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Yellow Fever: A Reemerging Threat

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Abstract

Yellow fever (YF) is a viral disease, endemic to tropical regions of Africa and the Americas. YF principally affects humans and nonhuman primates, and is transmitted via the bite of infected mosquitoes. The agent of YF, yellow fever virus (YFV), can cause devastating epidemics of potentially fatal, hemorrhagic disease. We rely on mass vaccination campaigns to prevent and control these outbreaks. However, the risk of major YF epidemics, especially in densely populated, poor urban settings, both in Africa and South America, has greatly increased due to: (1) reinvasion of urban settings by the mosquito vector of YF, *Aedes aegypti*; (2) rapid urbanization, particularly in parts of Africa, with populations shifting from rural to predominantly urban; and (3) waning immunization coverage. Consequently, YF is considered an emerging, or reemerging disease of considerable importance.

Keywords

Yellow fever virus; Mosquito-borne; Transmission; Vaccine; Emerging disease

A BRIEF HISTORY OF YELLOW FEVER RESEARCH

YF originated in Africa, and was imported into Europe and the Americas as a consequence of the slave trade between these continents.¹ In the Western Hemisphere, the first recorded epidemic of disease believed to have been YF occurred in the Yucatan in 1648.² Throughout the eighteenth and nineteenth centuries explosive YF outbreaks ravaged tropical South and Central America, as well as port cities on the eastern seaboard of North America and in Europe.³ Although it was realized early on that the disease was not contagious, the source was wrongly attributed to environmental miasmas. It was Carlos Finlay, a Cuban scientist, who first determined in the late 1800s that mosquitoes were responsible for disseminating the disease. Dispatched to Cuba by the United States government to investigate the cause of YF, Walter Reed and colleagues confirmed that the primary mode of YF transmission to humans was the *Aedes aegypti* mosquito (Fig. 1) and, in ground-breaking virologic studies, demonstrated that the disease was caused by an agent that could be filtered from the blood of infected individuals.⁴ Campaigns to eradicate *Ae aegypti*, the so-called yellow fever mosquito, from Cuba and Panama were highly successful in eliminating urban YF cases. Unfortunately, the goal of YF eradication was thwarted by YF being a zoonotic disease,

maintained by sylvatic mosquito species and nonhuman primates in the Amazon jungle. This aspect is discussed further in the Epidemiology section.

The causative agent of YF disease, YFV, was first isolated in 1927 from a Ghanaian patient named Asibi,⁴ and the Asibi YFV strain is still widely used by scientists today. In the 1930s, Max Theiler and colleagues⁵⁻⁷ produced a live-attenuated vaccine strain, designated 17D, which was attenuated for viscerotropic disease in monkeys and humans, but remained immunogenic. The YF vaccine used today derives from the original 17D strain, and Theiler was awarded the Nobel Prize for his life-saving research in 1951. Almost concurrently a second live-attenuated vaccine was developed from a YFV strain isolated in Senegal, in 1927. This vaccine was widely used from the 1940s to the 1960s in French-speaking African countries, virtually eradicating the disease, until its use was discontinued in 1980.

MICROBIOLOGY OF YELLOW FEVER VIRUS

YFV is the prototypic member of the genus *Flavivirus*, family *Flaviviridae*; *flavus* being Latin for yellow. The 3 genera in this family contain a large number of major human and veterinary pathogens,⁸ including dengue (DENV), Japanese encephalitis (JEV) and West Nile (WNV) viruses in the *Flavivirus* genus, bovine viral diarrhea (BVDV) and classic swine fever (CSFV) viruses in the *Pestivirus* genus, and hepatitis C virus (HCV) in the *Hepacivirus* genus. Here, descriptions of YFV structure and replication are extrapolated from studies with this and other *Flavivirus* genus members.

Virion Structure

Mature YFV virions are icosahedral and comprise a nucleocapsid, composed of capsid (C) protein subunits, surrounded by a lipid bilayer derived from host membranes. The viral envelope is studded with dimers of envelope (E) glycoprotein and membrane (M) protein (Fig. 2). The diameter of the virion is approximately 40 nm, with surface projections of 5 to 10 nm. The E glycoprotein is the major component of the virion surface⁹ and possesses most of the biologic activity, including cell-surface receptor binding, virion assembly and fusion activity at low pH, and immunogenicity.¹⁰

The genome packaged inside the YFV virion is a single-stranded, positive-polarity, infectious RNA molecule, approximately 11 kb in length¹¹ (Fig. 3). Similar to host messenger RNAs (mRNAs), the genome possesses a cap structure at the 5' terminus but, unlike most host mRNAs, it lacks 3' terminal polyadenylation.¹¹ Instead, the 3'-terminal nucleotides form a very stable, highly conserved stem-loop structure, serving to stabilize the genome and provide signals for initiation of translation and RNA synthesis,¹² discussed further below. All of the viral proteins are encoded in a single open reading frame (ORF), produced as a polyprotein and processed by proteolytic cleavage.^{9,11} The structural proteins (C, M, and E) that form the virion are encoded in the 5'-quarter of the genome, while the nonstructural (NS) proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) that form the viral replicase are encoded in the remaining three-quarters.^{9,11}

Virus Replication

At the molecular level, YFV and other flaviviruses appear simple, producing only 10 proteins in the infected cell (see Fig. 3). However, it is increasingly evident that the interaction between virus and cell is extremely complex, as the virus has adapted to exploit the host's machinery for macromolecular synthesis for its own propagation and to antagonize or circumvent antiviral responses.¹³ These pathogens modulate pattern recognition receptors, stress granules, and membranous structures to promote crucial steps in their life cycle.¹³ By elucidating these processes at the molecular level, scientists hope to identify points of vulnerability in the virus amplification cycle that can be targeted with antiviral drugs or used in the design of vaccine candidates.

Extracellular flavivirus particles bind to target cells by interaction with cell-surface receptors which have yet to be identified, and are internalized by receptor-mediated endocytosis. A conformational rearrangement of the E glycoprotein occurs in the lower pH environment of the endosome, which facilitates fusion of the viral lipid envelope with the endosomal membrane and release of the nucleocapsid into the cell's cytoplasm.^{14,15} After the nucleocapsid disassembles, replication proceeds with the immediate translation of the genome. Two short, conserved repeats (CS1 and CS2) are found 5' to the 3' putative secondary structure sequence. It has been postulated that base-pairing of these terminal sequences circularizes the genome to facilitate genome translation, replication, or packaging.¹⁶

Cap-dependent translation of the long ORF initiates at an AUG codon near the 5' end of the genome,⁹ for which the virus presumably borrows host eukaryotic translation initiation factors (eIFs) such as components of the eIF4F complex, membrane-bound ribosomes, and various other proteins (Fig. 4, step 1). Of note, flaviviruses may also use a novel, cap-independent translation mechanism under certain circumstances,¹⁷ perhaps to better compete with the translation of host mRNAs. Co- and posttranslational processing of the polyprotein into individual mature proteins involves tightly regulated, sequential cleavages mediated by proteases of both host and viral origin (see Fig. 4, step 2).^{9,13,18}

The NS proteins include the large, highly conserved proteins NS1, NS3, NS5, and the 4 small, hydrophobic proteins NS2A, NS2B, NS4A, and NS4B.¹¹ Current knowledge of NS protein functions is summarized here.

- The NS1 glycoprotein is an unusual protein that seems to be integral to virulence and pathogenesis. This protein can be cell associated,¹⁹ on the cell surface,^{19,20} and extracellular,²¹⁻²³ and plays a role in pathogenesis by inhibiting complement cascade activity.²⁴ On the other hand, NS1-specific antibodies provide protective immunity^{20,25,26} via Fc receptor-dependent and independent mechanisms.^{27,28}
- NS3 is an enzyme with central importance in the flavivirus life cycle. The N-terminal serine protease functions with its essential cofactor NS2B in the processing of the polyprotein, while the C-terminal NTPase/helicase performs ATP-dependent RNA strand separation during replication.²⁹⁻³⁷

- The NS5 protein has 2 distinct enzymatic activities, separated by an interdomain region: the *S*-adenosyl methyltransferase is located in the N-terminus and responsible for capping the nascent RNA,³⁸⁻⁴⁰ while the RNA-dependent RNA polymerase (RdRp) responsible for replicating the viral RNA genome is found in the C-terminus.⁴¹⁻⁴³
- The NS2A, NS2B, NS4A, and NS4B polypeptides consist mainly of multiple hydrophobic, potential membrane-spanning domains. These membrane-associated proteins are believed to participate in the assembly of viral replication complexes by localization of NS3 and NS5 to membranes via protein-protein interactions.¹⁸

The newly translated and processed viral NS proteins associate to form the replicase. The replicase recognizes secondary structure in the 3' terminus of the genomic RNA and the NS5 RdRp initiates the synthesis of full-length negative-sense RNA copies from the genome template (see Fig. 4, step 3). These negative-sense RNAs are rapidly transcribed to produce progeny positive-sense RNA genomes (see Fig. 4, step 4). RNA replication is asymmetric with positive-strand synthesis considerably more efficient than negative-strand synthesis, probably because the different stem-loop structures present at the 3'-terminal ends of the positive and negative strands may affect the efficiency of initiation of a replication complex.¹⁶

Flavivirus replication occurs associated with host cell membranes.⁴⁴ Infection causes dramatic proliferation of spherical invaginations known as vesicle packets (VP) in the perinuclear region of the endoplasmic reticulum (ER),⁴⁵⁻⁴⁸ at least in part through activity of the NS4A protein.^{44,49} The localization of several viral NS proteins and dsRNA replicative intermediates in VPs^{48,50} suggests they are the site of viral replication. Sequestration of the viral factory within these membranous structures may serve to concentrate viral and host components and improve the efficiency of replication, to anchor the viral replication complex, and to conceal the viral RNA replicative intermediates from host cell surveillance mechanisms.⁵¹ The potential importance of this immune evasion strategy to flavivirus pathogenesis remains to be elucidated.

Depending on the virus strain and cell type, the synthesis of YFV RNA is detectable within 3 to 6 hours after infection, and progeny virions are released by about 12 hours. Immature, noninfectious virions assemble within the ER, where viral RNA complexes with the C protein and is packaged into an ER-derived lipid bilayer containing heterodimers of the prM and E proteins,^{52,53} indicating that budding through the host cell membrane occurs intracellularly. Subcellular transport of immature flavivirus particles to the cell surface is thought to occur by the translocation of immature virion-containing vesicles from membranous components of the cell to the plasma membrane. Fusion of these vesicles with the plasma membrane then releases the vesicle contents including virions into the extracellular environment.⁵⁴ During assembly and transport of immature virions, the precursor to the M protein (prM) protects E proteins from undergoing irreversible conformational changes in acidic compartments of the secretory pathway.⁵⁵ Virion maturation occurs in the trans-Golgi network by a delayed furin-mediated cleavage of the prM to M^{56,57} triggering rearrangements in the E protein that promote infectivity.⁹

Infectious, mature virus particles are released by exocytosis into the extracellular medium (see Fig. 4, step 5).

EPIDEMIOLOGY OF YELLOW FEVER

Central to the epidemiology of YFV is the requirement for mosquito-borne transmission to primate hosts. The majority of flaviviruses are transmitted to man (and other vertebrates) by the bite of an arthropod vector, primarily mosquitoes and ticks. Despite the presence of virus in the blood and bodily secretions during acute infection, flavivirus infections are not contagious (ie, transmitted directly from human to human). Consequently, available reservoirs of infectious virus and high levels of vector populations are prerequisites for epidemic outbreaks. In addition, vertical or transovarial transmission of virus in mosquitoes is important in the maintenance of YFV.^{58,59}

Transmission Cycles of Yellow Fever Virus

YFV infects humans and nonhuman primates in tropical areas of Africa and the Americas, transmitted between primate hosts in the saliva of infected mosquitoes. The natural epidemiology of YF on both continents is a cycling of the virus between forest mosquitoes and wild primates. Secondary transmission to humans occurs in 3 cycles: sylvatic, intermediate, and urban (Fig. 5).⁶⁰ The *sylvatic (jungle) YF transmission cycle* occurs in the tropical rainforests of Africa and South America, where the virus is endemically transmitted between several monkey species and mosquitoes inhabiting the forest canopy. Occasionally, the virus is “accidentally” transmitted to humans who enter these areas, causing sporadic cases, usually in male forestry workers. The *intermediate YF transmission cycle* results in small-scale epidemics in rural villages of the African savannah when infected mosquitoes of semidomestic species indiscriminately feed on both monkey and human hosts. This type of outbreak has been the most common in Africa in recent decades.

The major cause for concern comes from the *urban YF transmission cycle*, initiated when virus is introduced into areas with high human population density and mosquitoes transmit YFV from human to human, resulting in explosive, large-scale epidemics. In the urban cycle, humans are the primary host and the urban mosquito species, *Ae aegypti*, is the vector. Urban YF occurs indiscriminately among naïve humans, spread either by the movement of viremic human hosts or by the accidental transportation of infected mosquitoes (eg, in used tires). Any outbreak in which *Ae aegypti* is the vector is classified as urban YF by the World Health Organization (WHO), regardless of the area, while outbreaks involving other mosquito species are classified as jungle YF.⁶¹ On the edge of the jungle the cycles intermingle, and infected workers returning from jungle areas often form the focus of an urban outbreak.

Epidemiology of Yellow Fever Today

Historically, devastating urban YF epidemics have occurred in Europe, Africa, and South, Central, and North America.⁶² In the absence of a sylvatic cycle, improved sanitation and mosquito abatement programs eliminated epidemic urban YF from North American and European cities, with the last outbreak occurring in New Orleans in 1905.³ However, it is

estimated that YF continues to affect over 200,000 persons annually in tropical regions of Africa, South America, and Central America, with at least 30,000 fatalities.⁶³ Forty-four countries in Africa (Fig. 6A) and South and Central America (see Fig. 6B) are within the modern YF endemic zone, with almost 900 million people at risk of infection.⁶⁴ This total includes an estimated 508 million people in 32 African countries, and the remainder in 12 South and Central American countries (Table 1).⁶⁵ Most YF cases occur in sub-Saharan Africa, including periodic, unpredictable outbreaks of urban YF. An alarming resurgence of virus circulation and expansion of the endemic zones have been detected in Africa^{66,67} and South America⁶⁸ in recent years, setting the scene for an explosion of urban epidemics.⁶⁹ The current situation in Africa, the Americas, and worldwide is described in more detail in this section.

Yellow fever in Africa—In Africa, YFV is endemic in many nonhuman primate species, causing a subclinical infection and a balanced relationship between the host and the virus. In the African sylvatic cycle, the virus is most frequently vectored by the *Aedes africanus* mosquito. Because *Ae africanus* is a night feeder, preferentially takes blood meals from monkeys, and rarely bites man, cases of true jungle YF in humans are uncommon in Africa. Infection of man with the forest virus typically occurs through the semidomesticated mosquito species *Aedes simpsonii*, known as a “bridging vector” because it feeds indiscriminately on nonhuman primates and humans. Although this appears to be a minor distinction, it has important epidemiologic implications. Exposure to infection results from the proximity of man’s habitation to the forest and consequently all members of the household are equally exposed.

Due to mass vaccination campaigns in the 1940s and efforts to remove *Ae aegypti* breeding sites, urban YF was dramatically controlled in Africa, particularly in French-speaking West African countries where vaccination with the French neurotropic vaccine (FNV) was made compulsory.⁷⁰ By the 1960s, however, in areas where vaccine coverage had waned or was absent, thousands of YF cases occurred in West Africa and in Ethiopia, where the disease had not previously been reported. A further expansion in the late 1980s produced approximately 120,000 cases with 20% fatality in Nigeria alone.⁷¹ These expansions were rooted in an amplification of the enzootic transmission cycle, exacerbated by poor or nonexistent vaccine coverage.

With each passing year, the risk of explosive urban YF epidemics in African cities becomes greater. The use of FNV was discontinued in the 1980s due to the risk of postvaccinal encephalitis, and current vaccination coverage with the 17D vaccine is inadequate. Another major contributing factor is the extremely rapid urbanization occurring in Africa, with cities becoming larger and more numerous and urban populations increasing by 4% annually. The effect of this rural to urban population shift is to crowd naïve populations into areas with poor housing, inadequate sanitation, and little access to running water. Drinking water is often stored near dwellings in large open containers; the preferred breeding sites of *Ae aegypti* mosquitoes.

The last decade has seen an increase in the number of African countries reporting YF activity to the WHO, particularly in West Africa; this reveals a disconcerting increase in the

circulation of the virus in nonimmune human populations and reemergence in geographic areas that have long been free of YF. As a result, several large cities have already experienced YF outbreaks in the past few years: Abidjan, Côte d'Ivoire (2001); Dakar, Senegal (2002); Touba, Senegal (2002); Conakry, Guinea (2002); and Bobo Dioulasso, Burkina Faso (2004). The outbreaks were rapidly controlled by emergency reactive vaccination campaigns involving the administration of millions of vaccine doses to at-risk populations, and the number of YF cases has remained low. For example, in the 2001 outbreak in Abidjan, 2.61 million people were vaccinated in a 12-day period. However, multiple outbreaks occurring simultaneously in different locations tremendously stress the response capacity of affected countries, as well as the support capabilities of the international community, and significantly deplete vaccine stockpiles.

Yellow fever in the Americas—Historically, urban YF was a major endemic and epidemic disease terrorizing cities in South and Central America and the southeastern United States. Similar to the situation in Africa, by the 1940s mosquito abatement programs to control and eradicate the domestic vector, *Ae aegypti*, and mass vaccination campaigns resulted in the disappearance of urban YF. However, it was clearly impossible to eradicate the jungle *Haemagogus* spp mosquito vectors or the nonhuman primate reservoir hosts and thus jungle YF continues to occur, primarily afflicting young male forestry and agricultural workers in the Orinoco and Amazon river basins. Probably reflecting the more recent introduction of YFV into the Americas, several nonhuman primates that are effective viremic hosts succumb to fatal disease. Large die-offs of these animals in the forests signal local YF activity and are helpful to surveillance efforts.⁷²

In the last few years, the Pan-American Health Organization (PAHO) reports intensely increased circulation of jungle YF in the Americas, affecting Argentina, Paraguay, and Brazil in the southern part of the continent, Colombia and Venezuela in the Andean region, and Trinidad and Tobago in the Caribbean. It is disturbing that *Ae aegypti* mosquitoes have reinfested most major urban centers in Central and South America, including cities that were historically centers of urban YF and are now inhabited by large nonimmune populations. Immunization coverage has dropped, placing these areas at greater risk of urban epidemics today than at any time in the past 50 years. The last documented urban YF epidemic in the Americas occurred in 1928 in Rio de Janeiro, Brazil.⁶⁸ Although there have been reports of sporadic cases in residents of urban areas in Brazil (1942), Trinidad (1954), and Bolivia (1999), verification of a true urban YF cycle in which humans serve as the primary host and virus is transmitted by *Ae aegypti* is controversial.⁷² Most recently, after a 34-year absence, YF returned to Paraguay in 2008, causing a cluster of possible urban YF cases in Asuncion.⁶⁵ Two million vaccine doses were urgently requested from the global stockpile (already sorely depleted by African outbreaks in the same year) and the outbreak was successfully contained by mass vaccination amidst public panic.

Yellow fever in travelers—YF poses a significant threat to unvaccinated travelers in the YF endemic zone.⁷³ The traveler's risk of YFV infection is dependent on immunization status, travel destination, season, length of visit, and activities. The ease of international travel also makes the introduction and spread of YF into new areas infested with competent

Ae aegypti vectors possible, theoretically placing parts of Asia, Australia, Europe, and North America at risk. During recent decades there have been several documented cases of the human importation of YFV to nonendemic areas. Since 1964, a total of 9 YF cases have been documented in European and North American tourists after returning home from visits to West Africa and South America,⁶⁶ but secondary transmission has not yet occurred. It is not certain why YFV has not spread to new regions infested with *Ae aegypti*, in particular Asia, but clearly the traditional geographic barriers to YF are breaking down. Although YF outbreaks in developed countries will probably be identified and controlled quickly, the impact on public emotion and the medical system would be significant.

Coping with the threat of future urban yellow fever epidemics—The strategies for YF control are routine infant immunization for children aged 6 months or older, mass vaccination campaigns to prevent epidemics, outbreak detection and rapid response, and control of *Ae aegypti* in urban centers. In 1988, the WHO recommended that vaccination against YF be included in routine infant immunization programs. As of 2009, 22 African countries and 14 South American countries have done so.

The Yellow Fever Initiative was launched in 2006 to procure the resources to confront the challenge presented by YF worldwide which, if not addressed, might result in large-scale urban epidemics, affecting millions of people. The Initiative assesses risk of YF outbreaks at the district level based on location in an ecological risk area, notification of suspected or confirmed cases since 1960 in this or an adjacent district, the number of these cases and the number of years since 1960 in which they have been reported, and the susceptibility of the population (ie, the proportion not covered by vaccination). During the first stage of the initiative in 2007, 12 African countries with large nonimmune populations are considered to be at high risk, and immunization efforts are being intensified. Vaccine coverage rates are high in South America and a few African countries, and are increasing in many others.

CLINICAL PRESENTATION OF YELLOW FEVER INFECTION

Described as “the original viral hemorrhagic fever (VHF),” severe YF is a pansystemic viral sepsis with viremia, fever, prostration, hepatic, renal, and myocardial injury, hemorrhage, shock, and 20% to 50% lethality.⁷⁴ However, human YF varies from an almost inapparent, abortive infection in which symptoms abate rapidly after the first phase to an invariably fatal, fulminating disease with symptoms following a biphasic course.¹⁰ YF shares clinical features with other VHFs such as Dengue hemorrhagic fever, Lassa fever, and Crimean-Congo hemorrhagic fever.

YFV is introduced subcutaneously into the primate host by injection of the saliva of an infected mosquito (Fig. 7). A 3- to 6-day incubation period is followed by the abrupt onset of symptoms. In mild, abortive YF cases, symptoms of infection are typically nonspecific, manifested as fever, headache and constitutional problems. In such cases, patients recover in a few days with no lasting sequelae. In more severe cases, patients experience fever, chills, malaise, headache, lower back pain, generalized myalgia, nausea, and dizziness, often manifesting Faget’s sign (increasing temperature with decreasing pulse rate). During this so-called period of infection, which lasts several days, viremic titers are sufficiently high for

transmission to biting mosquitoes. This stage is often followed by a “period of remission,” with rapid abatement of fever and other symptoms lasting up to 24 hours, and clearance of virus from the circulation. At this point, many YF infections resolve without further symptoms.

In approximately 20% of patients, illness reappears in a more severe form, the “period of intoxication,” with high fever, vomiting, epigastric pain, prostration, and dehydration. Hepatic-induced coagulopathy produces severe hemorrhagic manifestations including petechiae, ecchymoses, epistaxis (bleeding of the gums), and the characteristic “black vomit” (hematemesis; gastrointestinal hemorrhage). YF is distinguished from other VHFs by the characteristic severity of liver damage and appearance of jaundice (hence *flavus*; Latin for yellow). Moreover, damage to the kidneys frequently leads to extreme albuminuria and acute renal failure. Antibodies can be detected at this stage, while viremia is usually absent. Late central nervous system (CNS) manifestations, such as confusion, seizure, and coma, presage death, which typically follows within 7 to 10 days of onset.

Pathology of Human Yellow Fever Virus Infection

Gross pathology of fatal human YF reveals that the kidneys are generally grossly enlarged, congested, and edematous. The heart is also often enlarged. The liver, the characteristic organ of YF infection, is normal or slightly enlarged in size and icteric, with lobular markings obliterated.⁷⁵ Microscopic pathologic changes in the liver include swelling and necrosis of hepatocytes in the midzone of the liver lobule, with sparing of cells in the portal area and surrounding the central veins. The presence of Councilman bodies, coincident with disarray of the midzonal hepatocyte plate and microvesicular lipid accumulation, are considered to be hallmarks of fatal human YF infection.^{10,76} Viral antigen and RNA are demonstrable by immunocytochemistry and nucleic acid hybridization in cells undergoing these pathologic changes, and cytopathology appears to be mediated by direct viral injury^{10,76} via apoptosis.⁷⁷ Inflammatory changes, remarkably, are absent or minimal, and patients with hepatitis who recover do not develop residual scarring or cirrhosis.

The kidneys show acute tubular necrosis, probably the result of reduced perfusion of blood rather than direct viral injury. Focal degeneration of muscle cells may be present in the heart.⁷⁶ Spleen and lymph nodes show necrosis of B-cell areas.⁷⁵ The brain shows edema and petechial hemorrhages, but viral invasion and encephalitis are very rare events. Hemorrhage results principally from decreased synthesis of clotting factors by the liver and consequent disseminated intravascular coagulation (DIC). During acute YF, hemorrhagic symptoms and fatal outcome are strongly correlated with highly elevated pro- and anti-inflammatory cytokines,⁷⁸ suggesting a contribution to disease. Although the source of the cytokines is not known, hepatocytes,⁷⁷ endothelial cells,⁷⁹ or activated macrophages⁸⁰ may contribute. Furthermore, immune clearance attempts may exacerbate viral pathogenesis.

DIAGNOSIS OF YELLOW FEVER VIRUS INFECTION

YF surveillance is critical for the monitoring of the incidence of disease and to allow the prediction and early detection of outbreaks, and the monitoring of control measures and case reporting is required by International Health Regulations. The prompt detection of YF and

rapid response through emergency vaccination campaigns are essential for the control of outbreaks. However, underreporting is a tremendous concern and the true number of cases is estimated to be up to 250 times what is now being reported. One confirmed case of YF in an unvaccinated population should be considered an outbreak, and a confirmed case in any context must be fully investigated, particularly in any area where most of the population has been vaccinated. Investigation teams must assess and respond to the outbreak with both emergency measures and longer-term immunization plans. The WHO recommends that every at-risk country have at least one national laboratory where basic YF blood tests can be performed.

Clinical Diagnosis of Yellow Fever

YF should be suspected in patients in endemic areas (or with recent travel to endemic areas) who present with a sudden fever, relative bradycardia, and signs of jaundice (Table 2). Complete blood count, urinalysis, liver function tests, coagulation tests, viral blood culture, and serologic tests should be obtained. Leukopenia with a relative neutropenia, thrombocytopenia, prolonged clotting, and increased prothrombin time are common. Bilirubin and aminotransferase levels may be elevated acutely and for several months. Albuminuria occurs in 90% of patients and aids in differentiating YF from other forms of hepatitis.

Clinical diagnosis of YF in the field, particularly diagnosis of isolated cases, remains difficult for several reasons. Case-by-case differences in severity, and in the clusters of symptoms observed, make this a difficult disease to recognize and mild disease often escapes diagnosis. Although classic cases should be easily recognized, jaundice is more often absent than present, and YF may not be included in the differential diagnosis of patients presenting symptoms of headache, nausea, backache, and fever, especially during the early stages of the infection. YF is easily confused with Dengue fever, Lassa fever, Ebola fever, malaria, typhoid, hepatitis, and other diseases, as well as poisoning (Table 3).

Laboratory Diagnosis of Yellow Fever

Laboratory confirmation of YF is pivotal to diagnosis, but unfortunately requires highly trained laboratory staff with access to specialized equipment and materials (Table 4). Laboratory criteria for diagnosis are any one of the following: (1) the presence of YFV-specific IgM or a fourfold or greater increase in IgG levels between acute and convalescent sera in the absence of recent vaccination; (2) isolation of YFV; (3) positive postmortem liver histopathology; (4) detection of YFV antigen in tissues by immunohistochemistry; or (5) detection of YFV genomic sequences in blood or organs by polymerase chain reaction. Frequently YF is not diagnosed until the patient has either recovered or succumbed, if a diagnosis is ever made. Case definitions state that a suspected case is characterized by acute onset of fever followed by jaundice within 2 weeks of the onset of the first symptoms, while a confirmed YF case additionally requires laboratory confirmation or an epidemiologic link to a laboratory-confirmed case or outbreak.

TREATMENT OF YELLOW FEVER VIRUS INFECTION

There is no specific treatment for YF infection and consequently supportive care is critical. Of course, most patients with YF are unable to benefit from state-of-the-art medical care.⁸¹ Ideally a severely ill patient is admitted to the intensive care unit (ICU) and provided with vasoactive medications, fluid resuscitation, and ventilator support. Symptoms including DIC, hemorrhage, renal and hepatic dysfunction, and possible secondary infection are treated. The use of salicylates is contraindicated in YF cases because of the increased risk of bleeding. Although YFV cannot be transmitted person to person, viremic patients should be isolated with mosquito netting in areas with potential vector transmission and until differential diagnoses are eliminated.

Although no specific antiviral drug is available, several compounds with in vitro antiviral activity have been described, including ribavirin and interferon- α . However, trials with ribavirin in experimentally infected monkeys have yielded conflicting results.⁸² Interferon- γ treatment of monkeys resulted in delayed onset of viremia and illness, but had no effect on survival.⁸³

YELLOW FEVER VIRUS VACCINATION

Two YF vaccines were developed almost concurrently in the 1930s, but attenuation was achieved by distinctly different methods. Both FNV and 17D strains had lost the ability to cause visceral YF in primates, but retained their immunogenicity. Furthermore, neither vaccine strain is mosquito competent; an important consideration for a live-attenuated vaccine to prevent reversion to virulence and vector transmission.

Development of Live-attenuated Yellow Fever Vaccines

The FNV vaccine strain was derived by performing 128 serial passages in mouse brain of the wild-type French viscerotropic virus (FVV), isolated in Senegal in 1927.⁴ In the 1940s and early 1950s, nearly 40 million doses of FNV were administered (mostly by scarification of the skin along with the smallpox vaccine) in French-speaking countries of West Africa⁴ in a mandatory vaccination campaign. YF cases declined dramatically in these countries, but unfortunately administration of FNV was associated with a high incidence of encephalitis in children. In 1961, FNV vaccination in children under the age of 10 was abandoned and manufacture was discontinued in the 1980s.

The 17D vaccine was developed by Theiler and Smith in 1937.⁷ The original Asibi YFV isolate was empirically attenuated by 176 serial passages in murine and chick embryo tissue cultures, resulting in loss of viscerotropism and mosquito competence.⁷ Initial problems with over- or underattenuation were resolved by establishing of a virus seed lot system in 1945; WHO requirements are that no vaccine shall be manufactured that is more than one passage level from a seed lot that has passed all safety tests. The 17D vaccine strain is generally regarded as one of the safest and most effective live-attenuated viral vaccines ever developed.^{84,85}

Two substrains of 17D (17D-204 and 17DD) are used as vaccines today, and more than 500 million doses have been administered. At present there are 4 sites of vaccine manufacture worldwide (Institut Pasteur in Dakar, Senegal; Bio-Manguinhos, Fio Cruz, Rio de Janeiro, Brazil; and Sanofi Pasteur in the USA and France), which produce a total of 20 to 25 million doses annually. As described previously, for protection in areas at high risk of YF transmission, the WHO's dual strategy for prevention of YF epidemics relies on preventative mass immunization campaigns followed by infant routine immunization. One vaccine dose, containing approximately 10^5 plaque-forming units (pfu) of virus, provides protective immunity for at least 10 years, after which the WHO recommends revaccination. Approximately 90% of vaccinees seroconvert by 10 days and 99% of vaccinees seroconvert by 30 days post immunization.⁸⁵⁻⁸⁷ YFV-specific neutralizing antibodies are detectable for more than 35 years.^{88,89}

Immune Response to Vaccination

During the first few days after immunization with the live-attenuated 17D vaccine, low levels of circulating viral RNA and infectious virus are detectable (<100 pfu/mL) in blood.⁸⁷ The live-attenuated 17D vaccine strain infects human DCs very poorly in vitro,⁹⁰ but stimulates their activation and maturation via multiple Toll-like receptors.^{91,92} In vaccinees, proinflammatory cytokines interleukin- 1β ⁹³ and tumor necrosis factor- α ^{87,94} are released, and markers of the type I interferon (interferon- α/β) response are expressed by peripheral blood cells.^{95,96} Humoral immunity, involving CD4⁺ T-cell and B-cell activation, and rapid elicitation of YFV-specific IgM and neutralizing antibodies is believed to play a major role in host defense against YFV infection.^{83,95} Neutralizing antibodies are used as the correlate of protection by the WHO. However, the potential importance of cell-mediated immunity, especially class I-restricted CD8⁺ T cells, in controlling primary YFV infections should not be underestimated.^{87,97,98}

Molecular Determinants of 17D Vaccine Attenuation

Over the last 20 years, nucleotide sequence analyses of the genomes of wild-type YFV isolates and their attenuated derivatives have provided clues as to the molecular basis of virulence/attenuation,⁹⁹⁻¹⁰¹ but the large number of mutations and their distribution across the entire genome have complicated interpretation of these comparative data. Comparison of the complete genomic sequences of 17D-204 and 2 other 17D substrains, 17DD and 17D-213 (the original 17D virus is not available),^{11,102,103} with those of wild-type YFV isolates including the parental Asibi strain⁹⁹ has identified 48 nucleotide and 20 amino acid differences common to all 17D substrains, scattered throughout the genome.¹⁰² As these mutations arose before divergence of the 17D-204 and 17DD substrains from the common attenuated lineage at passage 176, these changes are considered to be the primary candidates that contribute to the molecular basis of attenuation. Five mutations clustered in the E protein occur at sites conserved in natural YFV isolates from Africa and South America, and consequently are likely to be implicated in the attenuation process. The location of these mutations relative to functional domains in the E protein lends credence to this hypothesis. Two of the mutated amino acids (E-52 and E-200) lie in the fusion domain.⁵⁶ Moreover, mutations at E-173 and E-305 are integral components of neutralizing epitopes mapped by monoclonal antibody escape mutants.^{104,105} The loss of a 17D-204 substrain-specific

epitope from the 17D virus, selected for neutralization escape, resulted in dramatic changes in neurovirulence ranging from avirulence to increased virulence.¹⁰⁴ Although less well studied, mutations in other locations, particularly in the nonstructural proteins, are also being considered as determinants of YFV virulence.^{101,106,107}

Vaccine-Associated Adverse Events

The 17D vaccine is often cited as one of the most successful vaccines ever developed, with only mild side effects reported by approximately 25% of vaccines, including injection site pain or redness, headache, malaise, and myalgia. However, in recent years several issues have arisen that must be addressed. Vaccine production technology has not changed since 1945 when the seed-lot system was introduced, contributing to limited production potential. The 17D virus is propagated in embryonated eggs, with each egg yielding around 400 vaccine doses. The eggs must be free of adventitious agents such as avian retrovirus. The inability to rapidly replace vaccine stockpiles has critical implications for availability.

In the last 10 years 2 categories of severe adverse events associated with vaccination have been recognized: (1) vaccine-associated neurotropic disease (YEL-AND)¹⁰⁸⁻¹¹⁰ and (2) vaccine-associated viscerotropic disease (YEL-AVD).¹¹¹⁻¹¹⁷ YEL-AND was first described in the 1940s as “post-vaccinal encephalitis,” most often in young children. Consequently, in 1945 vaccination was contraindicated for infants younger than 6 months and only recommended for those older than 9 months, which complicates inclusion in routine infant immunization programs. Moreover, a monkey neurovirulence test for vaccine seeds was introduced. Together, these measures resulted in a reduction in YEL-AND, but the development of the vaccine adverse event reporting system (VAERS) in the United States revealed that YEL-AND was not so rare a phenomenon, occurring at a frequency of 4 to 5 per million doses and with case fatality rates of less than 5%. The second category of adverse event, YEL-AVD, was first described in 1999 in Brazil, resembling wild-type YF with pansystemic disease and high virus titers in many organs, especially the liver.^{111,113,117,118} VAERS demonstrated that YEL-AVD occurred with a frequency of 3 to 4 per million doses administered but, significantly, the case fatality rate is 60%. The host genes and host immune response have been proposed to be at least in part responsible for YEL-AVD.¹¹⁹ However, there has been no one apparent causal factor that explains all cases. A recent cluster of 4 deaths and 1 nonfatal case of YEL-AVD in Ica, Peru, in October 2007⁶⁵ following administration of 42,742 doses from one vaccine lot (ie, 1 in 10,000 vaccinees died following administration of this vaccine lot) has caused this hypothesis to be reassessed.

At present, the US Advisory Committee on Immunization Practices and the WHO Global Advisory Committee on Vaccine Safety are reviewing the vaccine and contraindications for the vaccine,¹²⁰ and the WHO Expert Committee on Biological Standardization met in late 2008 to discuss the manufacturing and quality control processes for the vaccine. This committee last reviewed the vaccine in 1998.¹²¹ Given the above, there are discussions on the need to improve the current 17D vaccine. Is there a need for a new vaccine? The process from discovery to licensure for a new vaccine is at least 15 years. Furthermore, risk-benefit evaluations of the current 17D vaccine indicate that it is highly efficacious and that its

withdrawal would leave a huge number of unprotected people in disease endemic areas. Finally, there has been a considerable investment in using the current 17D vaccine virus as a backbone for chimeric vaccines that have the structural protein genes of a particular flavivirus in a 17D backbone. This so-called ChimeriVax platform is being used by Sanofi Pasteur and Acambis to develop candidate DENV, WNV, and JEV vaccines.¹²²

FUTURE PERSPECTIVES

There are many unanswered questions remaining about YF disease and its agent, YFV, while the threat of urban outbreaks and endemic zone expansion continues to increase. A major gap in our knowledge of YF and YFV is in management and treatment of patients with this disease or with serious vaccine-associated adverse events. Treatment of YF by supportive care is virtually ineffective, and even admission to the ICU does not seem to improve the prognosis or change the mortality rate. There is a desperate need for the development of specific antiviral drugs and improved rapid diagnostic tests for this and other flaviviral diseases. Finally, the 17D vaccine, on which disease control entirely rests, has been associated recently with fatal adverse events. Consequently, improvements to the vaccine's safety will be required. To address these issues, a greatly improved understanding of complex interactions between the virus and host cell factors that control replication, as well as innate and adaptive immune responses, will be required.

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Fig. 1.

The yellow fever mosquito. The *Aedes aegypti* mosquito is the primary vector responsible for the transmission of yellow fever virus (YFV) between humans. Known as the YF mosquito, this vector is responsible for explosive outbreaks of urban yellow fever (YF) in African, South American, and Central American cities. Image from the Public Health Image Library, Centers for Disease Control. Photo, James Gathany; contributor Frank Collins. (Courtesy of the Centers for Disease Control and Prevention; with permission.)

Fig. 2.

The yellow fever virus virion. The virus particle is small, icosahedral, and enveloped. (A) Photomicrograph showing multiple YFV virions (original magnification $\times 234,000$). Image from the Public Health Image Library, Centers for Disease Control. (B) The immature (intracellular) and mature (extracellular) infectious virion. The single-stranded, infectious RNA genome is packaged in an icosahedral nucleoside with a lipid envelope and viral spike proteins, prM/M and E. The prM protein is processed to M by furin-mediated cleavage immediately before egress. (Courtesy of the Centers for Disease Control and Prevention; with permission.)

Fig. 3.

The yellow fever virus genome. The genome is a single-stranded RNA molecule of positive polarity (ie, can be translated), with highly structured 5' and 3' nontranslated regions, a 5' terminal cap, and a single open reading frame encoding the 10 viral proteins, 3 structural and 7 nonstructural.

Fig. 4.

Yellow fever virus replication in a permissive cell. The replication cycle is depicted as a series of temporally regulated steps: (1) Translation of the polyprotein; (2) co- and post-translational processing to produce the structural proteins (C, prM, and E) and the nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5); (3) synthesis of complementary, negative-sense RNA by the RNA-dependent RNA polymerase NS5 and other viral replicase components; (4) synthesis of progeny genomes by transcription of the negative strand; (5) progeny genomes are packaged into nucleocapsids and bud intracellularly to acquire envelope.

Fig. 5.

Yellow fever transmission cycles. The yellow fever virus is transmitted between human and nonhuman primate hosts by mosquitoes in 3 cycles: the sylvatic (jungle) cycle in which mosquitoes of the forest canopy transmit virus to monkeys and secondarily to humans entering the jungle; the intermediate cycle (or zone of emergence) in which virus enters rural towns and villages bordering jungle areas; and the urban cycle in which humans serve as the viremic host and virus is transmitted from human to human by the domesticated *Aedes aegypti* mosquito.

Fig. 6. The yellow fever endemic zone. The maps depict the areas in (A) Africa and (B) the Americas that are at risk for yellow fever virus transmission in 2009. (*From Brunette GW, Kozarsky PE, Magill AJ, et al. CDC Health Information for International Travel 2010. Elsevier; 2009.*)

Fig. 7. The phases of yellow fever, indicating the clinical symptoms in the periods of infection, remission, and intoxication, alongside the pathogenesis of infection.

Table 1
Countries in Africa and the Americas at risk of yellow fever

Africa	
West Africa	Benin, Burkina Faso, Cape Verde, Côte d'Ivoire, Equatorial Guinea, Gambia, Ghana, Guinea, Guinea-Bissau, Liberia, Mali, Mauritania, Niger, Nigeria, Sao Tome and Principe, Senegal, Sierra Leone, Togo
Central Africa	Angola, Burundi, Cameroon, Central African Republic, Chad, Democratic Republic of the Congo, Gabon, Rwanda
East Africa	Ethiopia, Kenya, Somalia, Sudan, Tanzania, Uganda
The Americas	
Central America	Panama
South America	Argentina, Bolivia, Brazil, Colombia, Ecuador, Guyana, French Guyana, Paraguay, Peru, Suriname, Trinidad and Tobago, Venezuela

Table 2
Laboratory workup for suspected yellow fever

CBC count	<ul style="list-style-type: none"> • Leukopenia with relative neutropenia • Thrombocytopenia (associated with consumptive coagulopathy) • Early hemoconcentration with increased hemoglobin and hematocrit levels • Subsequent hemorrhage and hemodilution with decreasing complete blood cell counts
Coagulation analyses	<ul style="list-style-type: none"> • Reduced fibrinogen and clotting factors II, V, VII, VIII, IX, and X • Presence of fibrin split products (indicate disseminated intra vascular coagulation) • Decreased clotting factor synthesis • Elevated prothrombin time • Prolonged clotting time
Blood chemistry	<ul style="list-style-type: none"> • Elevated serum creatinine level • Hypoglycemia secondary to hepatic dysfunction • Metabolic acidosis
Urinalysis	<ul style="list-style-type: none"> • Elevated urinary protein levels • Elevated urobilinogen levels
Liver function tests	<ul style="list-style-type: none"> • Transaminitis precedes the appearance of jaundice, and the degree of liver dysfunction in the acute phase may be predictive of the clinical course • Serum aspartate transferase levels exceed alanine transferase levels • Elevated direct bilirubin levels
Other	<ul style="list-style-type: none"> • Hypoalbuminemia-albuminuria, decreased synthesis, and extravasation of albumin through damaged capillary endothelium • Chest radiography may show pulmonary edema or secondary bacterial pulmonary infections • Brain computed tomography scan may show intracranial hemorrhage late in the illness • Electrocardiogram and cardiac monitoring for arrhythmias

Table 3
Differential diagnoses for yellow fever

Viral hemorrhagic fevers	eg, Dengue hemorrhagic fever, Rift Valley, Venezuelan, Bolivian, Argentine, Lassa, Crimean-Congo, Marburg, and Ebola fevers
Viral hepatitis	eg, Hepatitis A, hepatitis B, hepatitis C, hepatitis E
Viral febrile syndromes	eg, Influenza, Chikungunya fever, Dengue fever, and many other viral infections
Other	Louse-borne relapsing fever Toxic hepatitis Malaria Relapsing fever Typhoid Typhus Acanthamoeba Toxin-mediated hepatitis Liver failure from other causes

Table 4
Laboratory diagnostic tests

Serologic/immunologic	<ul style="list-style-type: none">• IgM antibody-capture enzyme-linked immunosorbent assay to detect YFV-specific IgM; a single positive serum titer is diagnostic in the absence of recent vaccination• 4-fold increase in YFV-specific IgG antibody titer in a patient with no history of recent vaccination• Important to rule out cross-reactivity with other flaviviruses, often by likelihood of exposure• Lesions compatible with those of YF or detection of virus in tissue using immunohistochemical staining. Often post-mortem as liver biopsy is contraindicated due to high risk of hemorrhage
Molecular	<ul style="list-style-type: none">• Detection of YFV antigen by in serum specimens• Detection of viral genomic RNA in tissue, blood or other body fluid using reverse transcriptase-polymerase chain reaction