Yellow Fever: A Disease That Has Yet to Be Conquered

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Abstract
Yellow fever virus (YFV) is the prototype member of the genus Flavivirus, a group of viruses that are transmitted between vertebrates by arthropod vectors. The virus is found in tropical regions of Africa and South America and is transmitted to primates by mosquitoes: Aedes spp. in Africa and Haemagogus and Sabethes spp. in South America. Despite the availability of an effective vaccine, yellow fever (YF) is considered a reemerging disease owing to its increased incidence in the past 25 years. Molecular epidemiologic data suggest there are seven genotypes of YFV that are geographically separated, and outbreaks of disease are more associated with particular genotypes. In addition, the risk of urban YF, owing to transmission of the virus by Aedes aegypti, is increasing in Africa, as is the potential of urban YF returning to South America. Both present serious potential public health problems to large population centers.
INTRODUCTION

Yellow fever virus (YFV) was first isolated in West Africa in 1927. It is the prototype member of the family Flaviviridae and genus Flavivirus, which get their name from the Latin word for yellow (flavus). The genome is a single-stranded, positive-sense RNA, 10,500–11,000 nucleotides in length. The genus Flavivirus contains approximately 70 viruses, and the major flavivirus diseases are yellow fever (YF), dengue, West Nile, Japanese encephalitis, and tick-borne encephalitis (4). Ecologically the flaviviruses are termed arboviruses owing to most flaviviruses being arthropod-borne; YFV is transmitted between primates by mosquito vectors.

YF provided a critical impetus for the development of medical entomology. The devastating and widely distributed epidemics of the seventeenth, eighteenth, and nineteenth centuries demanded that physicians and scientists devote their attention to answering questions regarding what caused the disease, how it was transmitted, and what measures could be taken to reduce morbidity and mortality owing to the disease. Investigating each of these issues resulted in an improved understanding of vector-borne diseases, the development of scientific techniques to study pathogens and vectors, and the implementation of vector control as a component of large-scale public health programs. Following the successful isolation of YFV in 1927 by American (strain Asibi) and French (strain French viscerotropic virus) workers, researchers placed great effort on the development of vaccines. The development of two live vaccines in the 1930s (reviewed in 2) represents a milestone in the control of the disease. Strain Asibi was passaged through chicken tissue to develop the 17D vaccine strain, whereas strain French viscerotropic virus was passaged through mouse brain to develop the French neurotropic vaccine (FNV). Both vaccines are highly efficacious and dramatically reduced the number of YF cases in Africa. Unfortunately, the FNV caused cases of postvaccinal neurotropic disease in vaccinees and was discontinued in 1971, whereas 17D is still used today throughout the world.

A BRIEF HISTORY OF YELLOW FEVER VIRUS

The first disease outbreak that can reliably be regarded as YF was documented in 1648 and occurred in the Yucatan, Mexico (15). A Mayan manuscript described xekik (black vomit), which is a characteristic manifestation of severe YF. The term yellow fever was probably first used by Griffin Hughes in his book *Natural History of Barbados* (1750). An early description of a disease outbreak in Haiti in 1495 was likely YF. Most researchers generally agree that YFV originated in Africa, and shipping routes associated with commerce were most likely responsible for the spread of YFV from Africa and its introduction into the New World. The intensity of the international trade linking Africa, America, and islands in between inevitably resulted in YF epidemics in major cities. Outbreaks occurred as far south as Montevideo, Uruguay, and Tocopilla, Chile, and as far north as Quebec, Canada.

Between 1668 and 1870 there were at least 25 outbreaks in New York, and there were devastating outbreaks in such cities as Philadelphia, Memphis, and Charleston. The New Orleans epidemic of 1898 involved almost 14,000 cases with 4000 deaths, whereas the epidemic in the lower Mississippi valley in 1878 resulted in 20,000 deaths and economic losses of almost $200 million. The last outbreak in North America took place in New Orleans in 1905. Europe was not spared from YFV, with the first documented epidemic reported in Cadiz, Spain, resulting in over 200 deaths, and 5000–20,000 deaths occurred in the Barcelona epidemic of 1821. An epidemic occurred in Dublin, Ireland, in 1826, and an outbreak in Swansea, Wales, involved 27 cases with 17 deaths. Vainio & Cutts (69) give an excellent overview of the historical outbreaks by year and location.
ORIGINS OF THE VECTOR<ref>Aedes aegypti</ref>

The key factor in the development of YF into an important human disease was the mosquito component of the YFV-urban transmission cycle, namely <ref>Aedes aegypti</ref>. The biological and behavioral characteristics of <ref>Ae. aegypti</ref> enabled it to successfully expand its geographic distribution. These characteristics include an ability to breed in small temporary standing water, the production of desiccation-resistant eggs, and a preference for feeding on and closely associating with humans. The development of what is regarded as the domestic form of <ref>Ae. aegypti</ref> from a sylvan ancestor may have occurred in North Africa (51), perhaps as a result of gradual climate change to a drier environment beginning at approximately 200 B.C. (40). The spread of the domestic form to West Africa may have been facilitated by trans-Saharan trade routes at approximately 700 A.D. Exactly when domestic <ref>Ae. aegypti</ref> was introduced into the Americas is uncertain. Presumably mosquitoes breeding in water containers on ships were transported sometime during or after the fifteenth century when trade was developed between North and West Africa, Europe, and the Americas.

ECOLOGY OF YELLOW FEVER VIRUS: VECTORS AND VERTEBRATES

The Jungle Cycle

Until the 1930s, <ref>Ae. aegypti</ref> was considered the only mosquito vector of YFV. The description of a jungle cycle for YFV resulted from an outbreak in Vale do Canaã, Brazil, in 1932, where no <ref>Ae. aegypti</ref> mosquitoes were found. Subsequently, other foci of disease were identified in localities with no <ref>Ae. aegypti</ref> present (6). During a 1938 outbreak near Rio de Janeiro, YFV was isolated from <ref>Ae. leucocelaenus</ref> (later redesignated <ref>Haemagogus leucocelaenus</ref>), <ref>H. capricorni</ref>, and an unidentified sabethine species (60), but later studies have implicated <ref>H. janthinomys</ref> and <ref>S. chloropterus</ref> as important vectors in South America (reviewed in 3). Subsequently, a jungle cycle was described in Africa with <ref>Ae. africanus</ref> as the principal vector. It is now known that YFV is enzootic in rainforests and is transmitted between lower primates by canopy-dwelling mosquitoes, i.e., the jungle cycle (see Figure 1). Although transovarial transmission of YFV has been demonstrated (7a, 37a), the relative importance of this in maintaining the transmission cycle is unknown.

Although several vertebrate species are susceptible to infection and used in laboratory studies, in nature, only primates appear to be involved in the transmission cycle. One difference between the New and Old World cycles is that whereas in South America, primates often succumb to infection and die, in the Old World, primates typically display no signs of infection. In South America, howler monkeys (<ref>Aloutta spp.</ref>) have been implicated in the YFV-transmission cycle, suffering fatal disease. Although other vertebrates, such as marsupials, may be infected and develop viremias capable of infecting mosquitoes (5), their role in the cycle is unknown. In East and central Africa, <ref>Colobus abyssinicus</ref> is a major host, whereas in forests and savanna, <ref>Cercopithecus spp.</ref> are important. In East Africa the lemur <ref>Galago senegalensis</ref> is an important host, and unlike the other African primates, it develops fatal disease. Surprisingly, although <ref>G. senegalensis</ref> is widely distributed, it seems not to be involved in the transmission cycle in other parts of Africa.

The Intermediate Cycle

In addition to the jungle cycle, an intermediate or savannah cycle has been recognized in Africa (Figure 1). Geographically, this cycle takes place in moist savannah areas where there is some human activity. This area has been termed the zone of emergence because...
it probably reflects the mechanism by which YF evolved from the jungle cycle to become an important human disease. In this cycle, the vectors include *Ae. luteocephalus*, *Ae. furcifer*, *Ae. metallicus*, *Ae. opok*, *Ae. taylori*, *Ae. vittatus*, and members of the *Ae. simpsoni* complex (19). Although different species of mosquito may have specific host preferences, those that feed on nonhuman primates often feed on humans when the opportunity arises. Consequently, the transmission of YFV between primates and humans may require only that people intrude into an ongoing jungle cycle to become infected. Nonetheless, host preference is a complex subject. For example, *Ae. africanus* may exist in anthropophylic and nonanthropophylic forms (21). Mutebi & Barrett (46) have most recently described the relative importance of African vectors of YFV and the epidemiology of YF in Africa.

The Urban Cycle

The urban cycle involves transmission of YFV between humans by *Ae. aegypti* (Figure 1) and has its basis in relatively high-titer viremia and the ability of the virus to infect and disseminate in *Ae. aegypti* so it can be transmitted in the saliva. The urban cycle has not been described in tropical South America since 1942 in Brazil, although there is serologic evidence of a small urban outbreak in Santa Cruz, Bolivia, in 1999 (70). In comparison, urban outbreaks are still reported in Africa,
predominantly in Nigeria, a country with a large urban population.

YELLOW FEVER VIRUS INFECTIONS IN MOSQUITOES

The jungle and savannah cycles are difficult to study because some of the key sylvatic mosquito vectors cannot be reared under laboratory conditions. In contrast, the scale and location of urban epidemics and the relative ease with which *Ae. aegypti* can be reared for multiple generations in the laboratory have enabled detailed studies on YFV and made YFV one of the best-understood arboviruses.

Sequence of Infection and Dissemination

In general, the infection process and dynamics of YFV replication in the vector are similar for most mosquito-borne viruses (22, 24, 25, 33). The virus-vector relationship consists of a number of stages: (a) The mosquito must feed on a viremic host; (b) a minimum threshold of virus must enter the mosquito midgut lumen; (c) virions must bind to the membrane, enter, and multiply in midgut epithelial cells; (d) virions must disseminate from the epithelial cells via the basement membrane and enter the hemocoel; (e) virions must infect salivary glands; and (f) virions must be secreted in saliva when the mosquito feeds upon a host. These steps may be influenced by vector, viral, and environmental factors, although environmental factors tend to simply determine the replication dynamics of the virus.

The overriding consideration is that the virus and mosquito species must be compatible. From an entomological viewpoint, the mosquito must be a competent vector for the virus. This competence is determined by several factors that basically act through permissiveness to progress from one stage to the next (see above). In effect a number of barriers can affect the success or failure and rate of the transmission process. These include infection and dissemination (escape) barriers. Results from quantitative genetic studies with *Ae. aegypti* suggest that for the related flavivirus dengue, at least two gene sets control vector competence, one controlling a midgut infection barrier and another controlling a midgut escape barrier (10). Surprisingly, there is limited understanding of the molecular basis of vector competence. It is possible to annotate a mosquito chromosome map, but despite recent studies (9–11), we can conclude only that multiple host genes are involved, and different stages of the infection (such as midgut infection, midgut escape, and salivary gland infection) likely are influenced by different host genes.

Our knowledge of viral genes involved in the multiplication of arboviruses in mosquitoes is also limited. The initial infection of mosquito cells may be a receptor-mediated event; however, the identity of the receptor is unknown. A midgut infection barrier that effectively prevents the cells from ever being infected with the virus presumably reflects the lack of appropriate receptors on these cells. Several studies have demonstrated the presence of cellular molecules to which flaviviruses bind, but none have been identified definitively. The development of infectious clones of YFV facilitates ongoing studies to identify the role of specific YFV genes in the mosquito-infection process (39, 39a).

Yellow Fever Virus: Wild-Type and Vaccine Strains in Mosquitoes

Soon after the development of live-attenuated vaccine strains of YFV, scientists demonstrated that whereas wild-type YFV can infect, disseminate, and be transmitted by *Ae. aegypti*, the 17D vaccine strain can infect midgut epithelial cells but cannot disseminate and be transmitted when mosquitoes are infected orally (30, 41, 56, 79, 80). The FNV vaccine is similarly attenuated in mosquitoes (17, 50, 57). Dynamics of wild-type YFV infections in the vector are well described (6, 7, 24, 26, 27,
Early studies demonstrated that *Ae. aegypti* mosquitoes that had fed upon infected monkeys could transmit the virus to naïve monkeys within 9 days, and the mosquitoes remained infectious during their life span.

Current results indicate that an effective midgut escape barrier operates to prevent dissemination of the YFV 17D vaccine virus. Although the midgut escape barrier may act in a virus-mosquito strain-specific manner, the failure of 17D to disseminate in, for example, the Rexville D strain of *Ae. aegypti* is more likely a result of a virally encoded factor because the wild-type Asibi strain, parent to 17D vaccine, disseminates efficiently. Interestingly, wild-type YFV infects the midgut of sylvatic *Ae. aegypti formosus* 7D strain but fails to disseminate (42). The mechanism of this refractoriness is unknown, but in essence the wild-type YFV strain Asibi in *Ae. a. formosus* behaves similarly to YFV 17D in *Ae. a. aegypti*. The former is presumably under the control of mosquito genes, and the latter is believed to be virally encoded.

**Mosquito Strain Variation to Infection with Yellow Fever Virus**

Numerous studies have investigated intraspecific variation of *Ae. aegypti* with respect to susceptibility to infection with arboviruses (8, 9, 12, 36, 42, 63). Vector-competence studies reveal considerable variation between mosquito strains, often with poor reproducibility. Susceptibility to YFV is different in *Ae. aegypti* strains collected from different geographic locations (1, 7, 27, 31, 37, 52, 64, 76). In addition, colonization affects susceptibility to oral infection (36, 64), and genetic selection has been used to produce resistant and susceptible phenotypes (42). Interestingly, Beaty & Aitken (7) found that a resistant strain (Amphur) of *Ae. aegypti* transmitted wild-type YFV Asibi at a low rate, even when intrathoracically inoculated—an infection technique that bypasses the midgut and typically results in efficient infection of the salivary glands and therefore virus transmission. Recently, a total of seven genotypes of YFV have been identified (see below). Few virus strains have been used for vector-competence studies, and it is unknown if there are differences in vector competence between genotypes.

**CONTROL OF YELLOW FEVER: MOSQUITO CONTROL/ERADICATION PROGRAMS**

A number of approaches have been taken to control YF. Historically, the development of live vaccines was used to control the disease in Africa, whereas mosquito vector control was used in the Americas.

Following the demonstration that YFV was transmitted by *Ae. aegypti* came the realization that it should be possible to control the disease by controlling mosquito populations. The test ground for this theory was Havana, Cuba. In February 1901, Major William C. Gorgas, the chief sanitary officer for the city, instituted mosquito-eradication procedures, and by September of the same year, YF had been eliminated—accompanied by a significant decline in the incidence of malaria. From Havana, Gorgas moved to Panama where, using the same approaches of destroying mosquito-breeding sites, he again successfully broke the YFV-transmission cycle. This led to the initiation of the *Ae. aegypti*-eradication program by the Pan American Health Organization (61) to prevent urban YF outbreaks. This aggressive campaign was remarkably successful on the basis of source reduction to eliminate breeding sites and insecticide spraying to kill adults (58). *Ae. aegypti* populations were eliminated from several countries, and as a consequence, the incidence of mosquito-borne viral diseases, including dengue and YF, was significantly reduced. Unfortunately, the programs were not sustained, and today *Ae. aegypti* populations have returned to precampaign levels (20), and large dengue epidemics are now commonplace in the Americas.
EPIDEMIOLOGY OF YELLOW FEVER VIRUS

Geography

Clearly, the epidemiology of YFV reflects the geographical distribution of its mosquito vectors. YF endemic areas were primarily mapped in the early 1930s and 1940s by screening large numbers of human sera from natives residing in Africa prior to the widespread use of YF vaccination. Consequently, vaccination and seroprevalence of related flaviviruses make it difficult to accurately determine the geographic distribution of the virus today. However, accurate data are available from South America where YF surveillance is ongoing with annual reports.

Africa

Currently, the YF endemic region in Africa recognized by the World Health Organization (WHO) is approximately from 15° north to 15° south of the Equator. This region includes 32 countries in sub-Saharan Africa and stretches from the southern edge of the Sahara desert in the north to Angola in the south. Approximately 600 million people reside in this region and are at risk of acquiring YF infections, including 230 million in urban areas (Figure 2). The YF endemic region in Africa includes primarily three climatic regions: (a) equatorial rain forest, which extends from Guinea in West Africa to western Uganda in eastern Africa and south to northern Angola; (b) moist savanna, which is a zone that extends from the equatorial rain forest characterized by decreasing rainfall, and (c) dry savanna, which extends further away from the moist savanna. The moist savanna the zone of emergence is where most agricultural activity takes place and many YF outbreaks are initiated.

The vast majority of YF cases and epidemics are reported in Africa, indicating the disease burden is much more severe in Africa than South America. From 1965 to 2004, 33,381 cases of YF were reported to the WHO of which 83% were in Africa. Table 1 shows the number of YF cases reported in the past 25 years has increased considerably, indicating that the public health burden from YF infections is increasing. Furthermore, Nasidi et al.
Table 1  Number of yellow fever cases by country, 2000–2005

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(48) and Monath (44) have argued that the reporting of YF cases to the WHO may underestimate the actual number of cases, and the actual number may be 10 to 500 times higher. Overall, the WHO estimates that there are 200,000 cases, including 30,000 deaths annually, and over 90% of the cases are in Africa.

The 18,738 YF cases and 4522 deaths reported to the WHO between 1987 and 1991 compose the highest YF activity for any five-year period since reporting began in 1948. These figures demonstrate that YF should be classified as a reemerging disease (55). The tremendous increase in YF cases in Africa since the early 1980s reflects a major breakdown in YF-control measures.

Although the YF endemic zone in Africa is broad, the distribution of YF cases and epidemics is not uniform across all regions. From 1985 to 2005, 34 outbreaks were recorded; 29 were in West Africa, whereas only 5 were in East and central Africa. Epidemics in East and central Africa often occur during periods of civil unrest. For example, the 1940 outbreak in the Nuba Mountains in Sudan occurred during war conditions (32), when large numbers of nonimmune humans migrated into a YF endemic region. Similarly, the epidemic in Ethiopia from 1960 to 1962 was during a period of civil unrest (59), as were the recent outbreaks in 2003 and 2005 in Sudan. In contrast, epidemics in West Africa occur without the mass movement of people into highly endemic areas. Seroconversion rates obtained in surveys conducted before the widespread use of YF vaccines in Africa show comparable...
rates of seroconversion owing to natural infections throughout the YF endemic region in Africa (68). Therefore, the general lack of YF outbreaks in East and central Africa is not a result of lack of infections; rather, other factors must contribute to outbreaks.

Although climatic conditions vary across the YF endemic zone in Africa, the continual presence of YFV in the jungles of East and central Africa suggests that climate may not be the major factor regulating YF outbreaks. Genetic differences between YF genotypes may play an important role in the distribution pattern of YF outbreaks and cases in Africa. Different YFV genotypes possibly vary in virulence for humans. Unfortunately, virulence phenotypes of YFV are not well understood. Associations have been made between viruses in West Africa genotype I and frequent outbreaks, suggesting that these viruses may be more virulent or infectious to humans. In addition, West Africa genotype I is genetically heterogeneous (47), suggesting it is more adaptable to different transmission conditions, such as during YF outbreaks. In addition, genetic and behavioral variations have been noticed among YF vectors in Africa that may affect vector competence (see above). However, the role that mosquito vectors play in YF distribution is not completely understood owing to limited comparative studies between vector populations within the YF endemic zone in Africa.

South America

The majority of YF activity in South America is found in the Orinoco, Amazon, and Araguaia river basins, with occasional reports from surrounding areas, including Trinidad. On average, 160 cases of jungle YF are reported from South America each year, with a case fatality rate of 65%. This high figure partly results from the identification of the disease in some areas by histopathological examination of livers from fatal cases. This has led to speculation that the true incidence of YF may be tenfold greater than the reported number of cases. However, YF takes place in jungle areas where the human population density is low. YF usually occurs from December through May and peaks during the first three months of the year, when populations of *Haemagogus* mosquitoes are highest during the rainy season.

Historically, YF has been reported in many counties in tropical South America, from Panama in the north to Argentina in the south. However, most activity is reported in Bolivia, Brazil, Colombia, Ecuador, the Guianas, Peru, and Venezuela, with Bolivia, Brazil, and Peru accounting for 90% of the cases. In most situations, human cases have been sporadic and involve individuals who enter forest areas containing the mosquito vector *H. janthinomys*. Vaccination is used to control outbreaks and in recent years has been effective at reducing the number of human cases. In Brazil the vast majority of cases are reported in the Amazonian and central-western areas of Brazil. However, recent cases have been reported outside this region, in the Sao Paulo, Bahia, Tocantins, and Goias states. Also, in 1998, the first cases of YF were reported in French Guiana since 1902 (23). Thus YF is a reemerging disease in tropical South America.

In 1949, 10 countries in tropical Central and South America (Bolivia, Brazil, Guyana, Colombia, Ecuador, French Guiana, Panama, Peru, Surinam, and Venezuela) established a program to eliminate *Ae. aegypti*. This program was successful and resulted in the elimination of the mosquito from urban areas and, with it, urban YF (38). In addition, better-implemented vaccination programs contributed to fewer cases of YF in South America compared with Africa. However, the number of YF cases reported from South American countries has increased from 1985, including a 1995 outbreak in Peru involving at least 800 cases with a case fatality rate of 38%, which was the largest outbreak in South America since the 1950s. In addition to the increased numbers of cases in recent years, *Ae. aegypti* has reinfested many urban centers in South America, and there is now
the potential that urban YF will return to the Americas (see below).

Studies during the 1940s and 1950s in Brazil, Trinidad, and Colombia conducted by the Rockefeller Foundation established the role of nonhuman primates and forest canopy-breeding mosquitoes in the jungle cycle and the apparent cyclical nature of YF epizootics, in which virus appeared to cause outbreaks followed by four to seven years with no reported virus activity. These studies gave rise to the prevailing paradigm that YFV is maintained by wandering epizootics through the Amazon basin in a jungle cycle involving nonhuman primates and mosquitoes of the Haemagogus and Sabethes genera rather than the virus remaining in one locale. *H. janthinomys* is highly susceptible to YFV and is primatophilic. Thus, it is not surprising that humans entering the *H. janthinomys* ecosystem have a relatively high chance of being bitten and infected with YFV. Nearly all monkey species are susceptible to YFV, with the potential of a fatal outcome, and traditionally the finding of dead monkeys has been the marker for a YF epizootic. However, the paradigm of virus movement to sustain the jungle cycle has become the established hypothesis to explain YF activity in tropical South America given the death or immunity of monkeys from YFV infection, the long gestational period and low population turnover of monkeys, and the absence of alternative vertebrate hosts.

Subsequent studies have established a cyclical pattern of YF activity with interepidemic intervals required to establish susceptible populations of nonhuman primates. Recent studies by Vasconcelos et al. (71–73) of YF outbreaks in Brazil have suggested focal endemism of YFV in areas on the basis of annual isolation of the virus from *H. janthinomys* during the rainy season near the Trans-Amazon Highway. Vasconcelos et al. (74) have recently reported evidence implicating human movement as a mechanism to move YFV across large distances in short periods of time. The pattern of sylvan YF activity observed in the Amazon region of Peru and Bolivia suggests that the epizootic waves model may not be valid for eastern Peru and Bolivia. There are areas in eastern Peru and Bolivia where human cases of YF occur every year, suggesting that enzootic foci may exist in these countries, and a recent phylogenetic study of YFV isolates from Peru supports this hypothesis on the basis of concurrent appearances of multiple variants during the 1995 epidemic in Peru and the genetic stability of separate virus lineages (14). A subsequent study using phylogenetic analysis of 30 isolates from Brazil provided not only evidence for the traditional wandering epizootic paradigm, but also evidence of enzootic transmission (13).

**Why Has Yellow Fever Never Been Seen in Asia or Pacific Regions?**

The lack of YFV in Asia is not understood, although a number of hypotheses have been put forward (43). The mosquito vector *Ae. aegypti* is prevalent in Asia and Pacific countries and has been important in the rapid emergence of dengue as a major public health problem in the twentieth century (20). Laboratory studies indicate that Asian strains of *Ae. aegypti* can transmit YFV but are less competent than strains from the Americas. Demographic factors, including the remote location of sylvatic YF transmission and the cross-protective immunity provided by prior exposure to dengue and other flaviviruses, likely play a role in the lack of YF in Asia.

**BIOLOGY OF YELLOW FEVER VIRUS**

The pathogenesis and pathophysiology of YFV are poorly understood; however, a key feature of YFV is the high viremia in primates that is critical to the transmission of the virus by mosquito vectors. Laboratory research on YFV was difficult until Stokes et al. (62) successfully transferred YFV monkey to monkey, and Theiler (67) subsequently demonstrated the susceptibility of laboratory mice to YFV after intracerebral inoculation. With...
these techniques in place, it has been possible to undertake studies of YFV biology. However, there are ethical issues in using non-human primates for research, and although there are no antivirals to treat YF cases, the development of successful live vaccines has limited justification for the use of nonhuman primates to study the pathogenesis of YFV. The mouse has proved to be a useful model but is limited in application, as YFV only causes neurotropic disease in this host. Recently, Tesh and coworkers (66) developed a hamster model to study viscerotropic disease caused by wild-type YFV. This model has important applications in understanding the pathogenesis of YFV; however, wild-type strains have to be adapted to hamsters by serial passage. Thus, the hamster is not a good model for investigating differences in virulence between strains.

**MOLECULAR EPIDEMIOLOGY**

**Origins and Evolution of Yellow Fever Virus**

It is generally assumed that YFV evolved in Africa prior to its introduction in South America. On the basis of analyses of nucleotide sequences, several studies have provided evidence that supports this hypothesis. YFV is genetically more heterogeneous in Africa than in South America (35, 47), suggesting an African origin for YF. West African strains are phylogenetically closer to South American strains than to East or central African strains (16, 35, 77) (Figure 3), suggesting that South American strains evolved from West African strains. YFV is genetically more divergent in East and central Africa than in West Africa, indicating that the virus may have originated in jungle areas of East and central Africa. Overall, on the basis of nucleotide-sequence data and phylogenetic analyses, YFV may have originated in East and central Africa, extended its range to West Africa, and then was transported from West Africa to South America.

**Genetic Variation Among Yellow Fever strains**

Genetic studies of YFV strains using molecular techniques have revealed genetic variation among YF strains associated with different geographic regions. Initial studies by Deubel et al. (18) using oligonucleotide fingerprinting of the genomic RNA described three variants of YFV, of which two were found in Africa and one in South America. The same workers showed that geographically separate and epidemiologically unrelated YFV strains were genetically distinct and that YFV strains evolved slowly (18, 35). Subsequent nucleotide-sequencing studies identified a total of seven genotypes based on ≥9% nucleotide variation, of which five were in Africa (16, 47, 77) with each genotype circulating in a different geographic region (Figure 4). Two genotypes were found in West Africa. One genotype in West Africa (West Africa genotype I) was found from eastern Ivory Coast and Burkina Faso to Cameroon, including Nigeria, whereas the second genotype (West Africa genotype II) was found in western Ivory Coast and Mali to Senegal (35, 47). Additional genotypes were identified in eastern/central Africa [Central African Republic, central Sudan, Ethiopia, and the Democratic Republic of Congo (formerly Zaire)] and East Africa (Kenya, southern Sudan, and Uganda), plus one based on a single isolate from Angola (Angola genotype) (47, 49).

These studies demonstrated little sequence variation between YF strains isolated many years apart, indicating genetic stability of the genotypes over time. Apart from illustrating genetic variation between genotypes, and the genetic stability of YF strains within the genotypes, these studies demonstrate the distribution pattern of the genotypes. The Angola genotype of YFV was extremely divergent, 17.1%–25.0% nucleotide-sequence variation (75), from all other African genotypes, suggesting it diverged from other East and central African genotypes of YFV a long time ago and has evolved independently.
Strains in West African genotype I were genetically more heterogeneous than those in West Africa genotype II, possibly owing to frequent adaptation to different transmission cycles during frequent outbreaks and epidemics associated with this genotype. The East and central African genotype has a much broader distribution, including many countries, but YF outbreaks are rare in this region, and the cycle is generally silent.
Two genotypes were identified in South America. The largest is South America genotype I, which is found in Brazil, Panama, Columbia, Ecuador, Venezuela, and Trinidad, whereas South American genotype II is predominantly found in Peru, plus some isolates from Brazil and Trinidad (13).

Although there is extensive nucleotide variation between genotypes, there is no more than 7.6% amino acid divergence between genotypes (75). Interestingly, there is a codon-usage bias between genotypes, which is thought to be a result of a combination of factors, including mutational bias and translational selection (13, 47, 75).

REEMERGENCE OF YELLOW FEVER AS A MAJOR PUBLIC HEALTH PROBLEM

YF remains a major public health concern in sub-Saharan Africa and tropical regions, particularly in countries that historically have had high incidence rates. The resurgence of YF in recent years, especially in some African countries, highlights the need for continued vigilance and preparedness for the potential spread of the virus.

Figure 4
A map of Africa showing the yellow fever (YF) endemic region (orange and red) and the recognized distribution of YF genotypes (colored ellipses). Highlighted in red are countries that have reported YF outbreaks since 2000 (data from the World Health Organization).
South America, despite the availability of a safe and effective vaccine. Two concerns prevail: (a) the increased risk of urban YF in Africa owing to increased urbanization of the population and (b) the risk of urban YF returning to South America.

Increased Risk of Urban Yellow Fever in Africa

To understand the cause of the reemergence, one must look to the middle of the twentieth century. Until the development of the live-attenuated 17D and FNV vaccines, YF was considered a global public health problem, and the disease was subjected to a large research effort. The introduction of vaccination to control the disease had far-reaching consequences. On the positive side, the vaccine presented the opportunity to eliminate the disease from humans. YF 17D vaccine can be given to children aged nine months or older, the resulting immunity is lifelong and has long enjoyed a reputation as being one of the safest and most-effective live-attenuated vaccines (2, 45). As such, the incidence of YF drastically reduced following the introduction of vaccination in the late 1930s, and in the next 25 years there were relatively few cases of YF, with outbreaks limited to those countries that did not administer one of the vaccines. As the number of reported cases decreased, so did efforts to control the disease. Complacency and a feeling that YF was beaten set in.

However, YF is an epizootic disease, and vaccination cannot eliminate the nonhuman primate host of the virus. The potential was always present that the disease incidence would increase in humans if vaccination was not maintained to immunize susceptible individuals born after vaccination programs. Significantly, few countries, with the notable exception of Gambia in Africa, introduced the YF vaccine into their expanded program of immunization, and the YF vaccine was not included in childhood vaccination programs. Thus, as vaccination campaigns were not maintained, the number of YF cases has increased, particularly in children and young males who enter jungle areas for employment (68). In the late 1950s and early 1960s, there was a resurgence in the number of reported cases, and several outbreaks were reported in Africa. Although 32 of the 44 countries where YF is endemic have partial or national expanded programs of immunization for the YF vaccine, the number of cases continues to increase, as routine vaccination campaigns have been replaced by emergency vaccination campaigns once an outbreak has been identified. Once the outbreak ceases, so does vaccination. Clearly, this is not a cost-effective mechanism to control a vaccine-preventable disease.

The majority of YF outbreaks are reported in West Africa, and in the past five years 93% of countries in this region have reported YF cases, with multiple outbreaks taking place concurrently. This raises significant questions about the potential spread of virus among nonimmune populations. Presently, most epidemics in West Africa occur in endemic areas in the absence of mass movement of people. However, in terms of the increased risk of urban YF outbreaks in Africa, 60% of the African population is located in rural areas, but the urban growth rate is 4% per annum and consists mainly of nonvaccinated individuals. Currently 43 African cities have populations in excess of 1 million, and this will increase to 70 cities by 2015.

Risk of Urban Yellow Fever Returning to the Americas

Although *Ae. aegypti* was eliminated from many South American countries in the 1930s and 1940s (58, 61), control measures were not maintained, and *Ae. aegypti* soon returned to these countries. Today the mosquito infests a larger area of the Americas than before its temporary elimination (20). There are many reasons for this, including political decisions not to maintain mosquito-control programs and global warming, which causes increased temperatures and increased rainfall that favor increases in the populations of mosquitoes.
and other animals. However, the important point is that urban YF has not returned to the Americas, whereas *Ae. aegypti* infestation has been associated with ever-increasing rates of transmission of dengue viruses (20).

Many hypotheses have been proposed to explain the lack of urban YF, and these are similar to those proposed to explain the lack of YFV in Asia (see above). However, most agree it is only a matter of time before urban YF returns owing to increasing sizes of urban human populations and geographic spread of *Ae. aegypti*. In particular, highly populated coastal regions of Brazil have become reinfested with *Ae. aegypti*, leading to concerns about the reemergence of urban YF. As mass-immunization campaigns target residents of enzootic regions only, people living close by are at risk of infection.

It is worth comparing YF and the four dengue viruses. All are mosquito-borne flaviviruses; however, the evolution of these viruses has been different. All these viruses originated as sylvatic viruses putatively involving monkey-mosquito transmission cycles (reviewed in Reference 20). YFV has retained its sylvatic phenotype, whereas the dengue viruses have evolved into a human-mosquito (*predominantly Ae. aegypti*) transmission cycle. Furthermore, molecular epidemiologic studies show that YFV has undergone relatively little genetic variation and correspondingly few amino acid substitutions (13, 47, 75). In comparison, the dengue viruses have undergone extensive nucleotide and amino acid variation (28, 54). Thus, the lack of urban YF may be in part a result of YFV retaining its sylvatic character and not adapting to *Ae. aegypti*, whereas dengue viruses have evolved and followed *Ae. aegypti* into South American urban population centers.

PROSPECTS FOR THE FUTURE

Future Directions for Yellow Fever Research

The recent increases in our knowledge of the molecular biology of wild-type YFV and molecular entomology, together with a hamster model of viscerotropic disease (66), offer great opportunities to further our understanding of YFV-mosquito-vertebrate host interactions that should not only benefit YFV research, but also should illuminate fundamental aspects of the molecular basis of vector competence that can be applied to other arboviruses. Ultimately, this should result in countermeasures that can be used to prevent YF and other arboviral diseases. In particular, there are no antiviral agents available to prevent YF or any flavivirus.

Supply and Demand of Yellow Fever 17D Vaccine

The 17D vaccine is produced in embryo-ontated chicken eggs using technology that has changed little in the past 60 years (2). As such, this technology limits the capability to produce large quantities of vaccine at short notice to respond to outbreaks. Recent outbreaks have occurred concurrently, further stretching the limited stockpiles of vaccine. In the past 10 years we have seen the development of reverse-genetics techniques for YFV and other flaviviruses, and the 17D vaccine virus has been developed into a platform technology for expressing foreign antigens as chimeric 17D vaccine viruses in monkey kidney Vero cell culture (53). To date such candidate vaccine viruses are safe in phase II clinical trials, suggesting the possibility of transferring technology to manufacture 17D vaccine from eggs to Vero cells and producing vaccine at short notice. Similarly, chimeric vaccines have the potential to use the 17D vaccine backbone to develop vaccines against other flavivirus diseases for which no vaccine currently exists, such as dengue.

Unvaccinated Travelers and Ecotourism

Unvaccinated individuals who enter areas where YF is endemic have a significant risk of being infected by the virus. This includes
Table 2  Travelers acquiring fatal yellow fever infections while in yellow fever endemic countries

<table>
<thead>
<tr>
<th>Year</th>
<th>Home</th>
<th>Country visited</th>
</tr>
</thead>
<tbody>
<tr>
<td>1996</td>
<td>United States (Tenn.)</td>
<td>Brazil (Manaus)</td>
</tr>
<tr>
<td>1996</td>
<td>Switzerland (Basel)</td>
<td>Brazil (Manaus)</td>
</tr>
<tr>
<td>1999</td>
<td>Germany (Frankfurt)</td>
<td>Cote d’Azore (Comoe National Park)</td>
</tr>
<tr>
<td>2000</td>
<td>United States (California)</td>
<td>Venezuela (P. N. Canaima)</td>
</tr>
<tr>
<td>2001</td>
<td>Belgium (Ottignies)</td>
<td>Gambia</td>
</tr>
<tr>
<td>2002</td>
<td>United States (Texas)</td>
<td>Brazil (Manaus)</td>
</tr>
</tbody>
</table>

travelers who visit endemic areas owing to family or work and those who visit from other countries. One potentially important factor in the emergence of YF is ecotourism. People residing in developed countries are taking vacations to developing countries in increasing numbers, and residents of urban, coastal South America are vacationing in the endemic interior regions of the countries. Unfortunately, many tourists are not vaccinated before they leave for their vacations. There have been a number of incidences in which tourists going abroad have returned to their homes to succumb to YF (see Table 2). This has potentially important issues for public health in the twenty-first century. Firstly, air travel enables viremic travelers to reach any part of the world in less than 24 hours while they are still viremic. Secondly, few physicians in developed countries have seen a case of YF and are not trained in caring for patients. Thirdly, and most importantly, if the developed country has a mosquito vector that transmits YFV, such as *Ae. aegypti*, there is a potential to establish large outbreaks of human disease and/or YFV in the ecosystem. This potential is more than a theoretical risk as demonstrated by the introduction of other flaviviruses, West Nile virus, into North America in 1999 (34) and Usutu virus into central Europe (78).

**Transgenic Mosquitoes for Yellow Fever Virus Control**

Tabachnick et al. (63) suggested that, because populations of *Ae. aegypti* differ in parameters of vector competence, it should be possible to eventually focus control priorities on the more-dangerous populations, and then “attempts might be made to replace highly competent populations of *Ae. aegypti* with other naturally occurring field populations found to be less efficient.” With the development of new molecular and genetic techniques, scientists may eventually replace virus-competent populations with mosquitoes whose competence has been genetically manipulated in the laboratory. It is now possible to experimentally transform mosquitoes, and Higgs et al. (26) have demonstrated the proof of concept for preventing the transmission of YFV. Implementation of this approach is, however, not likely in the near future. Many issues have to be resolved, including mechanisms to ensure transgene fitness, drive mechanisms to facilitate population replacement and genetic stability (29), and, of course, the ethical issues pertinent to the release of genetically altered vectors.

**SUMMARY POINTS**

1. YFV was the first virus demonstrated to be transmitted by mosquitoes.
2. Mosquito control and public health programs have been implemented to control the disease.
3. Researchers have developed a live-attenuated 17D vaccine.
4. Mosquito vectors of YFV vary both in species and vector competence in different populations.
5. YFV is enzootic and as such cannot be eradicated, but a combination of mosquito control and vaccination is efficient in disease prevention.

6. YF is considered a reemerging disease and is still a major public health problem, with an increased risk of urban YF in Africa.

7. It is unclear why urban YF has not returned to South America.

8. There are seven genotypes of YFV that are geographically separated, and outbreaks of disease are more associated with particular genotypes.

LITERATURE CITED


**RELATED RESOURCES**
