despite the presence of specific immunity to rubella virus, reinfection with rubella virus can occur. This had been long suspected on clinical grounds alone.[37,38] Rubella reinfections have been documented by detection of a significant boost in rubella antibody titers in naturally immune individuals after reexposure to the virus. Most reinfections are asymptomatic.[39] It is likely that the virus can multiply locally in the upper respiratory tract, but that viremia occurs infrequently because the host's immune response eradicates the virus before it can invade the blood. However, in rare instances, patients have been reported to have proven rubella reinfection occurring years after naturally acquired rubella, with symptoms indicative of viremia (e.g., arthritis, rash).[40]

Rubella reinfection occurring months or years after the receipt of rubella vaccine has also been observed. Several investigators have documented reinfections in up to 80% of individuals who had received rubella vaccine previously and were subsequently exposed to rubella during an epidemic.[39,41] Most of these reinfections were not characterized by clinical illness but were identified only by a rise in antibody titer. Viremia is probably extremely rare in such cases,[41–43] although rubella virus has been recovered from throat secretions in reinfections.[39,42] In one study of eight seronegative adult vaccinees who were experimentally challenged with wild-type rubella virus, replication in the respiratory tract was found in seven subjects, and viremia was present in two.[44] However, these subjects experienced only a mild illness or remained asymptomatic.[44]

Reinfections are more common among vaccinees than among individuals who have experienced natural rubella, and they are most common among individuals with hemagglutination inhibition (HAI) antibody titers of 1:64 or less.[39,41,42] It has been suggested that there may also be qualitative differences in antibody between individuals with vaccine-induced immunity and those with natural immunity because in one study, even with similar HAI titers, vaccinees were 10 times more likely to be reinfected than individuals with natural immunity to rubella.[39]

Whether rubella reinfection that occurs during pregnancy can result in transmission of the virus to the fetus has been the subject of much debate. Several case reports in the older literature that ascribed fetal defects to maternal rubella reinfection likely actually involved primary maternal infections.[45,46] Viremia was documented in one woman with detectable rubella antibody before immunization.[47] Boué and coworkers[48] studied a small number of women with documented subclinical cases of rubella reinfection during pregnancy who carried their babies to term; all the babies were found to be normal. In a number of other case reports of rubella reinfection during pregnancy, infants born at term to the affected mothers had symptoms suggestive of congenital rubella.[49–54] Most of these reinfections occurred years after natural infection, although some occurred years after immunization.[53,54] However, these transmissions are acknowledged to be extremely rare, particularly considering the exceedingly low incidence of congenital rubella in the United States today.

It appears that individuals who are immune to rubella, either by having had the natural infection or by having received rubella vaccine, may be reinfected when reexposed. However, reinfection is usually asymptomatic and detectable only by serologic means. Viremia and congenital rubella in maternal reinfection appear to be very rare events.

The presence of large numbers of immune people in a community appears to be able to prevent rubella epidemics from occurring; this effect is termed *herd immunity.* Although it has been documented that herd immunity does not entirely eliminate the spread of rubella, it probably plays a major role in control of this infection, which is now rare in the United States.[5]

PATHOGENESIS

The incubation period for rubella ranges from 12 to 23 days (average, 18 days). As in measles (see Chapter 160), a primary and a secondary viremia are believed to accompany rubella. Rubella virus has been detected in leukocytes of patients 1 week before the onset of symptoms.[55] Also as in measles, the rubella rash appears as immunity develops and the virus disappears from the blood,[8] suggesting that the rash is immunologically mediated. Although circulating immune complexes are detectable during rubella, they do not appear to contribute to the development of rash.[8,56] Rubella virus has been isolated from involved skin,[57] but this does not preclude the possibility that the rash is secondary to an immune response to the virus.

CLINICAL MANIFESTATIONS

Age is the most important determinant of the severity of rubella. Postnatally acquired rubella is usually an innocuous infection, and, as

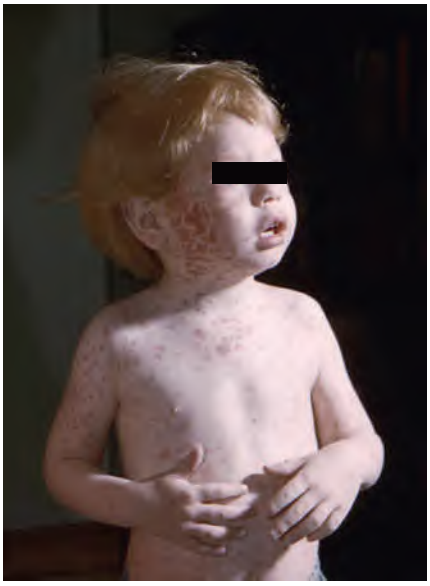

FIG. 152.1 Child with exanthem of acute rubella. *(Courtesy Centers for Disease Control and Prevention, Atlanta, GA. Vaccine Information. http://www.immunize.org/photos/rubella-photos.asp.)*

is true for many viral illnesses, children have milder disease than adults. In contrast, the fetus is at high risk for development of severe rubella, with serious sequelae if infected transplacentally in early pregnancy because of maternal rubella.

Postnatal Rubella

Many, if not most, cases of postnatal rubella are subclinical.[15,58] Among patients who are symptomatic, children do not experience a prodromal phase, but adults may have a prodrome of malaise, fever, and anorexia for several days. The major clinical manifestations of postnatal rubella are adenopathy, which may last several weeks, and rash. The lymph nodes involved include the posterior auricular, posterior cervical, and suboccipital chains. Splenomegaly also occasionally occurs.[59] These symptoms are not specific for rubella, and clinically the disease may resemble measles, toxoplasmosis, scarlet fever, roseola, parvovirus B19 infection, and certain enterovirus infections.

The rash of rubella begins on the face and moves down the body. It is maculopapular but not confluent, may desquamate during convalescence, and may be absent in some cases (Fig. 152.1). An enanthem consisting of petechial lesions on the soft palate (Forschheimer spots) has been described for rubella, but this enanthem is not diagnostic for rubella (in contrast to Koplik spots in measles). The rash may be accompanied by mild coryza and conjunctivitis. The rash usually lasts 3 to 5 days. Fever, if present, rarely lasts beyond the first day of rash.

Complications of Postnatal Rubella

Complications of postnatal rubella, in contrast to complications of measles, are uncommon. Bacterial superinfections after rubella are rare.

Arthritis or arthralgia has been reported in one-third of women with rubella; this complication is less common in children and in men.[60] The arthritis tends to involve the fingers, wrists, and knees, and it occurs either as the rash is appearing or soon afterward. It can be slow to resolve, taking 1 month. Chronic arthritis rarely develops.

The pathogenesis of rubella arthritis is not entirely understood. The frequency of detection and the quantity of circulating immune complexes are higher in rubella vaccinees who report joint complaints than in those with no joint involvement.[56,61] Rubella virus has been isolated from joint effusions in patients with acute or recurrent rubella arthritis associated with either previous natural infection or vaccination.[a] Rubella virus has been isolated from peripheral blood mononuclear cells in patients with chronic arthritis.[62,63] A persistent rubella virus infection of human synovial cells cultured in vitro was reported, and this was advanced as an explanation for the pathogenesis of chronic forms of rubella arthritis.[64]

Hemorrhagic manifestations occur as a complication in about 1 of every 3000 cases of rubella.[59,74] In contrast to other complications of rubella, hemorrhagic manifestations occur more often in children than in adults. This complication may be due to both thrombocytopenia and vascular damage, and it is probably immunologically mediated.[74] Some investigators have proposed that mild thrombocytopenia often goes undetected in apparently uncomplicated rubella.[75] Thrombocytopenia may last weeks to months and may cause serious problems if bleeding into vital areas (e.g., brain, kidney, eye) occurs.[59] Thrombocytopenic purpura as the single clinical manifestation of rubella in children has also been reported.[74]

Encephalitis is an extremely uncommon complication of rubella; its incidence during an epidemic was reported to be 1 in 5000 cases. It occurs more frequently in adults than in children, and it is associated with a mortality rate of 20% to 50%.[59,76,77] Survivors usually have no sequelae.[8] A fatal case of rubella encephalitis in a 2-month-old child whose mother had rubella in the last week of pregnancy has been reported.[78]

Mild hepatitis has been described as an unusual complication of rubella.[79]

Congenital Rubella Syndrome

Rubella can be a disastrous disease in early gestation and can lead to fetal death, premature delivery, and an array of congenital defects. The incidence of congenital rubella in a given population is quite variable, depending on the number of susceptible individuals, the circulation of virus in the community, and, in recent times, the use of rubella vaccine. The rubella epidemic of 1964 left 30,000 affected infants in its wake.[6] Between 1969 and 1979, however, an average of 39 cases per year were reported to the CDC.[80–82] Since the advent of the 21st century, congenital rubella has been essentially eliminated from the United States.[6,17,23,24]

The effects of rubella virus on the fetus depend to a large extent on the time of infection; in general, the younger the fetus when infected, the more severe the illness. During the first 2 months of gestation, the fetus has a 65% to 85% chance of being affected, with an outcome of multiple congenital defects or spontaneous abortion.[8] Rubella during the third month of fetal life has been associated with a 30% to 35% chance of developing a single defect, such as deafness or congenital heart disease. Fetal infection during the fourth month carries a 10% risk for a single congenital defect. Occasionally fetal damage (deafness alone) is seen if rubella occurs up to the 20th week of gestation.[83]

The specific signs and symptoms of congenital rubella may be classified as temporary (e.g., low birth weight), permanent (e.g., deafness), and developmental (e.g., myopia).[80] The most common manifestations are deafness, cataract or glaucoma, congenital heart disease, and mental retardation; the major clinical manifestations are listed in Table 152.1.[80]

Prospective studies of the congenital rubella syndrome suggest that it should not be considered a static disease. Some children whose mothers had rubella during pregnancy and who were considered normal at birth were found to have manifestations of congenital rubella when they reached school age.[84,85] Diabetes mellitus in late childhood has also been observed 50 times more frequently in children who had congenital rubella than in healthy children.[8,86] Insulin-dependent diabetes has been reported in 40% of adult survivors of congenital rubella from the 1942 epidemic.[87] In a follow-up study of 242 children who had congenital rubella, rubella virus–induced diabetes had genetic and immunologic features similar to those observed in other forms of insulin-dependent diabetes: the frequency of the human leukocyte antigen allele HLA-DR3 was increased, and the frequency of HLA-DR2 was decreased.[88] Antibodies to pancreatic islet cells or cytotoxic surface antibodies were present in 80% of patients with abnormalities in serum glucose concentration.[88] At autopsy of patients with congenital rubella, the virus was isolated from the pancreas, which was noted to have a subnormal number of glandular cells.[88] Progressive encephalopathy resembling subacute sclerosing panencephalitis was observed in children with congenital rubella.[89,90] In 1991, a group of 40 adults born with the congenital rubella syndrome between 1939 and 1943 were reexamined. Although they had multiple defects involving hearing, diabetes, growth retardation,

[a]References 62, 65, 66–68, 69, 70, 71, 72, 73.

TABLE 152.1 Congenital Rubella: Transient (T), Permanent (P), and Developmental (D) Manifestations

COMMON	UNCOMMON OR RARE
Low birth weight (T)	Jaundice (T)
Thrombocytopenic purpura (T)	Dermatoglyphic "abnormality" (P)
Hepatosplenomegaly (T)	Glaucoma (P)
Bone "lesions" (T)	Cloudy cornea (T)
Large anterior fontanelle (T)	Severe myopia (P, D)
Meningoencephalitis (T)	Myocardial abnormalities (P)
Hearing loss (P, D)	Hepatitis (T)
Cataract (and microphthalmia) (P)	Generalized lymphadenopathy (T)
Retinopathy (P)	Hemolytic anemia (T)
Patent ductus arteriosus (P)	Rubella pneumonitis (T)
Pulmonic stenosis (P, D)	Diabetes mellitus (P, D)
Mental retardation (P, D)	Thyroid disorders (P, D)
Behavioral disorders (P, D)	Seizure disorders (D)
Central language disorders (P, D)	Precocious puberty (D)
Cryptorchidism (P)	Degenerative brain disease (D)
Inguinal hernia (P)	
Spastic diplegia (P)	
Microcephaly (P)	

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and eye and heart abnormalities, most were well adjusted socially. There was no increased incidence of malignant disease in these 50-year-old survivors.[91]

Infants with congenital rubella develop high titers of neutralizing antibody that may persist for years.[92] However, these children may eventually lose detectable antibody.[93] Reinfection with rubella has also been documented in some of these children.[94] Impairment of cell-mediated immunity to rubella antigen was found in some children with congenital rubella.[95]

A number of pathologic mechanisms have been proposed to explain certain manifestations of congenital rubella. It has been suggested that persistent infection with rubella virus leads to a mitotic arrest of cells, which causes inhibition of cellular growth and, consequently, retarded organ growth.[96] Additional hypotheses put forth to explain the growth retardation associated with congenital rubella are that infection leads to angiopathy with placental and fetal vasculitis, which compromises growth,[97] and that tissue necrosis without inflammation or fibrotic damage leads to cellular damage.[98] Another possible explanation is that infection of various types of cells during gestation interferes with the normal balance of growth and differentiation, which leads to defects in organogenesis.[8] Human fibroblasts infected with rubella virus in vitro were found to produce a growth inhibitor, which might also account for fetal growth retardation.[99] An increased frequency of chromosomal breakage was found in cultured cells from children with congenital rubella compared with cells from healthy children.[100] Molecular evidence of cell cycle arrest caused by rubella virus has been obtained.[101] It has been postulated that lymphocyte abnormalities in patients with congenital rubella syndrome may predispose them to organ-specific autoimmunity.[102]

DIAGNOSIS

Because rubella is usually a mild disease with nonspecific symptoms, it is often difficult to diagnose clinically. The disease has been confused with other infections such as scarlet fever, mild measles, infectious mononucleosis, toxoplasmosis, roseola, erythema infectiosum, and certain enteroviral infections.[103,104] Routine laboratory studies are not helpful for diagnosis because they may reveal only leukopenia and atypical lymphocytes; more specific laboratory diagnostic techniques are usually necessary.

Virus isolation from throat swabs, urine, synovial fluid, or other body secretions is an acceptable method for diagnosis, particularly in the early days after rash onset.[6] The diagnosis of congenital rubella infection has been made by isolation of virus from amniotic fluid.[105] However, virus isolation is time-consuming and expensive,[8,10] and it is not used frequently today. Virus isolation for diagnosis of rubella and especially for congenital rubella has been largely supplanted by molecular methods such as reverse-transcriptase polymerase chain reaction.[6,9] Results of reverse-transcriptase polymerase chain reaction may be positive before the appearance of rubella immunoglobulin M (IgM) antibodies.

The laboratory diagnosis of postnatal rubella is most conveniently made serologically. At one time, HAI was the preferred means of measuring rubella antibody titers, but this technique has been supplanted by simpler, more accurate methods of similar sensitivity.[10,104,106–111,112] These include enzyme-linked immunosorbent assay, passive latex agglutination test, and radial hemolysis test. These tests have variable sensitivity. Most of these tests may be used to measure either IgG or IgM antibodies. A demonstration of specific IgG on one serum sample is evidence of immunity to rubella. Acute rubella infection may be diagnosed either by a demonstration of specific IgM in one serum sample or by a fourfold or greater increase in rubella antibody titer in acute and convalescent specimens assayed in the same test.[9,113] Positive rubella IgM antibody tests have been associated not only with primary infection but also with reinfection with rubella virus.[114] This phenomenon may explain, at least in part, why apparent false-positive results on IgM-rubella enzyme-linked immunosorbent assay testing in pregnant women have been reported.[115] Results of many of these serologic tests are available within a matter of minutes or hours and yield prompt, useful information. However, some commercial assays for rubella immunity may yield false-negative results (see "Vaccination Against Rubella").

For a serologic diagnosis of congenital rubella in the neonatal period, antibody to rubella virus should be measured in both infant and maternal sera. It may be necessary to perform several antibody determinations on serum from the infant to detect whether the titer of rubella antibody is falling, which indicates passively acquired maternal antibody, or rising, which suggests rubella infection. If rubella IgM is detected in a newborn infant's serum, transplacental rubella infection has occurred. Congenital rubella infection has been diagnosed by the following tests or procedures: placental biopsy at 12 weeks, demonstration of rubella antigen with monoclonal antibody, cordocentesis and detection of RNA by in situ hybridization,[116] and polymerase chain reaction.[117] It may also be diagnosed by the presence of specific IgM in fetal blood, but this may not be detectable until 22 weeks of gestation.[118,119] Because of public health concerns today, it is extremely important to confirm suspected congenital rubella by laboratory means.

THERAPY

There is no specific therapy, but for patients with fever and arthritis or arthralgia, the treatment of symptoms is indicated. At one time, immune globulin (IG) was advocated for the prevention or modification of rubella in susceptible pregnant women who were exposed to the infection. However, it was discovered that although IG might suppress symptoms, it would not necessarily prevent viremia.[120] Therefore indications for the use of IG for rubella prophylaxis are few. Possibly, IG may be given to a susceptible pregnant woman who is exposed to rubella and for whom abortion is not an option if she should develop the disease. With the advent of rubella vaccine, it is now recommended to immunize susceptible women of childbearing age against rubella before they become pregnant.

VACCINATION AGAINST RUBELLA

Rubella virus was isolated in 1962[1,2] and attenuated in 1966[121]; the live-attenuated vaccine was licensed for use in the United States in 1969. The rationale for use of the vaccine is to prevent congenital rubella by control of postnatal rubella (see Chapter 316). In the United States, the first strategy was to vaccinate prepubertal children to minimize exposure of susceptible pregnant women to rubella. More recently, there has been an emphasis on immunization of rubella-susceptible women of childbearing age who are not pregnant. Often, this is done just after delivery of an infant; nursing mothers who are vaccinated do not cause harm to their infants. In some other countries, the approach has been to vaccinate girls against rubella as they approach puberty.

Immunization programs in the United States have dramatically reduced the transmission of rubella in young children and prevented major epidemics of rubella. There have been no such epidemics for

almost 50 years, a phenomenon never previously observed in the United States. A mini-epidemic of congenital rubella in 21 infants occurred in 1990 in southern California. More than 55% of the mothers had a total of 22 missed opportunities for vaccination at the time of marriage or after previous delivery of a child; therefore more than half of these cases of congenital rubella were preventable.[122]

The incidence of postnatal rubella fell to an all-time low in 1988, but by 1991 it had increased threefold. There was a concomitant increase in cases of congenital rubella syndrome during the same period, although there was still a decline of more than 98% in cases of rubella compared with the prevaccine era. The observed increase in cases was attributed to failure to immunize rather than vaccine failure.[81] With improvements in vaccine delivery since 1991, the incidence of rubella in the United States again fell. As noted previously, endemic rubella has been eliminated from the United States. There remains, however, a continued need to emphasize the importance of immunization of susceptible women of childbearing age who are not pregnant, hospital employees, and infants and children.[121,122,123,124]

At the present time the only rubella vaccine available in the United States is RA 27/3. This vaccine has been widely used in Europe and is more immunogenic than the previously used vaccines. RA 27/3 vaccine stimulates the production of secretory and humoral IgA, which may account for its increased immunogenic potency.[125,126,127] Single antigen rubella vaccine is not available in the United States, so rubella vaccine is administered either as measles-mumps-rubella (MMR) vaccine or as measles-mumps-rubella-varicella (MMRV) vaccine. Because there is a second routine dose of measles vaccine in the United States, two doses of rubella vaccine are also routinely administered. Two doses of rubella are useful because they prevent primary vaccine failure. Second doses of MMR are not considered booster doses.

Presumptive evidence of rubella immunity according to the CDC is as follows: (1) written documentation of vaccination with one or more doses of live rubella virus vaccine on or after 12 months of age, *or* (2) birth before 1957, *or* (3) laboratory confirmation of disease or of rubella immunity by antibody testing. It is not uncommon for rubella antibodies to be undetectable using commercial antibody assays in immune individuals. Persons who do not have acceptable presumptive evidence of immunity should receive one dose of MMR vaccine.[128]

Complications of Vaccination

Rubella vaccine may cause viremia,[129,130] and therefore the main complications are fever, adenopathy, arthritis, and arthralgia. All the complications are more common in adults than in children, and they are most common in women older than 25 years.[131,132,133] In one study, 40% of such vaccinees developed joint complications[132]; however, all reactions were transient. It is uncommon for children to develop complications. In general, the incidence of joint complications, even in adults, is lower after vaccination than after natural rubella.[14,130] In 1991 a committee of the Institute of Medicine examined the issue of chronic arthritis after administration of RA 27/3 rubella vaccine and concluded that there is a rare causal relationship.[134] The risk for arthritis is increased in individuals with HLA alleles DR1, DR4, and DR6.[135] Evidence in vitro indicates that wild-type strains of rubella virus can be propagated more efficiently in joint tissues than vaccine strains.[136]

Efficacy of Vaccination

Since the introduction of rubella vaccine, the number of reported cases of clinical rubella has declined progressively. The vaccines available today, when properly administered, produce a seroconversion rate of about 95% after one dose.[14] Seroconversion in response to rubella vaccine is not impaired in children with upper respiratory tract infections.[137] Because antibody titers are lower after vaccination than after natural disease, the question has been raised as to whether the antibody titer, years after vaccination, will remain high enough to prevent clinical rubella. Only time and continued surveillance will provide an answer to this question, but at the present time there is little evidence of waning immunity,[35,36] as reflected by the low incidence of rubella in the United States. Therefore booster injections of rubella vaccine are not routinely indicated.

Effects of Rubella Vaccine on the Fetus

Since rubella vaccine was licensed in 1969, the CDC has monitored the outcome in infants born to women who were reported to have been inadvertently immunized against rubella during early pregnancy. As of late 1987, 812 such women who carried their infants to term had been included in the CDC study, with no cases of the congenital rubella syndrome attributed to rubella vaccine.[138] Therefore the observed risk for congenital rubella after immunization is reported as zero; however, the theoretical maximal risk could be 1% to 2%. This is in contrast to a 20% or greater risk after maternal rubella in the first trimester.[138] The vaccine-type virus can cross the placenta, and rubella virus has been isolated from both decidua and fetal tissue at abortion after inadvertent vaccination of pregnant women.[138–142] Rubella virus was isolated from the fetus of a woman given rubella vaccine 7 weeks before conception.[143] A single case of persistent infection of a fetus whose mother was inadvertently immunized in early pregnancy has been recorded; the infant had no signs or symptoms of congenital rubella syndrome.[144]

Based on an analysis of 293 normal infants born to rubella-susceptible mothers vaccinated 1 to 2 weeks before or 4 to 6 weeks after conception, for whom the theoretical risk to the fetus is 1.3%, the CDC recommends that women avoid pregnancy for 28 days after rubella vaccination.[145] Although it is not recommended that rubella vaccine be administered to women who are pregnant, the currently recognized minimal theoretical fetal risk does not mandate automatic termination of a pregnancy. Many, if not most, of such vaccinated women may wish to carry their baby to term.

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153 Flaviviruses (Dengue, Yellow Fever, Japanese Encephalitis, West Nile Encephalitis, Usutu Encephalitis, St. Louis Encephalitis, Tick-Borne Encephalitis, Kyasanur Forest Disease, Alkhurma Hemorrhagic Fever, Zika)

Stephen J. Thomas, Timothy P. Endy, Alan L. Rothman, and Alan D. Barrett

SHORT VIEW SUMMARY

Definition

- The Flaviviridae family incorporates the *Flavivirus, Pestivirus, Pegivirus,* and *Hepacivirus* genera, which cause systemic febrile, central nervous system, and plasma leakage and hemorrhagic fever syndromes, or a combination of these.

Epidemiology and Clinical Features

- Transmission and disease may occur with endemic, hyperendemic, or epidemic patterns, or a combination of these.
- Yellow fever (YF) is found in tropical South America and sub-Saharan Africa. Disease is severe, with high morbidity and mortality. Recent outbreaks have demonstrated new areas at risk for sustained YF transmission and the inadequacy of the current global vaccine production and supply chain.
- Dengue is widely distributed throughout the tropics and subtropics. Severe disease is infrequent (~2%–4% of clinically apparent sequential infections) but potentially fatal. Nonsevere disease (outpatient) accounts for much of dengue's global socioeconomic impact.
- Japanese encephalitis virus (JEV) is a significant cause of encephalitis in Southeast and South Asia. Chronic morbidity and mortality are high in symptomatic cases.
- West Nile virus (WNV), St. Louis encephalitis (SLE) virus, JEV, Usutu virus (USUV), and tick-borne encephalitis virus (TBEV) are the major neurotropic members of the genus *Flavivirus.* Infection with WNV is found worldwide, SLE virus is found in the Americas, and USUV is found in Africa and Europe, whereas TBEV occurs from Western Europe to Russia, Japan, and China. Most infections are subclinical. Clinically apparent cases with neurologic manifestations have a predilection for older individuals.
- Zika virus (ZIKV) infections have been documented since the 1950s in Africa and Asia. In 2007, a large outbreak occurred on Yap Island in the Pacific. In 2012, ZIKV transmission was documented with increasing frequency in Southeast Asia and French Polynesia. Beginning in 2015, ZIKV experienced an epidemic explosion in Central and South America. ZIKV infection generally causes a mild self-limited illness consisting of fever, maculopapular rash, arthralgias, and conjunctivitis. Recent outbreaks have been associated with increased incidences of neurologic complications such as Guillain-Barré syndrome and a spectrum of perinatal complications and developmental disorders such as microcephaly and motor deficits.
- Alkhurma hemorrhagic fever virus (AHFV) and Kyasanur Forest disease virus (KFDV) infections are emerging or reemerging in the Middle East, India, and Africa/Asia, respectively. Infections initially have nonspecific clinical findings; persons infected with AHFV and KFDV may develop hemorrhagic manifestations.

Microbiology

- Flaviviruses are icosahedral, approximately 50 nm in diameter, approximately 11-kb single-stranded, positive-sense RNA viruses consisting of a lipid envelope covered densely with surface projections consisting of M (membrane) and E (envelope) glycoproteins. Nonstructural proteins make up the remainder of the genome.
- Mutations and replication errors are high.

Diagnosis

- Diagnostic approaches are dictated by the disease course.
- During the viremic period, viral isolation and nucleic acid or antigen detection from blood are possible.
- During the subacute period, early-phase immunoglobulin M antibodies may be detected in sera or cerebrospinal fluid using various assay platforms (e.g., enzyme-linked immunosorbent assay).
- During the convalescent period, a rise in immunoglobulin G between acute and convalescent specimens is measured to identify a seroresponse (plaque reduction neutralization test, hemagglutination inhibition).
- Potentially unique to ZIKV, measurement of RNA in urine, saliva, and blood during the acute and remote period from infection has been documented and may enhance testing strategies.
- Cross-reactivity among the Flaviviruses adds complexity to interpreting serologic test results from areas where numerous Flaviviruses cocirculate.

Therapy

- No licensed or specific antiviral therapies are available.

SHORT VIEW SUMMARY—cont'd

- Treatment is supportive, and outcomes are variable depending on the experience of the practitioners and available critical care resources.

Prevention

- Personal protective measures to avoid exposure to the vector and the potential for infection is the first line of prevention.
- US-licensed vaccines exist for YF and JE. Vaccines also exist for TBE and Kyasanur Forest disease.
- A single dengue vaccine (Dengvaxia) has been licensed in 20 countries, but a safety signal in vaccine recipients who were dengue naïve at the time of vaccination has limited its potential widespread use. Two other vaccines (National Institutes of Health and Takeda Vaccines) are in advanced clinical testing.
- ZIKV is unique (except for hepatitis C) in its potential for sexual transmission and maternal-fetal infection. Pregnant women should weigh the risks of traveling to areas with active ZIKV transmission. Numerous Zika vaccine candidates are in preclinical and clinical development, with two DNA constructs in phase II clinical testing.

The *Flavivirus* genus consists of more than 70 viruses, most of which are arthropod-transmitted or zoonotic viruses, including some 30 known to cause human disease.[1–4] Many of the remainder have an unknown pathogenic potential but have been implicated in laboratory infections; several are veterinary pathogens. The agents are classified in the family Flaviviridae (Latin *flavus,* for "yellow" and for *yellow fever virus,* the type species), together with viruses in the *Pestivirus* genus (which are of veterinary importance) and the *Pegivirus* genus (11 species that cause persistent virus infections of primates, bats, rodents, pigs, and horses), and those in the genus *Hepacivirus* (hepatitis C–like viruses) on the basis of similar morphologic characteristics and genomic structures.[1–4] There are no significant antigenic relationships between viruses across genera.

Currently (2014–2018), numerous Flaviviruses pose a significant global public health threat. Zika virus (ZIKV) infections have spread extensively throughout the tropical and subtropical regions of the world to include Central and South America, the Caribbean, Mexico, the Pacific Islands, Africa, and Southeast Asia. ZIKV has been associated with devastating congenital complications (microcephaly and intracranial calcifications) and neurologic illnesses, such as Guillain-Barré syndrome (GBS; discussed in detail later).[2,3] From a global perspective, the public health and socioeconomic burdens of flavivirus infections such as dengue, yellow fever (YF), Japanese encephalitis (JE), Zika, and tick-borne encephalitis (TBE) have been of sufficient magnitude to warrant the development of vaccines to control these diseases.[5–7] The licensure and distribution of effective vaccines for YF (>80 years), JE (>50 years), and TBE (>30 years) have led to significant reductions in incidence and, in some locations, the effective disappearance of disease.[8] Dengue vaccine candidates have been under development for more than 75 years, two are in clinical efficacy trials and one has completed efficacy trials and is licensed in 20 countries. Demonstrating long-term safety and efficacy sufficient for licensure has been challenging for this vaccine (discussed in detail in the section "Prevention and Therapy" later).[9] Many of the other flaviviruses have the ability to cause considerable morbidity, but they occur too sporadically to support a concerted approach to prevention and control.

Flavivirus infections are important considerations in the differential diagnosis (especially in returning travelers) of a systemic febrile illness with headache, bone pain, and rash; central nervous system (CNS) infection; plasma leakage or hemorrhagic complications, or both; birth defects; and GBS.[10,11] By evaluating the epidemiologic history, including the places and dates of travel, incubation time of the viruses, activities during travel, and immunizations, in conjunction with clinical features of the illnesses, the clinician can obtain important clues to pursue or exclude a diagnosis. The diseases of chief importance in this group are described later in this chapter.

HISTORY

Yellow Fever

Although the historical record and molecular taxonomic studies of viral strains have indicated an African origin of YF virus, the disease was first recognized in an outbreak that occurred in the New World in 1648 (Fig. 153.1A).[12] The consensus is that the virus was introduced to the New World by *Aedes aegypti*–infested slave-trading vessels from West Africa. Through the next 2 centuries, similar outbreaks spread to port cities in the New World and in Europe. The resulting calamities were exemplified by the 1793 Philadelphia epidemic, in which one-tenth of the city's population died, and by the 1878 Mississippi Valley epidemic of 100,000 cases, the cost of which equaled the national budget. Sanitary measures, especially the introduction of piped water, inadvertently served to diminish transmission of the disease, although its mosquito-borne route of spread was not demonstrated until 1900 and its viral cause not until after 1928. Theiler's development of the attenuated 17D vaccine strain in the 1930s was recognized by a Nobel Prize, but more than 80 years later, vaccine implementation (through the World Health Organization [WHO] Expanded Program on Immunization) in areas with endemic transmission remains incomplete and outbreaks recur periodically.[13]

Dengue

Because of dengue's nonspecific clinical features, the interpretation of historical records for evidence of past epidemics is open to speculation.[14–17] However, Benjamin Rush's description of a 1780 Philadelphia epidemic was the earliest description in English of so-called breakbone fever. Subsequently, sporadic outbreaks were reported throughout the tropics and subtropics. Mosquito-borne transmission of the infection by *A. aegypti* was demonstrated in 1903, and its viral etiology was proven in 1906.[14,15] Outbreaks were common in the continental United States through the early decades of the 20th century, the last occurring in Florida in 1934 and in New Orleans in 1945. Dengue was generally considered a relatively benign disease (certainly in comparison to YF) with few, if any, complications or deaths, although cases of dengue complicated by hemorrhage, shock, and death were described in outbreaks in Australia in 1897, in Greece in 1928, and in Formosa in 1931.[14,16,17] While isolating the virus in 1944, Sabin demonstrated the failure of two viral strains to cross-protect in humans, first establishing the existence of distinct dengue virus (DENV) types; Hammon characterized two more types in 1956.[18] After World War II, intensified transmission of multiple DENV types began in Southeast Asia, and were associated with outbreaks of severe disease, which was given the name dengue hemorrhagic fever (DHF).

The program to control YF in the Americas in the 1940s by eradicating *A. aegypti* mosquitoes was also successful in controlling DENV transmission. The ending of these programs in the 1970s resulted in *A. aegypti* becoming widely reestablished in the region and dramatic increases in the occurrence of dengue and severe disease.[19–21] The introduction to Cuba in 1981 of a novel DENV-2 viral strain from Southeast Asia produced the first major DHF epidemic in the hemisphere, resulting in 116,143 hospitalizations, including 10,000 for shock. Since 1989, DHF outbreaks have been reported in most of Central and South America.[12,21,22] With the reintroduction of DENV-3 into Central America in 1994, all four DENV types now circulate in the Americas.[23,24] The most dramatic ascendance of dengue and severe disease over the past several decades has occurred in the Caribbean and in Latin America.[19–21] DHF outbreaks result in several thousand DHF cases each year throughout most of Central and South America and the Caribbean.[12,21,22]

In the past 30 years, this pattern of hyperendemic transmission of all DENV types and increased DHF incidence has become well established in South Asia, the Americas, and Oceania. Increased transmission and

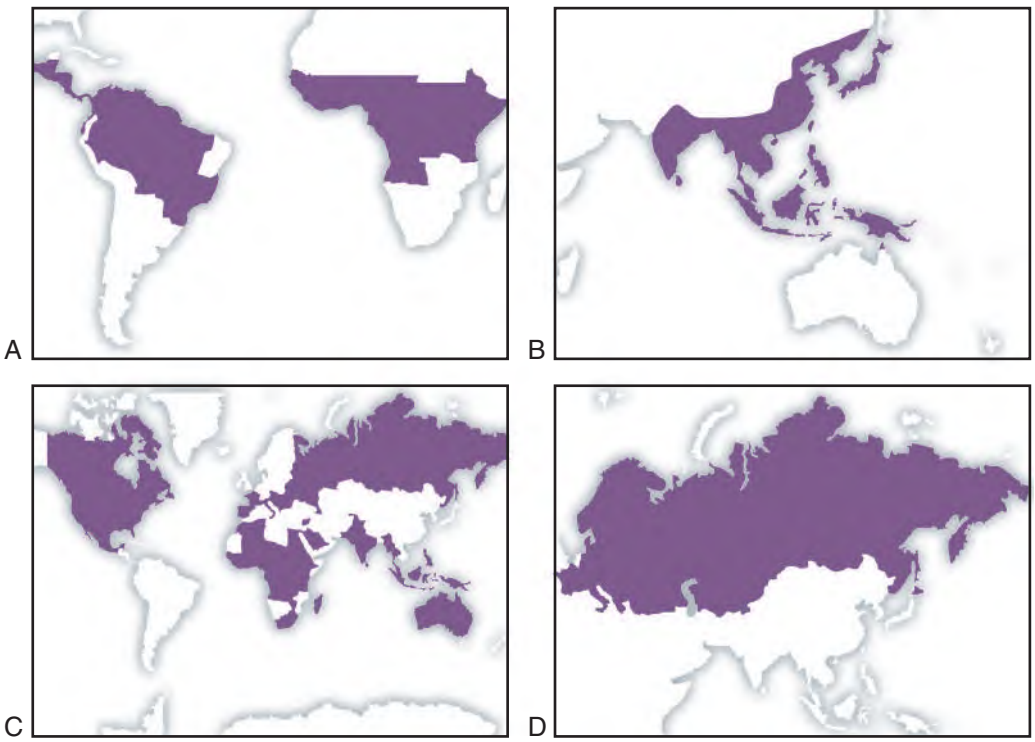

FIG. 153.1 Geographic distribution of medically important flaviviruses. (A) Regions with yellow fever virus transmission. (B) Regions with Japanese encephalitis virus transmission. (C) Countries with West Nile virus transmission. (D) Countries with tick-borne encephalitis transmission.

disease has been noted in Africa and the Middle East, as well as more temperate climates such as the southern parts of the continental United States and Europe.[25–29] DENV transmission and increasing global burden of disease is being fueled by population growth, urbanization, increasingly favorable ecologic and environmental habitats for *Aedes* mosquitoes compounded by poor vector control, and the ease of international travel.[30]

Japanese Encephalitis Virus

Japanese encephalitis virus (JEV) was isolated from a fatal human case in Japan in 1934, but summertime encephalitis outbreaks leading to thousands of cases were described previously and were called *Japanese B encephalitis,* differentiating it from von Economo's encephalitis lethargica, which was known as *type A encephalitis* (the qualifying *B* has fallen into disuse). Elegant studies conducted on the Kanto Plains outside Tokyo, Japan, established the complex ecosystem of JEV transmission among migrating birds, *Culex* mosquitoes, local pig populations, and accidental human hosts.[31–40] The burden of annual epidemics led to the introduction in the 1960s of vaccines that effectively eliminated the disease in Japan, Korea, and Taiwan and reduced its annual incidence in China by 10-fold. Since the 1970s, the incidence of the disease has increased in countries in Southeast Asia, India, Nepal, and Sri Lanka, probably owing in part to changes in agricultural productivity and increased recognition (see Fig. 153.1B). Smaller outbreaks have been reported in the Philippines, Indonesia, and the northern tip of Queensland, Australia.[41–43] Reported human cases vastly underestimate the burden of infection in endemic areas. An estimated 35,000 to 50,000 cases and 10,000 to 15,000 deaths are reported annually, making JEV the most important cause of epidemic viral encephalitis worldwide.[44] JE vaccines are effective, and their routine distribution and use are increasing but are not maintaining pace with transmission. Childhood immunization programs have been established in Thailand and areas of Vietnam, India, and Sri Lanka, but a great deal more remains to be done.

West Nile Virus

West Nile virus (WNV) was isolated from the blood of a febrile woman in the West Nile region of Uganda in 1937, and mosquito transmission between vertebrate hosts (especially birds) was demonstrated soon afterward. Although not associated with neurologic disease at that time, the virus was shown by serologic cross-reactivity to be closely related to the then–recently identified neurotropic viruses, JEV and St. Louis encephalitis (SLE) virus. Later, sporadic cases and larger outbreaks of febrile disease were reported in the Middle East and Africa, in some instances in association with arthralgia and a rash. In the 1950s, meningeal irritation and meningoencephalitis were noted in a few patients in Israel. Outbreaks of equine and human meningoencephalitis occurred in southern France during the 1960s, and a subtype of WNV (Kunjin virus) was isolated in Australasia. Since the 1990s, the epidemiology of WNV has changed, with increasing frequency and severity of outbreaks in southern Europe, Russia, and the Middle East and spread of the virus to the Americas (see Fig. 153.1C). The first outbreak in the Western Hemisphere occurred in the northeastern United States in 1999. The virus rapidly spread across the continent, reaching the Pacific Coast in 2002. During 2002 and again in 2003, more than 2000 cases of CNS disease and 200 deaths were reported from the United States, with additional cases in Canada and enzootic transmission reported in the Caribbean and Central America. Since 2003, the number of cases reported in the United States has decreased, but there are still more than 1000 neurologic cases per year. WNV activity has not been reported in tropical South America, but there was an outbreak in Argentina in equines in 2003.[45] In 2012, the United States experienced the worst outbreak of WNV since its introduction in 1999.[46] As of December 11, 2012, there were 48 states that reported WNV infections in people, birds, or mosquitoes, with a total of 5674 cases of illness in humans, the highest number of WNV cases ever reported in the United States, and 286 deaths. Of the human cases, 80% occurred in 13 states (Texas, California, Louisiana, Illinois, Mississippi, South Dakota, Michigan, Oklahoma, Nebraska, Colorado, Arizona, Ohio, and New York); one-third of all cases were reported from Texas, with an epicenter around the large metropolitan area of Dallas. The US Centers for Disease Control and Prevention (CDC) has reported more than 2000 cases of WNV disease each year from 2013 to 2017. WNV is now the leading cause of mosquito-borne and epidemic encephalitis in the United States.[47]

Usutu Virus

Usutu virus (USUV) is a mosquito-borne flavivirus that was first isolated in South Africa in 1959 and was subsequently identified in a number of African countries (Burkina Faso, Central African Republic, Côte d'Ivoire, Morocco, Nigeria, Senegal, Tunisia, and Uganda). As with many flaviviruses, it was not regarded as a public health problem until it emerged in Austria in 2001, where it caused a large number of fatal infections in birds, especially Euroasian blackbirds (*Turdus merula*); however, retrospective analyses suggest birds died from USUV infection in Italy in 1996. Subsequently, the virus has become endemic in a number of European countries as determined by serologic or reverse-transcriptase polymerase chain reaction (RT-PCR) analyses, or both, of samples from Austria, Belgium, Czech Republic, Croatia, England, France, Germany, Greece, Hungary, Italy, Netherlands, Poland, Serbia, Spain, and Switzerland (for a review, see Gaibani and Rossini[48]).

The virus exists in an enzootic cycle involving ornithophilic mosquitoes and birds and is a member the JE serocomplex. The main mosquito vector is *Culex pipiens*. Over 60 avian species have been described as vertebrate hosts from African and European countries, including a number of migratory bird species that are thought to have transported the virus from Africa to Europe. As with other members of the JE serocomplex, humans are dead-end hosts. Phylogenetic studies suggest that there are at least eight genetic lineages of USUV (Africa 1–3 and Europe 1–5), which suggests USUV originated in Africa in the 16th century and emerged in Europe in the middle of the 20th century via multiple introductions.[48,49] The first hypothesized introduction was to Spain in the 1950s, followed by introductions to Austria and Italy in the 1970s, and a further introduction in to Spain at the end of the 20th century.

The incidence of human USUV infections is difficult to estimate, but examination of blood donors in Germany, Austria, and Italy generated seroprevalence rates of 0.02%, 0.058%, and 1.1%, respectively. However, it is clear that awareness of the virus is steadily increasing over time, and this corresponds to increased identification of the virus in birds and clinical cases of USUV infection. To date, 21 human cases of USUV infection have been reported.[48] Clinical symptoms vary from febrile infections to neurologic disorders, including encephalitis and meningoencephalitis. Historically, USUV was shown to be the cause of two human febrile infections in Africa, one in Central African Republic in 1981 and the other in Burkina Faso in 2004.[48,50] The first human cases in Europe were described in Italy in 2009 and involved two human cases of encephalitis in immunocompromised patients.

St. Louis Encephalitis Virus

SLE was first reported as an epidemic of unknown cause in St. Louis, Missouri, in 1933, although outbreaks compatible with SLE had been described from the 1920s. A US Public Health Service investigation identified the viral cause and, on the basis of epidemiologic features, surmised a mosquito-borne mode of transmission, which subsequently was proved by viral isolations from *Culex* spp. mosquitoes in outbreaks in the Yakima Valley, Washington. The occurrence of more than 10,000 cases in more than 50 outbreaks through 1990 revealed the disease to be the most important cause of epidemic viral encephalitis in the United States until WNV became established. In 1975, there were 2900 cases of SLE in 31 states, and more than 200 cases occurred during an epidemic in Florida in 1990. SLE continues to be seen in the United States, particularly among elderly people in the southern states.[51] According to the CDC, there were 6, 19, and 7 neuroinvasive cases reported in 2014, 2015, and 2016, respectively.[51a] Interestingly, there is evidence that WNV is displacing the SLE virus in its ecosystem in California and Texas.[52,53]

Tick-Borne Encephalitis Virus

Descriptions of a disease compatible with TBE appeared in Austria in the early 1930s, although isolation of the virus responsible for this disease (then known as *Central European encephalitis* [CEE]) was not reported until 1948. Investigation of similar cases in the far eastern part of Russia in 1932 had led to descriptions of so-called Russian spring-summer encephalitis (RSSE), and in 1937 the virus was isolated from the blood of patients and from *Ixodes* spp. tick vectors. It is now recognized that three closely related subtypes of TBE virus (TBEV) exist, whose names reflect the geographic areas that they principally affect: European (TBEV-Eu), Siberian (TBEV-Sib), and Far Eastern (TBEV-FE). Across this vast geographic area, the disease has been given a range of different names, including CEE, RSSE, Far Eastern encephalitis, and biphasic milk fever. This last name reflects the transmission of TBEV through ingestion of unpasteurized milk from infected livestock, first confirmed during a 1961 common source epidemic in Czechoslovakia leading to 660 cases.

Disease is found across large geographic areas from Western Europe to Russia, Japan, and China (see Fig. 153.1D). During the past 2 decades, both new endemic foci and an increase in cases have been reported in many European countries.[54,55] The incidence of TBE varies according to location and year. It is estimated that there are 10,000 to 12,000 clinical cases of TBE per year. In Austria, where there had been several hundred cases annually, a formalin-inactivated vaccine was introduced in the 1970s. Administration of a purified form of the vaccine in mass campaigns since the 1980s and in subsequent routine immunization campaigns has led to a dramatic reduction in the number of cases such that the disease has nearly been eliminated from Austria. The TBEV group serocomplex also includes viruses that are rare causes of human neurologic disease (e.g., Powassan virus [POWV], first isolated from a fatal case in Ontario, Canada, in 1958, and louping ill virus, first isolated from a sheep in Scotland in 1930) and viruses that produce a hemorrhagic fever syndrome (e.g., Omsk hemorrhagic fever virus, Alkhurma hemorrhagic fever virus [AHFV], and Kyasanur Forest disease virus [KFDV]). Between 2007 and 2016 the CDC reported 88 cases of neuroinvasive POWV disease, with most cases found in Minnesota, Michigan, and New York.[56]

Alkhurma Hemorrhagic Fever Virus

AHFV was originally isolated from a male butcher who died of an acute febrile hemorrhagic fever in the village of Alkhurma district in Jeddah, Saudi Arabia.[57,58] It was confirmed as a unique flavivirus related to KFDV and believed to have diverged from a common ancestor flavivirus about 700 years ago.[59] AHFV along with KFDV are members of the mammalian tick-borne virus subgroup of the genus *Flavivirus,* family Flaviviridae.[60,61] In 2003 there were 20 confirmed cases in the city of Makkah, which is 75 km from Alkhurma. Cases have been sporadically reported from the southern region of Najran, and since 2008 there has been an increase in the number of reported cases. AHFV appears to be isolated to the Arabian Peninsula and Egypt and associated with the camel ticks: the soft-bodied tick *Ornithodoros savignyi* and the hard-bodied tick *Hyalomma dromedarii.* Seroprevalence in Saudi Arabia appears to be around 1%, and the case-fatality rate appears to be around 10%.[60]

Kyasanur Forest Disease Virus

In 1957, there were reports of an epizootic outbreak of a fatal disease in monkeys located in the forest areas of Shimoga District, Karnataka State, India. Simultaneously there was an outbreak in humans of what was thought to be enteric fever but locally known as "monkey disease," owing to its association with dead monkeys. The virus was isolated from dead langur monkeys and humans and demonstrated to be a new virus named for the locality of its isolation, Kyasanur Forest. KFDV is now known to be a tick-borne flavivirus in the antigenic complex of mammalian tick-borne flaviviruses related to AHFV.

Zika Virus

ZIKV was originally isolated at a YF surveillance station in a caged febrile rhesus monkey located in the Zika Forest, Entebbe, Uganda in 1947 and subsequently isolated from an *Aedes africanus* mosquito in 1948.[62–64] Cross-neutralization tests revealed that this was a unique arbovirus, and a serosurvey demonstrated evidence of human infection.[63,64] ZIKV is now known as a member of the Spondweni virus complex. Spondweni virus was first isolated in 1955 from mosquitoes (*Taeniorhynchus uniformis*) collected in Tongaland, South Africa. The name was derived from the location in which the mosquitoes were collected, the district of Spondweni.[63,64] ZIKV transmission was documented in Southeast Asia and French Polynesia in 2012, and had an

epidemic spread in Central and South America beginning in 2015 (see discussion in the "Epidemiology" section later).

PATHOGENS

Flaviviruses are icosahedral, are about 50 nm in diameter, and consist of a lipid envelope covered densely with surface projections consisting of 180 copies of the M (membrane) and 180 copies of the E (envelope) glycoproteins.[65,66] The latter are organized as dimers, paired horizontally head to tail, on the virion surface. The viruses are unstable in the environment and are sensitive to heat, ultraviolet radiation, disinfectants (including alcohol and iodine), and acid pH. The nucleocapsid joins the capsid (C) protein to a single strand of positive-sense RNA of 11 kb. The viral RNA sequence includes a 10-kb open reading frame that is translated as a single polyprotein precursor, which is cotranslationally and posttranslationally processed to yield 10 proteins. The order of protein gene products from the 5′ end is C, premembrane (preM, a precursor of the mature M protein), E, and a series of seven nonstructural (NS) proteins needed in the viral replicative process: NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5. The open reading frame is flanked by a short (about 100 nucleotides) 5′ noncoding region and a 3′ noncoding region that is variable in length (100–700 nucleotides).

The E protein exhibits important biologic properties, including attachment to host cell receptors, endosomal membrane fusion, and display of sites mediating hemagglutination and viral neutralization.[65] Its carboxyl terminus provides a membrane anchor, and the ectodomain is folded into three structural and corresponding antigenic domains (domains I, II, and III).[67] Domain II is involved in fusion, and domain III is involved in receptor binding. Viral-neutralizing epitopes are found in all three domains; because the protein is folded, they are nonlinear and conformationally dependent. PreM protein chaperones the E protein into the cell secretory pathway, preventing its misfolding, before it is cleaved to its M form in the mature virion. NS1 is expressed on the surface of infected cells and is also secreted as a complement-fixing antigen. Although antibodies to NS1 do not neutralize the virus, they contribute to protective immunity, probably by antibody-dependent cellular cytotoxicity and cell-mediated responses against infected cells.[68] Aside from their replicative functions, NS1, NS2A, NS3, and NS5 display epitopes mediating viral serotype and flavivirus cross-reactive human leukocyte antigen (HLA)–restricted lymphocytic responses.[69–72] Studies suggest that multiple NS proteins, including NS2A, NS2B, NS3, NS4B and NS5, are involved in interferon antagonism.[70,72]

Viral attachment to unidentified cellular receptors is followed by endocytic uptake of virus-containing vesicles. Acidic-induced changes of the viral envelope lead to fusion activity, uncoating of the nucleocapsid, and viral RNA release into the cytoplasm. Glycosaminoglycans and proteoglycans have been implicated as coreceptors in some studies, but proteinaceous receptors are believed also to be involved, and viral binding evidently varies with cell type.[73,74] The viral polyprotein is cotranslationally and posttranslationally processed by passage through the rough endoplasmic reticulum, providing the replicative complex for further viral RNA and protein synthesis and the assembly of nascent virions that mature through the Golgi complex and *trans*-Golgi network. Immature virions collect in the highly proliferated endoplasmic reticulum and secretory vesicles before release, although intracellular nucleocapsid accumulations have been observed in some virus cell systems.

Flaviviruses are adapted to grow in a wide variety of insect, tick, and vertebrate cells and at temperatures spanning the normal temperatures of their arthropod, reptilian, mammalian, and avian hosts. Cytopathologic changes and plaque formation develop in Vero, LLC-MK2 (rhesus monkey kidney), BHK-21 (baby hamster kidney), PS, and primary chick and duck embryo cells, whereas infection of mosquito cell lines (e.g., C6/36, AP61) is typically nondestructive and may persist.

A wide range of vertebrates, including mammals, birds, and reptiles, are naturally infected as amplifying hosts in the transmission cycle of alternating arthropod and vertebrate infection.[1] These infections are usually asymptomatic, but individual viruses may be pathogenic for domesticated or wild animals; for example, several neurotropic flaviviruses, including JEV, SLE virus, WNV, Kunjin virus, TBEV, and POWV, produce encephalitis in horses and certain TBEV strains are neurotropic for dogs, sheep, and goats. JEV is an important cause of swine abortion; louping ill virus is a manifestation of encephalitis in sheep, and YF virus and KFDV are lethal in some monkey species. Laboratory rodents are generally susceptible to neurotropic infection, with sensitivity inversely related to age.

With few exceptions, the flaviviruses can be classified by cross-neutralization assays into eight antigenic groups, of which the most important is the JE complex, which consists of JEV, SLE virus, WNV, USUV, and Murray Valley encephalitis virus; the dengue complex of DENV-1 through -4 viruses; the mammalian tick-borne virus complex, including TBEV, louping ill virus, POWV, KFDV, and Omsk hemorrhagic fever virus; YF virus; and a complex of non–vector-borne rodent- and bat-associated viruses.[1,75] Genetic studies largely support the antigenic classification and suggest the evolution of flaviviruses from viruses found only in insects to mosquito-borne viruses and subsequently non–vector-borne flaviviruses and tick-borne viruses.[76] Genomic sequencing studies of specific viruses have facilitated evolution studies and the tracking of viral movements historically and in epidemics. For example, YF virus strains have been divided into seven genotypes—three in East and Central Africa, two in West Africa, and two in South America—with a close relationship between West African and South American viruses supporting the hypothesis that YF virus was introduced to the Western Hemisphere from Africa.[77] Genotypic markers have been of particular help in understanding the emergence of dengue and JE epidemics in the wake of viral introductions from other regions.[78,79] Genotypes also have been correlated with viral biologic characteristics that underlie their transmission patterns. For example, SLE viral genotypes from the eastern and western United States exhibit distinct neurovirulence and transmission characteristics that are consistent with epidemiologic observations.[80–82] Four DENV types (also known as serotypes) are recognized and correspond to distinct virus species (DENV-1 to DENV-4) based on antigenic and genetic characteristics.[81,82] On a clinical level, structural distinctions between strains associated with classic dengue and with severe dengue (DHF) have been described, providing potential clues to molecular determinants of dengue viral virulence.[81,82]

EPIDEMIOLOGY

Yellow Fever

YF is transmitted in 48 countries in sub-Saharan Africa ($n = 34$) and tropical South America ($n = 14$) (see Fig. 153.1A). The disease has never been documented in Asia (although 11 infected travelers from Angola in 2016 had clinical disease in China, this did not lead to outbreaks in China), but, in principle, anthroponotic (vector-borne person-to-person) transmission of the virus could occur there and in other *A. aegypti*–infested locations, including the southern United States.[6,83–85] Epidemic ("urban") YF is transmitted by *A. aegypti* mosquitoes; the mosquitoes are infected after feeding on viremic humans and then spread the infection in subsequent feeding attempts. The threat of epidemic transmission arises when a person with a forest-acquired infection travels to an *A. aegypti*–infested location while viremic.

The fluctuating incidence of YF has been dominated by epidemics in Africa, with 90% of the 130,000 annual cases occurring there.[84,86] However, official reports considerably underestimate the true magnitude of those epidemics, which field studies estimated as 50-fold greater.[84–88] Epidemic attack rates ranged as high as 30 in 1000 persons, with case-fatality ratios of 20% to 50%. Since 1990, epidemics have been reported in a number of previously unreported areas, including Niger (1990), Kenya (1992), Gabon (1994), Central African Republic (2008), Cameroon (2012), and Sudan (2012), demonstrating why YF is classified as a reemerging disease. The variable size and frequency of epidemics in recent years may reflect cyclic changes in viral activity and in human immunity, acquired in recent epidemics and through vaccination in emergency campaigns and the WHO Expanded Program on Immunization. Over the last 15 years, a combination of mass vaccination campaigns followed by routine immunization have led to substantial reduction in cases of YF in West Africa, with no outbreaks reported in 2015, but the disease has resurged since then, including a large outbreak in Angola and the Democratic Republic of the Congo (DRC) in 2016. Currently, plans are underway to expand vaccination campaigns to other African countries and immunize a further 1.3 billion individuals through 2026 via the Eliminate Yellow Fever Epidemics (EYE) initiative.

In South America, an annual mean of about 100 cases has been reported in the past 25 years, reflecting the occurrence of forest-acquired infections in the greater Magdalena, Orinoco, and Amazon River basins.[89,90] However, as in Africa, YF has emerged in new areas in Brazil, with outbreaks spreading to Sao Paulo and Rio de Janeiro States for the first time in 2016 to 2018.[89,90] In its *jungle cycle,* the virus is transmitted from tree-hole *Haemagogus* and *Sabethes* spp. mosquitoes to forest monkeys in wandering epizootics that follow movements of the animals and of the virus to susceptible populations. Cases in humans predominate between January and March among men 15 to 45 years of age who are bitten incidentally by infected mosquitoes while employed as agricultural and forestry workers, soldiers, and settlers. Recent outbreaks frequently have occurred among nonimmune migrants from their coastal or Andean homes to at-risk locations in the tropical zone. An intensification of enzootic viral transmission frequently produces clusters of monkey deaths, indicating an increased transmission risk to humans. The last urban outbreak in the Western Hemisphere took place in Paraguay in 2008 after a period of 54 years since the penultimate urban outbreak in Trinidad in 1954. The growth of urban areas and their reinfestation by *A. aegypti* have renewed concern for the emergence of epidemics, especially in cities that border forested areas, which was exemplified by the outbreak in Paraguay in 2008. Since 1995, several patients with sylvatic disease have been hospitalized in Brazilian cities and in Santa Cruz, Bolivia, fortunately without urban spread. The imminent threat of epidemic transmission has prompted mass vaccination campaigns and other control measures, which have been very successful at controlling the disease.[89–91] In the moist savanna of Africa, a variety of tree-hole–breeding mosquitoes transmits infections among humans and monkeys during the end of the rainy season, leading to early infections in children and sporadic cases that are typically unrecognized. Infections are spread readily by migration, with the potential for involvement of *A. aegypti* in urban areas and in dry locations where stored water provides breeding sites. During the dry season, the virus survives in infected mosquito eggs that are resistant to desiccation.

Dengue

The four DENV types share similar mechanisms and patterns of transmission, with relatively small differences in the frequencies of different clinical manifestations.[92] They are transmitted in the tropics, in an area roughly between 35° north and 35° south latitude, corresponding to the distribution of *A. aegypti,* the principal mosquito vector, though this distribution is enlarging with global warming (Fig. 153.2).[93] *Aedes albopictus, Aedes polynesiensis,* and other species are also capable of transmitting the DENVs. Although enzootic transmission among forest monkeys in Asia and Africa has been described, anthroponotic viral transmission is sufficient to maintain the virus, and these animal infections could represent either epiphenomena or evidence for spread from sylvatic to endemic cycles.[94] The intensification of dengue transmission in tropical cities, where growing populations live under crowded conditions, can be understood in view of the close relationship of *A. aegypti* to humans.[95] After the female mosquito feeds on a viremic person, viral replication and dissemination must take place in the mosquito before it can transmit the virus on subsequent feeding attempts; this extrinsic incubation period is typically 1 to 2 weeks. Feeding attempts may occur several times a day over the insect's lifetime of 1 to 4 weeks. Mechanical transmission, without extrinsic incubation, has also been suggested. *A. aegypti* is adapted to breed around human dwellings, where the insects oviposit in uncovered water storage containers as well as miscellaneous containers holding water, such as vases, flower dishes, cans, automobile tires, and other discarded objects. Adult mosquitoes shelter indoors and bite during 1- to 2-hour intervals in the morning and late afternoon. In areas with endemic transmission, 1 of every 20 houses may contain an infected mosquito.[96,97] Cases often cluster in households, and human movements and the mosquito's peregrinations within a range of 800 m rapidly spread the infection.[98–103] DENV transmission between mosquitoes and humans appears closely linked to weather conditions in different regions, specifically rainfall, humidity, and temperature variation.[100,102,103]

When the virus is introduced into susceptible populations, usually by viremic travelers, outbreaks with significant infection and clinical attack rates are possible. Because cross-protective immunity among the four DENV types is limited, epidemic transmission recurs with the introduction of novel DENV types. Furthermore, because secondary infections predispose to severe disease (discussed in the "Pathogenesis" section later), the concurrent transmission of multiple DENV types establishes the conditions for endemic DHF. Under these circumstances, most DHF cases occur in individuals with secondary infections, with the relative risk for developing DHF, with shock in secondary compared with primary infection being as high as 100.[104–107]

DHF during primary DENV infection in the first year of life represents a special case. In dengue-endemic areas, infants passively acquire DENV-specific antibodies from their DENV-immune mothers, and the age distribution of DHF parallels the expected decline of maternal antibodies, a phenomenon taken to support a central role for antibodies in causing more severe disease.[108–111]

As noted earlier, intensified DENV transmission and epidemics of DHF evolved in Southeast Asia after World War II. Today, Asia and the Western Pacific bear nearly 75% of the global dengue disease burden, with the highest incidence during childhood.[112] In Thailand, for example, DENV infection rates have been documented in the range of 5% to 10% of schoolchildren per year, with DHF incidence rates of 10 to 300 per 100,000 persons per year, and dengue accounts for one-third of acute febrile illnesses in children seeking medical attention.[113,114] Although DHF still is principally a disease of children younger than 15 years of

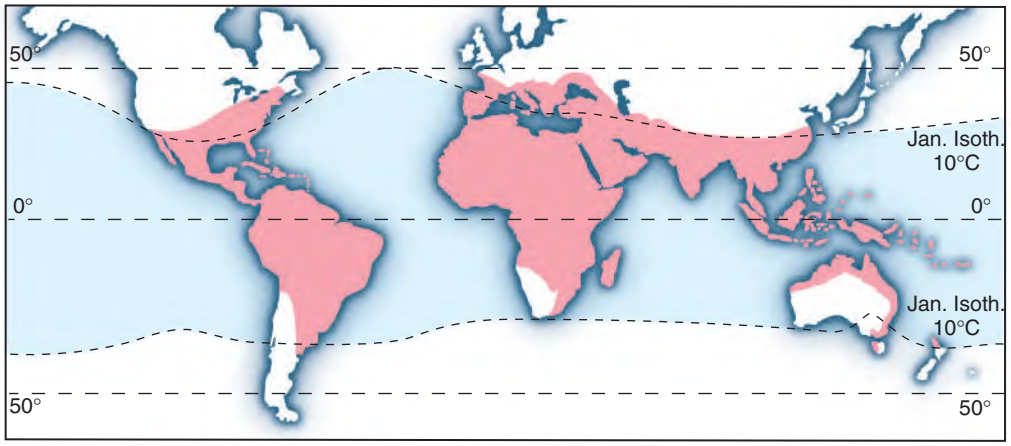

FIG. 153.2 Approximate actual and potential distribution of *Aedes aegypti.* The band between the 10°C isotherms represents potential distribution. *(From World Health Organization.* Technical Guide for Diagnosis, Treatment, Surveillance, Prevention, and Control of Dengue Haemorrhagic Fever. *2nd ed. Geneva: World Health Organization; 1997.)*

age in hyperendemic areas, the peak age of risk has risen in recent years as DENV transmission has declined due to increased use of screens and air-conditioning and lowering of birth and death rates.[115–118] In contrast, dengue occurs in all age groups when relatively newly introduced to an area or reintroduced after a significant interval, and severe disease may occur only in previously immunologically primed adults.[119]

The dissemination of DENVs by viremic travelers has been facilitated by the increased mobility of people living within endemic areas and internationally by burgeoning air travel.[115–118] In several studies, DENV infection was documented in 7% to 45% of febrile returned travelers.[120–122] Small numbers of autochthonous cases acquired in Texas towns bordering Mexico were recognized in 1980, 1986, 1995, 2005, and 2013.[123,124]

Infection can be transmitted by accidental needlestick.[125–127] The high incidence of infection in endemic areas suggests that transfusion-associated DENV transmission may be a risk. Despite this being an infrequent occurrence, guidelines for blood product donation have been developed.[125–127]

The continental United States has regularly experienced autochthonous DENV transmission and occasional small outbreaks in Texas, along its border with Mexico.[128] In Key West, Florida, expansion of DENV has continued into previously DENV-free areas with establishment of a vector population.[129] Sequencing of DENV-1 strains isolated in Key West, Monroe County, Florida, indicated that the viruses were undergoing endemic transmission for more than 2 years before being recognized.[26] Southern areas of Europe have also experienced periodic DENV introduction and local transmission.[27,29,125]

Japanese Encephalitis

JE is transmitted in Asia over an area spanning one-third of the world's circumference, from Pakistan at the westernmost edge to far eastern Russia (Table 153.1; see Fig. 153.1B). The disease is endemic and periodically epidemic in Southeast Asia, China, and the Asian subcontinent.[130] During 2005 and 2006, there were large outbreaks in Northern India that caused several thousand deaths.[131] Sporadic cases are reported in

TABLE 153.1 Estimated Risk for Japanese Encephalitis by Country and Season

COUNTRY	AFFECTED AREAS	TRANSMISSION SEASON	COMMENTS
Australia[b]	Islands of Torres Strait	February–April peak; year-round transmission risk	Localized outbreak in Torres Strait in 1995 and sporadic cases in 1998 in Torres Strait and on mainland Australia at Cape York Peninsula
Bangladesh	Few data, but probably widespread	Possibly July–December, as in northern India	Outbreak reported from Tangail District, Dacca Division; sporadic cases in Rajshahi Division
Bhutan	No data	No data	—
Brunei	Presumed to be sporadic-endemic, as in Malaysia	Presumed year-round transmission	—
Cambodia	Endemic-hyperendemic countrywide	Presumed to be May–October	Cases in refugee camps on Thai border and from Phnom Penh
Democratic Republic of Korea	Presumed to be countrywide in rural areas <800 m altitude	July–October	Epidemics in 1970s; few recent data
India	Reported cases from all states except Arunachal, Dadra, Daman, Diu, Gujarat, Himachal, Jammu, Kashmir, Lakshadweep, Meghalaya, Nagar Haveli, Orissa, Punjab, Rajasthan, and Sikkim	South India: May–October in Goa, October–January in Tamil Nadu, August–December in Karnataka Second peak: April–June in Mandya District Andhra Pradesh: September–December[b] North India: July–December	Outbreaks in West Bengal, Bihar, Karnataka, Tamil Nadu, Andhra Pradesh, Kerala, Assam, Uttar Pradesh, Manipur, and Goa; urban cases reported (e.g., in Luchnow)
Indonesia	Kalimantan, Bali, Nusa, Tenggara, Sulawesi, Moluccas, Irian Jaya, and Lombok	Probably year-round risk; varies by island; peak risks associated with rainfall, rice cultivation, and presence of pigs Peak periods of risk: November–March, June and July in some years	Endemic on Bali, Java, and possibly in Lombok; sporadic cases recognized elsewhere
Japan[b]	Rare; sporadic cases on all islands except Hokkaido	June–September, except April–December on Ryuku Islands (Okinawa)	Vaccination not routinely recommended for travel to Tokyo and other major cities; enzootic transmission without human cases observed on Hokkaido
Laos	Presumed to be endemic-hyperendemic countrywide	Presumed to be May–October	—
Malaysia	Sporadic-endemic in all states of Peninsula, Sarawak, and probably Sabah	Year-round transmission; October–February in Sarawak	Most cases from Penang, Perak, Selangor, Johor, and Sarawak
Myanmar	Presumed to be endemic-hyperendemic countrywide	Presumed to be May–October	Repeated outbreaks in Shan State in Chiang Mai Valley
Nepal	Hyperendemic in southern lowlands (Terai); sporadic cases in Kathmandu Valley	July–December	Vaccination not recommended for travelers visiting only high-altitude areas
Pakistan	May be transmitted in central deltas	Presumed to be June–January	Cases reported near Karachi; endemic areas overlap those for West Nile virus; lower Indus Valley might be an endemic area
Papua New Guinea	Normanby Islands and Western Province	Probably year-round risk	Localized sporadic cases
People's Republic of China[b]	Cases in all provinces except Xizang (Tibet), Xinjiang, Qinghai Temperate areas: endemic to periodically epidemic Southern China: hyperendemic Hong Kong: rare cases in new territories	Northern China: May–September Southern China: April–October (Guangxi, Yunnan, Guangdong, and southern Fujian; Sichuan, Guizhou, Hunan, and Jiangxi provinces) Hong Kong: April–October	Vaccination not routinely recommended for travelers to urban areas only

Continued

TABLE 153.1 Estimated Risk for Japanese Encephalitis by Country and Season[a]—cont'd

COUNTRY	AFFECTED AREAS	TRANSMISSION SEASON	COMMENTS
Philippines	Presumed to be endemic on all islands	Uncertain; speculations based on locations and agro-ecosystems West Luzon, Mindoro, Negros, Palawan: April–November Elsewhere: year-round, with greatest risk April–January	Outbreaks described in Nueva Ecija, Luzon (including January 2004), and Manila
Republic of Korea[b]	Sporadic-endemic with occasional outbreaks	July–October	Last major outbreaks were in 1982–1983
Russia	Far eastern maritime areas south of Khabarovsk	Peak period July–September	Sporadic transmission in rural and sylvatic cycles
Singapore	Higher rates of enzootic transmission in western rural areas of island	Year-round transmission with April peak	Vaccination not routinely recommended; two sporadic cases in 2001
Sri Lanka	Endemic in all but mountainous areas; periodically epidemic in northern and central provinces	October–January; secondary peak of enzootic transmission in May–June	Outbreaks in central (Anuradhapura) and northwestern provinces
Taiwan[b]	Endemic, sporadic cases island-wide	April–October; June peak	Cases reported in and around Taipei and the Kaohsiung-Pingtung River Basins
Thailand[b]	Hyperendemic in north; sporadic-endemic in south	May–October	Annual outbreaks in Chiang Mai Valley; sporadic cases in Bangkok suburbs
Vietnam[b]	Endemic-hyperendemic in all provinces	May–October in the North, year-round in the South	Highest rates in and near Hanoi
Western Pacific	Two epidemics reported in Guam and Saipan since 1947	Uncertain; possibly September–January	Enzootic cycle may not be sustainable; epidemics have occurred after introductions of virus

[a]Assessments are based on publications, surveillance reports, and personal communications. Extrapolations have been made from available data. Transmission patterns may change.
[b]Locally reported incidence rates may not reflect risks to nonimmune visitors because high immunization rates in local populations may obscure ongoing enzootic transmission.
Modified from references 2 and 99 and updated from ProMED Mail (http://www.promedmail.org) and after the Global Alliance for Vaccines and Immunizations, Southeast Asia and Western Pacific Regional Working Group's Japanese Encephalitis Meeting: Setting the Global Agenda on Public Health Solutions and National Needs, Bangkok, Thailand, 2002.

tropical Asia, including the Indonesian and Philippine archipelagoes, but field studies suggest a higher incidence.[132,133] Twice, in 1947 and 1990, the virus was introduced to the western Pacific, resulting in outbreaks on Guam and Saipan. The virus invaded the Torres Strait Islands of Australia in 1995 and the Australian mainland in 1998.[79,134]

Worldwide, 160,000 cases of JE were reported to the WHO in 1966 and 16,000 cases in 1996, the 10-fold decline reflecting widespread childhood immunization in China, Japan, Korea, and Taiwan as well as regional economic development and the declining emphasis on agriculture. In the latter three countries, few cases are reported currently, although enzootic viral transmission persists. In areas with endemic transmission, an annual incidence of 2.5 per 10,000 children younger than age 15 years is estimated, with a case-fatality rate of 25% and disability in 45% of surviving patients.[6,130] Extrapolating this incidence to the population of 700 million children younger than age 15 years in the region, an estimated 175,000 JE cases, 45,000 deaths, and 78,000 cases of newly disabled children would occur annually in the absence of immunization.[132] Allowing for the countries where there is immunization, the expected number of cases annually is greater than 125,000. The fact that only one-fifth of these are officially recorded probably reflects the lack of reporting from many countries where no surveillance currently exists.[132] In an era in which polio has declined to the point of eradication, JE is now preeminent among causes of pediatric CNS infections in the region. The introduction of new WHO surveillance guidelines in many Asian countries should improve detection.[135]

Within temperate areas, JE is transmitted sporadically from July to September at a relatively low incidence and with periodic seasonal epidemics. In subtropical Asia, viral transmission extends from March to October in a hyperendemic pattern, resulting in cases throughout the year and the absence of easily detected seasonal epidemics. The geographic distribution of different JEV genotypes was postulated to explain the differences in clinical epidemiology, but it is now believed to be best explained by the virus's evolution in the Indonesia-Malaysia region and its subsequent spread as more recently evolved genotypes.[134,136]

The virus is transmitted by *Culex tritaeniorhynchus* and related ground- and pool-breeding mosquitoes to pigs and aquatic birds, which are the principal viral-amplifying hosts.[137] Viremic adult pigs are asymptomatic, but infected pregnant sows abort or deliver stillbirths. Infected horses and humans are symptomatic but incidental hosts. Because rice paddies provide favorable breeding habitats for vector mosquitoes, the risk for infection is highest in rural areas. However, both pigs and rice paddies are found at the edges of some Asian cities, resulting in isolated cases and, rarely, urban outbreaks. The mosquito vectors chiefly feed outdoors, in the evenings, and prefer animal to human hosts.

More than 99% of infections with JEV are subclinical; consequently, in areas with endemic transmission, infections acquired naturally at an early age result in immunity in more than 80% of young adults. Cases occur chiefly in children between 2 and 10 years of age, with a slight predominance of boys. In Japan, Korea, and Taiwan, children are protected by immunizations and cases occur principally in elderly people, reflecting waning immunity or other biologic factors associated with senescence.[138]

Expatriate and traveler cases have been recognized since 1932, and outbreaks among American, British, and Australian soldiers in World War II, the Korean War, and the Vietnam war were considered militarily important. Travelers of all ages without naturally acquired protective antibodies are at risk for acquisition of the illness. The risk is slight, estimated to be 1 in 150,000 person-months of exposure, reflecting low vector mosquito infection rates (0.5%) and the small case-to-infection ratio (0.3%).[139] However, cases of JE among travelers, even on short trips to Southeast Asia, serve as a reminder of the unpredictable risk for acquiring this disease.[140–142]

West Nile Encephalitis

WNV is one of the most widely distributed arboviruses, being found across much of Africa, southern Europe, the Middle East, Asia, Australia (Kunjin subtype), and, more recently, the Americas.[143,144] Recent outbreaks have included almost 400 confirmed cases in Romania in 1996, almost 200 cases in the Volgograd region of Russia in 1999, and more than 200 cases in Israel in 2000 (Table 153.2).[144] In 1999, the virus appeared in North America for the first time in the New York area, with 62

TABLE 153.2 Outbreaks of West Nile Virus Infection

YEAR OF OUTBREAK	COUNTRY	NO. OF SUSPECTED CASES	NO. OF CASES INVESTIGATED	NO. OF CONFIRMED CASES	NO. OF DEATHS	NOTES
1957	Israel	419	247	About 180	4	Included first naturally occurring encephalitis cases (12 patients)
1974	South Africa	18,000	558	307	0	Estimated 18,000 cases of WN fever
1962–1966	France (Camargue)	—	—	14	1	Many horses also affected
1994	Algeria	50	18	17	8	—
1996	Romania	835	509	393	17	Continuing cases in 1997–1999
1997	Tunisia	173	129	111	8	—
1998	Democratic Republic of Congo	35	35	23	0	Military personnel newly arrived in this area
1999	USA (New York)	719	719	62	7	—
1999	Russia (Volgograd region)	826	318	183	40	—
2000	Israel	—	—	233	33	91 nonhospitalized patients with WN fever also identified
2000–2001	USA	—	—	85	24	—
2002	USA	—	—	4156	284	2956 with CNS disease
2003	USA	—	—	9862	264	2866 with CNS disease
2004	USA	—	—	2539	100	1142 with CNS disease
2005	USA	—	—	3000	119	1294 with CNS disease
2006	USA	—	—	4269	177	1459 with CNS disease
2007	USA	—	—	3630	124	1217 with CNS disease
2012	USA	—	—	5674	286	2873 with CNS disease
2013	USA	—	—	2469	119	1267 with CNS disease
2014	USA	—	—	2205	97	1347 with CNS disease
2015	USA	—	—	2175	146	1455 with CNS disease
2016	USA	—	—	2149	106	1309 with CNS disease

[a]Only details of selected outbreaks are shown. Criteria for hospitalization, case definitions, and diagnostic methods varied among outbreaks, and some numbers are approximations.
CNS, Central nervous system; *WN,* West Nile.

confirmed human cases and 25 equine cases. The virus rapidly established itself in North America with a peak in 2003 of nearly 10,000 clinical cases, including 286 cases of neurologic disease. Since 2004, the number of cases has varied from 1300 to 2900 neurologic cases per year and there has been a consistent mortality rate of 10% among neurologic cases. As with JEV, many of the survivors of WNV neurologic infection suffer from neuropsychiatric impairment that may last as long as 3 to 4 years. Surprisingly, there are few reports of human disease south of the United States, with a few equine cases reported in Argentina.

WNV is transmitted in an enzootic cycle between birds by mosquitoes (Fig. 153.3). Recent studies in the United States have demonstrated infection in at least 300 different bird species and 62 mosquito species. In addition, at least 30 vertebrate species are infected, but they have insufficient viremia to infect a feeding mosquito and are considered "dead-end" hosts. Some, however, may have clinical infection (e.g., in humans). Members of the order Passeriformes (jays, blackbirds, finches, warblers, sparrows, and crows) appear to be important in transmission of the virus in nature; the family Corvidae (crows and blue jays) is particularly susceptible. In some areas, arrival of the virus was heralded by dying birds falling from the sky. The lack of resistance and fatal infection of avian intermediary hosts provided evidence for the novel introduction of the virus to the ecosystem of the Western Hemisphere, unlike the asymptomatic infection of autochthonous birds by the closely related SLE virus. Of the many mosquito species from which WNV has been isolated, *Culex* spp. appears to be important in the enzootic cycle, although the species varies by geographic location. Transovarial transmission of the virus in mosquitoes probably provides for viral overwintering. During the 2002 outbreak in the United States, it became clear that on rare occasions viral transmission could occur by transplantation of infected organs, from infected blood products, transplacentally, and, possibly, through breast milk.[145,146] During the height of the 2002 epidemic, the risk for infection by transfusion was estimated to be as high as 21 per 10,000 donations. Blood screening using real-time PCR assay has been instituted and has dramatically decreased the risk for contaminated blood.[145,146]

The means by which WNV is introduced to new areas are not completely understood. Migratory birds are thought to be important for movement of the virus from Africa into southern Europe. They may have been involved in its introduction into North America, although importation of viremic exotic birds or amphibians and inadvertent transport of mosquitoes are alternative explanations.[147] Molecular genetic evidence suggests a single introduction into the United States of a strain, possibly from the Middle East.[148]

Most human infections with WNV are asymptomatic. Epidemiologic surveys taken after the 1999 outbreak in New York suggested that about 1 in 5 people infected with WNV develops fever and that only about 1 in 150 develops CNS disease.[145,146] These rates are similar to the attack rates for the Romanian 1997 outbreak, but they appear to be much higher than those reported from Egypt and South Africa, most likely because of preexisting antibodies in people in Africa.[149–151] In New York, Romania, and Israel, the risk for neurologic disease increased with age, which may explain, in part, the different epidemiologic patterns seen in parts of Africa. In Egypt, most of the population is infected during childhood and neurologic disease is rare.[150] In South Africa, a large outbreak affected an estimated 18,000 people of all ages, yet only a single West Nile encephalitis (WNE) case was reported.[151]

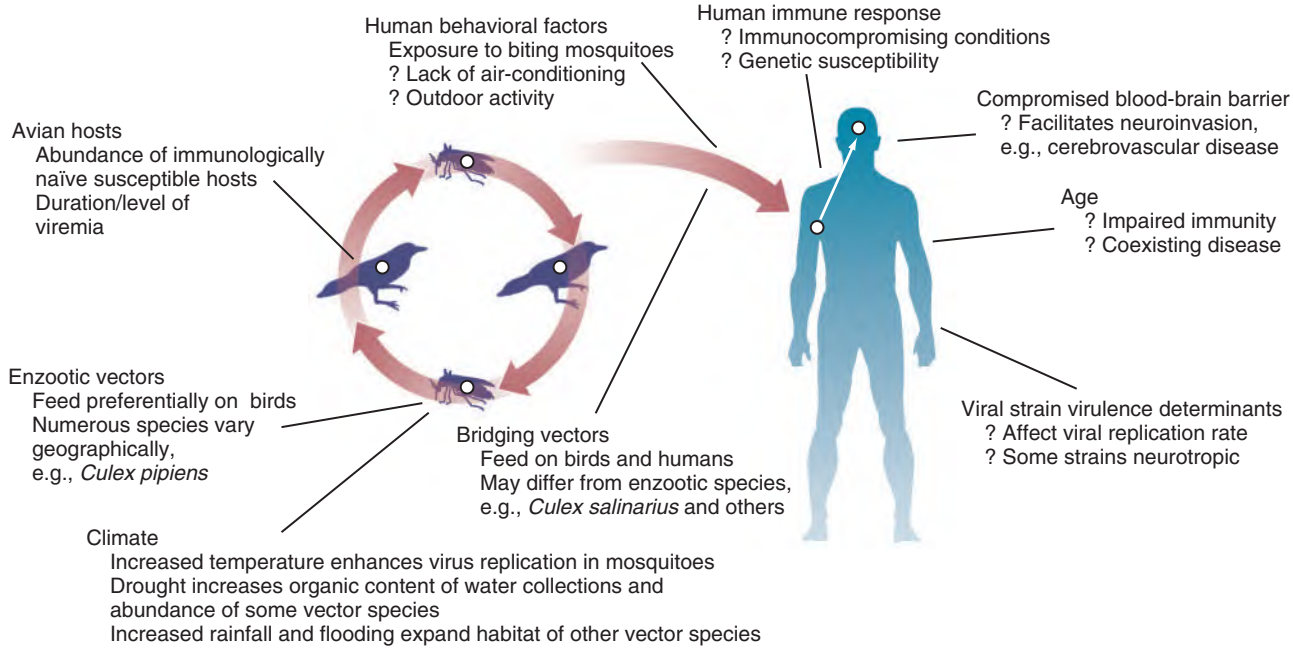

FIG. 153.3 West Nile virus transmission cycle as an exemplar of mosquito-avian-virus transmission cycle and the ecologic, virologic, vector, and human factors impacting exposure, infection, and infection outcomes.

St. Louis Encephalitis

Outbreak-associated cases of SLE have been reported from virtually all US states, the provinces of Ontario and Manitoba in Canada, and Sonora State in Mexico, whereas only sporadic cases have been reported from Argentina, Brazil, Panama, Trinidad, French Guiana, Surinam, Curaçao, Jamaica, and the Dominican Republic. Enzootic viral transmission has also been recognized in Alberta and British Columbia in Canada and in Ecuador, Guatemala, and Haiti and may occur elsewhere in the hemisphere.[152,153]

In the United States, the virus is transmitted to birds in three distinct cycles overlapping those of WNV: by *Culex pipiens* and *Culex quinquefasciatus* in the Midwestern and Eastern states, by *Culex nigripalpus* in Florida, and by *Culex tarsalis* in the Great Plains and farther west. Humans are infected incidentally from the enzootic cycle and, as with WNV infection, humans are dead-end hosts. Characteristics of the vectors and their respective transmission cycles define epidemiologic features in each location.

In the eastern states, SLE is transmitted periodically in localized and regional outbreaks at lengthy intervals without significant transmission in intervening years. Outbreaks in the late summer and fall occur in urban areas, often in older neighborhoods, where polluted wastewater provides a breeding habitat for *C. pipiens* and *C. quinquefasciatus,* the northern and southern house mosquitoes, respectively. More than 50 epidemics, ranging in the hundreds of cases, have been recognized in small towns or cities, including Houston, Dallas, Memphis, New Orleans, Chicago, and Detroit. The largest epidemic, in 1975, led to more than 3000 cases of neurologic infection nationally, similar in scale and geographic location to recent WNE outbreaks. In three outbreaks since 1991, disproportionate risk was reported among homeless persons infected with the human immunodeficiency virus (HIV), probably reflecting their increased vulnerability to mosquito bites in the evening, when the vectors are most active.[154] Between 1992 and 2000, much smaller outbreaks and sporadic cases occurred (median, 14 cases; range, 2–26 cases annually). The 2001 outbreak in Louisiana produced 71 cases, and was a reminder of the continued enzootic transmission and epidemic potential of this virus.[155]

Outbreaks in Florida occur more diffusely in suburban and urban locations, where swales and ground pools provide breeding sites for C. *nigripalpus.*[156] In the western states, SLE is transmitted perennially and at a low level in rural areas, frequently in association with irrigated farms and pastures. Forty years ago, outbreaks in agricultural areas occurred at regular intervals; more recently, cases have been more sporadic and frequently have involved vocational exposures or have occurred in proximity to cities. Small urban outbreaks have also occurred. The decline in cases has been attributed to secular changes in land use, air-conditioning of residences, and other factors leading to reduced exposure. Diminished exposure to infection has been confirmed in rural California populations, in whom seroprevalence rates now range from 0.1 to 11%.[153]

The risk for illness is associated most strongly with advanced age, but a slightly elevated risk is also seen in infants. The importance of age is reflected in the declining ratio of asymptomatic to symptomatic infection, which ranges from 800:1 in children to 85:1 in adults older than age 60 years.[157] Between 1999 and 2007, 75% of all patients were older than age 40 years, with an increased risk in males and in blacks.[51]

Tick-Borne Encephalitis

TBEV is classified as one species within the mammalian group of tick-borne flaviviruses and is further subdivided into three subtypes: Far Eastern (TBEV-FE; previously RSSE), Siberian (TBEV-Sib; previously west-Siberian), and European (TBEV-Eu; previously CEE). There is evidence that these three virus subtypes have overlapping geographic areas.

The three subtypes of TBEV, as well as other related mammalian tick-borne complex viruses, are transmitted across the Holarctic, with some evidence for their dissemination from an Asian source.[76,158] The TBEV-FE subtype is transmitted in eastern Russia, Korea, China, and parts of Japan and the Baltic states; the TBEV-Eu subtype and related viral strains are found in Scandinavia, Europe, and eastern states of the former Soviet Union; and the TBEV-Sib subtype is found in all TBE-endemic areas of Russia (see Fig. 153.1D). The geographic distributions overlap in Eastern Europe, where both the TBEV-Sib and TBEV-FE subtypes may be isolated.[54] Louping ill virus is found in the British Isles, and POWV is found in North America and northern Asia. Closely related tick-borne flaviviruses include Turkish and Spanish sheep encephalitis viruses, which are found in southern Europe, and three viruses that cause hemorrhagic fever—KFDV in India, AHFV (which is a subtype of KFDV) in Saudi Arabia and Egypt, and Omsk hemorrhagic fever virus in Siberia.[59,159,160]

Surveillance systems for TBE exist in 20 of the 30 European Union and European Free Trade Association countries. TBE is recognized throughout Europe, except in Portugal and the Benelux countries, but

endemic transmission is most intense in Austria, areas of Sweden, Switzerland, Slovenia, the Czech Republic, Estonia, Lithuania, Latvia, and Russia.[159,160] From 2000 to 2010, the overall number of reported TBE cases demonstrated increases in 2003, 2006, 2009 and 2010, with the Czech Republic reporting 25% of all cases, Lithuania 15%, Latvia 11%, Germany 11%, and Slovenia 10%. Incidence rates in unvaccinated populations have approached 20 in 100,000 persons. In Austria, national vaccination programs have reduced the incidence of disease to fewer than 1 in 1 million.[159–161] Sporadic cases are reported from France, Liechtenstein, Sweden, Switzerland, Italy, and Greece. In the Far East, TBE cases occur principally among people working or living in sylvatic locations in Russia, Korea, northern China, and Hokkaido Island, Japan. New models based on environmental factors and satellite data suggest that climate change is partially responsible for the increased incidence in Europe.[162] Longitudinal studies show the virus now circulates at greater altitudes in the Czech Republic and Switzerland.

The viruses are transmitted horizontally between ticks and vertebrates and through the winter by vertical transmission in the ticks and latent infections in hibernating animals. The virus passes transovarially and transstadially, from egg to larva, nymph, and adult, so all stages of the tick and both male and female ticks transmit infections to animals and humans. In addition, it appears that virus may be transmitted between ticks, as they feed on the skin of the same host, through infected host reticuloendothelial and inflammatory cells, without the need for host viremia.[163] Larval and nymphal ticks feed principally on birds and small mammals, and adult ticks on larger mammals such as roe deer, deer, domestic goats, sheep, cows, dogs, cats, and humans. Human infections are incidental to the transmission cycle. Animal movements can spread ticks and the virus to new foci.

Within the ranges of *Ixodes ricinus* and *Ixodes persulcatus* (the principal tick vectors of the TBEV-Eu and the TBEV-FE plus TBEV-Sib subtypes, respectively) and additionally *Ixodes ovatus* and *Haemaphysalis* spp. (vectors in Japan and Korea, respectively), the ticks are distributed focally in sheltered microhabitats with high humidity and moderate temperatures and can be found at elevations up to 2100 m. Landscape ecology studies have characterized forest ecotones to fields or meadows and low stands of deciduous trees and brush with a thick canopy as high-risk biotopes that correlate with foci of human cases.[164] Transmission foci tend to be highly stable but are subject to human environmental modifications and possibly climate change.[165] In central Europe, cases occur from April until November, peaking in June and July, with a secondary rise in October. Climate change has produced a dramatic change in the distribution of *Ixodes* spp. tick vectors and TBEV transmission.[166–169]

Cases occur mainly in adults 20 to 50 years of age, with a male predominance, reflecting occupational exposure in forestry and farming. Nevertheless, children at outdoor play and persons with vocational exposure while hiking, berry picking, or mushroom gathering also may be at risk, depending on the location and season. However, the risk for most persons with short-term exposures is low. Among American soldiers stationed in central Europe, no cases and a low rate of seroconversion (0.1%–0.4%) have been reported.[170] Louping ill is principally a rare occupational disease of veterinarians, sheepherders, and butchers.[171]

TBEV is stable at acid pH, and consumption of unpasteurized milk or milk products from infected goats, sheep, or cows previously accounted for 10% to 20% of cases in some parts of central Europe. The possibility that POWV can be transmitted from raw milk products in the United States has been suggested.[172] Slaughter or butchering of infected animals or meat is a principal mode of transmission for louping ill virus to humans and also has been reported in TBEV and in outbreaks of AHFV.[173] Infection has also been acquired from infected ticks carried to households on fomites.

In addition to TBEV, *I. ricinus* also transmits several *Borrelia* spp. responsible for Lyme disease (as well as *Anaplasma phagocytophilum, Babesia microti,* and several species of *Rickettsia*), and dual infections of ticks and humans are observed. However, in at-risk areas, Lyme disease is far more common than the other diseases. This difference reflects the low proportion of virus-infected ticks (0.1%–5%) and the 10-fold higher borrelial infection rates of ticks in the same location. This distinction may result from the brevity of viremia in animal hosts, which provides an opportunity for tick infection of only a few days; in contrast, persistent borrelial infections of rodents offer a higher likelihood of tick infection during feeding. An analogous situation occurs in the United States, where *Ixodes scapularis* transmits Lyme disease, babesiosis, human anaplasmosis, and a genotype of POWV represented by deer tick virus.[174] However, *Ixodes cookei* ticks (the principal vector of POWV) and *I. scapularis* differ somewhat in their host range, which may further limit opportunities for the viral and borrelial transmission cycles to intersect. The main enzootic cycles for *I. cookei* involve woodchucks and for *Ixodes marxi* involve squirrels (POWV lineage 1). For *I. scapularis,* they involve deer (POWV lineage 2).[55,56]

Alkhurma Hemorrhagic Fever

Since its original isolation in 1995, Alkhurma hemorrhagic fever has occurred in sporadic outbreaks in Saudi Arabia, usually in limited numbers and characterized by a mortality that ranged from 1.3% to 25%.[57] From August 2003 to December 2009, there were 148 suspected cases of Alkhurma hemorrhagic fever in Najran, Saudi Arabia, of which 78 (53%) were laboratory confirmed. There were 2 cases in 2003, 1 in 2004, 4 in 2005, 12 in 2008, and 58 cases in 2009.[57] Cases occurred year-round, although more than half occurred during the summer months. Transmission is thought to be tick-borne and occurs as a zoonotic disease in domestic livestock, with outbreaks associated with livestock contact and familial clustering. A seroprevalence study of 1024 Saudi soldiers deploying to Jazan Province was conducted in 2009.[175] Thirteen (1.3%) of the soldiers were seropositive for AHFV and came from Tabuk and Eastern Directorates not known for cases of AHFV, which suggests a wider geographic distribution of this virus in the Arabian Peninsula. This was confirmed in 2010 when two tourists from Italy on vacation in southeastern Egypt near the Sudanese border became infected with AHFV.[176] It is now believed to have wider geographic distribution than once thought, is considered an emerging infectious disease, and is potentially spread by the transportation of cattle, goats, and camels and their potentially infected ticks throughout the Arabian peninsula and Egypt.

Kyasanur Forest Disease

As previously noted, KFDV was first isolated from sick monkeys in the Kyasanur Forest of Shimoga district of Karnataka state of India. This is a heavily rain-forested region with a large nonhuman primate population of *Presbytis entellus* (black-faced langur) and *Macaca radiata* (red-faced bonnet) monkeys.[177] Monkeys become ill from KFDV presumably through the bite of infected ticks, primarily *Haemaphysalis spinigera,* although the reservoir is not known but thought to be a rodent species. KFDV was originally thought to be geographically localized to the Kyasanur Forest area. Between 1964 and 1973, 1046 wild monkeys had died of KFDV, with a seasonal activity between December to May coinciding with the immature stages of the *Haemaphysalis* tick.[177] The epizootic geographic areas have changed from the 1960 location of Kyasanur Forest to the Yakshi and Gudavi forests in the 1970s and to further distances in Shimoga district. Serologic surveys detected the presence of antibodies in humans located in Gujrat state, 1200 km from the Kyasanur Forest area.[177] Outbreaks in humans occur during epizootic outbreaks in wild monkeys and humans working in forested areas for lumber or clearing of areas for farming. From December 2011 to March 2012, 215 suspected cases from 80 villages (total population, 22,201) in Shimoga were identified as potential infections from KFDV, representing an attack rate of 9.7 cases/1000 persons.[178] Of these, 61 (28%) cases were laboratory confirmed. Cases began occurring in the last week of December 2011, peaked during the first 2 weeks of February, and then declined gradually. Risk factors for infection included handling of cattle, frequent visits to the forest, and piles of dry leaves within the compounds of their house. Of the 51 case-patients, 20 had received two doses of vaccine and 2 had received one dose. KFDV was discovered in Yunnan Province, China, in 1989, where it was originally called Nanjianyin virus and is now known as a subtype of KFDV.[179] A seroepidemiologic investigation in Yunnan Province from 1987 to 1990 demonstrated that residents in the region of the Hengduan Mountains had been infected with this virus. This suggests that KFDV may have a wider geographic distribution than previously appreciated and that migratory birds with

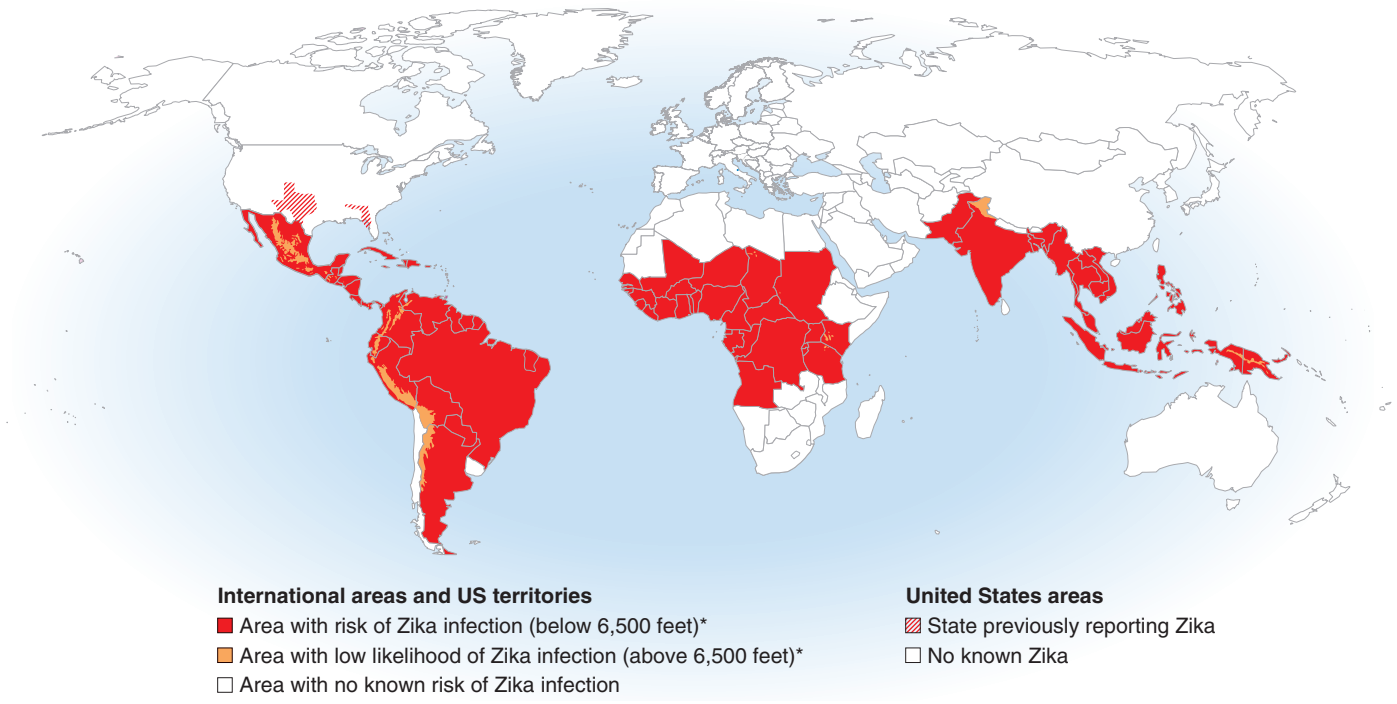

FIG. 153.4 World map of areas with risk of Zika as of March 2018. *Mosquitoes that can spread Zika usually live in locations below 6500 feet. The chances of acquiring Zika from mosquitoes living above that altitude are very low. *Data from the Centers for Disease Control and Prevention.*

attached infected ticks may be responsible for its geographic distribution.[179] A vaccine for KFDV is available and licensed in India. It is a formalin-inactivated virus vaccine produced in chick embryo fibroblasts with a vaccine efficacy of 79.3% with one dose and 93.5% with two doses.[177,180–182]

Zika

ZIKV was demonstrated to be a human infection soon after its discovery in a febrile monkey through serologic surveys and in a case of human infection in a field worker. An experimental infection of a volunteer resulted in a febrile illness associated with headache, myalgias, and symptoms of prostatitis.[183] Subsequently, ZIKV has been isolated from a variety of *Aedes* mosquitoes and is a cause of human infections throughout equatorial Africa, Indonesia, Malaysia, and Cambodia, with *Aedes aegyptii* being the primary vector in Southeast Asia.[184] In 2007, a large outbreak of ZIKV infection occurred in Yap Island, Micronesia.[185] In total, there were 49 confirmed and 59 probable cases of ZIKV illness. Patients developed rash, fever, arthralgia, and conjunctivitis. *Aedes hensilli* was the predominant mosquito species identified. A large outbreak of ZIKV infection occurred in French Polynesia in 2013 and 2014 and subsequently moved to the Western Hemisphere, first on Chile's Easter Island in February 2014 to June 2014, followed by detection in Brazil in May 2015.[186] Within a year, the Brazilian epidemic resulted in ZIKV spread through the majority of states within Brazil to a total of 205,578 reported cases in 2016; a total of 13,353 cases were reported in 2017 with a peak seroprevalence of 63% in Northeast Brazil.[187] By February 2016, ZIKV had spread from Brazil and was detected in 31 countries and territories in the Americas.[186] According to the WHO, as of March 2017 ZIKV had spread to 84 countries and territories where vectors are present, to include 61 areas with ongoing transmission throughout Central America, South America, the Caribbean, Africa, and Southeast Asia; 13 countries with person-to-person transmission; 31 countries with reports of microcephaly and other CNS abnormalities associated with congenital infection; and 23 countries reporting an increase in GBS.[188] In the United States ZIKV became a nationally notifiable infection in 2016.[189] According to the CDC, from 2015 through June 5, 2019 there have been 5,470 travel-related cases in the United States, 231 locally acquired cases, and 55 cases related to other routes of transmission such as sexually acquired Zika. In the United States and territories, there have been 148 travel-related cases and 37,206 locally acquired Zika cases with zero cases accounting for transmission via other routes. Clinicians should be aware of areas at risk of ZIKV transmission when conducting pretravel counsel or when seeing the ill returning traveler (Fig. 153.4).

ZIKV transmission appears to be primarily by *A. aegyptii* mosquitoes, but *A. albopictus,* which lives in temperate regions, may also be able to transmit the virus.[190] ZIKV has been associated with possible sexual transmission, transmission through blood transfusion, and perinatal transmission.[191,192] On February 5, 2016, the CDC issued interim guidelines for prevention of sexual transmission of ZIKV.[191,193] These include the recommendation that men who travel to or reside in an area of active ZIKV transmission and who have a pregnant partner should abstain from sexual activity or consistently and correctly use a condom. Men with such travel histories and nonpregnant sex partners should also consider abstinence or proper use of condoms.[191,192,194,195]

PATHOGENESIS

The early stages of infection are quite similar for all of the flaviviruses, with an initial stage of local replication followed by dissemination and viremia. Subsequent events in disease pathogenesis likely depend to a greater extent on the clinical syndrome than on the specific virus. As a result, the pathogenesis of infection by those viruses that have not been characterized in detail are presumed to be similar to one of the prototype pathogens; for example, hemorrhagic fevers caused by ALKV, KFDV, and Omsk hemorrhagic fever virus are expected to share characteristics similar to YF. ZIKV appears unique among the flaviviruses for its robust tropism for placenta, neuroprogenitor cells, and other cells, which results in a spectrum of congenital and other defects.[191,196] The tissue tropism of ZIKV is presented in Fig. 153.5.

Yellow Fever

Early stages of YF infection can be inferred from human vaccine studies and from experimental wild-type viral infections of primates. Two days after inoculation of the attenuated 17D vaccine, levels of tumor necrosis

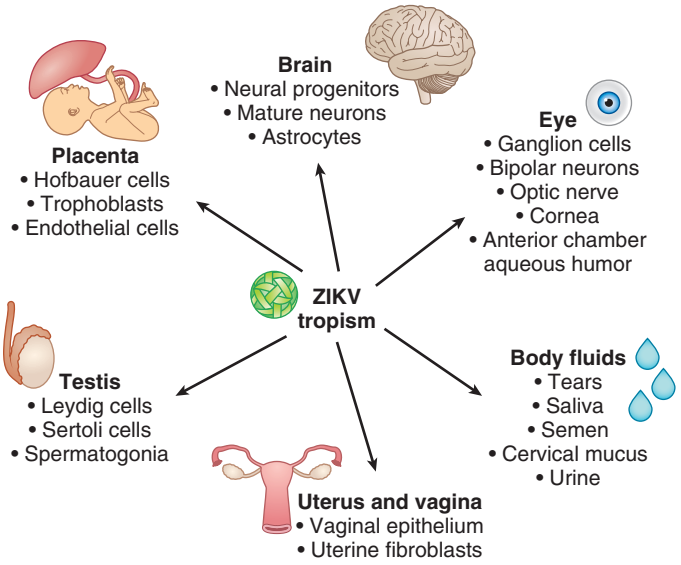

FIG. 153.5 Observed Zika virus *(ZIKV)* tissue and cell tropism. Human studies and studies in animal models (mice and nonhuman primates) have detected ZIKV in cells of the placenta, including Hofbauer cells (in vitro and in explanted human placental tissue), trophoblasts (mice, nonhuman primates, and humans), and endothelial cells (in vitro in explanted human placental tissue and in vivo in placenta of mice). Other ZIKV cellular targets include neuronal cell types, including neural progenitor cells and mature neurons (mice, nonhuman primates, and humans) and astrocytes (in vitro human cell cultures). In addition, ZIKV infects ocular tissues, including the cornea, neurosensory retina, and optic nerve (mice), as well as the aqueous humor of the anterior chamber (humans). ZIKV also targets cells of the reproductive tract, including spermatogonia, Sertoli cells, and Leydig cells (in the testis of mice); sperm (samples from mice and humans); and the vaginal epithelium (mice) and uterine fibroblasts (in vitro infection of human samples). The extensive tropism results in ZIKV detection in multiple body fluids, including conjunctival fluid or tears (mice and humans), saliva (nonhuman primates and humans), semen (mice, nonhuman primates, and humans), cervical mucus (humans), vaginal washings (mice and human), and urine (nonhuman primates and humans). *(Modified from Miner JJ, Diamond MS. Zika virus pathogenesis and tissue tropism.* Cell Host Microbe. *2017;21:134–142.)*

factor-α (TNF-α), interleukin (IL)-1 receptor antagonist, and, to a lesser extent, IL-6 increase, with a secondary TNF-α peak 7 days later.[6,83,197,198] The cytokines are synthesized in response to local spread of the vaccine and again as a response to viremia, which peaks between days 3 and 6.[6,83,199] TNF-α elevations correspond to declines in the lymphocyte count. After wild-type viral infection, the grippe phase of early YF presumably is associated with a similarly timed elaboration of cytokines. In experimentally inoculated rhesus monkeys, the virus replicates initially in local lymph nodes, followed rapidly by bloodborne infection of fixed macrophages, especially Kupffer cells in the liver, and further spread and replication in liver, lung, kidney, and adrenal glands, and most prolifically in regional lymph tissue, spleen, and bone marrow.[6,83] Infection by mosquito feeding, which introduces salivary proteins, is believed to differ from experimental needle inoculation in the outcome of local viral replication and distribution, but the importance of these factors in modulating human flavivirus infections is unknown.

Pathologic changes are most pronounced in the liver and kidneys, but widespread hemorrhages are found on mucosal surfaces, in the skin, and within various organs. Numerous petechial hemorrhages and erosion of the gastric mucosa contribute to the hematemesis that typically introduces the illness. Hepatocellular damage is characterized by a patchy mid-zonal distribution, sparing cells around the central vein and portal triad. The extent of lobular necrosis is variable, with an average of 60%, but even with confluent lobular necrosis, the reticular architecture is preserved. The preservation of the reticulin network, minimal inflammatory changes, and the morphology of degenerating hepatocytes are consistent with apoptosis as the principal pathway of cell death. Early changes in infected hepatocytes consist of glycogen depletion and cloudy swelling, followed by accumulations of fat and of ceroid pigment. Necrotic cells finally undergo coagulation, with the formation of characteristic eosinophilic Councilman bodies, which correspond to apoptotic cells. Viral antigen is identified initially in Kupffer cells and appears later in hepatocytes, Councilman bodies, and endothelial cells.[200–202] Healing occurs without fibrosis.

Albuminuria and renal insufficiency reflect prerenal factors, including vomiting and myocarditis, as well as parenchymal invasion and, in advanced illness, acute tubular necrosis.[83,203] Viral antigen can be identified in the kidney and also in the heart, in which degenerative fatty infiltration of the myocardium and of the conduction system contributes to decreased output and arrhythmias.[202] Neurologic findings probably reflect metabolic disturbances, cerebral edema, and hemorrhages rather than encephalitis. The cause of the bleeding diathesis is ill defined but probably represents a combination of reduced hepatic synthesis of clotting factors, intravascular coagulation, thrombocytopenia, and endothelial and platelet dysfunction. A combination of direct parenchymal damage and a systemic inflammatory response–like syndrome appears to contribute to shock and a fatal outcome. Neutralizing antibodies elaborated within the first week of illness clear the virus, and recovery is followed by lifelong immunity.

Heterologous flavivirus immunity (e.g., previous dengue) is believed to provide partial protection against infection, which may contribute to the absence of YF in Asia.[204] However, in contrast to the situation with DHF, there is no evidence of antibody-mediated immune enhancement. Genetic selection has been described in survivors of YF epidemics, and youth and advanced age have both been implicated as risk factors for symptomatic illness.[6,205] Hepatitis B carriage, which is prevalent in areas of Africa with endemic YF, is not a risk factor for symptomatic disease.[206]

Dengue and Dengue Hemorrhagic Fever

Most DENV infections are subclinical. Self-limited dengue fever is the usual clinical outcome of infection. The syndrome of DHF (Fig. 153.6) occurs in a small percentage of cases, apparently as an immunopathologic response to infection and usually in the setting of heterologous immunity from prior infection with a different DENV type or possibly another flavivirus.[207,208]

As with YF, early stages of DENV infection are inferred from experimental infection of humans or other primates. After an infectious mosquito bite, the virus replicates in local lymph nodes and within 2 to 3 days disseminates through the blood to various tissues. Virus circulates in the blood typically for 4 to 7 days in plasma and infected monocytes.[209] Viral RNA is also readily detected in circulating B cells.[210] Virus likely also replicates in skin, reactive spleen lymphoid cells, and tissue macrophages.[211,212] Viral antigen can be demonstrated more widely in liver Kupffer cells and endothelia, renal tubular cells, and alveolar macrophages and endothelia, although active viral replication at these sites has been difficult to document. Almost all patients are viremic at the point of clinical presentation with fever and clear the virus from the blood within days after defervescence.[213,214] The malaise and influenza-like symptoms that typify dengue probably reflect patients' cytokine response; however, myalgia, a cardinal feature of the illness, may also indicate pathologic changes in muscle, typified by a moderate perivascular mononuclear infiltrate with lipid accumulation.[215] Musculoskeletal pain (breakbone fever) conceivably could reflect viral infection of bone marrow elements.[216,217] Suppression of erythropoiesis, myelopoiesis, and thrombopoiesis is reflected in peripheral cytopenias. Histopathologic examination of skin from patients with rash discloses a minor degree of lymphocytic dermal vasculitis and, variably, viral antigen.[211,212] Elevated hepatic aminotransferase concentrations have been reported in most cases of dengue, with the aspartate aminotransferase (AST) level initially higher than that of alanine aminotransferase (ALT) and with levels higher in DHF compared with dengue fever.[114] In fatal cases, histopathologic findings resemble those of early mild YF, with hypertrophy of Kupffer cells, focal ballooning, and necrosis of hepatocytes in a mid-zonal distribution, with occasional Councilman body formation, mild fatty changes, and a scant periportal mononuclear cell response.[218] Neurologic complications have been attributed chiefly to metabolic

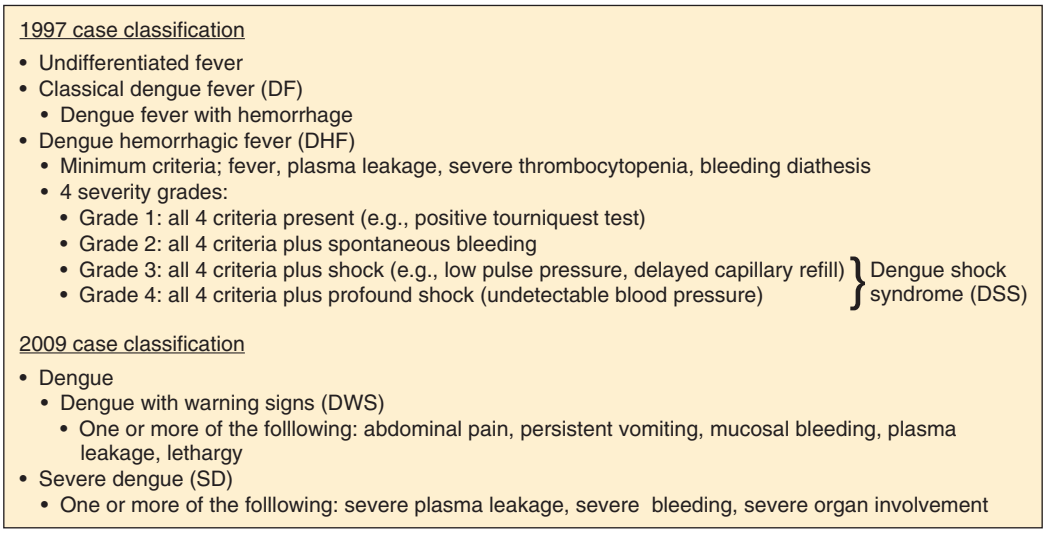

FIG. 153.6 Clinical spectrum, pathophysiology, and case classification of dengue hemorrhagic fever. For the 1997 case classification, severe thrombocytopenia refers to a platelet nadir of 100,000 cells/mm^3 or lower; evidence of bleeding diathesis includes at a minimum a positive tourniquet test; evidence of plasma leakage includes hemoconcentration of 20% or greater, pleural effusion, or ascites; and criteria for shock include a pulse pressure of 20 mm Hg or lower. For the 2009 case classification, evidence of severe plasma leakage includes either shock requiring fluid resuscitation or fluid accumulation impairing respiratory function; severe bleeding and severe organ involvement are determined by the clinician's judgment. *(From World Health Organization.* Technical Guide for Diagnosis, Treatment, Surveillance, Prevention, and Control of Dengue Haemorrhagic Fever. *2nd ed. Geneva: World Health Organization; 1997.)*

alterations and to focal, and sometimes massive, intracranial hemorrhages, but viral CNS invasion and encephalitis have been demonstrated to occur.[219,220]

Shock in dengue shock syndrome (DSS) occurs after the sudden extravasation of plasma into extravascular sites, mainly the pleural and abdominal cavities, usually coincident with the disappearance of fever.[207,221] The extensive increase in vascular permeability is associated with immune activation, as manifested by increased plasma levels of soluble tumor necrosis factor receptor (sTNFR/75), IL-8, interferon (IFN)-γ, and vascular endothelial growth factor, among other mediators.[222–225] In addition, immune complex formation activates the complement system, with increases in C3a, C5a, and soluble C5b-9 complexes.[226,227] The rapid, predictable reversibility of the syndrome within 48 hours and the paucity of histopathologic correlates—usually perivascular edema with diapedesis of red cells and widespread focal hemorrhages—suggest that the inflammatory response produces a vasculopathy. Reduced cardiac output may contribute further to shock.[228,229] The hemorrhagic diathesis is complex and not well understood, reflecting a combination of cytokine action and vascular injury, viral antibodies binding to platelets or cross-reacting with plasminogen and other clotting factors, reduced platelet function and survival, and a mild consumptive coagulopathy.[230–233]

The increased frequency of DHF associated with sequential DENV infections of different DENV types has suggested a role for adaptive immunity in the pathogenesis of severe dengue disease. Serotype cross-reactive "heterologous" antibodies from an earlier DENV infection have been shown to enhance viral uptake and replication in Fc receptor–bearing cells (antibody-dependent enhancement) in vitro and in a mouse model.[234,235,236–238a]

Passively acquired antibody has been proposed to have the same pathologic effect during primary DENV infection in infants born to dengue-immune women.[239] Simultaneously, memory cellular responses to heterologous antigens also may contribute to immunopathology during secondary infections.[240] Elevated levels of plasma-soluble CD8 and soluble IL-2 in patients with DHF and flow cytometry of circulating peripheral blood lymphocytes indicate an activation of memory CD4$^+$ and CD8$^+$ T cells in response to a second infection.[223,241] Cells responding to cross-reactive antigens predominate over primary responses to the infecting virus, following the paradigm of original antigenic sin, but may be ineffective in viral clearance, and instead may be a source of cytokines with a negative clinical effect.[241] The resulting production of IL-2, IFN-γ, TNF-α, and other lymphokines is reinforced by the increased abundance of infected target cells and flavivirus-induced expression of major histocompatibility complex type I and II molecules that further activate T lymphocytes.[242–246] Activated infected monocytes (and possibly endothelial cells) produce and release with their lysis TNF-α, IL-1, platelet-activating factor, IL-8, and RANTES (regulated on activation, normal T-cell expressed and secreted), which act in synergy with lymphokines, histamine, and C3a and C5a to produce the transient vascular endothelial dysfunction that leads to plasma leakage.[242,243] Additional direct and indirect effects on vascular endothelial function have been ascribed to secreted NS1 protein and to the activation of other cell types, including platelets and mast cells.[242,244–246]

Although infection with any of the four DENV types can produce DHF, there is some indication of a greater propensity after second infections with certain DENV types or with specific strains of putatively greater virulence in a given partially immune population.[214,247,248] Trends toward an increased or fluctuating severity of illness during prolonged outbreaks have been attributed to the evolution of viral quasispecies.[249] Various genetic polymorphisms in the human host also influence the risk for DHF, particularly in HLA and *MICB* genes, which control immune responses, as well as in the *PLCE1* gene, the mechanism for which is unknown.[250,251]

The induction of innate immune responses and a rise in levels of serum neutralizing antibodies are correlated with the clearance of viremia,[213,252] but protective immunity is associated with both humoral and cellular immune responses.[240,253–255] The cross-protective immunity induced by infection with one DENV type is limited and brief because infection with a second DENV type during the same transmission season is not uncommon. Illness after infection with two or more DENV types (i.e., a third or fourth clinically apparent DENV infection) occurs infrequently, which is thought to reflect the higher titers of anti-DENV antibody after secondary infection and a greater complement of cross-reactive antibodies.[256]

During outbreaks in Cuba in 1981 and 1997, DHF cases occurred only in the age cohorts exposed during the previous epidemic (older than 3 years and older than 17 years, respectively), further underscoring sequential/secondary infection as a critical precondition for DHF, at least for some DENV types.[119,257] However, DHF cases have been well documented in persons with primary infections, pointing to other

contributory factors such as viral strain and host factors.[258,259] Race and specific HLA haplotypes have been implicated in the risk for acquiring DHF, and a variable predominance of severe cases has been observed in girls and in children with good nutrition, indicating the contributions of both genetic and acquired host factors to susceptibility to the syndrome.[250,260–266]

Encephalitis

The variable and potentially lengthy incubation period of 4 to 21 days may reflect the interval for viral replication in the skin Langerhans cells and local lymph nodes, with a subsequent brief viremia before the virus invades the CNS.[267] Virus can rarely be recovered from blood, usually less than 1 week after the onset of illness and before the onset of neurologic symptoms, but sometimes later in an immunosuppressed patient; for WNV, detection by real-time PCR assay is more common.[268,269] The large proportion of infections that are asymptomatic, about 100 times the number of symptomatic cases, is striking and remarkably consistent among SLE, JE, and WNE. Subclinical infection presumably reflects the peripheral clearance of virus before neuroinvasion.

Innate responses are thought to be critical to this clearance. Genetic susceptibility to flaviviral encephalitis in laboratory mice was first associated with an allelic variant in the IFN-induced gene 2′-5′-oligoadenylate synthetase 1 (*OAS1*) that produced a truncated protein,[69,71] and a polymorphism in the same gene has been associated with WNV infection in humans.[270] Knockout mice deficient for other genes in the type I IFN-induced pathway similarly show increased susceptibility to lethal encephalitis.[271] Other host response factors have also been linked to susceptibility to flavivirus encephalitis. A functional Toll-like receptor 3–dependent inflammatory response has been associated with WNV neuroinvasion and neuronal injury in mice and with TBE in humans.[272,273] A defective allele in the chemokine receptor CCR5 (CCR5Δ32) was found to be significantly associated with symptomatic WNV infection and with severe disease in TBE.[274,275]

The mechanisms of viral neuroinvasion remain incompletely understood. In animal models of arboviral encephalitis, virus enters the CNS by crossing the vascular endothelium or through the olfactory epithelium, where the blood-brain barrier is impaired. However, in humans, the evidence suggests transmission across the vascular endothelium, either by passive transfer or by replication in endothelial cells.[267,271] Infected monocytes have also been proposed to carry virus across the blood-brain barrier.

Within the brain, virions spread from cell to cell. Pathologic changes consist of meningeal congestion and inflammation, brain edema, and widespread encephalitis with a predilection for the hippocampus and temporal cortex, thalamus, substantia nigra, cerebellum, periventricular areas of the brainstem, and anterior spinal cord.[267] Focal neuronal degeneration and necrosis with neuronophagia evolve to the formation of glial nodules and, with healing, spongiform changes. Viral antigen appears in neuronal bodies and their processes and later in phagocytic cells.[276] Perivascular inflammatory infiltrates consist of activated $CD4^+$ and $CD8^+$ T cells, macrophages, and B cells. Activated T cells predominate in the cerebrospinal fluid (CSF).[277]

A variety of processes may contribute to neuronal cell death, including apoptosis, cytoplasmic swelling, vacuolation, and membrane breakdown. In one study of TBE pathology, viral antigen was identified predominantly in large neurons; there was a poor topographic relation between inflammatory infiltrates, mainly T cells and macrophages, and distribution of antigen, suggesting an immune-mediated neuronal cell death rather than direct viral lysis.[278]

The rare recovery of virus from CSF, usually in patients with fulminant and fatal disease, is associated with the absence of intrathecal antibodies, indicating an important role of viral neutralization in recovery.[279] T cells are also essential for clearance of virus in experimental infections of mice, and low $CD4^+$ T-cell IFN-γ responses to JE viral NS3 protein correlated with more severe disease in one human study.[280–282] On the other hand, intrathecal immune complex formation and antineurofilament and anti–myelin basic protein antibodies have been reported in association with a poor outcome, suggesting immunopathologic injury.[283,284] Immunopathology is also implicated in animal models of flavivirus encephalitis and is supported by the observation that in some immunocompromised patients infected with WNV there is a delayed onset of clinical features despite high levels of viremia.[271,285] Regulatory T cells may play an important role in controlling this response to protect against severe disease.[286]

Subacute and progressive paralysis of the limb musculature and chronic epilepsy are well-known features of TBE, and CNS viral persistence has been demonstrated.[158] CSF neopterin, β_2-microglobulin, and intrathecal immunoglobulin G (IgG) synthesis remain elevated for weeks and pleocytosis persists considerably longer than in other CNS infections, consistent with a protracted inflammatory reaction.[287] In some studies, mutations in the virus NS1 gene and defective T-cell responses have been reported.[158,288] Delayed CNS clearance of JEV has also been suggested by the presence of infectious virus, antigen, or immunoglobulin M (IgM) antibodies in CSF several weeks after the onset of illness.[289]

Advanced age is preeminent among the risk factors for development of neurologic infection. The biologic basis for this age-related susceptibility is ill defined. Although immunosenescence affecting innate or adaptive immune responses or both is a likely scenario, other observations indicate roles for functional or structural CNS changes that facilitate neuroinvasion. As examples, in some studies, severe encephalitis has been associated with neurocysticercosis, hypertension, and concurrent viral, bacterial, or parasitic infections.[290–304]

Congenital Malformations and Guillain-Barré Syndrome

ZIKV is unique among the flaviviruses for its association with a range of congenital defects to include microcephaly and neurologic conditions such as GBS.[293–294] The pathogenesis of fetal infections and resulting congenital defects likely involves insult within the placenta or developing fetal neural tissue or both.[293,295,296] During the first trimester the placenta barrier is developing and the fetus is at its most vulnerable, as illustrated by rubella infection, when the risk of fetal infection during maternal rubella infection is 20% if it occurs during the first trimester.[292,297] In a postmortem pathology study of infants born with microcephaly, ZIKV was present in both neuronal and nonneuronal cells with high infectivity in intermediate progenitor cells and immature neurons with neuronal apoptosis.[293,298]

GBS is an autoimmune disorder affecting the peripheral nervous system, resulting in an ascending progressive polyradiculoneuritis and paralysis, and may require ventilator support.[294,299] GBS has been associated with the flaviviruses, including JEV and DENV, as well as an atypical polio-like syndrome with WNV.[300–302] ZIKV infections have also been associated with GBS and the pathophysiologic mechanisms have been evaluated.[294,299,303,304]

CLINICAL MANIFESTATIONS

Yellow Fever

YF illness ranges in severity from an undifferentiated, self-limited grippe to hemorrhagic fever that is fatal in 50% of cases.[6,83,203] In addition, between 5% and 50% of infections are inapparent. After an incubation period of 3 to 6 days, fever, headache, and myalgias begin abruptly, accompanied by few physical findings except conjunctival injection, facial flushing, a relative bradycardia (Faget sign), and, on laboratory examination, leukopenia. In most cases, resolution of this *period of infection* concludes the illness; in others, the remission of fever for a few hours to several days is followed by renewed symptoms, including high fever, headache, lumbosacral back pain, nausea, vomiting, abdominal pain, and somnolence (*period of intoxication*). Profound weakness and prostration ensue, compounded by poor oral intake and protracted vomiting, but the severe multisystemic illness is dominated by icteric hepatitis and a hemorrhagic diathesis with prominent gastrointestinal bleeding and hematemesis, epistaxis, gum bleeding, and petechial and purpuric hemorrhages. Albuminuria is a constant feature that aids in the differentiation of YF from other causes of viral hepatitis. Deepening jaundice and elevations in aminotransferase levels continue for several days, at the same time that azotemia and progressive oliguria ensue. Whereas direct bilirubin levels rise to 5 to 10 mg/dL, alkaline phosphatase levels are only slightly raised; not infrequently, AST may be elevated above ALT because of myocardial damage.[305] Ultimately, hypotension,

shock, and metabolic acidosis develop, compounded by myocardial dysfunction and arrhythmias as late events and acute tubular necrosis in some patients. Confusion, seizures, and coma distinguish the late stages of illness, but CSF examination discloses an increased protein level without pleocytosis, consistent with cerebral edema or encephalopathy. Death usually occurs within 7 to 10 days after onset. If the patient survives the critical period of illness, secondary bacterial infections resulting in pneumonia or sepsis are common complications. Recovery has not been followed by chronic hepatitis.

Clinically, severe YF resembles other viral hemorrhagic fevers occurring in Africa and South America, so laboratory confirmation is required to make the diagnosis. Early exclusion of other causes with the potential for person-to-person spread is important to prevent nosocomial transmission. Other forms of viral hepatitis, particularly hepatitis E (which frequently appears in outbreaks), leptospirosis, malaria, typhoid, typhus, relapsing fever, acute fatty liver of pregnancy, and toxin-related hepatitis, are alternative diagnoses.

Dengue Fever and Dengue Hemorrhagic Fever

The classification of dengue illness (see Fig. 153.6) has traditionally focused on the distinction between classic dengue fever and DHF, the latter reflecting more severe disease manifesting with plasma leakage, bleeding complications, or both.[306] In 2009, an alternative classification defined criteria for dengue, dengue with warning signs, and severe dengue; the revised classification has been adopted for epidemiologic reporting in most countries, but both systems continue to be used in the literature.[306,307] Classic dengue fever is an acute febrile disease with headaches, musculoskeletal pain, and rash, but the severity of illness and clinical manifestations vary with age and DENV type. Infection is often asymptomatic or presents as a nonspecific syndrome consisting of fever, malaise, pharyngeal injection, upper respiratory tract symptoms, and rash, particularly in children.[12,308–310] DENV-2 and DENV-4 may be more likely to cause inapparent infections in flavivirus-naïve persons.[214] Disease severity may be increased among infants and elderly people.[311] After an incubation period of 4 to 7 days, fever—often with chills, severe frontal headache, and retro-orbital pain—develops abruptly with a rapid progression to prostration, severe musculoskeletal and lumbar back pain, and abdominal tenderness. Anorexia, nausea, vomiting, hyperesthesia of the skin, and dysgeusia are common complaints. Initially, the skin appears flushed, but within 3 to 4 days and with the lysis of fever, an indistinct macular and sometimes scarlatiniform rash develops, sparing the palms and soles. As the rash fades or desquamates, localized clusters of petechiae on the extensor surfaces of the limbs may remain. A second episode of fever and symptoms may ensue ("saddleback" pattern). Recovery may be followed by a prolonged period of listlessness, easy fatigability, and even depression.

Although virtually all cases of dengue fever are uncomplicated, minor bleeding from mucosal surfaces (usually epistaxis, bleeding from the gums, hematuria, and metrorrhagia) is not uncommon, and gastrointestinal hemorrhage and hemoptysis can occur (see Fig. 153.6).[312] In patients with preexisting peptic ulcer disease, severe, even fatal, gastric bleeding can be precipitated.[313] Subcapsular splenic bleeding and rupture, uterine hemorrhage resulting in spontaneous abortion, and severe postpartum bleeding have also been reported.[314,315] It is important to differentiate these phenomena from the bleeding diathesis that accompanies the life-threatening syndrome of hypotension and circulatory failure in DHF-DSS.

Hepatitis frequently complicates dengue fever.[114,316] In Taiwan, transaminase levels raised 10-fold above normal were observed in 11% of cases, with rare deaths due to hepatic failure. Neurologic symptoms associated with dengue fever have been reported sporadically and attributed to hemorrhages or cerebral edema, but recovery of virus from the CSF, intrathecal viral-specific IgM, and immunohistochemical evidence of infection in the brain indicate the possibility of primary dengue encephalitis in some cases.[219,317–319] Myositis with rhabdomyolysis has also been reported.

Vertical transmission of DENV to neonates whose mothers had an onset of primary or secondary dengue fever up to 5 weeks before delivery has resulted in acute neonatal dengue manifesting as fever, cyanosis, apnea, mottling, hepatomegaly, and reduced platelet counts (as low as 11,000/mm^3).[320] One infant died of intracerebral hemorrhage, but others, although ill, did not have other signs of DHF, and they recovered without incident. DENV was isolated from the neonates in some cases. The outcome of infection acquired earlier in pregnancy has not been addressed satisfactorily. Anecdotal reports have described spontaneous abortion (see earlier discussion) and a variety of birth defects and, in a postepidemic investigation, an increase in neural tube defects.[321] Other investigations have found no increases in abnormal pregnancy outcomes.[320,322,323] In a study of cord blood samples from infants delivered 5 to 9 months after an outbreak, 4 of 59 samples had viral-specific IgM but all the infants appeared normal.[324]

The central clinical features of DHF-DSS are hemorrhagic phenomena and hypovolemic shock caused by increased vascular permeability and plasma leakage.[325–327] The early clinical features in children who ultimately develop DHF-DSS are indistinguishable from those of ordinary dengue fever, namely, fever, malaise, headache, musculoskeletal pain, facial flushing, anorexia, nausea, and vomiting. However, with defervescence, typically 3 to 7 days after illness onset, reduced perfusion and early signs of shock are manifested by central cyanosis, restlessness, diaphoresis, and cool, clammy skin and extremities. Abdominal pain is a common complaint. In cases with a benign course of illness, blood pressure and pulse may be maintained but a rapid and weak pulse, narrowing of the pulse pressure to less than 20 mm Hg, and, in the most extreme cases, an unobtainable blood pressure establish DSS. The platelet count declines, and petechiae appear in widespread distribution with spontaneous ecchymoses. Bleeding occurs at mucosal surfaces from the gastrointestinal tract and at venipuncture sites. The liver is palpably enlarged in up to 75% of patients, with variable splenomegaly. Increased amylase levels and ultrasonographic evidence of pancreatic enlargement are found in up to 40% of patients. Pleural effusions can be detected in more than 80% of cases if a decubitus film is taken; in combination with an elevated hematocrit and hypoalbuminemia, reflecting hemoconcentration, these studies provide objective measures of plasma loss. In some studies, ultrasonography has been more sensitive in detecting pleural effusions, ascites, and gallbladder edema in more than 95% of severe cases, and pararenal and perirenal effusions in 77%, as well as hepatic and splenic subcapsular and pericardial effusions.[328,329] The presence of pleural and peritoneal effusions is associated with severe disease. In contrast, pulmonary edema is uncommon except in cases with prolonged shock.[330] In untreated patients, hypoperfusion complicated by myocardial dysfunction and reduced ejection fraction results in metabolic acidosis and organ failure. However, with support through the critical period of illness, spontaneous resolution of vasculopathy and circulatory failure usually can be expected within 2 to 3 days, with complete recovery afterward. The duration of illness ranges from 7 to 10 days in most cases. Fatality rates have reached 50% in underserved populations, but in experienced centers, less than 1% of cases are fatal. Encephalopathy, prolonged shock, and hepatic or renal failure infrequently complicate the illness and are associated with a poor prognosis. As would be expected in areas where DENV infects 10% of children each year, concurrent infection with bacteria, parasites, and other viral pathogens occurs frequently. Dual infections, principally gram-negative sepsis, have been reported in 1 of 200 children hospitalized with dengue, resulting in prolonged fever and hospitalization.[331]

Attempts to differentiate dengue fever from other acute febrile illnesses on clinical grounds are unreliable, although laboratory findings of leukopenia, neutropenia, thrombocytopenia, or mildly elevated AST levels are supportive.[11,114,249] A positive tourniquet test, one of the criteria in the DHF case definition, is obtained more often in children with dengue than in children with other febrile illnesses, but its specificity is low. In comparison with chikungunya, another *A. aegypti*–borne viral infection, dengue patients are less likely to have conjunctivitis, rash, and joint pain.[332] The difficulty of differentiating dengue from rubella, measles, and even influenza has been underscored by the early misrecognition of entire epidemics. The clinical picture of DHF is more distinct, but overlaps with YF and other viral hemorrhagic fevers.

Clinical or laboratory differentiation, at the time of first presentation, of children destined to develop DHF would facilitate intervention

before the sudden onset of shock.[333] In one study, AST elevations greater than 60 U/mL, leukocyte counts less than 5000/mm^3, and absolute neutrophil counts less than 3000/mm^3 had higher predictive values than the tourniquet test in differentiating dengue from other febrile illnesses.[114,249] In another study, an elevated sTNFR/75 level had a sensitivity of 93% and a negative predictive value of 95% in foretelling shock.[222] Other cytokines, and combinations of markers, have been evaluated as predictors of mild versus severe disease, but results have varied and there have been no prospective validation studies. Furthermore, implementation of useful measures at the point of care may be a challenge.[208,334]

Japanese Encephalitis

Infection with JEV is symptomatic in less than 1% of cases of JE, but the illness is usually a severe encephalitis, leading frequently to coma and to a fatal outcome in 25% of cases. The spectrum of clinical illness is probably broader than is appreciated from an evaluation of hospitalized patients. JE cases are found among hospitalized children with acute pyrexia of undetermined origin, and, undoubtedly, many patients with febrile illnesses and headache or aseptic meningitis do not present to a hospital. Studies have drawn attention to patients presenting with spinal paralysis without encephalitic signs, initially misdiagnosed as poliomyelitis cases, and, conversely, acute behavioral changes mimicking psychosis without motor signs.[1,5,335–337] The earliest symptoms are lethargy and fever and, frequently, headache, abdominal pain, nausea, and vomiting. Lethargy increases over several days, when uncharacteristic behaviors associated with an agitated delirium, unsteadiness, and abnormal motor movements may develop, advancing to progressive somnolence and coma. Although the prodrome may evolve over several days to 1 week, some children present with a sudden convulsion after a brief febrile illness.

The chief findings are high fever and altered consciousness, ranging from mild disorientation or a subtle personality change to a severe state of confusion, delirium, and coma.[1,5,335] Mutism has been a presentation in some cases. Nuchal rigidity is a variable finding, present in one-third to two-thirds of the cases. Cranial nerve palsies resulting in facial paralysis and disconjugate gaze are detected in one-third of the cases. Muscular weakness can be associated with decreased or increased tone and can be generalized or asymmetrical, with hemiparesis or unusual distributions of flaccid and spastic paralysis. Hyperreflexia, ankle clonus, and other abnormal reflexes may be elicited. Disordered movements such as nonstereotypical flailing, ataxia, or tremor may be present initially. Not uncommonly, choreoathetosis, rigidity, masked facies, and other extrapyramidal signs appear later in the illness. Focal or generalized seizures develop in up to 85% of children and 10% of adults.[338] Multiple seizures and status epilepticus are associated with a poor outcome. Subtle motor status epilepticus, in which the only clinical manifestation might be the twitching of a finger or eyebrow, may also occur but is easily overlooked.[338]

Signs of increased intracranial pressure, such as papilledema and hypertension, are detected in a minority of patients, although some fatal cases show evidence of uncal or tentorial herniation, and clinical signs consistent with brainstem herniation syndromes are not uncommon.[338] More than one-third of patients in coma need ventilatory support. Fulminant cases may be rapidly fatal. More typically, improvement can be expected after 1 week with the defervescence of fever. Neurologic function is regained gradually over several weeks, with further recovery after hospital discharge over intervals of months to years.[339,340] Infections from stasis ulcers, urinary tract infection, pneumonia, and bacteremia frequently complicate the lengthy recovery from coma and paralysis and may be secondary causes of death. The virulence of the infection is underscored by contemporary fatality rates of 25% in locations with intensive care facilities. Neurologic abnormalities such as seizure disorders, motor and cranial nerve paresis, cortical blindness, and movement disorders persist in up to one-third of patients after 5 years. A greater proportion, perhaps even 75% of recovered children, exhibit behavioral and psychological abnormalities. Anecdotal cases of clinical relapse weeks after hospital discharge, with recovery of virus from peripheral blood, have alluded to delayed viral clearance or persistence, but the significance of these observations is uncertain.[341] In illness acquired during the first or second trimesters of pregnancy, the virus can infect the fetal-placental unit and precipitate abortion.[342] Cases acquired in the third trimester have not been reported to interrupt pregnancy. Congenital infections have been reported only when the virus was newly introduced to a susceptible adult population because almost all women in endemic areas have acquired immunity. Nonimmune travelers may have an increased risk.

Laboratory studies disclose peripheral leukocytosis, as high as 30,000/mm^3 with a left shift, and hyponatremia. The CSF opening pressure is elevated in about 50% of patients.[338] Pleocytosis ranges from less than 10 to several thousand cells per cubic millimeter, with a median of several hundred cells of a predominantly lymphocytic composition. CSF protein may be normal or elevated up to 100 mg/dL. The electroencephalogram discloses a pattern of diffuse slow waves (theta or delta) with superimposed seizure activity, including periodic lateralized epileptiform discharges.[338,343] Brain imaging reveals diffuse white matter edema and abnormal signals mainly in the thalamus (often with evidence of hemorrhage), basal ganglia, cerebellum, midbrain, pons, and spinal cord.[344,345] Electromyography shows changes of chronic partial denervation consistent with anterior horn cell destruction.

In rural Asia, tuberculosis, cerebral malaria, and bacterial meningitis (especially partially treated) are the principal alternative diagnoses.[11,132,346,347] Typhoid fever with tremors and ataxia, dengue infection with encephalopathy, lead poisoning, heat stroke, and enterovirus 71 encephalitis have all been confused with JE. In JE-endemic areas, any encephalitis outbreak is initially assumed to be JE, but the outbreak of the previously unknown Nipah virus in Malaysia in 1999 and outbreaks of Chandipura virus infection in India since 2003 have shown how easy it is to be misled.[348] WNV and JEV infections in particular may be mutually misrecognized because the viruses overlap in their distribution in Southwest Asia and can produce clinically indistinguishable illnesses in contemporaneous outbreaks, and because differentiation in laboratory tests may be difficult.

West Nile Virus Fever and West Nile Encephalitis

Most infections with WNV are asymptomatic; when symptoms do occur, they develop after an incubation period that typically lasts 2 to 6 days but may extend to 14 days, or even longer in immunosuppressed persons. In recent outbreaks, the syndrome of WNV fever occurred in about 20% of infected individuals, who developed a sudden onset of an acute, nonspecific, influenza-like illness lasting 3 to 6 days, with high fever and chills, malaise, headache, backache, arthralgia, myalgia, and retro-orbital pain, without overt neurologic signs.[143,144,335] Other nonspecific features include anorexia, nausea, vomiting, diarrhea, cough, and sore throat. In some epidemics, a flushed face, conjunctival injection, and generalized lymphadenopathy were common, and a maculopapular or pale roseolar rash was reported in about 50% of patients, more frequently in children. In one outbreak, 20% of patients with WNV fever were reported to have hepatomegaly and 10% had splenomegaly.[349] Myocarditis, pancreatitis, and hepatitis have also been described occasionally in severe WNV infection.

Neurologic disease occurs in less than 1% of infected individuals. Patients typically have a febrile prodrome of 1 to 7 days, which may be biphasic, before developing neurologic symptoms (Fig. 153.7). Although in most cases the prodrome is nonspecific, 15% to 20% of patients have features suggestive of WNV fever, including eye pain, facial congestion, or a rash; fewer than 5% have lymphadenopathy.[350,351] In recent outbreaks, approximately two-thirds of hospitalized patients had encephalitis (with or without signs of meningeal irritation), and one-third had meningitis.[149,352,353] Acute flaccid paralysis caused by virus infection of the anterior horn of the spinal cord (myelitis) has been recognized in recent outbreaks.[354–356] The clinical picture suggests poliomyelitis; paralysis is frequently asymmetrical and may or may not be associated with meningoencephalitis. Once paralysis is established, little long-term improvement has been described. Although convulsions occurred in about 30% of patients in the early descriptions of WNE, they did not appear to be an important feature in the more recent outbreaks.[353,356] Other neurologic features include cranial neuropathies, optic neuritis, and ataxia. Stiffness, rigidity, spasms, bradykinesia, and tremors,

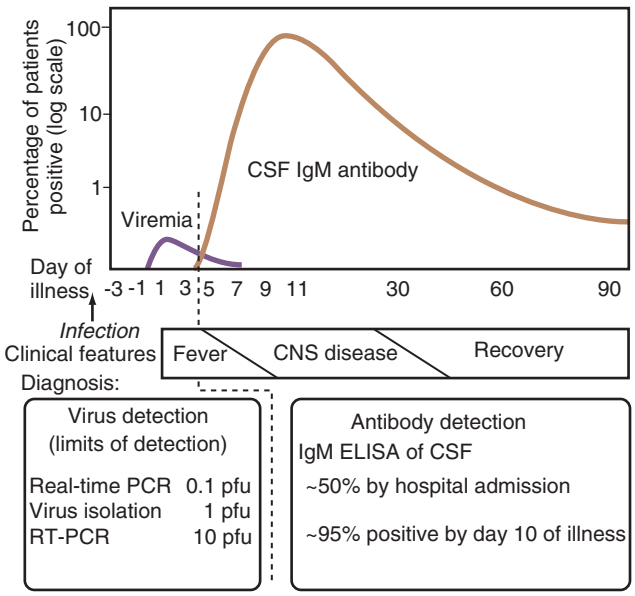

FIG. 153.7 Schematic representation of the clinical course of West Nile encephalitis. Schematic includes viremia, development of antibody, and implications for diagnosis (as approximate percentage of patients). Limits of virus detection are expressed as plaque-forming units *(pfu)* per 100 µL; human viremia is believed to be less than 10 pfu/100 µL. The first day of fever is taken as the first day of illness; most patients are not admitted to a hospital until day 3 to 5 of illness. *CSF,* Cerebrospinal fluid; *CNS,* central nervous system; *ELISA,* enzyme-linked immunosorbent assay; *IgM,* immunoglobulin M; *PCR,* polymerase chain reaction; *RT,* reverse transcriptase. *(From Solomon T, Ooi MH. West Nile encephalitis.* BMJ. *2003;326:865–869.)*

associated with basal ganglia damage, have also recently been recognized in WNE.[356,357]

In recent outbreaks, overall case-fatality rates for hospitalized patients ranged from 4% to 14% but were higher in older patients.[352,353,356] Other risk factors for death include the presence of profound weakness, deep coma, failure to produce IgM antibody, impaired immunity, and coexisting illness such as hypertension or diabetes mellitus.[353,358] Neurologic sequelae are common among survivors. In one study, one-half of hospitalized patients still had a functional deficit at discharge and only one-third had recovered fully by 1 year.[359]

About 50% of patients have a peripheral leukocytosis, and 15% have leukopenia.[353,359] Hyponatremia sometimes occurs in patients with WNE. Examination of the CSF typically shows a moderate lymphocytic pleocytosis, although sometimes there are no cells, or neutrophils may predominate. The protein is moderately elevated, and the glucose ratio is typically normal. Magnetic resonance imaging (MRI) may show high signal intensities in the thalamus in T2-weighted images and diffusion-weighted images in some patients, although this is a late finding and occurs in the more severely ill patients.[356,357] Electroencephalograms show diffuse slowing and, in some cases, focal seizure activity. Nerve conduction studies typically show the reduced motor axonal amplitudes consistent with anterior horn cell damage, although there may also be some slowing of conduction velocities and some changes to sensory nerves.[335]

St. Louis Encephalitis

SLE has been classified into three syndromes, characterized respectively by constitutional symptoms and headache (febrile headache), aseptic meningitis, and fatal encephalitis.[1,152,157] The proportion of cases in each category is age dependent, with increasing proportions of encephalitis and fatal cases in adults, especially in elderly people. The illness usually begins with a febrile prodrome of malaise, fever, headache, and myalgias, sometimes with upper respiratory or abdominal symptoms, that evolves over several days to more than 1 week with progressive lethargy, periods of confusion, and the onset of tremors, clumsiness, and ataxia. Vomiting and diarrhea are common, and some patients complain of dysuria, urgency, and incontinence.

Altered consciousness, marked by confusion, delirium, or somnolence, is the predominant presenting feature, and generalized motor weakness is more usual than are focal signs. Indications of meningeal irritation are inconstant and are elicited more often in children. Mental clouding may be subtle and manifested only by slight disorientation. Most patients do not progress to deep coma. Tremulousness involving the eyelids, tongue, lips, and extremities is usual, and cerebellar and cranial nerve signs are common.[155,360] Various abnormal movements may be present, including myoclonic jerks and nystagmus. Convulsions are infrequent and signal a poor prognosis, except in children; subtle motor seizures also have been reported. Most patients improve over several days; however, pneumonia, thrombophlebitis and pulmonary embolism, stroke, gastrointestinal hemorrhage, and nosocomial infection can complicate recovery. The mortality rate is 8% overall and 20% among patients older than 60 years of age. In recovered adults, asthenia, emotional lability, anxiety, irritability, forgetfulness, tremor, dizziness, and unsteadiness may persist for months, accompanied by tremor, asymmetrical deep tendon reflexes, and visual disturbances.[361] No cases of clinical relapse or progressive illness have been described. Infants and young children frequently exhibit significant neurologic sequelae when discharged, but psychomotor function is usually recovered on later follow-up.[362] Little is known of the risk or outcome of congenital infection. HIV-positive individuals appear to be at greater risk for acquisition of SLE, but whether this is related to their immune status or to other factors (e.g., increased risk for exposure due to homelessness) is not clear.[154]

The peripheral white cell count may be slightly elevated. In some patients, microscopic hematuria, proteinuria, and pyuria have been reported. Hyponatremia due to syndrome of inappropriate secretion of antidiuretic hormone (SIADH) occurs in more than one-third of patients, and the concentrations of ALT and creatine phosphokinase may be slightly elevated. One-third of patients have an increased CSF opening pressure, and there is typically a moderate mononuclear pleocytosis, with an elevated protein concentration. The electroencephalogram shows diffuse slowing and seizure activity, including periodic lateralized epileptiform discharges. MRI may show high signal intensity in the substantia nigra.[363] The diagnosis should be suspected if the case is one of a cluster in the summer or early fall, especially if the patient is an elderly or homeless person. A cerebral ischemic event, heat stroke, medication or drug toxicity, or other cause of delirium or encephalopathy has frequently been the initial diagnosis in confirmed cases. Other infectious causes of aseptic meningitis or acute encephalitis, including WNE, which can be transmitted contemporaneously, cannot easily be distinguished on a clinical basis.

Tick-Borne Encephalitis (Including Powassan Encephalitis)

Infection leads to symptoms of TBE in as many as 1 in 3 persons. Three-fourths of patients are able to recall a tick bite occurring a median of 8 days (range, 4–28 days) before symptoms developed.[364–366] The illness usually begins with a nonspecific grippe of fever, malaise, headache, nausea, vomiting, and myalgias that may be accompanied by fasciculation. Within 1 week, these symptoms resolve spontaneously. In most patients who have the "febrile form" of the disease, there are no further symptoms; in others who progress to more severe illness, the remission of symptoms is temporary, usually 2 to 8 days (range, 1–20 days), before high fever, headache, and vomiting resume.[158] The second phase may be limited to a "meningeal form" with aseptic meningitis (commonly in children), or it may manifest as a "meningoencephalitis form," a "poliomyelitic form" with poliomyelitis-like flaccid paralysis, or a "polyradiculoneuritic form" with a Guillain-Barré–like paralysis, which usually resolves spontaneously. Neurologic infections usually are benign in children, whereas severe disease occurs more often in elderly persons. The TBEV-FE form of TBEV is reported as more severe, with fatalities occurring in 20% of hospitalized patients and residual neurologic sequelae in up to 60% of recovered patients.[367] Six percent to 8% of the TBEV-Sib subtype and 1% to 2% of the TBEV-Eu subtype infections are fatal. Although differences in hospital admission rates may have confounded some of

these observations, intrinsic differences in the neurovirulence of different TBEV subtypes have been shown in experimental animal infections.

Early prodromal symptoms may be undetected in children, whose illness in more than two-thirds of cases consists of aseptic meningitis. Altered consciousness, ataxia, tremor, paresthesias, focal signs, and, less often, seizures characterize the presentation with encephalitis. Limb weakness and paralysis usually represent lower motor neuron lesions caused by myelitis or radicular neuritis; paresis may be transient, or it may evolve to permanent weakness and muscular atrophy. The shoulder girdle and upper limb musculature are affected most frequently, and urinary bladder continence and other autonomic functions can also be disturbed. Involvement of cranial nerves III, VII, IX, X, and XI produces gaze and peripheral facial paralysis and dysphagia. The outcome generally is good, especially in children, but the prognosis varies with age. A hemorrhagic syndrome has been reported in some cases and also in a laboratory-acquired louping ill infection.[368]

Sequelae are reported in up to 40% to 60% of patients, most frequently consisting of psychological disturbances such as asthenia, headache, memory loss and decreased concentration, anxiety, and emotional lability. Residual motor abnormalities include ataxia and incoordination, tremor, dysphasia, and in fewer than 5% of cases, specific cranial or spinal muscular paralysis.[364,369] Progressive motor weakness and epilepsia partialis continua (Kozhevnikov epilepsy) are specific syndromes that may reflect a chronic encephalitic process. Pneumonia, heart failure, and other complications associated with prolonged hospitalization have been reported.

Powassan encephalitis is a severe encephalitis with a case-fatality rate of less than 10%. Focal features have occurred in more than 50% of reported cases; in one patient, the clinical presentation with olfactory hallucinations and temporal lobe seizures mimicked herpes encephalitis.[370,371] Significant residua of hemiplegia, quadriplegia, or aphasia may result, and spinal paralysis with residual muscular wasting is similar to the myelitis associated with TBE. Powassan encephalitis continues to emerge; the CDC reported 1 human case in 2001 and 21 human cases in 2016.[55,56] The highest total number of cases and highest incidence continue for the northern United States, with the majority occurring in Minnesota, Wisconsin, and New York. In New York, POWV has spread to the lower part of the state in Westchester County. Mortality rates from neuroinvasive disease in 2015 and 2016 were 17% and 14%, respectively.

Examination of the peripheral blood in TBE discloses leukopenia in the initial phase of illness, and leukocytosis up to 20,000/mm^3 during the second phase, with a transition to leukopenia again before recovery. Thrombocytopenia can also occur in the initial viremic phase.[372] An elevated erythrocyte sedimentation rate and C-reactive protein concentration are common by the time patients present with neurologic disease, and elevated ALT and AST levels and electrocardiographic abnormalities have been reported anecdotally.[365] There is a moderate lymphocytic CSF pleocytosis (average, <100 leukocytes/mm^3). The CSF protein level, although normal at the onset of neurologic symptoms, rises during the next 6 weeks, with an increase in the IgG index, indicating intrathecal synthesis. The albumin CSF-to-serum ratio, indicating disturbed blood-brain barrier permeability, can remain abnormal for as long as 1 year.[364] The number and distribution of CSF lymphocytes and their cell markers differ in TBE and neuroborreliosis, a fact that may have diagnostic value in rapidly identifying patients for antibiotic treatment.[277] Abnormal MRI signals are found most consistently in the thalami and basal ganglia.[373] Electroencephalograms show diffuse slowing in 90% of patients with encephalitis, with or without focal abnormalities.[365]

Although a history of tick bite is not given in all cases, exposure to an endemic focus during the transmission season should trigger suspicion. TBEV is transmitted under the same circumstances as *Borrelia burgdorferi,* and clinically their radicular and aseptic meningitis syndromes can overlap. Anecdotal observations suggest that neurologic symptoms of Lyme disease may occur more often in the context of concurrent TBE, and, conversely, that TBE may be more severe in a dual infection.[292–304,374] TBE results in a more prolonged course of illness and hospitalization than do the other acute encephalitides of presumed viral origin; this pattern and the presence of spinal paralysis may aid in making the diagnosis.

Alkhurma Hemorrhagic Fever

The largest case series of AHFV occurred in patients from 23 districts in Najran Province, Saudi Arabia.[375] From August 2003 to December 2009, there were 148 cases that fulfilled the clinical case definition of AHFV infection. Of the clinical cases, 78 (52.7%) were laboratory confirmed. Sixty-nine (88.5%) patients were diagnosed as inpatients hospitalized for acute febrile illness, and 9 (11.5%) patients were identified by contact tracing. The mean age was 30 years (range, 4–85 years). The majority of patients were male (63%), and the duration of illness prior to hospitalization was 1 to 10 days (mean, 4.9 days). Hospital stay ranged from 1 to 19 days (mean, 6.2 days). Patients were febrile, and two had a biphasic febrile illness. Clinical symptoms included headache (85.9%), malaise (85.9%), arthralgia (83.3%), myalgia (82.1%), nausea and vomiting (71.8%), diarrhea (51.3%), abdominal pain (48.7%), and hemorrhagic manifestations (25.6%). CNS manifestations occurred in 23% and included altered sensorium, confusion, disorientation, and neck stiffness, with four in a coma. Of the 78 patients with laboratory-confirmed infection, 70 (89.7%) were positive for virus by PCR assay. Laboratory abnormalities include elevation in serum creatinine concentration, with one patient having acute renal failure, an increase in liver function test results, hyperbilirubinemia, thrombocytopenia, and leukopenia. The overall mortality in this case series was 1.3%, compared with a mortality of 25% in previous outbreaks.[57]

Kyasanur Forest Disease

The incubation period for Kyasanur Forest disease is believed to be 3 to 8 days, after which illness begins abruptly with fever, headache, chills, vomiting, myalgia, photophobia, and conjunctival suffusion.[376] Facial and conjunctival hyperemia, lymphadenopathy, hepatosplenomegaly, and petechiae are found on examination. Diffuse hemorrhages from the nares, gums, and gastrointestinal tract develop, with hemorrhagic pulmonary edema in 40% of cases and renal failure in severe cases. A biphasic course of illness can occur with neurologic manifestations during the second and third week of illness. Neurologic manifestations include severe headache, stiff neck, changes in sensorium, and CSF pleocytosis.[376] Laboratory findings are similar to those of DHF and Alkhurma hemorrhagic fever, with leukopenia, thrombocytopenia, and elevated hepatic transaminase levels. Patients have detectable viremia up to 12 days after the onset of illness, which is unusual and prolonged compared with other viral hemorrhagic fevers.[177] The case-fatality rate in KFDV infection is 2% to 10%, which is higher than in DHF and lower than was observed in Alkhurma hemorrhagic fever.[177] Convalescence in survivors can be prolonged—up to 4 weeks after acute illness.

Zika Virus Infection

The clinical presentation and course of infection with ZIKV is most consistent with a dengue fever–like illness without hemorrhagic manifestations. The first large outbreak of ZIKV infection occurred on Yap Island, Micronesia, in 2007.[185] During this outbreak, there were 49 confirmed and 59 probable cases of ZIKV illness. The median age was 36 years (range, 1–76 years), and the majority of patients were female (61%). Rash was common in all cases of acute infection, seen in 90%, followed by fever (65%), arthralgia (65%), nonpurulent conjunctivitis (55%), and headache (45%).[185] No deaths were associated with acute infection. Based on an experimental infection in a human volunteer in the 1950s, the incubation period of ZIKV infection was 3 days, with the first symptoms a frontal headache followed by fever.[377] Clinical illness lasted for 5 days and then resolved. Laboratory findings included a mild leukopenia and minimal changes in liver function test results and platelet counts. The clinical signs and symptoms of the majority of cases seen in the 2013 to 2015 outbreaks of ZIKV infection are generally similar to these.[377,378] Gastrointestinal symptoms and mucous ulceration have been observed less commonly. Generally, illness has been mild and self-limited, and lasted for several days to 1 week.[377,378] It is estimated that approximately 80% of ZIKV infections are asymptomatic.

The recent outbreaks of ZIKV infection appear to be more frequently associated with certain neurologic syndromes, particularly GBS.[63,64] The outbreak in French Polynesia had 74 patients who presented with various neurologic syndromes, 42 of whom were classified as having

GBS. In 2015 and 2016, increased rates of GBS were reported in patients with recent histories of ZIKV infection in Brazil and El Salvador.[63,64] The association between cases of ZIKV infection and GBS is currently being investigated in multiple other countries in Central and South America.

Perinatal complications have also been noted in association with the recent outbreaks of ZIKV infection,[63,64] as well as retrospectively in the Yap Island outbreak.[377,379] Most notably, a marked increase in microcephaly was first noted in northeast Brazil in October 2015. As of the first week of January 2016, 3530 cases of microcephaly had been reported in Brazil, compared to an average of 163 per year between 2010 and 2014.[63,64] Cases of microcephaly had extensive intracranial calcifications, lissencephaly, and pachygyria.[377,380] Macular abnormalities have also been noted in children with microcephaly.[63,64]

Vertical transmission with ZIKV has also been reported. ZIKV has been detected in amniotic fluid of live fetuses with microcephaly[377,381] and in tissue specimens from fetal losses,[377,382] including brain tissue.[377,383] The impact and potential pathogenic mechanisms of ZIKV fetal infection are incompletely understood at present. Important questions remain to be addressed, including the effect of pregnancy stage on the fetal risk, and these are now the subject of intense investigation. Based on information available in January 2016, the CDC[377,384] and the European Centre for Disease Prevention and Control issued guidelines for pregnant women during a ZIKV outbreak.[377,385]

As stated, an increase in cases of microcephaly was noted during the outbreak of ZIKV infection in Brazil in 2015. A comprehensive understanding of the mechanisms for this association remains elusive. ZIKV was detected by immunohistochemistry in chorionic villi of one case of spontaneous first-trimester miscarriage and in amniotic fluid sampled at gestational week 28 in two cases of microcephaly.[377,383,386] In two cases of microcephaly with death within the first day after birth and one case with medical termination of pregnancy at gestational week 32, ZIKV RNA was detected only in fetal brain tissue.[377,383,387] These data suggest that initial fetal infection via placental tissue is followed by localization of ZIKV replication in the fetal CNS. Much has been learned but much remains unknown about ZIKV's tissue tropism and pathophysiologic potential (see Fig. 153.5).

ZIKV is unique among the flaviviruses for being concentrated in semen, with documented sexual transmission and infection of the partner.[377,388,389] ZIKV was detected by RT-PCR in semen up to 62 days after acute infection.[377,388] Sexual transmission of ZIKV changes the nature of the transmission dynamics of this virus from a seasonal vector-borne disease to potentially a continuous sustained sexual transmission in both the heterosexual and the men who have sex with men populations.[377,390]

LABORATORY DIAGNOSIS

Viral isolation is relevant to the diagnosis of suspected flavivirus infection during the early illness phase when patients are viremic and viral infectivity titers in blood are sufficiently high. In the case of the flavivirus encephalitis-producing viruses such as JEV and WNV, mammalian hosts are considered dead-end hosts, because viral load is minimal and titers are insufficient for isolation. However, real-time RT-PCR has become the technique of choice to detect viremia for all the flaviviruses.[268,391,392] In the case of DENV, identifying the infective dengue viral type is important chiefly for public health reasons, but an individual patient also may benefit because future exposure to other DENV types places the patient at higher risk for DHF. In general, viral recovery from blood is successful only before an antibody response develops. Contrary to expectation, the isolation of neurotropic viruses from the CSF is usually unsuccessful except in the early stages of fulminant illness (Fig. 153.8).

Tissue samples, whether from biopsy or autopsy, ideally should be divided into aliquots that are frozen at −70°C for viral isolation and fixed in buffered formalin and glutaraldehyde for light and electron microscopy. Viscerotomy liver samples are frequently taken after death as a means of postmortem YF diagnosis, but because pathologic changes are not pathognomonic, a purely histologic diagnosis should be considered presumptive and should be supplemented with immunohistochemical staining using virus-specific antibodies. Liver biopsy should never be attempted from patients with suspected YF, because they are at risk for fatal hemorrhage. SLE virus, WNV, TBEV, and JEV have been isolated from brain, lung, liver, spleen, and kidney with varied success, depending on the duration of illness and the day of death. Diverse areas of the brain and spinal cord should be sampled. JEV is rarely cultured from peripheral blood using conventional techniques, but culture of virus from white blood cells separated from blood clots may be positive.[393] SLE virus was also isolated from vitreous humor in one case. Suckling mice and C6/36 or AP61 mosquito cell cultures are the most sensitive systems for viral isolation, but Vero, LLC-MK2, PS, and other continuous vertebrate cell lines are also used.[394]

Multiplex PCR assays in various formats that simultaneously identify the presence of DENV and its serotype in serum samples are used only in specialized laboratories in the United States and several Asian, African, and South American countries.[395] YF virus genomic sequences have also been detected in blood, but clinical experience is limited. In patients with flavivirus encephalitis, RT-PCR assay of the CSF has not proved very useful, although it may have a role in the detection of TBEV in acute serum. However, real-time (TaqMan) PCR assay has proved sensitive in detecting WNV in the CSF and serum and has been used for screening of blood products.[146,395] PCR assays have been developed

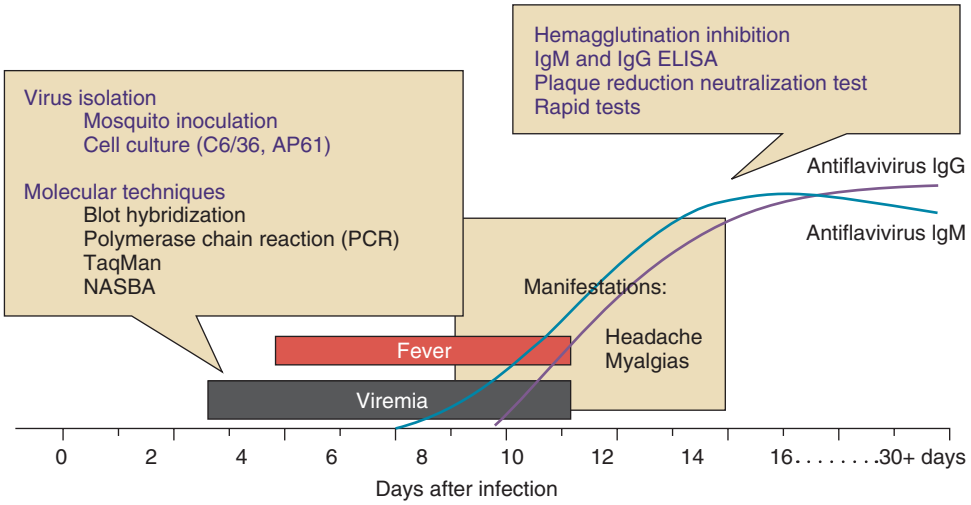

FIG. 153.8 Schema for the types of diagnostics appropriate for day after infection in most flavivirus infections. *ELISA,* Enzyme-linked immunosorbent assay; *IgG,* immunoglobulin G; *IgM,* immunoglobulin M; *NASBA,* nucleic acid sequence–based amplification.

for the detection and quantification (viral load) of all the flaviviruses and in many ways are the preferred diagnostic tests of choice during the viremic period owing to speed (<4 hours in many assays) and high degree of sensitivity and specificity.[395]

Laboratory diagnosis of most cases, especially in travelers who come to clinical attention after viremia has cleared, depends principally on the serologic testing of serum and, in the case of neurologic infections, the CSF.[396] IgM detection by antibody capture enzyme-linked immunosorbent assay (ELISA) is the preferred technique for a specific diagnosis, although some laboratories successfully detect IgM and IgG antibodies by indirect immunofluorescence assay. The assay is more than 95% sensitive when serum specimens obtained between 7 and 10 days after the onset are tested. In secondary flavivirus infections, a combination of IgM and IgG ELISA is 100% sensitive as early as 4 to 5 days after the onset of illness. Both CSF and serum should be examined in cases of flavivirus encephalitis because IgM may appear earlier in the CSF. If both specimens are tested, positive results are obtained in almost all patients by 10 days after the onset of illness, with, in general, a 10% increase in cumulative positivity per day (see Fig. 153.7). However, some patients die without making an antibody response.[335] Serum IgM in DENV infection declines to undetectable levels within 60 days, but antibodies persist for up to 9 months in recovered SLE and TBE patients and for longer than 16 months in some WNE patients, potentially limiting the specificity of tests.[397]

Heterologous reactions with other flaviviruses are problematic where numerous flaviviruses cocirculate, owing to extensive serologic cross-reactivity between flaviviruses. In Asia, five flaviviruses—DENV, JEV, ZIKV, and in specific locations WNV and KFDV—may cocirculate with a high degree of serologic cross-reactivity; in Africa, YF virus and DENV cocirculate; and in South America, YF virus, DENV, and SLE virus cocirculate. Recent vaccination against JE or YF, or recent infection with dengue or SLE virus, may cause a false-positive WNV IgM antibody test result; neutralization assays such as the plaque reduction neutralization test and ELISA that detect antibody to NS5 are more specific.[398] Fractionation of IgM before hemagglutination inhibition testing and competitive epitope-blocking ELISA improve specificity. However, among sera from African patients, all serologic approaches frequently fail to resolve previous and recent infections. Heterologous flavivirus antibodies have become an issue even among specimens submitted in the United States for arboviral diagnosis. Previous DENV infection, reflecting prior exposures in persons who have resided abroad, is now a frequent finding that can interfere with interpretation of the serologic diagnosis of a recent flavivirus infection. These heterologous dengue antibodies frequently pose difficulties in the interpretation of tests for SLE, WNE, and Powassan encephalitis. Although neutralization tests provide the greatest specificity, they are time consuming, are expensive to perform, are offered only in specialized laboratories, and require control reference sera to obtain reliable results. Hemagglutination inhibition and complement fixation tests are now used infrequently, but they still have utility under some circumstances. Complement fixation antibodies, which recognize NS1 protein, are relatively specific in distinguishing between antigenic complexes, and because they rise rather late (often 4–6 weeks after the onset) and decline with a half-life of 3 years, positive reactions indicate infection in the intermediate period after the disappearance of IgM antibodies. Hemagglutination inhibition and neutralizing antibodies can persist for decades after infection. Rapid immunochromatographic tests formatted as small folders to detect dengue and JE IgM and IgG have demonstrated high sensitivity and specificity in field evaluations (100% and 90%, respectively, for the dengue test) and should facilitate laboratory confirmation of cases in clinical facilities.[399] NS1 protein is present in serum early in DENV infection and can be detected by antigen capture ELISA or rapid immunochromatographic tests.[400]

In a patient with a compatible illness, a case is confirmed by a fourfold change in the serum antibody titer or, alternatively, in encephalitis patients, by the demonstration of viral-specific IgM in CSF, reflecting intrathecal immune response. An elevated serum IgM antibody level alone is considered presumptive evidence of recent infection if high IgM prevalence rates in the population prevail because of frequent asymptomatic infections and because antibodies may persist beyond a single transmission season.

Serologic testing for SLE virus, WNV, DENV, and other selected arboviruses is performed at several private laboratories, most state laboratories, the CDC, the US Army Medical Research Institute of Infectious Diseases, and other reference laboratories. In addition, an indirect immunofluorescence assay kit for the domestic arboviruses that includes an SLE antigen can be purchased in the United States, and a TBEV ELISA kit can be obtained in Europe. Dengue immunochromatographic folders and ELISA kits are sold in Asia and Australia.[401–404]

A single IgM assay (ZIKV *Detect* 2.0 IgM Capture ELISA [InBios, Seattle, WA]) has been granted a marketing authorization by the US Food and Drug Administration (FDA) (May 2019).[404a] Laboratory testing is available through the CDC Arbovirus Diagnostic Laboratory, various state health laboratories, and the Pan American Health Organization/WHO. Some state health departments in the United States also perform ZIKV laboratory testing. Detection of ZIKV can be established by RT-PCR, and virus is present in the bloodstream within the first few days of illness.[401,402] ZIKV RNA is present in the blood for only a relatively short period after onset of illness (3–7 days). Saliva or urine samples collected 3 to 5 days after onset of illness may also be positive.[401,403] ZIKV may be present in urine samples up to 20 days or longer after the onset of illness.[401,404] ZIKV has also been detected in semen,[191,405] and possible sexual transmission has been reported.[191,406] Serologic tests can also be used to detect ZIKV infection and may become positive 4 or more days after onset of illness. IgM antibody determinations by immunofluorescence, ELISA, or neutralizing antibody assays are being used. Neutralizing antibodies should be greater than or equal to fourfold higher than anti-DENV antibodies to indicate the presence of specific anti-ZIKV antibodies.[401,404] Because of cross-reactions with other flaviviruses, antibody testing to DENV, chikungunya virus, and WNV should also be carried out.

PREVENTION AND THERAPY

Yellow Fever

Hospitalization in an intensive care facility where the patient can be sequestered from mosquitoes is recommended to provide close clinical monitoring and supportive care and to prevent anthroponotic transmission. Blood in the acute phase of illness is potentially infectious. No antiviral therapy is available, and specific supportive interventions have not been clinically evaluated. Exploratory studies in small animal models and nonhuman primates of various compounds (e.g., interferons, ribavirin) have had varied results.[407] Recent efforts to develop a therapeutic agent have focused on directly targeting viral proteins or key host proteins involved in viral replication.[408,409] Supportive care with oxygen, fluids, and pressors is indicated to treat and prevent hypotension and metabolic acidosis. Histamine type 2 receptor antagonists and sucralfate may be of value in preventing or ameliorating gastric bleeding. Avoidance of sedatives and drugs dependent on hepatic metabolism is prudent, and the medication dosing intervals should be adjusted with reduced renal function. Encephalopathy should be investigated for treatable metabolic causes, particularly hypoglycemia. Fresh-frozen plasma and vitamin K have been administered to replenish clotting factors. The effect of heparin therapy is unproven. Secondary infections should be pursued and treated.

YF is preventable with the attenuated 17D vaccine, which produces immunity in more than 95% of recipients and long-term (at least 10 years and possibly lifelong) protection with a single 0.5-mL subcutaneous dose.[6] The WHO updated the recommendations for vaccination in 2013. It was recommended for healthy immunocompetent individuals that a single dose of vaccine is sufficient to confer lifelong protective immunity against YF disease and that a booster dose every 10 years is not necessary. The recommendations for immunocompromised and other special groups were not changed. Subsequently, some countries have adopted this recommendation, including Brazil, which updated its policy in 2017. In 2015 the US Advisory Committee on Immunization Practices updated its recommendations so that most travelers do not require booster vaccinations. However, certain groups still require booster doses, including travelers who had received a vaccine dose more than 10 years previously and plan to spend a prolonged period in endemic areas or those traveling to an area with an ongoing outbreak. In addition, booster doses should be given to laboratory workers and immunocompromised

individuals, and women who were pregnant when they received their first dose of vaccine should receive a booster dose before they travel.[408,409]

Vaccination has been associated with anaphylaxis in 13 of every million doses distributed, but the relative roles of hypersensitivity to chicken eggs and gelatin are unclear because the vaccine is produced in chick embryos, and gelatin has been removed.[410,411] Equally serious, however, are rare but potentially fatal cases of vaccine-associated CNS infection or systemic illness, mimicking wild-type infection, which have been reported from multiple countries. To date, 100 suspected or probable cases of yellow fever vaccine–associated viscerotropic disease (YEL-AVD) have been reported. All known cases occurred in primary vaccinees who ranged in age from 10 months to 81 years (mean, approximately 45 years). Clinical signs of high fever, arthralgia, myalgia, headache, and vomiting usually occur within 2 to 5 days after immunization and are followed by elevated liver enzyme and bilirubin levels and thrombocytopenia and lymphocytopenia. Subsequently, combinations of fulminant hepatitis, shock, renal failure, and disturbances of coagulation, all consistent with YF, have been observed. When laboratory tests for virus have been undertaken, large quantities of vaccine virus are detected in tissues or blood. The clinical and laboratory picture, plus the histology and immunohistochemistry at autopsy, is similar to those seen with wild-type YF infection. The case-fatality rate is over 60%. There is little evidence in support of mutation in the vaccine virus as a cause of YEL-AVD; rather, host factors are thought to be responsible. Since 1989 there have been 113 cases of yellow fever vaccine–associated neurotropic disease (YEL-AND), including cases of meningoencephalitis, acute disseminated encephalomyelitis, and GBS, but only 1 has been fatal, and the majority of cases are aseptic meningitis. Clinical signs of headache and fever (>38.6°C [101.5°F]), focal neurologic dysfunction, and altered mental state are first seen from 2 to 30 days after immunization and are accompanied by CSF pleocytosis or elevated protein.[412–414]

The reported rates of YEL-AVD and YEL-AND vary in publications. In part, this is due to differences between travelers and those who live in endemic areas. In travelers, the rate of YEL-AVD is 2.5 to 4 cases per million doses and that for YEL-AND is 2.5 to 8 cases per million doses.[413,415] Both rates are low and have resulted in no change in vaccine recommendations for travelers. Not surprisingly, rates are lower in endemic areas at 0.13 to 3 cases per million doses for YEL-AVD and 0.16 to 11 cases per million doses for YEL-AND. Because of the well-established risk for vaccine-associated encephalitis in infants, YF vaccine is contraindicated in infants younger than 4 months of age and is recommended for 4- to 9-month-old infants only under situations of high risk. YF and measles vaccines are coadministered at the 9-month Expanded Program of Immunization visit under WHO–United Nations Children's Fund recommendations in 35 African countries. The potential risk of administration of vaccine in pregnancy is unclear. Cord blood IgM viral antibodies indicating congenital infection were reported in one case without evidence of birth defects.[416,417] In one study the vaccine was less immunogenic in pregnant women (39% seroconversion),[421] whereas in another it was as immunogenic (95% seroconversion)[417a] as in the general population. A recent study reported 65 of 78 (83%) HIV-infected adults develop neutralizing antibodies compared with controls (64 of 66; 97%), and this has been followed up with a study of the same cohort 10 years postvaccination that showed long-term immunity is largely dependent on whether or not HIV replication was controlled at the time of vaccination.[418,419] For those taking a successful combination antiretroviral therapy regimen (CARTR), the immune response up to 10 years was comparable to that of non–HIV-infected adults. Thus vaccine boosters were recommended after 10 years for individuals who are on a successful CARTR, while individuals whose HIV RNA levels are uncontrolled may need a booster earlier. No adverse events were reported in pregnant women, but a fatal CNS infection has been reported in one HIV-infected vaccinee.[416,417,420–426]

The YF vaccine is only manufactured by six producers worldwide and so periodically vaccine supplies are exhausted when large outbreaks of disease take place, requiring a response with large quantities of vaccine.[422,423] In 2016, there was a large outbreak of YF involving Angola and subsequently the DRC. During the first 6 months of 2016, the world's supply of vaccine was exhausted twice, and in the summer of 2016 the disease spread to the DRC, including urban YF in the capital Kinshasa. It was clear there was insufficient vaccine to immunize everyone and so fractional dosing of the vaccine was undertaken.[422,424] Previous studies with the 17DD substrain vaccine manufactured in Brazil had shown that fractional dosing would work. Seven hundred forty-nine adult males in the Brazilian army were recruited to a double-blind, randomized clinical trial to test for immunologic noninferiority, over 12 months of different doses of 17DD vaccine. A "full dose" of 27,476 international units (IU) was compared to five lower formulations: 10,447, 3013, 587, 158, and 31 IU, respectively.[422,425,426] No serious adverse events were reported from any groups. Seroconversion was 97% or higher, except in those who received fractions lower than 587 IU. Analysis of serum cytokines/chemokines in association with neutralization titers and viremia supported the use of a 10-fold lower subdose (3013 IU) of vaccine that would be equivalent to that given with a full dose of vaccine. Based on these data and availability of the Brazilian 17DD vaccine, fractional dose YF vaccination, an off-label use of the product, was considered in response to an emergency situation in which current vaccine supply was insufficient. A preventive vaccination campaign was launched in the DRC on 17 August 2016. The campaign aimed to immunize over 8 million people in 32 Health Zones in Kinshasa province using the fractionated dose strategy, in which the vaccine was administered at one-fifth of the standard vaccine dose; this strategy was only recommended for use in an emergency situation in the context of limited vaccine availability, and in those over 2 years of age and excluding pregnant women. One-fifth of a dose was selected for practicality because a full dose is given in a volume of 0.5 mL, and 0.1 mL could be given using tuberculin syringes without the need for dilution of the vaccine. The vaccination campaign proved successful and the outbreak was controlled.

The 17DD substrain vaccine is only produced in Brazil, and the other producers use 17D-204 or 17D-213 substrain vaccines. Consequently, clinical trials are ongoing to evaluate fractional dosing for vaccine made by all the producers to ensure that all vaccines can be fractional dosed in emergency situations.

As stated earlier, a large outbreak of YF took place during later 2016 to mid-2018 in Brazil, where the virus has spread to new regions where there are large population centers. Vaccine supply was exhausted and, for the second time in the history of the vaccine, a fractional dose vaccination regimen was employed. In January 2018, Brazil launched a mass immunization campaign to give fractional doses of 17DD vaccine to approximately 24 million residents of 69 municipalities in the states of Rio de Janeiro and São Paulo.

Travelers to at-risk South American and African countries should receive the vaccine either once or at 10-year intervals to meet current national requirements. The vaccine can be given concurrently with measles, oral polio, hepatitis A or B, meningococcal polysaccharide, oral or intramuscular typhoid, or oral cholera vaccines; chloroquine; or immune serum globulin.

Prevention of epidemic *A. aegypti*–borne YF follows the approach for dengue control, with the reduction of peridomestic breeding sites. In dry savanna and urban locations where drinking water frequently must be stored, the simple expedient of covering the containers or reservoirs eliminates a principal source of breeding. Surveillance of viral activity by monitoring of viral infection rates in sylvatic mosquitoes has been proposed as an early warning system for West and Central Africa, where outbreaks frequently emerge in a region-wide distribution. The discovery of intensified viral activity, even in a small number of sentinel sites, may be a sufficiently sensitive predictor of viral activity in a broader area to trigger timely and effective mass immunization. In South America, surveys to detect dead monkeys on the forest floor are conducted to monitor viral transmission and risk for its spillover to humans.

Dengue and Dengue Hemorrhagic Fever

Management of dengue is straightforward and highly effective at reducing morbidity and mortality if properly executed. The key is to recognize the disease phase the patient is experiencing at presentation or is transitioning to (i.e., febrile, critical, recovery) and to implement, in a judicious and controlled manner, the required interventions to comfort and address key pathophysiologic mechanisms, mainly plasma leakage

and hemorrhage.[112] When fatalities do occur, one or more of the following issues is often at fault: late presentation to medical care, delayed escalation of care, and overaggressive provision of intravenous fluids. Therefore it is essential that patients, parents or guardians of patients, and clinicians are cognizant of the warning signs and symptoms for plasma leakage and intravascular volume depletion, such as abdominal pain, nausea, vomiting, lethargy, mental status changes, decreased urine output, tachycardia, and decreasing pulse pressure, so that supportive therapy can be administered promptly. Clinicians must also follow the clinical and biochemical clues of volume status to avoid contributing to iatrogenic volume overload. Comorbidities such as hypertension, diabetes, and pulmonary and cardiac disease increase the risk for severe disease and a poor outcome.[427,428] Evidence-based management algorithms exist and are highly effective.[112,429]

Acetaminophen-based products may be used to reduce fever and treat myalgias and arthralgias. Acetylsalicylic acid (aspirin), ibuprofen, or other nonsteroidal antiinflammatory agents may aggravate hemorrhagic complications or induce Reye syndrome and should be avoided. Oral rehydration with fluids containing electrolytes and sugars is encouraged to counter vomiting and diarrhea.

Attentive clinical monitoring of patients with suspected severe dengue (DHF-DSS) and anticipatory and supportive care, especially at the time of defervescence, are lifesaving and have reduced fatality rates 50- to 100-fold. The critical activities are monitoring the adequacy of circulation and vascular leakage, by serial clinical assessments of pulse, blood pressure, skin perfusion, urine output, and hematocrit, to trigger intravenous fluid therapy. An increase in hematocrit of greater than 20% (e.g., from 35% to 42%) indicates a significant loss of intravascular volume and the urgent need for fluid resuscitation. Normal saline is administered to maintain circulation and, under continued monitoring, to treat recurrent shock. Shock necessitates rapid intervention with isotonic crystalloid or colloid solutions or, if needed, plasma or whole-blood transfusions.[430] As a result of the danger of acute respiratory distress syndrome (ARDS) due to excessive fluid administration, DHF-DSS has been reported to be the third most common cause of ARDS in hospitalized children in Malaysia.[330] Whole-blood, platelet, and fresh-frozen plasma transfusions may be needed if there is significant hemorrhage, but caution is indicated in the administration of heparin except in patients with clear signs of disseminated intravascular coagulopathy.[431] Preventive transfusions may be harmful and should be avoided, and invasive procedures should be minimized to avoid hemorrhagic complications. Treatment to end virus replication could be beneficial, although viremia levels usually are already decreasing dramatically at the time of presentation to health care providers.[432] Controlled clinical trials have shown no benefit for corticosteroids.[433,434] Numerous other antiviral and immunomodulatory drugs have been proposed for use, such as intravenous immune globulin, pentoxifylline, and chloroquine, and some have been in wide use in dengue-endemic areas, but none has been established to have clinical benefit.[435–442]

In the absence of a vaccine suitable for widespread use, dengue prevention relies on community-based *A. aegypti* control programs to remove and destroy mosquito breeding sites.[98] The ubiquity of containers that potentially provide breeding habitats in urban neighborhoods and individual houses makes this a formidable challenge. Although a combination of vector surveillance, area treatment, and monitoring can be effective, it has rarely been successful for prolonged periods. Insecticidal fogging is considered unhelpful, but indoor residual insecticidal sprays should be effective in sealed houses.[443–445] Insecticide-treated curtains may be effective; bed nets are not useful because the mosquitoes are most active during the day.[443–445]

A live-attenuated tetravalent vaccine against dengue, containing four recombinant chimeric flaviviruses each constructed from the YF virus 17D genome with PreM and E gene segments replaced by the corresponding segments of one DENV serotype (Dengvaxia), has been licensed in 20 countries for prevention of dengue in healthy individuals 9 to 45 years of age. In two large phase III clinical trials, the vaccine showed overall efficacy of 56.5% and 60.8% in Asia and Latin America, respectively, against virologically confirmed dengue during the first year after completion of the three-dose vaccination schedule (0, 6, and 12 months).[446–449] There were higher rates of efficacy for preventing hospitalized dengue (80.3%) or severe dengue (95.5%).[446,448] However, efficacy against DENV-2 (47%) was significantly lower than against DENV-1 (58%), DENV-3 (74%), or DENV-4 (83%), and overall efficacy was low in seronegative individuals. Furthermore, there was an increase in the relative risk of hospitalized dengue in the youngest vaccinated cohort (2- to 5-year-olds) during the second year following vaccination.[446,450] Further analyses have confirmed a lack of efficacy, and a higher risk for hospitalized dengue, among subjects in the targeted age group (9- to 16-year-olds) who were seronegative for dengue at baseline.[446,451] As a result, this vaccine is not recommended for use without laboratory confirmation of prior DENV infection.

Two other live-attenuated tetravalent dengue vaccines are currently in phase III trials. These vaccines differ in composition from Dengvaxia. One vaccine contains a recombinant attenuated DENV-2 strain and three recombinant chimeric viruses constructed from the DENV-2 genome with pre-M and E gene segments of the other DENV types. The second vaccine contains recombinant strains of DENV-1, DENV-3, and DENV-4 containing engineered attenuating mutations and a chimeric virus constructed from the DENV-4 genome with pre-M and E gene segments of DENV-2. It is hoped that inclusion of DENV nonstructural gene segments in these vaccines will eliminate the increased risk observed with Dengvaxia. Several vaccines based on other platforms, including purified inactivated viruses adjuvanted with novel adjuvants, recombinant proteins, and plasmid DNA, are in earlier phases of development.[9,452–457]

Zika Virus Infection

Vaccines or antivirals are not available against ZIKV infection, although several vaccines are in clinical development and undergoing human clinical trials.[458,459] DNA and purified inactivated Zika vaccine candidates that have reported phase I human data indicate the safety profile is acceptable, and immunogenicity, as measured by neutralizing antibody, is also supportive of advancing clinical development.[458,460–462] It is unclear, however, if the current diminution of Zika cases will support pursuit of a clinical end point study.

Treatment of acute Zika cases is generally supportive and may include rest, fluids, and use of analgesics and antipyretics. Fever should be treated with acetaminophen. Aspirin and nonsteroidal antiinflammatory drugs should be avoided until dengue can be ruled out, to reduce the risk of hemorrhage.

For prevention of infection, measures to avoid mosquito exposure in areas of potential transmission should be taken, including wearing long sleeves and pants, applying insect repellent, and staying indoors in areas well protected by windows, screens, and mosquito nets. Environmental control measures to eliminate mosquito breeding areas should be implemented, particularly reduction of standing water containers and tanks.

The CDC and the European Centre for Disease Prevention and Control have issued guidelines for pregnant women during ZIKV outbreaks.[377,384,385] The CDC recommends that pregnant women consider postponing travel to an area where ZIKV transmission is ongoing. If travel is necessary, steps to prevent mosquito bites should be taken.

Recommendations for pregnant women with a history of travel to an area of ZIKV transmission have also been formulated.[377,384] Women who traveled to such areas should be evaluated for ZIKV infection. Women who report a travel history and who have two or more symptoms consistent with ZIKV infection (fever, onset of rash, arthralgias, or conjunctivitis) should have laboratory tests for ZIKV infection (see "Laboratory Diagnosis" earlier). Women with a travel history and fetal ultrasound findings of microcephaly or intracranial calcifications should also be tested. If laboratory testing is inconclusive, an amniocentesis can be considered. Testing is not recommended for women with a travel history but no other findings.

For pregnant women with laboratory evidence of ZIKV infection, serial ultrasounds should be considered to monitor fetal anatomy and growth every 3 to 4 weeks. Referral to a maternal-fetal medicine or infectious disease specialist with expertise in pregnancy management is recommended.

For a live birth with evidence of maternal or fetal ZIKV infection, the following tests are recommended: histopathologic examination of

the placenta and umbilical cord, testing of frozen placental and cord tissue for ZIKV RNA, and testing of cord serum for ZIKV and DENV IgM and neutralizing antibodies. If a pregnancy results in fetal loss in a woman for whom laboratory testing is recommended, ZIKV RT-PCR and immunohistochemical staining should be performed on fetal tissues. Because of the potential sexual transmission of ZIKV, the CDC has formulated guidelines for prevention of sexual transmission (discussed earlier).[191,193]

Flavivirus Encephalitis

No specific antiviral therapy for flavivirus encephalitis has been developed; current treatment options are supportive. Anecdotal use of IFN-α in the prophylaxis and treatment of JE and SLE cases has been reported, but a phase III randomized, double-blind, placebo-controlled trial of IFN-α2a in children with JE showed that it did not improve the outcome at hospital discharge or at 3 months' follow-up.[463] A randomized controlled trial of oral ribavirin versus placebo in children with JE in Uttar Pradesh, India, failed to demonstrate a difference in outcome.[464] Ribavirin was employed during a WNV outbreak in Israel in 2000, with a high mortality (41%) observed in the ribavirin group.[352] Corticosteroid therapy for TBE resulted in a more rapid reduction of fever but prolonged hospitalization, whereas in JE a small trial of corticosteroid treatment failed to show benefit or harm.[465] In a small controlled study, tetracycline administered as an immunomodulator was shown to reduce elaboration of proinflammatory cytokines and to hasten recovery from TBE.[466] Antisense oligomers that inhibit WNV replication in vitro have been reported, and an RNA interference screen approach has examined in detail virus and human host cell interactions, identifying potential targets for antiviral treatments.[467,468] In addition, cell-based high-throughput assays have been developed to screen compound libraries for agents effective against WNV and other flaviviruses.[469]

A mixture of JEV-neutralizing monoclonal antibodies was reportedly beneficial in a clinical trial in China, but considerable experience with TBE immune globulin therapy has highlighted the potential hazards of immunotherapy.[470,471] Humanized monoclonal antibodies against WNV envelope protein have shown efficacy in mice and hamsters, even when given as a single dose on day 5 after infection and when the virus has reached the CNS.[472,473] A phase I safety study of a recombinant humanized monoclonal antibody targeting the E protein of WNV was well tolerated.[474] Hyperimmune gamma globulin has been used on a small scale in severe WNV infections with varied clinical outcomes.[475,476] Additional WNV treatment candidates are being explored in small animal disease models and early-phase human trials.[477–485]

Supportive care should focus on controlling seizures, providing ventilatory support in respiratory failure, and monitoring and reducing cerebral edema. In many parts of Asia, mannitol, corticosteroids, or both are given to patients with severe JE. Fluid and electrolyte administration should balance circulatory needs, the avoidance of cerebral edema, and SIADH. Secondary infections should be anticipated and treated, and careful nursing attention should be paid to minimize complications such as bedsores and contractures.

JE vaccines have been available since the 1950s (Table 153.3).[5] Inactivated mouse brain vaccines, containing either Nakayama-NIH

TABLE 153.3 Landscape and Other Details of Japanese Encephalitis Vaccines Licensed to Date

TYPE OF VACCINE	VIRUS STRAIN AND PHENOTYPE	SUBSTRATE	GENERIC NAME, IF ANY	MANUFACTURER AND TRADE NAME	REGIMEN	COUNTRY
Inactivated	Nakayama-NIH; wild-type	Mouse brain	JE-MB	BIKEN, Japan (Biken, JE-VAX); other local producers	Days 0, 7, 28 at 12–24 mo of age; booster after 12–14 mo, then every 3–5 yr	European Union, India, Japan, Malaysia, North Korea, South Korea, Sri Lanka, Taiwan, Thailand, United States, Vietnam
	Beijing-1 (P-1); wild-type	Mouse brain	JE-MB, BK-VJE	GCC, Korea (JEV GCC); other local producers	Days 0, 7, 28 at 12–24 mo of age; booster after 12–14 mo, then every 3–5 yr	European Union, India, Japan, Malaysia, North Korea, South Korea, Sri Lanka, Taiwan, Thailand, United States, Vietnam
		Vero cells	BK-VJE	BIKEN, Japan (JEBIK-V); KAKETSUKEN, Japan (ENCEVAC, JEIMMUGEN); Boryung Pharma, Korea (TC-JEV)	Days 0, 7, 28 at 12–24 mo of age; booster after 12–14 mo, then every 3–5 yr	Japan, South Korea
		Mouse brain		China	Days 0, 7 at 12 mo of age; then 2, 6 and 19 yr of age	China
	Beijing-3 (P-3); wild-type	PHK cells		China		China
		Vero cells		China		China
	SA-14-14-2; live attenuated	Vero cells	JE-PIV, IC51, JE-VC	Intercell/Novartis/Valneva, Austria (IXIARO); CSLB, Australia (JESPECT), BEL (JEEV)	Days 0, 28 as early as 2 mo of age; booster after 1 yr	Australia, Bangladesh, Bhutan, Canada, European Union, Hong Kong, India, Japan, Latin America, Nepal, New Zealand, Pacific Islands, Papua New Guinea, Singapore, South Korea, Switzerland, United States
	821564XY; wild-type	Vero cells		BBIL (JenVac)	Days 0, 28 as early as 6 mo of age	India
Live attenuated	SA-14-14-2; live attenuated	PHK cells	SA-14-14-2	CDIBP (CD.JEVAX)	Single dose at the age of 8–9 mo; if needed, booster 3–12 mo later, and at 6–7 yr of age	China, Hong Kong, India, Japan, Nepal, South Korea, Sri Lanka, Thailand
Chimeric	YFV 17D containing JEV proteins; live attenuated	Vero cells	ChimeriVax-JE; JE-CV	Acambis/Saofi-Pasteur (IMOJEV, THAIJEV)	A single dose for those >18 yr, with a booster, if needed, after 5 yr; for those ≥9 mo and ≤18 yr, primary dose followed by booster after 12–24 mo	Australia, South Korea, Thailand

BBIL, Bharat Biotech International Ltd., India; *BEL,* Biological Evans Ltd., India; *BIKEN,* The Research Foundation for Microbial Diseases of Osaka University, Japan; *CDIBP,* Chengdu Institute of Biological Products, China; *CSLB,* Commonwealth Serum Laboratories Biotherapeutics, Australia; *GCC,* Green Cross Corporation, Korea; *JEV,* Japanese encephalitis virus; *KAKETSUKEN,* Chemo-Sero-Therapeutic Research Institute, Japan; *NIH,* National Institutes of Health, Japan; *PHK,* primary hamster kidney; *YFV,* yellow fever virus.

Note: The mouse brain– and the PHK cell–derived inactivated vaccines have been discontinued.

From Hegde NR, Gore MM. Japanese encephalitis vaccines: Immunogenicity, protective efficacy, effectiveness, and impact on the burden of disease. Hum Vaccin Immunother. *2017;13:1–18.*

or Beijing-1 virus strains, were developed in Japan and produced by several manufacturers.[44] For several decades, this JE vaccine was used in the United States and Europe (BIKEN; distributed as JE-VAX by Sanofi Pasteur, Lyon, France).[486–490] Routine vaccination led to decreases in JE incidence in Thailand, India, Korea, Taiwan, Vietnam, and areas of Malaysia and Sri Lanka.[491,492]

The safety profile of mouse brain–derived JE vaccines has been questioned. Acute disseminated encephalomyelitis (ADEM) temporally related to vaccination in Japan prompted the Japanese government to suspend strong recommendations for routine childhood JE vaccination. However, ADEM has been infrequently reported as a severe drug reaction.[493] The Global Advisory Committee on Vaccine Safety found no definite evidence of an increased risk for ADEM temporally associated with JE vaccine.[488–490] Production of JE-VAX by the BIKEN laboratories in Japan has since ceased.[494]

A live-attenuated vaccine (SA-14-14-2) developed by Chinese researchers has been used extensively in China; because issues over the primary hamster kidney cells used in its production have been resolved, it has also been used in South Korea, Nepal, India, and Sri Lanka.[495] Two doses are administered during spring campaigns to children older than 1 year. The live vaccine, however, is highly efficacious after even one dose.[5,495,496] Since its licensure, more than 500 million doses have been produced and administered to more than 200 million Chinese children.[497]

IXIARO is a purified, formalin-inactivated, whole-virus JE vaccine. The product is licensed and distributed in the United States, Europe, Canada, Hong Kong, and Israel and distributed in Australia under the trade name JESPECT.[498] The vaccine construct, developed at the Walter Reed Army Institute of Research (Silver Spring, MD), is based on an SA14-14-2 virus strain passaged in primary dog kidney (PDK) cells, cultivated in Vero cells, and formulated with 0.1% aluminum hydroxide.[499] Several clinical studies established its safety and immunogenicity profile.[500–509] Booster doses given 11 or 23 months after primary vaccination series in recipients whose neutralizing antibody titers had waned to below detection led to 100% seroconversion.[505,506] The initial indication was for adults 17 years of age or older. In May 2013 the FDA extended the age range to include infants, children, and adolescents ages 2 months to younger than 17 years for active immunization.[504,510] The primary vaccination schedule is two doses delivered at day 0 and 28 in a 0.5-mL suspension given intramuscularly. The dosing series should be completed at least 1 week before travel or potential exposure.

Sanofi Pasteur has developed a JE vaccine (JE-CV; IMOJEV) by inserting pre-M and E genes from the SA14-14-2 JEV into the YF 17D viral strain "backbone" containing the YF nonstructural genes.[511–513] The vaccine is currently available in Australia; additional filings for approval have been submitted in Asia. IMOJEV has demonstrated safety and seroprotective immunogenicity after a single vaccine dose.[514–519]

The Advisory Committee on Immunization Practices suggests that physicians and others should counsel travelers to be aware of the low-level, but almost unavoidable, risk for acquiring JE when traveling to JE-enzootic countries.[498] JE vaccination should be considered as an important option to reduce this risk. At-risk individuals, even if exposure is short term (<1 month), should consider vaccination. High-risk activities include those that occur outdoors, near agricultural areas, or during evening hours, and where lodging is in the open without use of bed nets. Vaccination should be considered for travel to a JE-endemic area without a defined destination or to an area with a known JE outbreak. JE vaccine is not recommended for short-term travelers (<1 month) whose visit will be restricted to urban areas or outside the transmission season. Lastly, JE vaccine is recommended for laboratory personnel who work with live, wild-type JEV strains. At-risk laboratory personnel should receive booster doses of JE vaccine or be evaluated regularly for JEV-specific neutralizing antibody titers.[498,520] Although data are lacking, individuals partially or completely immunized with any first-generation inactivated JE vaccine should likely receive the complete immunization series of IXIARO to ensure a protective immune response.[497]

No human vaccines against WNE or SLE viruses are licensed; however, formalin-inactivated and canarypox-vectored WNV vaccines for horses are commercially available in the United States. A live-attenuated chimeric WNV/YF 17D vaccine has advanced to phase II clinical evaluation.[521–523] Chimeric vaccines of WNV combined with DENV-2 or DENV-4 have also been developed and provide complete protection against WNV in challenge experiments.[524]

TBE vaccines currently in use are FSME-Immun (Baxter, Austria), Encepur (Novartis Vaccines, Germany), EnceVir (Scientific Production Association Microgen, Russia), and TBE vaccine Moscow (Federal State Enterprise of Chumakov Institute of Poliomyelitis and Viral Encephalitides, Russian Academy of Medical Sciences, Russia).[525] FSME-Immun and Encepur are available in the European Union and have been authorized by the European Medicines Agency.[526] They are all safe and immunogenic with considerable uptake rates in areas that have a high transmission risk.[55,56,161] All four vaccines seem to give protective immunity against all three subtypes of TBE. Mass vaccination has been practiced in Austria since the 1990s.[527] Administration in three doses over a period of 1 year, with an additional booster 3 years later, has been highly effective in reducing rates of disease. An abbreviated 0-, 7-, and 21- or 28-day immunization schedule also is immunogenic.[528] Cross-protection against TBEV-FE has been shown in animals and in clinical trials. Chiefly, mild adverse events (fever and local reactions) are reported; however, neurologic adverse events, including GBS, have been noted, albeit without a proven causal association, in about 1 of every 1 million vaccinees. TBE vaccine is not licensed in the United States but is available in Canada. For most travelers, the risk for acquiring the disease is extremely low and personal protective measures (e.g., avoidance of risky habitats, wearing protective clothing, using repellents) are appropriate. Expatriates may choose to be immunized abroad. Live-attenuated and recombinant vaccines are under development.

Vaccines for KFDV infection have been in development since the 1960s using varied approaches.[182,529–531] Annual rounds of vaccination using formalin-inactivated tissue-culture vaccine have been conducted in India in the Karnataka State since 1990. Unfortunately, vaccine coverage and effectiveness appears lower than previously reported.[532]

OTHER FLAVIVIRUS INFECTIONS

Murray Valley Encephalitis

Murray Valley encephalitis virus is a member of the antigenic complex of JEV, SLE virus, and WNV, and, like them, it is transmitted in a mosquito-avian cycle, chiefly by *Culex annulirostris*. Foci of perennial viral transmission are maintained in Western Australia, where sporadic cases and small outbreaks occur. Most sporadic cases occur in Aboriginal children living in areas where they are exposed to the virus, but cases have also occurred among travelers to these areas, including a visitor from Europe.[533–535] At infrequent intervals since the initial recognition of the disease in 1917, the virus has spread to the heavily populated southeastern river valleys, where it has produced larger outbreaks, most recently in 1981. Sporadic cases also have been recognized in Papua New Guinea. About 350 cases have been reported in total, with a case-fatality rate of 20% in the most recent outbreak. The onset of encephalitis is preceded by a prodrome of headache, nausea, vomiting, photophobia, and neck stiffness, followed within 2 to 5 days by changes in sensorium, stupor, and motor signs. Coma, limb paralysis, and respiratory depression necessitating ventilatory support develop in severe cases. Recovery is followed by motor paralysis in severe cases and by milder motor disturbances and emotional and psychological symptoms in a higher proportion of survivors. Serologic diagnosis is potentially encumbered by cross-reactive antibodies to Kunjin, Kokobera, JE, Edge Hill, Alfuy, Sepik, dengue, and other flaviviruses in the region. Supportive treatment has reduced mortality and morbidity. Regional surveillance of sentinel chicken infections is maintained as an early warning system.

Rocio Encephalitis

Rocio virus was recognized to be the novel cause of a series of encephalitis outbreaks that occurred from 1975 to 1977 in the Ribiera Valley and Santista lowlands in coastal São Paulo and Paraná States, Brazil.[536] More than 1000 cases were identified, chiefly in fishermen and others with outdoor occupations. The virus was isolated from human brain, and its relationship to SLE virus was shown antigenically and, later, by genomic sequencing. The virus is transmitted from *Psorophora* spp.

TABLE 153.4 Less Commonly Recognized Flavivirus Infections

VIRUS	CLINICAL SYNDROME	GEOGRAPHIC DISTRIBUTION	TRANSMISSION CYCLE	MODE OF TRANSMISSION
Alkhurma	Hemorrhagic fever, encephalitis	Saudi Arabia, in Africa near the Egypt-Sudan border and Djibouti	*Ornithodoros savignyi* and *Hyalomma dromedarii*–camels	DC, V
Bussuquara	Fever, arthralgia	Argentina, Brazil, Colombia, Panama	*Culex melanoconion* spp.–rodent	V
Edge Hill	Fever, polyarthritis	Australia	*Aedes vigilax*–marsupial	V
Ilhéus	Fever, myalgia, encephalitis	Argentina, Brazil, Colombia, Guatemala, Panama, Trinidad	*Psorophora ferox*–bird	V, E
Kokobera	Fever, polyarthralgia	Australia, Papua New Guinea	*Culex annulirostris*–? marsupial	V
Koutango	Fever, rash, arthralgia	West and Central Africa	Tick–rodent	L
Kunjin	Fever, polyarthralgia, encephalitis	Australia, Malaysia, Thailand	*Culex annulirostris*–bird	V
Modoc	Aseptic meningitis	Western United States, Canada	Rodent–rodent	Z
Rio Bravo	Nonspecific febrile illness, meningitis	Western United States, Canada	Bat–bat	Z, L
Sepik	Nonspecific febrile illness	Papua New Guinea	*Mansonia* spp.–?	V
Usutu	Fever, rash	South and Central Africa, and Europe	*Culex* spp.–bird	V
Wesselsbron	Fever, arthralgia, rash, encephalitis	Sub-Saharan Africa, Thailand	*Aedes* spp.–?	V, L, DC

DC, Contact with infected domestic animals or livestock; *E,* experimental infection; *L,* laboratory-acquired infection; *V,* vector-borne; *Z,* zoonotic infection.

mosquitoes to birds, and human infections are incidental. Sporadic asymptomatic infections have been detected in field studies, but outbreaks have not recurred. In 1996, serologic evidence of infection was reported in Bahia State, far to the north, but the virus has not been isolated outside the original focus. A prodrome of fever, headache, malaise, vomiting, and conjunctivitis precedes the onset of altered consciousness, motor weakness, and, frequently, cerebellar signs. Neurologic infection progresses to coma in one-third of cases and death in 10%. Neurologic and psychological sequelae have been reported in 20% of survivors. Supportive treatment is potentially lifesaving. Emergency applications of insecticides have been implemented in outbreak control.

Omsk Hemorrhagic Fever

Omsk hemorrhagic fever virus is transmitted between *Dermacentor* spp. ticks and small mammals in forest-steppe zones of the Omsk, Novosibirsk, Kurgan, and Tyumen regions of western Siberia, but the disease emerged in significant form only after muskrats were introduced to the region to establish a fur industry.[458–462,537,538] Outbreaks between 1945 and 1958 led to muskrat epizootics and 1500 human cases, chiefly among trappers, their family members, and laboratory workers. Infection is transmitted directly from infected animal tissues or by tick bite, with a peak in spring or early summer and another peak in autumn. The illness resembles Kyasanur Forest disease, but neuropsychiatric sequelae have been reported more often. The case-fatality rate is less than 3%. Inactivated TBE vaccine (produced in Russia) has been reported to offer cross-protection against the disease.

Less Commonly Recognized Flavivirus Infections

Small numbers or even single cases of the diseases listed in Table 153.4 have been reported. In some instances, experimental human infection (evaluated as cancer therapy) provides the only knowledge of their pathogenicity.

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154 Hepatitis C

Susanna Naggie and David L. Wyles

SHORT VIEW SUMMARY

Definition

- Hepatitis C virus (HCV) is an enveloped, positive-strand RNA virus that is a member of the Flaviviridae family, *Hepacivirus* genus.
- It is a major worldwide cause of chronic liver disease, including cirrhosis and hepatocellular carcinoma (HCC).

Microbiology

- HCV is roughly spheroid and approximately 65 nm in diameter (see Fig. 154.1).
- The RNA genome is 9.6 kb in length and contains a single large open reading frame, which is processed into at least 10 proteins, including 3 structural proteins and 5 proteins of the viral RNA replicase complex (NS3, NS4A, NS4B, NS5A, and NS5B (see Fig. 154.2).
- HCV has seven major genotypes and provisionally an eighth.
- Extensive quasispecies variation is present in each infected individual.

Epidemiology

- An estimated 71 million persons are actively infected (viremic) with HCV worldwide.
- Transmission occurs most commonly by percutaneous exposure to blood.
- In developed countries, two epidemics of incident HCV infections are occurring: one in persons who use intravenous drugs and a second in men who have sex with men (MSM).
- In the developing world, most transmission occurs from unsafe medical practices.
- HCV may be transmitted sexually, but this appears to be infrequent except in select high-risk populations such as human immunodeficiency virus (HIV)–positive MSM.

Clinical Manifestations

- Acute illness caused by HCV infection is unusual, but it is typical of acute hepatitis and consists of malaise, nausea, and right upper quadrant pain, followed by dark urine and jaundice.
- Fulminant hepatitis due to HCV infection is uncommon.
- Chronic infection with HCV occurs in approximately 75% of patients after acute infection with HCV.
- Once established, chronic infection persists indefinitely and can be associated with cirrhosis, metabolic disorders such as insulin resistance and steatosis, and HCC.

Diagnosis

- Diagnosis of HCV infection is usually made by means of assays of serum antibody to HCV followed by testing for HCV RNA.
- RNA tests are also used to assess the effects of treatment.
- Noninvasive liver tests have now replaced biopsy as the preferred methods to assess the stage of liver disease.

Treatment (Table 154.4)

- The primary aim of treatment is to eradicate HCV in blood and liver (cure) and thus prevent complications of HCV infection.
- The American Association for the Study of Liver Diseases (AASLD) and Infectious Diseases Society of America (IDSA) provide regularly updated treatment recommendations for hepatitis C (see the online guidance for recommendations for re-treatment and for treatment of special populations, including those who are HIV-infected or have renal failure).
  - Current recommendations for initial treatment were issued in September 2017.
  - See Table 154.4.
    - Genotype 1a
      - Elbasvir-grazoprevir for 12 weeks if no NS5A resistance-associated substitution (RAS) for elbasvir is detected (no cirrhosis or compensated cirrhosis)
      - Glecaprevir-pibrentasvir for 8 weeks if no cirrhosis and 12 weeks in patients with compensated cirrhosis
      - Ledipasvir-sofosbuvir for 12 weeks (no cirrhosis or compensated cirrhosis)
        - 8 weeks for patients without cirrhosis and baseline HCV RNA <6 million IU/mL
      - Sofosbuvir-velpatasvir for 12 weeks (no cirrhosis or compensated cirrhosis)
    - Genotype 1b
      - Elbasvir-grazoprevir for 12 weeks (no cirrhosis or compensated cirrhosis)
      - Glecaprevir-pibrentasvir for 8 weeks if no cirrhosis and 12 weeks if compensated cirrhosis
      - Ledipasvir-sofosbuvir for 12 weeks (no cirrhosis or compensated cirrhosis)
        - 8 weeks for patients without cirrhosis and baseline HCV RNA <6 million IU/mL
      - Sofosbuvir-velpatasvir for 12 weeks (no cirrhosis or compensated cirrhosis)
    - Genotype 2
      - Glecaprevir-pibrentasvir for 8 weeks if no cirrhosis and 12 weeks if compensated cirrhosis
      - Sofosbuvir-velpatasvir for 12 weeks (no cirrhosis or compensated cirrhosis)
    - Genotype 3
      - Glecaprevir-pibrentasvir for 8 weeks if no cirrhosis and 12 weeks if compensated cirrhosis (treatment naive)
      - Sofosbuvir-velpatasvir for 12 weeks if no cirrhosis or if compensated cirrhosis; testing for NS5A RAS for velpatasvir is recommended and an alternative approach recommended if present
    - Genotype 4
      - Elbasvir-grazoprevir for 12 weeks (no cirrhosis or compensated cirrhosis)
      - Glecaprevir-pibrentasvir for 8 weeks if no cirrhosis and 12 weeks if compensated cirrhosis
      - Ledipasvir-sofosbuvir for 12 weeks (no cirrhosis or compensated cirrhosis)
      - Sofosbuvir-velpatasvir for 12 weeks (no cirrhosis or compensated cirrhosis)
    - Genotype 5 or 6
      - Glecaprevir-pibrentasvir for 8 weeks if no cirrhosis and 12 weeks if compensated cirrhosis
      - Ledipasvir-sofosbuvir for 12 weeks (no cirrhosis or compensated cirrhosis)
      - Sofosbuvir-velpatasvir for 12 weeks (no cirrhosis or compensated cirrhosis)
  - Clinicians are urged to consult online guidelines for the latest recommendations on HCV treatment (www.hcvguidelines.org).

Prevention

- Strategies are primarily designed to reduce exposure to contaminated blood through screening of blood products, application of precautions in health care settings, and reduction of intravenous drug use risks.
- In settings where sexual transmission appears prominent (HIV-positive MSM), barrier protection methods should be recommended.

SHORT VIEW SUMMARY—cont'd

- High-coverage screening and treatment approaches in high-risk populations are emerging as another approach to prevention (so called "treatment as prevention").
- Development of vaccines against HCV is challenging because of extensive viral diversity.

Elimination

- The introduction of highly effective therapy has fostered the discussion, planning, and implementation of HCV elimination programs.
- The World Health Organization has put forth goals for HCV elimination by 2030, including 90% diagnosed and 80% of eligible persons treated, to reach 90% reduction in HCV incidence and 65% reduction in mortality.
- Localized examples of progress toward elimination are beginning to emerge in select populations (HIV-positive MSM) and countries (Georgia).
- Major barriers to elimination currently include suboptimal diagnosis rates, poor or variable access to direct-acting antiviral (DAA) therapy, and lack of governmental support and/or coordinated efforts.

NON-A, NON-B VIRAL HEPATITIS AND HEPATITIS C

When serologic tests for hepatitis A virus (HAV) and hepatitis B virus (HBV) were developed during the 1970s, it became evident that most cases of transfusion-associated hepatitis must be caused by yet another agent, leading to the term *non-A, non-B hepatitis* (NANB).[1,2] Studies in chimpanzees confirmed that bloodborne NANB hepatitis was transmissible and due to a relatively small, lipid-enveloped virus.[3,4] In the late 1980s, Michael Houghton's laboratory at Chiron Corporation, working with Daniel Bradley's laboratory at the Centers for Disease Control and Prevention (CDC), identified a virally encoded antigen associated with NANB hepatitis and called the agent *hepatitis C virus* (HCV).[5] This finding rapidly led to the molecular cloning of the complete viral genome[6] and other major discoveries, including the proclivity of this virus to establish persistent infection and its strong association with chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC).

HEPATITIS C VIRUS

Virology and Classification

HCV is a roughly spherical, enveloped, positive-strand RNA virus approximately 65 nm in diameter with a broad size range (40–100 nm) (Fig. 154.1).[7–10,11] Its structure, genomic organization, and replication cycle support classification as a member of the family Flaviviridae, yet it is sufficiently distinct from the type genus *Flavivirus* (e.g., yellow fever virus and dengue virus) to merit classification within a separate genus, *Hepacivirus*. Sibling genera also include *Pestivirus* (e.g., bovine viral diarrhea virus and classic swine fever virus) and *Pegivirus* (human pegivirus [HPgV; formerly GBV-C] and related viruses).[12,13] In addition to HCV, the *Hepacivirus* genus includes GB virus B (GBV-B), and nonprimate hepaciviruses (NPHVs) found in rodents, dogs, horses, and bats.[14–16] Studies of infectious plasma, sera, and culture supernatants suggest that the HCV envelope may associate with and be partially masked by low-density lipoproteins in a "lipo-viro-particle" or "lipovirion."[17,18]

Organization of the Hepatitis C Virus Genome

The genome of HCV is a positive-sense, single-stranded RNA molecule approximately 9.6 kb in length. Unlike a typical eukaryotic mRNA, the HCV genomic RNA is neither 5′ capped nor polyadenylated and contains a large open reading frame (ORF) encoding a single large polyprotein (approximately 3010 amino acids). A frame-shifting event occurring near the 5′ end of the ORF has been suggested to shift some translating ribosomes into production of an alternative reading frame protein (ARFP) that is of uncertain function in the viral life cycle (see later). The large ORF is flanked by highly conserved 5′ and 3′ untranslated regions (UTRs) that function in both translation and viral RNA replication (Fig. 154.2).[6]

Nontranslated RNA Segments

The HCV 5′UTR is approximately 341 nucleotides in length, demonstrates extensive secondary and tertiary RNA structure (see Fig. 154.2), and contains two overlapping functional regions. The 5′ terminal 125 nucleotides are essential for viral RNA replication, probably for recognition of the RNA by the viral replicase, whereas the remainder of the 5′UTR appears to play an accessory role in this process.[19] An overlapping approximately 300-nucleotide segment acts as an internal ribosomal

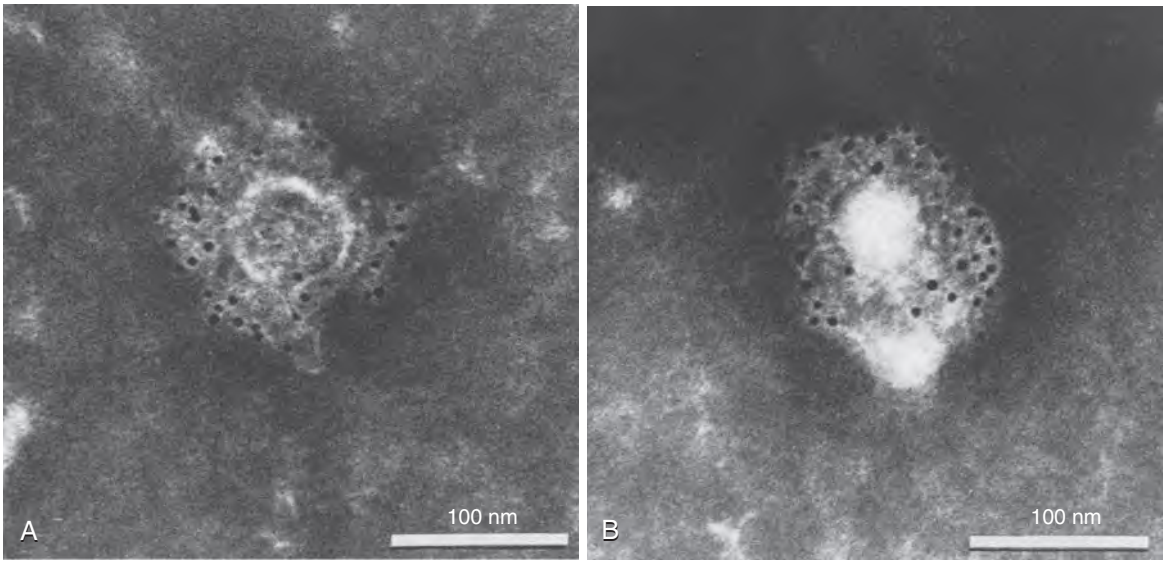

FIG. 154.1 (A and B) Electron microscopic images of hepatitis C virus (HCV) virions concentrated from human plasma by means of high-speed centrifugation. The virions are identified by staining with gold-labeled antibodies to the HCV envelope proteins. *(From Kaito M, Watanabe S, Tsukiyama-Koham K, et al. Hepatitis C virus particle detected by immunoelectron microscopic study.* J Gen Virol. *1994;75:1755–1760.)*

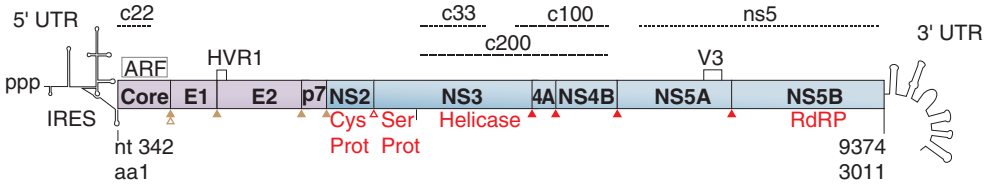

FIG. 154.2 Organization of the hepatitis C virus (HCV) genome and viral polyprotein. The 5′ and 3′ untranslated region *(UTR)* RNA structures *(curves)* flank the major open reading frame (ORF). Unlike eukaryotic mRNA, the viral RNA genome has 5′ triphosphate *(ppp)* and no 3′ polyadenylation. The 5′ UTR contains an internal ribosomal entry site *(IRES). Triangles* indicate sites of cleavage by host cellular signal peptidase (*brown,* open indicating additional processing by signal peptide peptidase), and viral (*red,* open indicating cleavage by the NS2/NS3 cysteine protease) proteases are shown as triangles. Putative structural *(purple)* and nonstructural *(blue)* mature proteins *(boxes)* generated by these cleavage events are labeled, below which in *red* are viral enzymatic functions NS2/NS3 cysteine protease *(Cys Prot),* NS3 serine protease *(Ser Prot),* NS3 helicase, and the NS5B RdRP. Positions of the first and last nucleotides *(nt)* and amino acids *(aa)* of the polyprotein ORF are shown, based on reference genome H77 (GenBank accession number AF009606). Above the ORF are positions of the alternate reading frame protein *(ARF),* variable regions HVR1 and V3, and (indicated by *horizontal lines*) the antigens included in the enzyme immunoassay serologic assay. See text for additional details.

entry site (IRES), directing the cap-independent translation of the viral ORF.[19–24] The HCV IRES and the closely related IRES elements of pestiviruses are unique among eukaryotic RNAs in that they are capable of binding directly to the 40S ribosome subunit in the absence of any protein translation initiation factor.[25–27] The binary complex formed by the 5′UTR RNA and the 40S subunit appears to involve specific macromolecular interactions around the initiator AUG codon of HCV.[28] Thus, HCV initiates translation of its proteins via a unique prokaryotic-like mechanism that may prove to be a useful target for future antiviral drug development. A highly unusual feature of HCV replication that involves the 5′UTR is the complementary binding of liver-specific microRNA 122 (miR-122), an interaction that has been found to enhance translation of HCV RNA, be necessary for HCV replication, prevent degradation of cytoplasmic viral RNA, contribute to the liver tropism of HCV, and represent a therapeutic target.[29,30–33] HCV RNA sequestration of miR-122, through a molecular sponge effect, derepresses endogenous mRNAs normally downregulated by miR-122 including those involved in inflammation and oncogenesis.[34–36] Although demonstrated in vitro, to what extent this occurs in vivo and contributes to fibrogenesis or HCC development clinically is unknown.[34]

The 3′UTR consists of a relatively variable 30- to 60-nucleotide segment downstream of the termination codon that is followed by a highly variable poly-U/UC tract of 50 to 100 nucleotides. Downstream of the poly-U/UC tract there is a highly conserved 98-base sequence (the "3′-X" region).[37–39] This highly structured 3′ terminal 98-base sequence is the most conserved segment of the HCV genome. Experiments in vitro (discussed later) indicate that "kissing loop" interactions between RNA structures in the 3′-X region and NS5B coding region, and 33 consecutive U residues in the poly-U/UC tract, are absolutely required for viral RNA replication.[40–42] The poly-U/UC tract may be the principal pathogen-associated molecular pattern (PAMP) of HCV sensed by the human cytoplasmic pattern recognition receptor (PRR) retinoic acid inducible gene-I (RIG-I, see "Viral Persistence").[43]

Outside of these well-defined untranslated structural regions, data increasingly point to a functional role of RNA structure in other areas of the HCV genome (core, NS4B, and NS5B). These structural elements appear to further enhance viral replication and aid in innate immune evasion and may undergo conformational changes which modulate replication.[44–46]

Polyprotein

The approximately 9-kb ORF encodes a polyprotein that is cotranslationally processed into at least 10 proteins. These include 3 "structural" proteins: the nucleocapsid protein, core (C), and two envelope proteins (E1 and E2); two proteins that are essential for virion production but not required for viral RNA replication (p7 and NS2); and 5 nonstructural proteins that form the viral RNA replicase complex (NS3, NS4A, NS4B, NS5A, and NS5B) (see Fig. 154.2). Processing of the polyprotein is directed by both cellular and viral proteases. Four distinct signal sequences within the amino third of the polyprotein direct the translocation of the nascent protein into the endoplasmic reticulum (ER), with the result being that signal peptidase cleaves the polyprotein at the C/E1, E1/E2, E2/p7, and p7/NS2 junctions. The nascent NS2/NS3 protein is a cysteine protease that cleaves the NS2/NS3 junction, whereas the NS3 protein contains a second (serine) protease activity that catalyzes the remaining in *trans* polyprotein cleavages among the nonstructural proteins. Full expression of NS3 protease activity requires formation of a complex with the NS4A protein.

Structural Proteins

The 191–amino acid segment at the amino terminus of the HCV polyprotein is cleaved from the nascent polypeptide by signal peptidase, forming the highly basic core protein, which has RNA-binding activity.[47–50] A second cleavage occurs just upstream of the signal peptide sequence, directed by signal peptide peptidase within the membranes of the ER, producing a mature core protein of 173 amino acids that is trafficked to lipid droplets, where it associates with NS5A.[51–53] The core protein is immunogenic; both core protein and antibody to it are typically present in the serum of infected individuals.

Many biologic activities have been associated with the core protein, including suppression of HBV replication, alterations in regulation of the cell cycle and transcription of cellular protooncogenes, either induction or suppression of apoptosis, and transformation of rat embryo fibroblasts.[54–64] The core protein has also been suggested to interfere with anti-HCV immune responses through a variety of mechanisms, including NK cell inhibition via upregulation of major histocompatibility complex (MHC) class I expression, inhibition of T-cell proliferation via interaction with complement receptor gC1qR, and interaction with the cytoplasmic tail of several cellular receptors belonging to the tumor necrosis factor (TNF) receptor family.[60,61,63,65,66] However, these data are derived largely from studies in which core has been overexpressed from recombinant cDNA. It is not clear whether core exerts any of these biologic effects when expressed by replicating virus within the liver, and in many cases there is contradictory evidence.

Yellow fever virus and other flaviviruses have a single major envelope protein and a glycosylated, cell-associated NS-1 protein that can elicit neutralizing antibodies. In contrast, hepaciviruses have two major envelope glycoproteins (E1 and E2) and no comparable NS-1 protein. Signal peptidases direct cleavage of the HCV polyprotein at amino-acid residues 383 and 746 (numbering based on the prototype strain H77), producing the E1 and E2 proteins, respectively (see Fig. 154.2).[48] These are secreted into the ER as type 1 transmembrane proteins, fold cooperatively, and remain anchored to the membrane by a hydrophobic carboxyl-terminal anchor sequence (Fig. 154.3). The E1 and E2 proteins are heavily glycosylated, with sugar moieties representing about 50% of the mature mass of each. Although initially associated as a noncovalent heterodimeric complex because of oligomerization determinants in the transmembrane segments, covalently linked complexes have been identified in mature, infectious virions.[67,68] In HCV cell culture, E1 and E2 are localized to the ER compartment, perhaps owing to ER-retention signals located in the transmembrane domain.[52,69–71] This suggests that, like other members of the Flaviviridae, HCV particles assemble and exit the cell by budding into intracytoplasmic vesicles and then follow the secretory pathway for release.

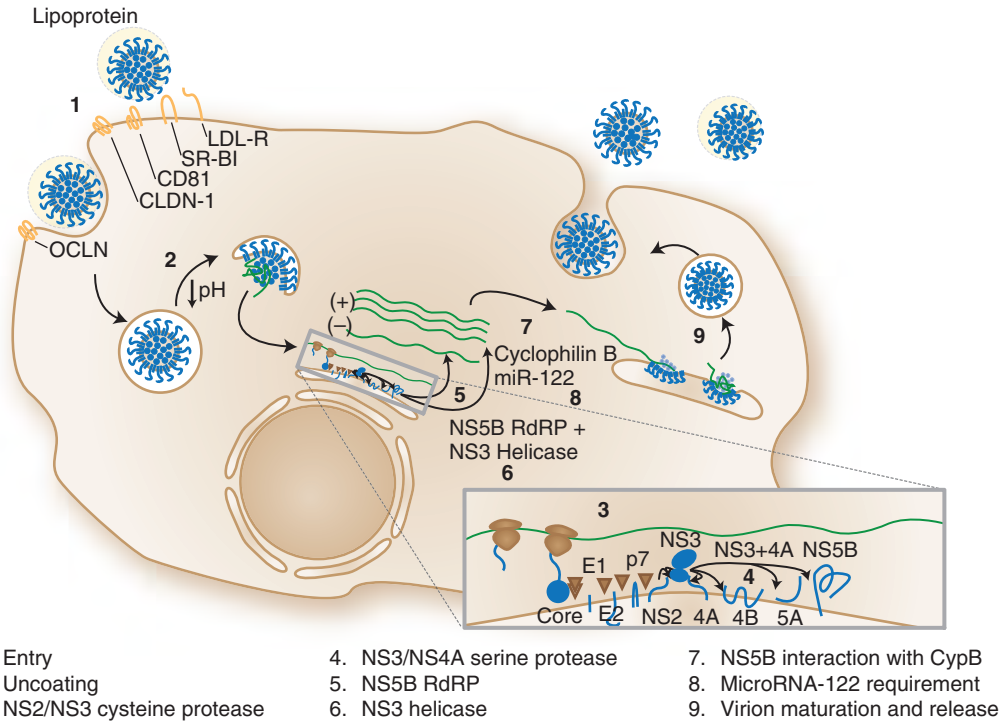

FIG. 154.3 Enhanced understanding of hepatitis C virus replication leads to new therapeutic targets. The life cycle has been labeled with key steps for which therapeutic agents are approved or have been investigated. *(Graphic art by Jocelyn Ray.)*

Detailed structural information regarding the HCV envelope proteins continues to be refined, with partial structures of the E1 and E2 ectodomains now resolved.[72–74] Although further details remain to be elucidated, some domains and putative functions are known. Major functions of the E1/E2 heterodimer include viral attachment through key interactions with cellular receptor and fusion with endosomal membranes. E1 modulates E2 interactions with CD81 and also appears to impact other coreceptor usages such as shifts from claudin-1 to claudin-6 entry dependence.[75,76] Recent data also implicate a role for E1 in recruiting viral genomic RNA during virion morphogenesis.[76] E2 is responsible for primary interactions with viral receptors such as SR-B1 and CD81. It also contains major epitopes for several highly neutralizing antibodies.[77,78] A highly variable segment approximately 30 amino-acid residues in length near the amino terminus of E2 has been called hypervariable region 1 (HVR1).[79–81] HVR1 is the most genetically variable segment of the envelope proteins, and the extent of sequence heterogeneity within HVR1 indicates that there are few sequence-related constraints on its function; however, some constraints have been observed (see "Humoral Immunity").[82–84] It has been suggested that HVR1 may function as an immunologic decoy during infection by masking a deeper, more highly conserved structure within the envelope, such as a recognition site for the cellular receptor.[85] Important to note, deletion of this region reduces but does not eliminate virus infectivity.[86] Downstream of HVR1 are HVR2 and the intergenotypic variable region (igVR), which in spite of their variability may be more directly involved in viral entry.[87] These regions are interspersed among determinants of binding to CD81 and other host molecules that are necessary for viral entry (see "Viral Replication").[88–90]

p7 and NS2 Proteins

The proteins p7 and NS2 are required for viral particle assembly or egress from the cell, but neither is required for viral RNA replication.[91] A signal peptidase cleavage near the carboxyl terminus of E2 generates the p7 protein (see Fig. 154.2). Hexamers of this small 63–amino acid hydrophobic polypeptide form a transmembrane viroporin that is cation selective.[92–95] This activity is essential for production of infectious virions and inhibited in vitro by amantadine and long-alkyl-chain iminosugar derivatives, thereby representing a possible therapeutic target.[91–93,96,97]

The NS2 protein is a membrane-associated dimeric cysteine protease with two composite active sites that mediate cleavage at the NS2/NS3 junction.[98–100] The transmembrane and protease domain structures of NS2 are essential for production of infectious virions in cell culture, whereas the protease activity is not.[91,101] Structural and in vitro analyses suggest that NS2 may serve as a lipid droplet–associated bridge linking the envelope proteins with p7 and NS3.[100,102–104]

Nonstructural Proteins Involved in RNA Replication

Proteins spanning the region within the polyprotein from NS3 to NS5B (see Fig. 154.2) are required for RNA replication and assemble into a membrane-associated replicase complex within the cytoplasm of infected cells. The NS3 protein possesses serine protease activity localized to its amino-terminal third and an RNA helicase with NTPase activity in its carboxyl-terminal domain. The mature, fully active NS3 protease requires the noncovalent association of NS3 with the NS4A protein, which becomes an integral part of the protease structure.[105–108] Atomic-level resolution structures of these domains have been solved separately and together, and these have been exploited for antiviral drug discovery (see "Treatment of Chronic Hepatitis C").[105,109,110] The NS3 serine protease is dependent on zinc and is responsible for the NS3/NS4A *cis* cleavage, and for the NS4A/NS4B, NS4B/NS5A, and NS5A/NS5B cleavages that follow in the processing of the polyprotein; all except the *trans* cleavage sites share a canonical (D/E)xxxxC∧(A/S) recognition motif (with "∧" indicating the site of cleavage). The carboxyl-terminal 465 amino acids of NS3 contain the NTPase and RNA helicase activities, which are likely to direct the unwinding of duplex RNA molecules during genome replication. The NS3 helicase can bind to the 3′ poly-U/UC sequence and has 3′ to 5′ directionality.[111,112] No cleavage site has been identified between the NS3 protease and helicase, and functional studies suggest that these domains are interdependent.[113] In addition, the NS3 helicase domain directly interacts with the core protein during virion production.[114]

NS3 protease activity has been shown to interfere with interferon (IFN)-mediated signaling by blocking the virus-activated phosphorylation of IFN regulatory factor 3 (IRF3) via cleavage of host TIR-domain-containing adapter-inducing IFN-β (TRIF) and mitochondrial antiviral

signaling (MAVS) proteins, providing a mechanism by which HCV might evade innate cellular antiviral defenses (see "Viral Persistence").[115,116] The multifunctional nature of NS3, including polyprotein processing, its role in the RNA replicase, and its contribution to immune evasion, is typical for the proteins of small, positive-stranded RNA viruses such as HCV.

The NS4A protein acts as a cofactor for the NS3 protease, as described earlier. An amino-terminal segment of the protein anchors the NS3/4A complex to intracellular membranes, while NS4A also interacts with NS5A as a critical component of the replicase complex. NS4B is a hydrophobic, membrane-associated protein. It appears to mediate modifications of the ER membranes that occur in association with replicase assembly, and in doing so may also inhibit normal ER-to-Golgi secretory pathways.[117–120]

NS5A is a membrane-anchored cytoplasmic RNA-binding phosphoprotein that appears to play a role in RNA replication, although its exact function remains obscure.[121–123] It contains an amino-terminal amphipathic alpha helical membrane anchor followed by three domains (D1–D3) separated by low-complexity sequences (LCS1 and LCS2). RNA replication depends on RNA binding and dimerization mediated by the region extending from D1 to D2; D3 interacts with core protein on lipid droplets and is essential for viral assembly.[124] Portions of D2 and D3 interact with multiple proteins, including cyclophilin A and a lipid kinase, and each of these functions has been targeted therapeutically (see "Medications").[125–127] Sequence polymorphisms within a short segment of NS5A (the "IFN sensitivity determining region" [ISDR]) have been correlated in some studies with resistance to IFN therapy for some HCV genotypes, and this may be mediated by the interaction of NS5A with the catalytic domain of IFN-induced dsRNA-activated protein kinase R (PKR).[128–131] Inactivation of PKR by NS5A could mitigate both the antiviral and antiproliferative activities of IFN, though this remains unproven. In addition, NS5A may induce expression of interleukin (IL)-8, which antagonizes expression of IFN-stimulated genes.[132]

NS5B is a membrane-bound protein that contains a Gly-Asp-Asp motif characteristic of RNA-dependent RNA polymerases and is considered to be the catalytic core of the replicase complex. As with the enzymatic activities of the NS3 protein, the NS5B RNA polymerase has proven to be a useful target for antiviral drug development with nucleoside and nucleotide analogues and nonnucleoside small molecule inhibitors, and cyclosporine A analogues (see "Medications").

In addition to the polyprotein described earlier, ribosomal frameshifting may generate the ARFP from a short ORF found within the core region of some genotype 1 isolates.[133–135] Some individuals with HCV infection, but not those with HBV monoinfection, have been found to have serum antibody and T-cell reactivity to in vitro synthesized ARFP, suggesting that the protein can be expressed in vivo. The role of this protein remains speculative, and it may be a related RNA element, rather than the ARF protein itself, which is required for infection in vitro and in vivo.[136]

Viral Replication

Details of the viral life cycle were initially examined through use of replication-competent subgenomic RNA replicons in cultured cells, and then more recently with a cell culture system that completes the viral life cycle from cell entry to release of infectious virions (see "Experimental Models"). These studies and reasonable analogies with other positive-strand RNA viruses suggest the following scenario (see Fig. 154.3). The virus enters the cell through interaction with multiple cell surface receptor molecules including CD81, the LDL receptor, NPC1L1, the EGF receptor, DC-SIGN, L-SIGN, human scavenger receptor SR-B1, and the tight junction proteins claudin-1 (CLDN1) and occludin (OCLN).[137–146] Following attachment, penetration, and uptake into a cellular endosome, acidification alters the conformation of the envelope proteins, resulting in fusion with the endosomal membrane. The viral RNA is released into the cytoplasm, where it acts as messenger RNA directing the cap-independent translation of the viral polyprotein.[147,148] Viral translation occurs in association with the rough ER by the process of internal ribosome entry, and the polyprotein undergoes a series of further cotranslational proteolytic cleavages, as described in the preceding section.

The core protein remains within the cytoplasm following cleavage by signal peptide peptidase from the signal sequence at its carboxyl terminus, while E1 and E2 are secreted into the lumen of the ER, remaining attached to the membrane and becoming heavily glycosylated. A replicase complex, composed of NS3, NS4A, NS4B, NS5A, and NS5B, forms cytoplasmic clusters of "membranous webs" derived from the ER associated with lipid droplets.[119,120] This replicase complex recognizes the 3′ end of the genomic RNA and subsequently directs the synthesis of a negative-strand copy of the genome. The resulting duplex RNA most likely serves as a template for the subsequent synthesis of multiple copies of the positive-strand genomic RNA, following recognition of the opposite end of the genome by the replicase. The genomic RNA is packaged into new viral particles, which are likely to be extruded into the ER, leading to the release of the virus via the vesicular secretory pathway.

Although the liver appears to be the primary source of virus present in blood, there are few data that directly support this conjecture. HCV-specific antigens and both negative- and positive-strand HCV RNA have been identified within hepatocytes, indicating that replication does occur in this cell type via a negative-strand intermediate as outlined earlier.[149–151] However, additional data suggest that the virus may also replicate within peripheral mononuclear cells of lymphoid or perhaps bone marrow origin (see next section).[152,153]

Mathematical models of viral kinetics suggest a half-life of approximately 45 minutes for virions in the bloodstream and that up to 10^{12} virions are produced each day in a chronically infected human.[154,155] Compared with human immunodeficiency virus (HIV), the rate of HCV production is 10- to 100-fold higher; however, similar half-lives suggest that the mechanism of clearance could be similar.[155,156]

Genetic Diversity

Quasispecies Variation

The high level of virion turnover, coupled with the absence of proofreading by the NS5B RNA polymerase, results in the rapid generation of viral mutations. Multiple HCV variants can be recovered from the plasma and liver of an infected individual at any time, and this "swarm" of closely related but distinct variants infecting a person is called a "quasispecies."[81,157] During RNA replication, mutations occur in a nearly random fashion throughout the genome, whereas fixation of a substitution within the quasispecies population (evolution) depends on how that substitution influences viral "fitness" as related to its effect on functional protein and RNA structures, the capacity of the virus for replication, and the host-viral interaction.

Strong selective forces such as immunologic responses drive HCV evolution in vivo. For example, spontaneous mutations within the HVR1 segment of the E2 protein may be favored for survival in the host when they reduce the binding of neutralizing antibodies to the viral envelope, whereas they occur slowly or not at all in those who do not generate antienvelope antibodies.[158–163] There is also evidence that cellular immune responses may drive the selection of specific quasispecies variants, although they may also incur a viral "fitness cost" such as reducing the efficiency of a viral enzyme.[84,164–170] Thus, quasispecies variants recovered from blood reflect the balance of production and selective forces.

Although the nucleotide substitutions identified in circulating virus represent only a fraction of all mutations generated during viral replication, these mutations are estimated to occur at an overall rate of 0.9 to 1.92×10^{-3} base substitutions per site per year during chronic infection.[171–173] Variation within an HCV quasispecies instantaneously and over time may be linked to the extent of disease and the duration of infection.[174,175] This is consistent with the hypothesis that immunologic responses affect both the extent of disease progression and the pace of sequence change. Differences in the HCV quasispecies present in the blood and liver have been described, suggesting that differences in tissue tropism may also influence genetic variation, though these studies have suffered from limited sampling.[153,176,177]

The extent of genetic diversity varies markedly throughout the HCV genome, being highest in the segment that encodes the amino terminus of the second envelope protein, E2, within HVR1, and lowest in the core gene and the 5′ and 3′ nontranslated segments of the genome.[178–182] High conservation at some loci suggests functional constraint (mutations

may be lethal or sufficiently disadvantageous to replication to be undetectable among surviving virus populations).

Hepatitis C Virus Genotypes

In addition to the impressive heterogeneity that often exists among HCV sequences present in a single infected individual (i.e., quasispecies variation), there is also remarkable genetic heterogeneity and divergence among HCV sequences recovered from different individuals (i.e., strain and genotype variation). Phylogenetic evaluation of HCV sequences recovered from multiple geographic regions suggests that there are at least seven major genotypes or clades, with provisional identification of an eighth genotype in a specimen originating from the Punjab region of India.[183–186] These genotypes are even more diverse than those causing the worldwide HIV-1 pandemic (Fig. 154.4), in part because of a much longer epidemic history for HCV.[187,188] Depending on the genomic region evaluated, HCV sequences assigned to different genotypes may have less than 50% nucleotide sequence identity. Significantly, this level of nucleotide sequence divergence usually correlates with substantial serotypic differences among other RNA viruses (e.g., poliovirus type 1 vs. poliovirus type 2, which are not cross-neutralizable in assays of antibody-mediated neutralization). The extent of serotypic variation among HCV strains is poorly understood, although some human monoclonal antibodies have broad neutralizing activity.[189–191] Despite extensive study, there is little evidence that HCV genotypes differ in transmissibility or level of replication. However, genotype-specific difference in the rate of progression of liver disease and complications such as HCC are now recognized, with genotype 3 in particular being associated with a more aggressive clinical phenotype (see "Natural History and Pathogenesis").[192–194] Genotype differences were also recognized in response to IFN-based treatments and early direct-acting antiviral (DAA) regimens. The arrival of highly potent pangenotypic regimens has negated much of the impact of genotype on response (see "Medications").

Within individual HCV genotypes, strains can be further grouped into subgenotypes (subtypes) that generally share 75% to 85% nucleotide sequence identity within the core-E1 and NS5B regions of the genome.[183,195] Currently, 86 confirmed subtypes have been described, with genotype 6 followed by genotype 4 having the most confirmed subtypes (https://talk.ictvonline.org/ictv_wikis/flaviviridae/w/sg_flavi/56/hcv-classification).[185] In contrast, the quasispecies variants that exist within a single person generally have 91% to 99% identity in these regions.[196] The phylogenetic grouping of HCV strains is largely independent of the segment of the genome that is analyzed (i.e., in contrast to HIV, recombination is rarely observed for HCV).[183,197–199]

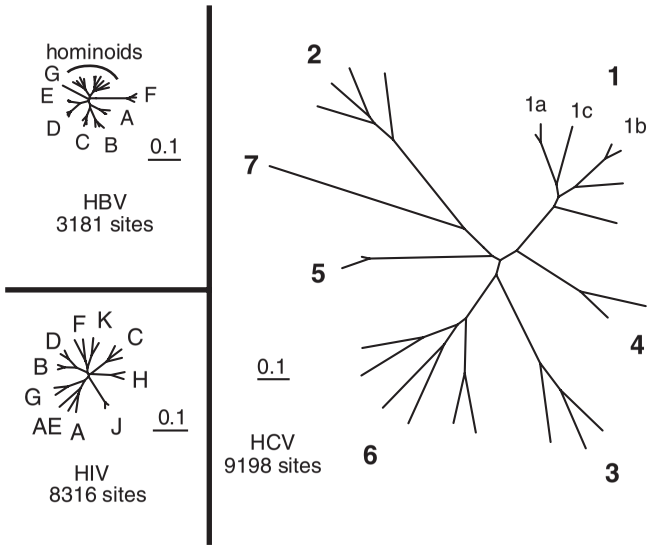

FIG. 154.4 Phylogenetic trees for hepatitis B virus *(HBV)*, human immunodeficiency virus *(HIV)*, and hepatitis C virus *(HCV)* shown to the same scale in terms of nucleotide genetic distance, based on full-length genome nucleotide sequences. Sequences of representative strains for major genotypes were obtained from GenBank and aligned using ClustalX with minor manual adjustment, and then sites containing gaps were removed, resulting in an alignment of 3181 sites for HBV, 8316 sites for HIV, and 9198 sites for HCV. Maximum likelihood trees were inferred using PAUP* version 4b10, with the model (GTR+I+G in all three cases) and parameters selected by ModelTest 3.7 using the AIC criterion.

Globally, HCV recombination is a rare event, with eight of the nine identified recombinants limited to a single isolate. The notable exception is the HCV 2k/1b recombinant virus, which has been well characterized and is increasingly recognized in parts of Eastern Europe, Georgia, and Russia.[185] The 2k/1b recombinant variant was first recognized in 2002 in samples from St. Petersberg.[200] Initial epidemiologic studies suggested a low prevalence of this recombinant (1%–2%) largely confined to the former Soviet Union; however, more detailed contemporary analyses have found the 2k/1b recombinant in up to 20% of HCV-infected persons in the country of Georgia.[201,202] Identification is complicated by the fact that line probe assays that rely on NS regions for genotyping (5′UTR with or without core) will misidentify this recombinant as genotype 2.[203,204] Detailed phylogenetic analyses from DAA treatment trials have indicated an increased spread, including isolation from patients in the Netherlands and the United States.[203] Patients harboring the 2k/1b virus respond to DAA therapy in a fashion similar to those with genotype 1 infection, with lower responses to sofosbuvir plus ribavirin therapy.[203,205] As pangenotypic therapies are more widely used, this distinction will be of less clinical importance.

Our understanding of the geographic distribution of HCV genotypes has improved significantly with the increased availability of sequencing technologies aided by detailed clinical virology studies accompanying DAA therapeutic trials. However, data are still biased by the large amount of sequence data from North American and Western Europe compared to the rest of the world. Genotype 1 is the most widely dispersed worldwide accounting for 44% of infections with subtype 1b predominating globally.[206] In the United States, roughly 70% of infections are genotype 1 with subtype 1a predominating (~60% of genotype 1 sequences) (Fig. 154.5).[207–209] Genotype 2 is widely dispersed but is most diverse (suggesting the location of its origin) in central and west Africa.[210] Genotype 3 is the second most prevalent globally (25%) and is most diverse in Asia but has been linked in other geographic regions, including North America, to illicit drug use.[206,211–213] Genotype 4 infections are most prevalent in northern Africa and the Middle East.[214,215] Types 5 and 6 have been reported in South Africa and Southeast Asia, respectively.[216–218] The four isolates of genotype 7 all originated from the Democratic Republic of Congo.[219] Differences in genotype nomenclature may cause some confusion in interpreting older studies; however, there is general acceptance of a revised nomenclature.[14,185,220]

Viral Tropism

HCV replicates within the hepatocyte, and the liver-specific expression of miR-122 may contribute to this specificity. Differential expression of receptors may also contribute to the hepatotropic nature of HCV, with LDL-r (nonspecific) and SR-B1 (specific) contributing to liver tropism. However, replication may also occur in other cell types. Some studies have suggested the presence of negative-strand (replicative intermediate) HCV RNA in T cells, B cells, and monocytes, especially in patients with chronic infection.[221–223] Others have suggested that this occurs rarely,[152] or not at all,[224,225] but, as mentioned earlier, differences in dominant quasispecies populations in these various compartments are supportive of extrahepatic infection. Substantial evidence also supports the ability of HCV to replicate at low levels in cultured human cells of T- and B-cell origin.[226]

HCV RNA also has been detected in cutaneous lesions of persons with HCV-related cryoglobulinemia and vasculitis,[227] in renal biopsy specimens of patients with HCV-associated membranoproliferative glomerulonephritis,[228] and (with variable success) in various body fluids including saliva, semen, tears, urine, cerebrospinal fluid, and ascitic fluid.[229–233] Unlike HBV, HCV does not replicate through a DNA intermediate and does not have the ability to integrate its genetic information into chromosomal DNA. Because of this, the detection of HCV sequences in these tissues and fluids may indicate the presence of infectious virus, although transmission via these fluids appears to be rare (see "Incidence and Transmission of Hepatitis C Virus").