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ABSTRACT

Yellow fever, a mosquito-borne flavivirus disease occurs in tropical areas of South America and Africa. It is a disease of major historical importance, but remains a threat to travelers to and residents of endemic areas despite the availability of an effective vaccine for nearly 70 years. An important aspect is the receptivity of many non-endemic areas to introduction and spread of yellow fever. This paper reviews the clinical aspects, pathogenesis, and epidemiology of yellow fever, with an emphasis on recent changes in the distribution and incidence of the disease. Recent knowledge about yellow fever 17D vaccine mechanism of action and safety are discussed.

1. Introduction

Yellow fever (YF) is caused by the prototype member of the genus *Flavivirus* (family *Flaviviridae*), which contains approximately 70 positive-strand, single-strand RNA viruses, the majority of which are transmitted by arthropods (mosquitoes and ticks). Yellow fever is endemic in tropical regions of Africa and South America, and many review articles describe its epidemiology in the two continents [1–3]. A recent analysis of country-by-country geographic risk re-defined the borders of the YF endemic zone, but emphasized the fluid nature of virus activity and the occurrence of periodic expansions and retractions [4]. The virus has a relatively narrow host range for productive infection, and is maintained in nature by transmission between non-human primates and blood-feeding mosquitoes mainly belonging to the genera *Haemagogus* and *Aedes* (*Stegomyia*) in South America and Africa, respectively, and by transovarial transmission in these vectors. Humans are infected sporadically when bitten by sylvatic mosquitoes that previously fed on a viremic monkey (so-called **jungle yellow fever**), but may also serve as the viremic host for inter-human transmission, mainly by *Aedes aegypti*, a species that breeds in water-containing vessels inside dwellings or in close proximity to them (so-called **urban yellow fever**). The epidemiology of YF in Africa is often mixed, involving both sylvatic and domestic vector species in inter-human transmission. Consequently, the force of infection in Africa is higher (generally 20–30×) than in South America, the consequence being large epidemics. In recent years, new efforts have been made to vaccinate the populations of high-risk countries in West Africa; the long-term consequences of this effort will be a reduction in major epidemics.

Yellow fever was a major threat to human health from the 18th Century to the early 20th Century, with repeated epidemics following introductions to coastal towns and cities distant from endemic areas in North America, the Caribbean and Europe. The identification in 1900 of *A. aegypti* mosquitoes as the agency whereby YFV was transmitted, and subsequent efforts to control the vector, resulted in a decline in yellow fever outside the tropical, endemic zone. The development of two live, attenuated YF vaccines in the 1930s, and their wide deployment in the 1940s, led to a further decline of the disease. Subsequently, there have been periodic upsurges of YF activity in endemic regions without routine immunization programs.

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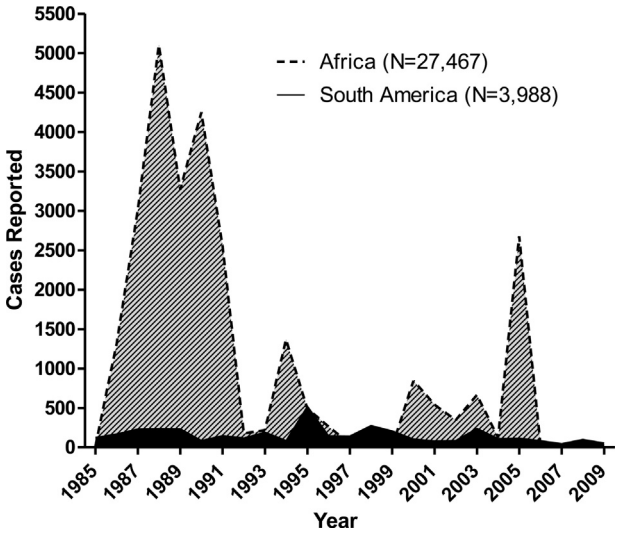

Fig. 1. Cases of yellow fever in Africa and South America, 1985–2009, officially notified to the World Health Organization.

The annual reporting rate of officially reported cases in South America and Africa shown in Fig. 1, but these rely on passive surveillance and thus significantly underestimate the true incidence. The latter remains unknown, except in some discrete epidemics that have been actively investigated. In non-epidemic periods, estimates of 200,000 cases derived from serosurvey data and the rate of inapparent to apparent cases (7–12:1) have been cited from paper to paper for over 15 years, without supporting evidence. Since 2008, more active laboratory-based surveillance activities suggest that, of several thousand suspect clinical cases in Africa investigated, only 1–2% have laboratory evidence for YF. The World Health Organization (WHO) appropriately treats the detection of a case of YF as an emergency, since it reflects transmission of the virus and a risk of a further spread; if the affected region is an area of low vaccination coverage, a regional mass vaccination campaign is generally conducted in response. Between 2007 and 2010, 57 million people were vaccinated against YF in 10 countries at risk in Africa, and during the same period, 17 million people were protected through emergency vaccination [5]. In the 1990s, despite 50 years of use and over 500 million doses distributed, new safety concerns about the live attenuated YF 17D vaccine have come to light, revealing that in rare circumstances the vaccine can cause a disease similar to parental wild-type virus. This fact has modified vaccine policy and regulations in some circumstances, as will be discussed later on.

Despite the availability of vaccines since the 1940s, large epidemics occurred in areas without a background of naturally acquired or artificial immunity. Dramatic upsurges in YFV activity occurred in Africa in the 1960s and the late 1980s each involving >100,000 cases, and recent outbreaks affected southern Brazil, Paraguay and Argentina (2007–2009), Uganda (2010), and Sudan and Ethiopia (2012–2013). Although the absence of an immune barrier in the human population is a key factor, the underlying reasons for virus amplification remain unclear, and are multifactorial, involving deterministic (vector density and competence, viral virulence), and stochastic factors. Expansions of YFV activity have sometimes been associated with the emergence of a new virus lineage [6], but the lack of information about biological correlates of genetic change, make it difficult to assign causality. Perturbations of weather, particularly prolonged increases in rainfall and high temperatures have been associated with outbreaks of YF in Africa and South America.

In humans, YF is a severe acute illness with fever, nausea, vomiting, epigastric pain, hepatitis with jaundice, renal failure, hemorrhage, shock and death in 20–60% of cases. Yellow fever is the prototypical viral hemorrhagic fever, and shares many pathophysiological features with unrelated diseases associated with a similar syndrome, except that the severity of hepatic dysfunction is generally greater in YF patients. The lower case fatality in Africa (~20%) than in South America (40–60%) [7], suggests that genetic factors determine lethality of the infection, a subject that deserves further study. Interestingly, the neutralizing antibody response to YF 17D vaccine is statistically higher in Caucasians than in African-Americans [8] possibly indicating genetic resistance to YF in the latter. Some New World monkeys, notably *Alouatta* (howling monkeys), are also susceptible to lethal infections, and epizootics associated with monkey deaths may precede the occurrence of human cases, a useful surveillance tool [9]. In contrast, almost all African nonhuman primates have inapparent, viremic infections. This reflects the origin of yellow fever virus (YFV) in Africa several thousand years ago, and a balanced co-evolution of virus and hosts. Based on these factors and genetic analyses, YFV was introduced into the Americas from West Africa during the slave trade about 400 years ago [10], and rapidly invaded a new ecological niche involving local hosts and vectors, much like another flavivirus, West Nile, did after its more recent introduction into the Americas.

A serious concern for the future is whether YFV could be introduced by a viremic air traveler to *A. aegypti*-infected areas outside the endemic zone, and particularly India and Southeast Asia. The recent spread of another virus transmitted in a human-*Aedes* cycle—Chikungunya—in islands of the Indian Ocean, India, southern Europe, and the Caribbean illustrates the threat. Although the WHO maintains an emergency YF 17D vaccine stockpile, an extensive outbreak could create a significant shortfall in vaccine supply.

2. Advances in epidemiology

2.1. Geographic distribution

2.1.1. Yellow fever outbreaks in the Americas

Beginning in 1997 and extending throughout the years of the first decade of the present century, intense YFV circulation was observed in Brazil (Pará and Goiás states) which has extended to areas in contiguous states of Goiás and Mato Grosso do Sul (Central Brazil), and then outside endemic region, as well as to countries such as Paraguay and Argentina, which had not identified YFV circulation for the previous 34 and 41 years, respectively. Additionally, many outbreaks were reported in Colombia and Peru, both being endemic countries, the latter being responsible for almost 50% of all YF cases reported in the Americas [11].

In Paraguay, YF was recognized in 2008 when cases of jungle yellow fever were diagnosed in San Izidro and San Pedro Departments. A few weeks later, a cluster of cases was diagnosed in the district of Laurelty in the metropolitan area of Asunción, the Paraguayan capital. This was only the second instance of urban YF in South America since the early 1940s. Urban transmission was limited to 14 recognized cases (8 fatal), though undoubtedly there were many more people infected. After vector control measures and a mass vaccination campaign, no further cases were reported. Before the occurrence of cases, *A. aegypti* Breteau and house indices were approximately 30% in the affected area.

In Argentina, 5 jungle YF cases and a single death were reported in Misiones Province. An interesting finding in this country was the implication of a new vector, *Sabethes albiprivus* in yellow fever transmission [12].

In Brazil, the spread of YF in 2008–2009 was impressive. Initial cases were reported in State of Pará (municipalities of Afua and

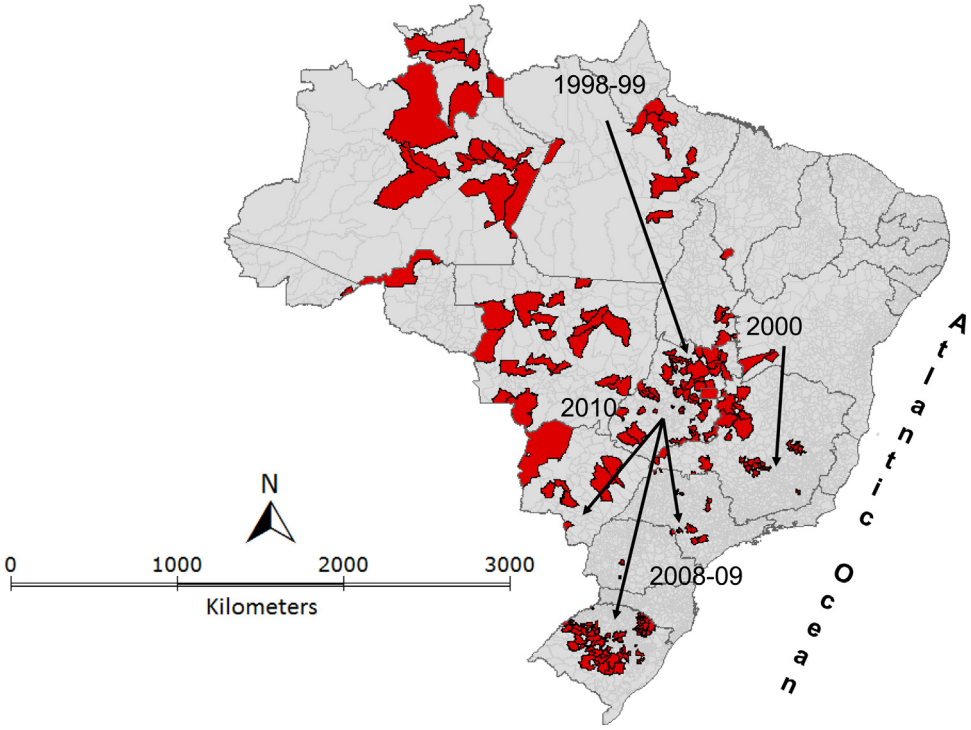

Fig. 2. Map of Brazil showing the widespread of yellow fever between 1998 and 2012 and the movement of yellow fever virus (YF) to the southeast threatening densely populated areas on the Atlantic coast. Areas in red represent epizootic and or epidemic. The arrows indicate the directional movement of YFV and the years of the major epidemics/epizootics.

Breves), in the first months of 2008, and later in Tocantins and Goiás states. In 2009, a large epizootic and epidemic wave occurred in Goiás and Mato Grosso do Sul states (Central Brazil), and reached as far south as Rio Grande do Sul; a lower level of YFV activity was reported in the years following this episode (Fig. 2).

In the State of São Paulo a total of 28 cases and 11 deaths (CFR 39%) were reported in 2009. Many cases were reported outside the recognized endemic/enzootic area, where routine YF vaccination had not been performed. A molecular study showed implicated a new YFV lineage (designated genotype 1E) [6] which evolved from the 1D lineage circulating in Brazil in the 1990s [13a]. Two important facts should be emphasized, first, that YFV in São Paulo State was only recovered from areas considered free of virus circulation, i.e. without recommendation for vaccination; second, YFV was isolated less than 100 km from São Paulo city with approximately 16 million people almost all of whom are unvaccinated.

In Rio Grande do Sul State, a region where YFV circulation occurs only at long intervals, epizootic circulation was observed in 2008 and 2009. In fact, thousands of monkey deaths, mainly *Alouatta caraya* (howler monkeys), were documented in 2008, and these occurred almost at the same time as transmission of YFV was documented in Argentina and Paraguay. Before this episode, two small epizootics without reported human cases had been registered in 2001 [13a], and during that episode two YFV strains were recovered from *Haemagogus leucocelaenus*, a mosquito considered as secondary vector of YFV [14a]. In Brazil more than 200 epizootic foci were reported in 2008. An entomological study during the epizootic wave in the Ijuí River basin resulted in the isolation of YFV from *H. leucocelaenus*, with a high minimal infection rate (MIR) of 3.70% [14b]. YFV was also recovered from *Aedes serratus*, which had not previously been recognized as a potential vector. Seven YFV strains including one obtained from *A. serratus* were sequenced, confirming the circulation of the YFV lineage 1E in southern Brazil. It is noteworthy that almost all cases and epizootics were reported outside the endemic area, and thus the virus moved toward the Atlantic coast, the most populated area in Brazil. YFV was found around 50 km west of Porto Alegre, the state capital, whose metropolitan area has approximately 3.5 million persons not vaccinated against YF. During the 2008 episode the CFR reported was 43% [15].

2.1.2. *Yellow fever in Africa*

Yellow fever is endemic in tropical and subtropical regions of Africa (Fig. 3A). A consideration of the geospatial and temporal distribution of cases during the era after 1960 (when organized vaccination generally ceased in francophone countries) provide the following general conclusions: (1) outbreaks occur more frequently in West Africa than elsewhere (Fig. 3B); (2) large epidemics have been reported from West and East Africa, but are infrequent and small in Central Africa; (3) there is a periodicity of YF activity, with upsurges at highly irregular intervals of 5–20 years in West Africa and much longer intervals, e.g. 45 years in parts of East Africa; (4) some areas have sustained epidemics across multiple years, exemplified by Ghana (1977–83), Guinea (2000–2005), and Nigeria (1986–1994); and (5) there are years of intensification of YFV activity across multiple countries and geographic regions of Africa, such as 1987 and 2005, involving multiple virus lineages and different vector species. The latter undoubtedly reflect continent-wide abnormal rainfall patterns favoring transmission. Where sustained YFV activity across sequential years is evident (pattern 4), this has been associated with movement of virus activity, generally from south to north, and involvement of urban (*A. aegypti*) transmission in West Africa, with the result that transmission continues through the dry season. In East Africa, YFV activity may persist in a pattern of up and down years across a short interval, for example in Sudan (2003, 2005) and Ethiopia (1961, 1962, 1966), but here sylvatic vectors have been responsible for transmission, transmission slows or stops during the dry season, and the intensity of transmission across years is highly variable.

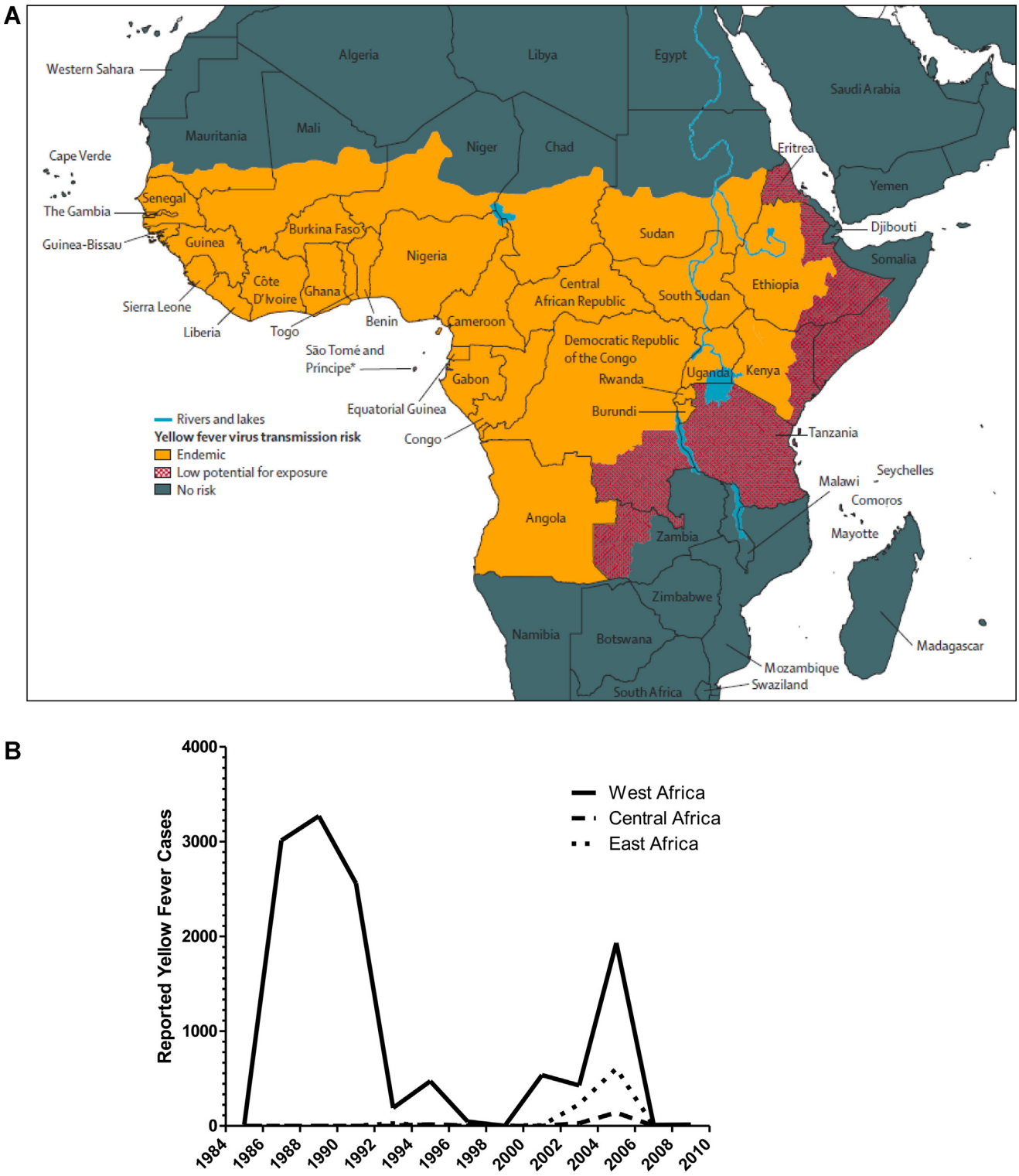

Fig. 3. (A) Map of Africa showing the area considered endemic for YF (yellow) and having low risk of exposure (red based on the absence of human case reports and minimal data for transmission), from Jentes et al. [4] with permission. (B) Yellow fever (YF) cases officially reported, by region in Africa 1985–2009, showing the preponderance of reports from West Africa. However, very large epidemics have occurred in East Africa in the remote past (e.g. 300,000 cases in Ethiopia in 1961–1962), and there have been recent notable outbreaks in Uganda (2010), Sudan (2003, 2005, 2012–2013) and Ethiopia (2013).

The annual reporting rate in Africa has varied widely, with the virtual absence of reported cases in some years to over 5000 cases. The CFR in Africa is quite consistently around 20%, though this is likely an underestimate, since patients with severe YF are frequently removed from hospitals by relatives, so that the outcome of disease is not recorded. In Nigeria, during the upsurge of YF in the early 1990s, the incidence (based on official reports) was 3–4 per 100,000. During large outbreaks in countries with smaller populations, such as Liberia (1995) and Guinea (2000), incidence rates are 10–14 per 100,000. Where direct epidemiological investigations

of epidemic locales have been conducted, for example in Nigeria (1986–1987) and the Gambia (1978–1979), attack rates of 3000 per 100,000 were noted [1].

In the last decade, YF epidemic activity has occurred in Burkina Faso (2004), Côte d'Ivoire (2001–2003), Ethiopia (2013), Ghana (2003), Guinea (2000–2001, 2005), Liberia (2000–2001, 2004), Mali (2004), Senegal (2002–2003, 2006), southern Sudan (2003, 2005, 2012–2013), Togo (2006), and Uganda (2010). Starting in 2008, an increased number of cases have been reported from Central African countries such as the Central African Republic, Congo, and Chad. Many of these countries have infrequently reported YF cases in the past and it is unclear if the recent reports in these countries are due to improved surveillance or increased disease activity either locally or an extension from neighboring areas. In 2010, an epidemic was recognized in East Africa (Uganda) for the first time in 15 years. This signaled the beginning of YF re-emergences in neighboring countries, Sudan and Ethiopia in 2013 [16].

Surveillance has been enhanced in Africa, starting in 2002, when a network of national laboratories was established to diagnose cases, and a system of case investigation put in place. This system has identified "outbreaks" (defined as one or more confirmed cases) and led to vaccination campaigns designed to control spread. With improved surveillance, reporting and the availability of laboratory diagnosis, estimates of an endemic burden of 130,000 [17] to 200,000 [18] cases of YF in Africa, are open to question. The surveillance efforts in both West and Central Africa have annually turned up a few thousand cases meeting a broad case definition of fever with jaundice, of which only 1–2% have laboratory evidence for YF infections [19]. Interestingly, a 1970 study in which active surveillance for hospitalized cases with fever and jaundice conducted in an inter-epidemic period in Nigeria, found only 1% of such cases with laboratory evidence for YF infection [20]. A recent study of patients with viral hemorrhagic fever syndrome and hepatitis in 18 hospitals in central and northern Ghana (2009–2011)—an area with repeated, fluctuating YF virus activity in the past—turned up no cases of YF [21].

The vectors and associated ecological patterns of YFV transmission vary across regions of Africa. Central Africa, the presumed evolutionary origin of the virus [22], is characterized by rain forest. Yellow fever virus is maintained in a primary cycle involving tree-hole breeding vectors (*Aedes africanus* and a closely related species, *A. opok*) and non-human primates. Humans may become infected, generally by exposure to vectors that have acquired infection from monkeys, although inter-human transmission may also occur. The force of infection in these cycles is relatively low, accounting for the relative paucity of human cases. In West Africa and in parts of East Africa, a different condition prevails, linked closely to the forest-savanna ecotone where other tree-hole breeding anthropophilic *Aedes* reach high densities during the rainy and early dry seasons. In West Africa, a variety of vector species, including A. furcifer-taylori, *A. luteocephalus*, *A. metallicus* (as well as *A. africanus*) amplify the virus. Non-human primates play a role in transmission, particularly in gallery forests, but humans may become the dominant host. *A. aegypti* is generally present also, and transmission shifts to this domestic vector as the dry season takes hold, a transition that was clearly shown in the Gambia in 1978–1979 [23]. Virus spread from the forest-savanna ecotone to moist savanna and dry savanna may occur as a continuum or in a saltatory way by means of rapid movement of viremic humans, with *A. aegypti* becoming the dominant vector in dry areas with water stockage in domestic containers providing ample breeding sites. Explosive *A. aegypti*-borne outbreaks in such conditions are well described, e.g. Senegal (1965), in Mali and western Nigeria (1987), northern Ghana (1970s) and northern Nigeria (1990s). In contrast, in East Africa, *A. aegypti* is not anthropophilic and does not appear to play a role in transmission. The principal vector in both delimited and very large outbreaks is *A. bromeliae* (a member of the *A. simpsoni* complex), which breeds in tree holes, banana leaf axils and other natural sites [3].

A number of investigators have compared partial or complete genome sequences of African strains of YFV. The general conclusions from these studies have been quite consistent, showing (i) that the South American virus genotypes are derived from West African strains and diverged around the time of the slave trade [10]; (ii) that there are 5 major lineages (genotypes) in Africa representing discrete but overlapping geographic regions of the continent; (iii) that the East African genotype is the most divergent; (iv) that there is considerable micro-heterogeneity so that within a region, a number of clades may be distinguished; and (v) that phylogenetic clock models show dispersal at discrete intervals within fairly recent time. The 5 main African lineages are designated West Africa I (or West/Central) representing strains from Nigeria, Cameroun, and Gabon; West Africa II (or West), representing strains from Senegal, the Gambia, Guinea, Ghana, and Cote d'Ivoire; East Africa, representing strains from Uganda and Sudan; East/Central Africa, representing strains from Central African Republic, Democratic Republic of the Congo, and Ethiopia; and Angola (Fig. 4). The common ancestor of all lineages emerged (by various estimates) between 700 and 1200 years before the most recently defined genotype [10,22,24]. The West and East African lineages appear to have diverged by dispersal from Central Africa, and the outlying lineages (e.g. West Africa II and East Africa appear to have arisen more recently, indicating an outward dispersal of the virus. Thus the West African I lineage shows more heterogeneity than West Africa II [25]. Where multiple strains have been examined over time in the same region [26], the data support the concept that YF virus circulates in discrete foci, with periodic emergences limited by accessible corridors containing vectors and hosts.

Relatively few emergences of YF have been characterized with respect to the genotype involved in transmission, generally because virus strains are not preserved for study. However, the existing molecular data allow some insights into YF epidemiology. For example, two lineages co-circulate in some areas, for example Burkina Faso, where the West Africa II and West Africa I viruses were associated with outbreaks in 1983 and 1985, respectively. West Africa I isolates have appeared occasionally as far west as Cote d'Ivoire and Senegal. A major emergence of YF in 1987 was associated with large concurrent outbreaks in Nigeria and Mali caused by two distinct genotypes (West Africa I and II, respectively), indicating coincident factors favoring transmission in two distinct foci. In East Africa, both East and East/Central strains have emerged in Uganda and Sudan. In Sudan, for example, the 1940 outbreak in the Nuba Mountains was associated with the East/Central lineage. The outbreak in 1959 which spread to neighboring Ethiopia (1961) was also likely caused by this genotype. The Ethiopian outbreak expanded in 1962, with over 300,000 cases, the largest epidemic ever reported in Africa. However, the 2003 and 2005 epidemics in Southern Sudan were caused by a different lineage (East Africa). The 2003 outbreak occurred in Eastern Equatorial State, bordering the East African country of Uganda. The long interval between these events in Sudan and shifting genotypes may indicate that YF is periodically introduced from separate foci, possibly in Uganda [3].

2.1.2.1. Factors associated with yellow fever emergence. A recent review, details the several factors that were associated with reemergence of yellow fever in Brazil in areas previously free of virus circulation were revised [2].

2.1.2.2. Factors implicated in yellow fever emergence. A severe and prolonged rainy season is associated with an abundance of vectors, and may be linked to enhanced YFV circulation. Clear examples occurred in Nigeria in 1987 and in the Goiás State (Brazil) in 2000. In

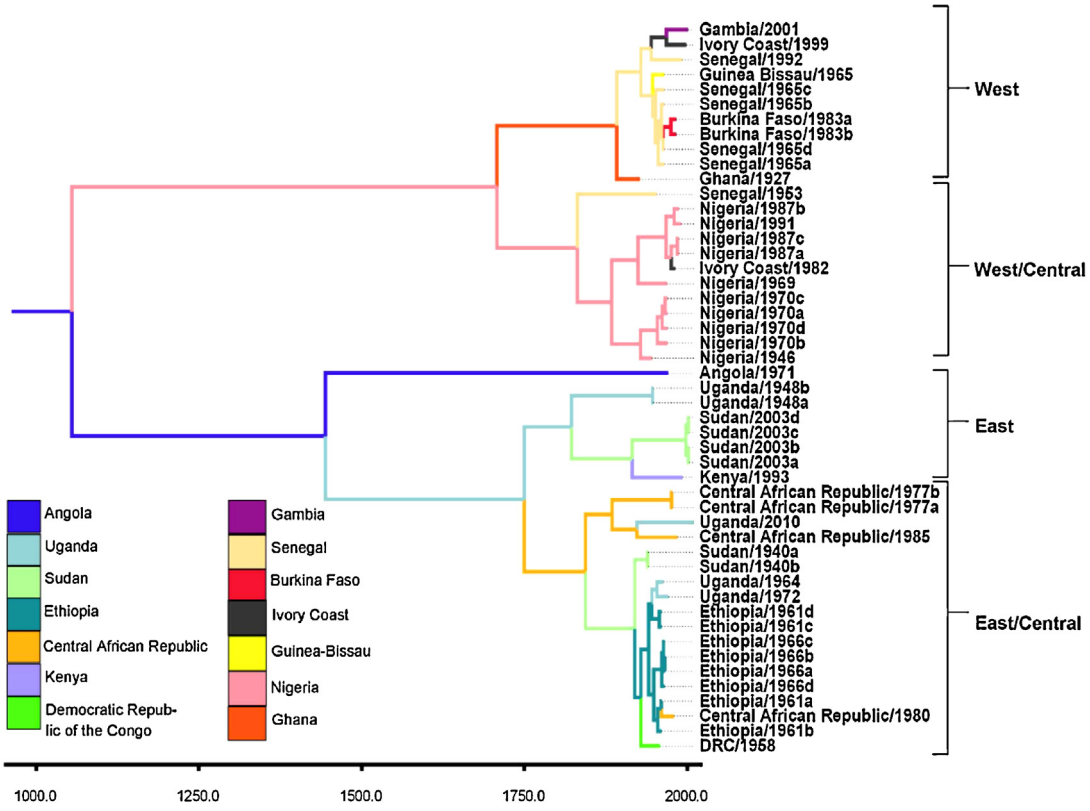

Fig. 4. Phylogenetic relationships of African strains of yellow fever virus, from Beck et al. [22], with permission.

Brazil, excessive rainfall and an increase of two degrees centigrade in average temperature were followed by an impressive number of epizootics with many monkey deaths reported, and almost simultaneous occurrence of human cases [27]. Similar rainfall aberrations were responsible for the spread and amplification of YFV in São Paulo State [6] and Rio Grande do Sul State in 2007 [14b].

Deforestation for land use, especially agriculture and cattle grazing, have been associated with emergence of YF outbreaks due to the resulting ground-level biting activity of canopy-dwelling vectors, and this is important to infection in new settlements inside or near the forest. Deforestation related to lumber for exportation has also resulted in an emergence of outbreaks in endemic areas. A recent study conducted by PAHO showed a strong association of deforestation and land use with places with appearance of YF cases [11].

Colonization of areas within the endemic zone by unvaccinated migrant populations has accounted for a high proportion of YF cases in South America. In Peru, in the 1990s, a marked increase in YF cases was directly associated with migration from coastal or mountainous areas to the Amazon region. Vaccination programs in Peru in recent years have been aimed at increasing coverage in non-endemic bordering provinces that were the source of population movements into the Amazon region.

In Brazil, a new lineage of YFV, genotype 1D, was responsible for all cases reported between 1998 and 2000. The genotype was isolated simultaneously in the north (Pará State), northeast (Bahia) and southeast (Minas Gerais and São Paulo) and the distance between Pará and the southeast states is more than 2000 km, distances too large to be explained by movement in monkey populations. The authors proposed that migrant viremic humans carried the virus to new areas where YFV established local epizootic transmission [13a].

Autochthonous cases of YF have never been reported in places 2300 m above sea level in South America (Fig. 5), and this is due to the incapacity of the YF vectors to survive above this altitude [4,11]. Low-lying rain forest, the forest-savanna ecotone, and savanna are the most important ecosystems associated with YF in both South America and Africa. These ecosystems are rich in river basins and riverine (gallery) forest habitat for non-human primates, and exposure to tree hole-breeding YF vector species to YFV [28]. In Africa vector density and longevity in the moist savanna and gallery forests reaches highest levels during the late rainy season and early dry season.

Finally, it important to emphasize that the complex interactions between virus, vector, host, weather and the environment remains only superficially understood, largely due to the senescence of longitudinal field research programs. Cyclical expansion and retraction of YFV circulation was documented during long-term studies in West Africa (eastern Senegal); continuation of such studies would present opportunities to define the underlying factors for emergence.

2.2. Advances in basic virology

Yellow fever was the first flavivirus to have been studied by genomic sequencing. In the last decade, there have been extraordinary advances in our understanding of the flavivirus genome and translated proteins, tertiary structure, replication, and assembly of new virus particles (for excellent reviews, see Refs. [29,30]). Differences at the molecular level between virulent YFV and the attenuated 17D vaccine strain are partially understood. These subjects are beyond the scope of this review, but pertinent aspects will be mentioned in the sections below.

Direct studies on pathogenesis of YF in humans are limited and much of our knowledge is derived from comparative medicine studies in experimental models [31,32]. Wild-type YFV is primarily viscerotropic, with liver being the most affected organ; however,

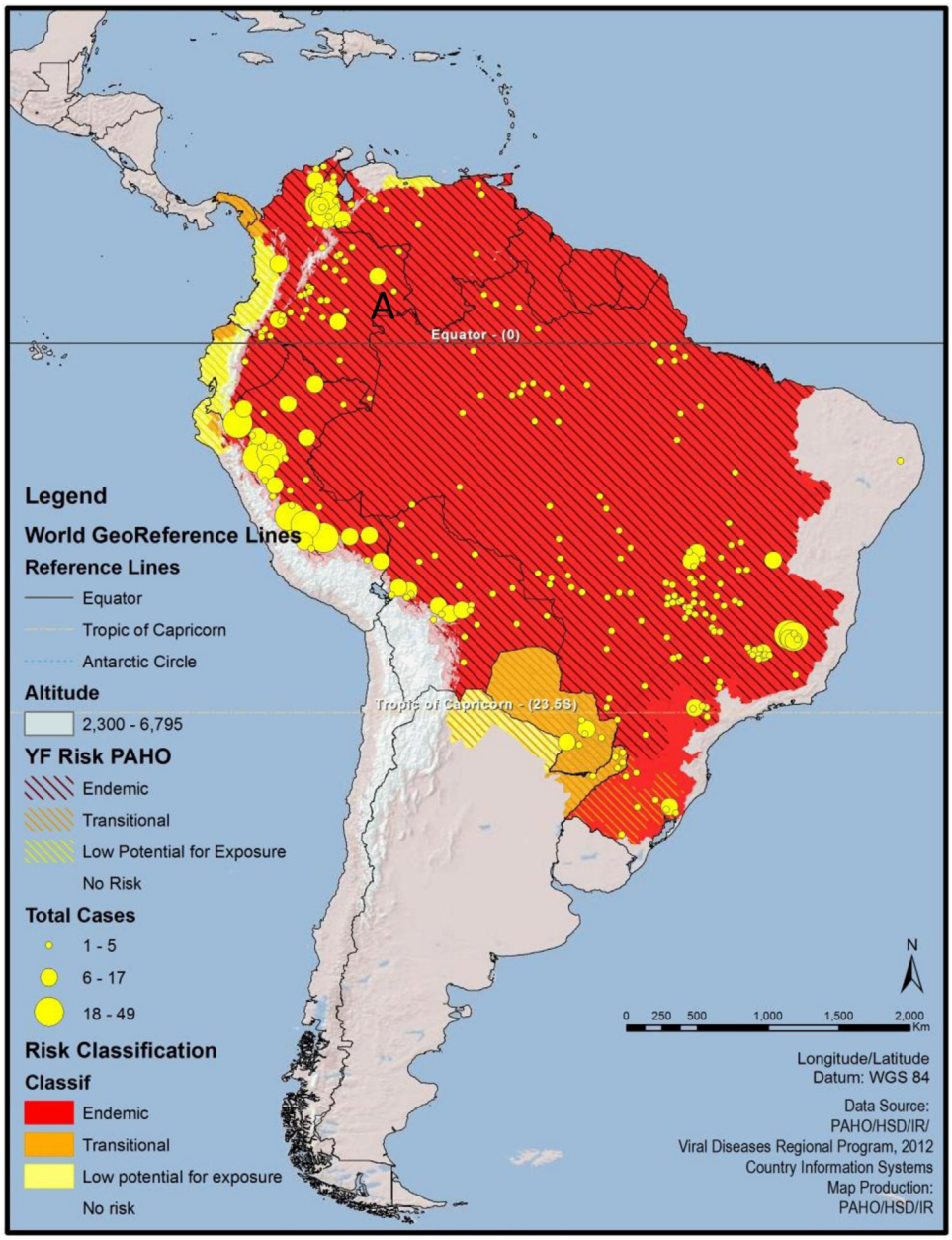

Fig. 5. Map of South America showing the endemic region for yellow fever virus transmission (in red) and the geographic distribution of cases. Source: Pan American Health Organization, 2013. Cases are found in areas below 2300 m altitude, predominantly at the western fringe of the Amazon and Orinoco river basins.

the kidney, spleen, lymph nodes and heart, and probably other tissues are also injured by YFV.

As for many other infectious diseases, YF infection presents with a broad spectrum of severity, with clinical presentation ranging from asymptomatic (inapparent) infection to fatal disease. The ratio of inapparent to apparent infection was estimated in field studies in Africa to approximate 7–12:1 [1]. The prototype Asibi strain of YFV isolated in 1927 is a highly virulent strain whereas the 17D vaccine derived from Asibi, is highly attenuated, although it retains the capacity to replicate and, more importantly, induce an immune response (see Section 2.4). An interesting and intriguing observation is the isolation of naturally attenuated strains, obtained from patients with wild-type YFV infections in Brazil. Recently a comparative experimental study in golden hamsters (an animal susceptible to lethal infection with YFV) demonstrated that these strains cause minimal liver damage when compared with strains having high virulence (Martins LC, Vasconcelos PF et al., 2014 – unpublished information). Similarly, older studies in non-human primates with unmodified YFV strains showed remarkable differences in virulence [33]. These observations suggest that virus strain differences in virulence could influence clinical presentation, but host factors and in particular prior immunity to heterologous flaviviruses represent other possible explanations.

It has been hypothesized (Vasconcelos, P, unpublished) that after multiple cycles of mosquito-human transmission, the virulence of YFV for humans is increased. This hypothesis was based on the observation that disease severity appears to be higher after an outbreak or epidemic is established in a region, after human involvement in the transmission cycle may have occurred. This observation could also be due to improved case detection (counting of milder cases) late in an outbreak. In future epidemiological studies, it will be important to establish case detection methodology

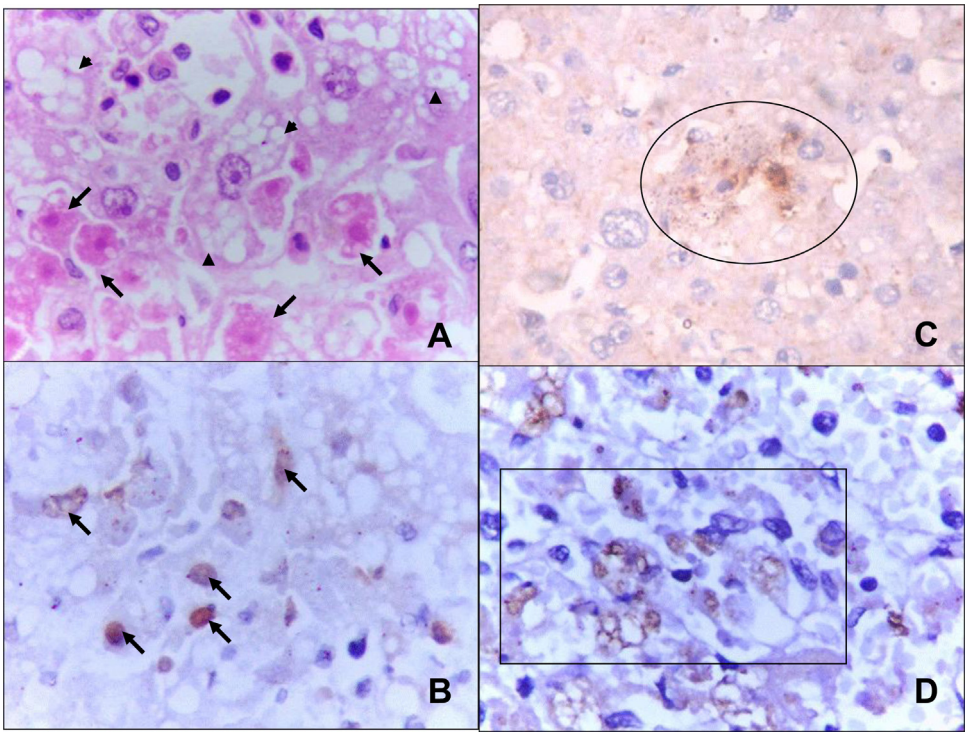

Fig. 6. Human liver in fatal yellow fever. (A) Councilman bodies (arrows) and steatosis (arrowheads) stained by hematoxylin and eosin; (B) Immunohistochemical staining showing hepatocytes in apoptosis (arrows) which correspond to Councilman bodies in A; (C) Apoptotic hepatocytes expressing FAS/APO-1 (circle) by immunohistochemical assay; (D) Immunohistochemical assay showing TGF-β expressed in hepatocytes (rectangle). Figure magnification (400×). Microphotographs' are courtesy of Dr. Juarez Quaresma.

early, to assess case-fatality rates early and late in an epidemic, and to develop sensitive assays for biological characterization of recovered virus strains without confounding effects of laboratory passages.

The lethality and severity of hepatic disease is associated with YF virus load detected in the blood. This has been demonstrated in the hamster model [34]. The development of real-time PCR, has improved the feasibility to quantify titers of YFV in both experimental and natural infections. Nevertheless there are few data on quantitative viremia levels in humans with yellow fever, and such data would be exceptionally valuable in assessing the potential for vector infection, the relationship between virus load and disease outcome, and correlations between viremia and markers of viral virulence.

In the last decade, Quaresma and colleagues focused on the role of the host immune response in pathogenesis [32,33–39] including details of in situ response in livers from of fatal YF cases. Apoptosis is the main mechanism of cellular death in severe yellow fever. The transforming growth factor beta (TGF-β), a potent apoptotic inductor and anti-inflammatory cytokine prominent during the course of disease, is probably responsible for the induction of apoptosis and for down regulation of inflammatory infiltrates, a feature observed in the livers of fatal cases despite the extensive area of liver tissue infected and showing necrosis.

Immunostaining of tumor necrosis factor alpha (TNF-α) and interferon gamma (IFN-γ) is found in livers of fatal YF cases, but was less evident than TGF-β [36]. The immune response is prominently Th1 oriented and the pro-inflammatory cytokine IL-6 is also markedly expressed. The inflammatory infiltrate is typically constituted by CD4+ helper lymphocytes and CD8+ cytotoxic lymphocytes, and also by NK cells with rare neutrophils and plasmocytes [37]. Importantly, the cellular inflammatory response is more intense in the portal tract followed by the midzone and is scarce in the central vein area, and the presence of antigens in the liver cells is a strong signal of a direct cytopathic (apoptotic) effect of YFV on hepatic cells, which results in damage to hepatocytes and Kupffer cells [38].

Apoptosis in affected liver tissue is much more prominent than necrosis, and as previously mentioned, is induced by TGF-β and expressed by FAS/FASL [36,37]. Different figures of apoptotic cells representing different stages of the apoptosis process are observed in the microscopic field [35,36]; it is noteworthy that apoptotic cells correspond to the well-known histopathologic feature of YF, Councilman bodies, representing hepatocytes that have under gone eosinophilic degeneration (Fig. 6).

Previous immunity to other flaviviruses. A great mystery in the epidemiology of YF is why this disease has never circulated in Asia, despite the continent having all epidemiologic elements to maintain both jungle and urban transmission. One hypothesis is that cross-immunity to other flavivirus, particularly dengue, could preclude establishment of YF virus in Asia. Some evidence for this hypothesis was obtained in a study of non-human primates immunized to dengue and subsequently challenged with YFV [40]. More recently, Xiao and colleagues re-investigated this hypothesis in hamsters infected with one, two and three different flaviviruses (JEV, SLE, WNV, and/or DENV-1) followed by challenge with lethal hamster-adapted YFV; they observed protection against death and viremia associated with heterologous flavivirus immunity [41]. Epidemiological observations are supportive, including an increase in the ratio of inapparent:apparent infections in persons with preexisting flavivirus antibodies [1]. Overall, the experimental and epidemiological data support the hypothesis that antibodies to heterotypic flaviviruses may protect against YF by diminishing of the virus titers in blood, below the threshold for infection of mosquito vectors.

The clinical outcome of YF appears to correlate with the degree of liver damage, which can be measured by levels of hepatic aminotransferases. In a series of cases in Brazil, prognosis was guarded

when alanine aminotransferase (ALT) exceeded 1200 IU or aspartate aminotransferase (AST) level levels were 1500 IU or greater [7]. AST levels tend to be higher than ALT in yellow fever, the reverse of findings in viral hepatitis, possibly reflecting a degree of myocardial or skeletal muscle injury. Severe liver damage is accompanied by reduced synthesis of clotting factors, and with consumption of coagulation factors which in turn explain the hemorrhagic diathesis in yellow fever, a sign associated often with fatal outcome. Renal failure is also a hallmark of severe and fatal yellow fever, including azotemia, albuminuria, anuria and acute tubular necrosis of renal tubules. BUN levels over 100 mg/mL were associated with elevated risk of death [7]. Lymphoid tissues (spleen and lymph nodes) are likely the primary sites of replication of YFV, and histopathological damage of these tissues in humans has long been noted.

Many of these clinical observations are recapitulated by experimental data in the rhesus monkey, showing hepatocellular degeneration suggestive of apoptosis, necrosis of B cell areas in spleen and lymph nodes, renal failure which was initially prerenal (due to low flow hypoxia) followed by acute tubular necrosis, hypoxia, hypotension and shock [42].

The final stage of YF disease in humans and experimentally infected monkeys is characterized by rapid deterioration, multi-organ failure and circulatory shock, similar to other viral hemorrhagic fevers. Immune clearance of infected cells, associated with release of cytokines, might play a role in the pathogenesis of capillary leak and shock. ter Meulen et al. [43], found a pattern of elevated pro- and anti-inflammatory cytokines (IL-6, TNF-α, monocyte chemoattractant protein-1, IL1-receptor antagonist, IL-10), resembling bacterial sepsis in patients with fatal YF whereas patients surviving the disease had anti-inflammatory cytokine elevations. Given the array of immune response genes activated by the 17D vaccine (see below), it is likely that in wild-type infection a severe systemic inflammatory syndrome contributes to lethality. In the hamster model of viscerotropic YF, the mid-course of the infection when replication of virus occurred in tissues was characterized by suppression of interferon-γ, IL-2 and TNF-α and elevation of regulatory cytokines, whereas the late stage of the disease process was characterized by enhanced expression of pro-inflammatory cytokines [44a]. These findings suggested that the innate immune system was initially down-regulated in favor of the virus and that end-stage immunopathological mechanisms contributed to death.

2.3. Advances in diagnostic virology

Presently the most widespread diagnostic procedure is detection of anti-YF antibodies by IgM-ELISA. The test is performed in a few hours and provides a presumptive diagnosis of yellow fever. The limitation of this procedure is the well-known cross-reactivity among flaviviruses, including dengue [44b]. Indeed, in endemic areas immunity to of other flaviviruses is widespread. Moreover, some dengue hemorrhagic fever and dengue shock patients present with a clinical picture resembling YF. Another problem is that IgM antibody production may be low in secondary flavivirus infections. In fact, during secondary infections the IgM is only produced in the first two-to-three days and in low titers, sometimes in the threshold of detection (cut-off) of IgM-ELISA. Finally, following YF infections, IgM may persist for long periods, up to a year or more, and is not a reliable marker of a recent YF infection.

The development of molecular tools has significantly advanced the diagnosis of YF and the ability to distinguish severe infections caused by wild-type virus vs. the 17D vaccine strain. The high sensitivity and specificity of the RT-PCR to YF underlies current approaches to YF diagnosis. Quantitative real time RT-PCR (qRT-PCR) Sybr green and TaqMan based protocols and an isothermal method based on helicase-dependent amplification technology constitute major advances in rapid and more accurate diagnostic approaches [45–47]. These protocols can detect YFV in clinical specimens at a low virus concentration (~1–10 virus particles). The protocol developed by Nunes et al. [47] can be also used to amplify YFV RNA from paraffin embedded samples. Since histopathological examination represented the primary means of diagnosing YF in South America, the use of PCR now opens the possibility to perform phylogenetic studies on archived specimens to describe the phylogeographic and evolutionary history of YFV.

Recently a LAMP RT-PCR test was used to detect YFV genome in the field, since this methodology did not use sophisticated devices for amplification. RNA amplification and genome detection is based on very simple and colorimetric method without complex machines and is performed in a previously defined temperature in a microtube [48]. Another recent paper has established an alternative protocol for detection of YFV in a region lacking capacity for freezing samples or during field investigation; the approach is based on recombinase polymerase amplification (RPA) assay, and can be performed real-time with or without a microfluidic semi-automated system and lateral-flow assay. Sensitivity and specificity were good, and the approach promises to be useful for YFV detection in remote areas in the endemic region, especially Africa [49].

NS1 antigen (which is secreted from infected cells) may be detected in acute-phase blood using ELISA methods. Preliminary results have demonstrated high sensitivity and specificity and good predictive values (Vasconcelos et al., 2014 – in preparation). This approach has previously been validated for the diagnosis of dengue and represents an advance in rapid and early diagnosis of YF.

Additional, new tools for diagnosis based on the nanotechnology and biochip approaches certainly will be developed in the near future to improve diagnostic of YF and other infectious diseases.

Despite the array of molecular tools for detecting YF RNA, it is important to attempt virus isolation if adequately preserved specimens are available. Recovery of infectious virus permits the assessment of biological attributes of virus strains, assessment of virulence in experimental models, and infectiousness for and transmission by mosquito vectors. In general two different systems have been used to isolate YFV: cell culture and suckling mice (or hamsters). Several cell lines are susceptible to YFV, but, Vero cells and C6/36 *Aedes albopictus* cells are most widely used. YFV replicates abundantly and quickly, with resultant cytopathic effect. The detection of YFV is done by several approaches but immunofluorescent staining by monoclonal antibodies is preferred. Suckling mice inoculated by the intracerebral (IC) route are highly susceptible to YFV; animals usually develop signs of encephalitis in the first week after inoculation.

2.4. Advances in prophylaxis and therapy

2.4.1. Vaccines

The history of development of YF vaccines has been extensively reviewed [1]. The live, attenuated 17D vaccine was developed in 1936. Two different substrains are used to manufacture vaccines (17DD in Brazil, and 17D-204 in all 5 other manufacturers), reflecting different passage series. All vaccines are manufactured as extracts of infected chicken embryos to the same standards [50]. There are no significant differences in safety or immunogenicity across 17D vaccines. Yellow fever 17D is widely used for the prevention of yellow fever in travelers, for routine immunization of infants in endemic areas, for catch-up campaigns in Africa, and for emergency response during outbreaks. Twenty to 60 million doses are distributed annually.

The 17D virus retains a degree of neurovirulence demonstrable by IC inoculation of laboratory mice of all ages, which develop fatal encephalitis and of rhesus or cynomolgus macaques, which develop minimal symptoms but consistent histopathological changes in brain and spinal cord. Infant mice are more susceptible

on a dose–response basis, than adult animals. Neurotropic adverse events are rare in humans following vaccination [occurring at a rate of between 0.2 (Europe) and 0.8 (US) per 100,000] [51,52] presumably because the 17D virus causes very low, transient viremia [with peak titers <2.0 $\log_{10}$ plaque-forming units (PFU)/mL], insufficient to cause neuroinvasion, and because the latter, if it occurs, generally causes subclinical infection. Young infants with an immature blood-brain barrier are more susceptible to neuroinvasion and 17D encephalitis, and the vast majority of the adverse events seen in the early years after introduction of the vaccine were in infants less than 7 months of age; in 1960 recommendations were made contraindicating vaccine use in infants ≤6 months of age.

The sites of replication of YF 17D vaccine in humans may be inferred from studies in non-human primates, and include skin at the site of inoculation, but principally lymphoid and reticuloendothelial tissues [53]. Virus is found in the blood of vaccines during the first week after inoculation, but RNA genomes may persist in blood during the second week. There are recent reports of finding YFV RNA in urine for 6 months or more after vaccination [54], suggesting that persistent infection or expression of viral genes may contribute to the remarkably durable immune response to 17D vaccine. A single report of recovery of YF 17D virus from nasal secretions in a subject who had a respiratory tract symptoms 8 days after 17D vaccination [55], suggests that virus may be shed as a result of active infection of mucosal tissues or transudation from serum. There are no reports of direct contact spread of 17D virus, but no specific studies of this phenomenon have been performed. However, the wild-type YF virus is known to have cross-infected monkeys in animal facilities, suggesting the possibility of aerosol infections.

2.4.2. Immune responses to 17D vaccine

Yellow fever 17D vaccine is given in a volume of 0.5 mL by the subcutaneous route in a dose of no less than 1000 International Units [50], based on an international reference standard, which equates to approximately 4.0–5.0 $\log_{10}$PFU, depending on the laboratory performing the potency assay. This is a substantial excess of the dose required for effective immunization, based on dose-ranging studies which indicate that >90% of subjects given doses as low as 100–200 PFU develop neutralizing antibodies [1]. Not surprisingly, Roukens et al. reported that 0.1 mL given by intradermal injection (1/5th dose) induced a similar antibody response compared to the standard dose given subcutaneously [56]. Such dose sparing methods or dilution of vaccine could be an important measure in an emergency, where vaccine shortages might limit containment of an epidemic, but require more robust data than are currently available. A large dose–response study is in progress in Brazil, but results have not been reported as yet.

Yellow fever 17D vaccine elicits a rapid, exceptionally strong, and markedly durable (essentially life-long) adaptive immune response, and has attracted the interest of basic immunologists who wish to understand the basis for such responses, and vaccinologists who have harnessed 17D as a vector for foreign genes. Neutralizing antibodies are directed to a small number of highly conserved conformational epitopes present in the envelope (E) glycoprotein, some of which have been mapped by means of escape mutants. A recent study of human vaccine sera reacting with a set of recombinant proteins found that complex quaternary epitopes on the E protein surface contributed predominantly to neutralization [57], consistent with similar findings for dengue viruses.

Since neutralizing antibody is the accepted mediator of protective immunity against YF, antibody responses have long been the focus of study. Antibodies are found in nearly 90% of subjects by day 10 (when the certificate of immunization for travel becomes valid), and in nearly all subjects by day 30 [1]. The level of neutralizing antibodies has been measured using both constant serum-varying virus (log neutralization index, LNI, the log reduction in virus titer by minimally diluted serum) and constant virus-varying serum (plaque-reduction neutralization) assays. The methodology is relevant to the question of the seroprotective level of neutralizing antibodies. In a study in rhesus monkeys actively immunized with 17D, it was determined that an LNI of ≥0.7 correlated with protection against challenge with virulent virus [58]. Human subjects developed a mean LNI of 2.2 [8], indicating a power of neutralization approximately 30-fold higher than that needed for protection. Passive immunization is a more reliable means of dissecting the role of antibodies in protection. In the hamster model of YF immune or control serum was administered 24 hours before lethal challenge. Full protection was observed in animals with passive antibody levels of ≥40 by $PRNT_{50}$ and partial protection in animals with passive titers of 10–20 [59]. In laboratory workers requiring yellow fever vaccination, a $PRNT_{80}$ ≥40 has also been used as a minimal protective level indicating the need for re-vaccination [60]. In addition to neutralizing antibodies targeting E glycoprotein epitopes, antibodies against the YF nonstructural protein NS1 may also play a role in protection, but little is known about anti-NS1 immune responses of humans following primary immunization with 17D.

Yellow fever 17D vaccine provokes a rapid and strong innate immune response, preceding the adaptive response, including NK cells [61], interferons and multiple interferon-stimulated genes, the inflammasome, and the complement system. Activation of antiviral molecules, early activation of a balanced Th1/Th2 CD4+ response, and CD8+ T cells lead to rapid containment of and recovery from 17D infection in normal subjects. The strong activation of innate immunity probably explains why 17D vaccine generates such strong and durable immunity [62]. A large array of genes are up-regulated and innate immune sensors activated following 17D inoculation, including Toll-like receptors (TLR2, 3, 7, 8 and 9), RIG-I and expression of proinflamatory cytokines such as IL-6, IL-12, TNF, IP-10, MCP-1, RANTES, type I interferons [63–65]. These molecules likely underlie the mild systemic reactions seen in the first week after vaccination in many normal subjects. The early predominantly pro inflammatory cytokine responses in the first week after vaccination change to a mixed pro-inflammatory and regulatory cytokine pattern in the second week [66]. It is not surprising that some cases of viscerotropic adverse events, caused by a runaway infection with 17D virus (described below), are associated with defects in innate immune antiviral responses.

Because of the strong immunological memory induced by 17D vaccine, and a preponderance of evidence suggesting that immunity is life-long (reviewed in Ref. [67]), the requirement for booster doses of YF 17D has been questioned. Considerable effort, expense and vaccine supplies are expended on revaccinations in endemic countries without evidence that waning immunity is associated with vaccine failures. The outcome of a recent analysis was removal of the recommendation for revaccination at 10 year intervals after primary vaccination in endemic areas [67]. However, the 10-year revaccination remains in place for travel under the International Health Regulations. It should be noted, as a precaution to the conclusions described above, that primary vaccine failures of up to 10% occur in children [68] and that immune response to 17D in this age group is lower than in adults, indicating that there could be a benefit of revaccination in endemic or epidemic circumstances particularly for persons immunized in infancy. Moreover a study in laboratory workers suggested that antibody titers may wane below putatively protective levels over time [60], suggesting that persons with occupational exposure to wild-type virus may benefit from revaccination.

In addition to neutralizing antibodies, robust T cell responses are evoked by 17D vaccine. T cell responses appear within the first week after immunization, including CD4+ T cells with mixed Th1/Th2 phenotype, CD8+ cytotoxic T cells and, subsequently

central memory CD45RA+ T cells. T cell responses are both broad (representing a diverse oligoclonal repertoire of cells) and long lasting [69]. A high percentage of CD4+ and CD8+ T cells are activated and functionally cytotoxic, peaking between day 11–14 after immunization [46] and then subsequently differentiate into polyfunctional memory cells [70]. Memory CD8+ cells, together with preformed antibody, contribute to protection against exposure to wild-type YFV, and blunt the immune response to revaccination [60].

2.4.3. Vaccine safety

A revolution of thinking about the safety of 17D vaccine occurred after the publication in 2001 of 7 cases (6 fatal) of acute multi-organ failure resembling wild-type yellow fever caused by overwhelming infections with 17D virus (yellow fever vaccine associated viscerotropic disease, or YEL-AVD) [71]. Over the next 10 years, a total of 65 cases were recorded [1], with a high CFR of 63%. Retrospective studies have documented the occurrence of YEL-AVD as far back as 1973 [72]. Case definitions for YEL-AVD have been recently revised [73]. Fortunately YEL-AVD is rare. The reporting rate of YEL-AVD in the US has been estimated at 0.4 per 100,000 overall, but is higher with advancing age, reaching 1.0–2.3 per 100,000 in persons over 60 years [51,52]. Similar rates have been estimated during mass campaigns in areas of Brazil in populations without previous vaccination [74]. Despite the higher reporting rate in the elderly, severe disease and deaths have been more frequent in young persons and in women [75]. The lethality of these adverse events is higher than for wild-type YF disease, reflecting the fact that many patients affected had underlying conditions associated with immune dysregulation. These events are believed to be due to host susceptibility rather than 17D mutations causing an increase in virus virulence. Risk factors for YEL-AVD (in addition to advance age) include thymus disorders/thymectomy, auto-immune diseases (reviewed in Ref. [1]) and genetic factors. Elderly subjects have a delayed antibody response and prolonged viremia following 17D vaccination [76], possibly indicating some acquired impairment of innate immune responses.

The most intriguing aspect of the pathogenesis of YEL-AVD in young subjects without acquired risk factors is the possibility that rare genetic defects in innate immunity are responsible. One young adult patient with YEL-AVD was found to have polymorphisms in two oligo adenylate synthetase (OAS) alleles encoding a critical enzyme in type 1 interferon-mediated innate immunity [77]. Biological plausibility comes from studies in mice showing that susceptibility to flavivirus infection maps to a mutation in an OAS gene, and association of single nucleotide polymorphisms in OAS genes in humans associated with increased susceptibility to West Nile disease [78]. Another YEL-AVD patient was found to have polymorphisms in the innate immune effector for RANTES and was also heterozygous for the CCR5-Δ32 mutation [79a]. Biological plausibility is again based on the observation of increased susceptibility to West Nile disease associated with CCR5 deficiency and CD4+ dysfunction [80]. However, other patients with YEL-AVD have been studied without finding genetic defects, and far more needs to be done to clarify the basis for severe 17D susceptibility.

Yellow fever vaccine associated neurotropic disease (YEL-AND) has been known since the 1940s [79b]. Approximately half to two-thirds of cases are manifest by meningitis or encephalitis attributed to neuroinvasion and direct viral injury, and the remainder have clinical or radiological evidence for demyelinating inflammatory syndromes (Guillain Barré or acute disseminated encephalomyelitis), which likely have auto-immune basis, possibly triggered by preceding neural infection. Individual case reports describe a wide variety of other neurological and ocular syndromes temporally associated with 17D vaccine. Neurological adverse events are rare, possibly slightly more frequent than viscerotropic disease, with an overall reporting rate of 0.8 per 100,000 in the US. As for YEL-AVD, reporting rates of YEL-AND are higher in persons over 60 years (1.6–2.3 per 100,000) [51,52]. The prolonged viremia and delayed antibody response in the elderly has been mentioned earlier as a possible factor in neuroinvasion [76]. There is a single case report of fatal encephalitis in a patient with HIV/AIDS, who was severely immunosuppressed. The most discriminating diagnostic method is examination of the cerebrospinal fluid for YF IgM antibodies, which reflects local synthesis of antibody in the central nervous system. Both neurotropic cases and those with auto-immune features (signs of demyelination on neuroimaging) often have IgM in the cerebrospinal fluid [1]. Case definitions and assessments of causality have been developed, and are available in a WHO report [81]. Recovery is the rule, deaths are rare (<1.0%), and neuropsychiatric sequelae are thought to be unusual, but systematic long-term follow up studies have not been performed. A recent case that drew international attention in the media was characterized by a rapid onset of fever and severe, persistent delusionary manic psychosis [82]. The authors are aware of a few other puzzling and complex organic brain syndromes temporally associated with YF 17D vaccination but not reported or published, and increased vigilance for such events is encouraged.

Recent reports have documented yellow fever 17D virus transmission, with resulting YEL-AND in 3 newborn babies breast-fed on mothers who had been recently vaccinated [83,84]. The interval between maternal vaccination and onset of symptoms in the infants was 3–4 weeks. Secretion of flaviviruses in breast milk in humans or animals is known for other flaviviruses (West Nile, tick-borne encephalitis) and the orogastric route of infection of YFV has been documented in nonhuman primates [33].

2.4.4. Antiviral treatment

A specific treatment for YF and YEL-AVD would have considerable value, but none are available. In experimental models, interferon may prevent disease if administered within 24 h of infection (reviewed in Ref. [85]). Administration of interferon-α (or possibly intravenous globulin of US origin containing YF antibodies) would be a reasonable post-exposure prophylactic treatment in an unvaccinated laboratory or hospital worker accidentally exposed to YFV or blood of an acutely ill (potentially viremic) YF patient if administered within ~24 h. In the hamster model, recombinant adenovirus expressing interferon-α protected animals against lethal YFV challenge when administered intranasally up to 2 days after challenge [86]. As is the case for interferon, passive administration of antibodies may prevent disease only if given in a very short window after infection.

A long list of novel small molecules, as well as RNAi, has shown activity against YF and other flaviviruses *in vitro* or in animal models, but none are available clinically. Ribavirin (1-β-D-ribofuranosyl-1H-1,2,4-triazole-3-carboxamide), a purine nucleoside, is active against YFV *in vitro*, but at relatively high concentrations. In studies in nonhuman primates, ribavirin treatment was not effective in prolonging survival (reviewed in Ref. [85]). In the hamster model, however, ribavirin initiated at a high loading dose (80 mg/kg) followed by daily doses of 40 mg/kg and initiated up to 120 h after infection showed reduced mortality and hepatocellular dysfunction [87]. There is no clinical experience with use of ribavirin or other active antiviral compounds in yellow fever or YEL-AVD.

In a retrospective analysis, patients having YEL-AVD accompanied by shock were found to have improved survival when treated with stress-dose corticosteroid (200–300 mg per day) [88]. The clinical experience is insufficient to determine whether stress-dose steroids have clinical benefit in this setting. However, as discussed previously the shock phase in YF is likely to have an immunopathological basis, involving an exaggerated

pro-inflammatory cytokine response [89]. Aside from stress-dose corticosteroids, other modalities to counteract cytokine storm and shock, including extracorporeal cytokine filters, activated protein C, and angiotensin II receptor blockade have not been investigated.

3. Prospects for the future

The risk of introduction of YFV to receptive (*A. aegypti*-infested) areas of the world remains a major concern. This risk is enhanced by urbanization and the juxtaposition of the endemic zone in South American coastal areas with unvaccinated populations. The large scale vaccination of previously unimmunized adults in these areas with YF 17D vaccine is precluded based on the risk of rare but serious adverse events. Since YF is a dramatic disease in its full-blown clinical presentation, it is likely that it would be recognized quite quickly after an introduction, but even a small event in highly vulnerable populations such as India or SE Asia would signal considerable alarm. Under such circumstances, there would be an emergency need for vaccine. Fortunately the WHO retains an emergency stockpile, but nevertheless a substantial shortage of vaccine supply can be easily envisioned. To address this problem, dose sparing techniques could be applied, as it is well known that the current vaccine formulations contain approximately 100-fold excess virus than needed for effective immunization [1]. Definitive clinical studies are needed to establish whether a simple reduction in volume of injection could stretch vaccine supplies without compromising effectiveness.

Vaccine safety remains a concern, especially for elderly persons, those with contraindications or precautions, and under the circumstances of mass vaccination, as described above. An inactivated YF 17D vaccine was developed in the US and tested clinically [90], but not pursued commercially. Such a vaccine would likely elicit relatively short term immunity. However, the inactivated vaccine could be boosted with live 17D vaccine, providing a strategy for avoiding adverse events while inducing durable responses. Efforts to develop an inactivated or recombinant subunit vaccine are in progress in Brazil. While market forces are not particularly favorable, these efforts are clearly needed in the long-term.

Experimental studies in non-human primates should be a priority to clarify aspects of disease pathogenesis which are impossible to obtain from human patients. The priority should be to define the role of systemic inflammatory syndrome (cytokine storm) in the pathogenesis of YF, the detailed role of in situ action of cytokines like TGF-β, TNF-α, IFNγ, and IL-6 in liver and other affected organs, and to investigate possible means of intervention. Such studies are directed not only at understanding how wild-type YFV causes fatal outcomes, but is also directed at the future clinical management of cases of YEL-AVD. It is likely that other viral hemorrhagic fevers have similar pathways and those studies in the YF monkey model, which can be performed under BSL3 conditions, could shed light on generalizable pathophysiological mechanisms.

Great strides have been made in developing rapid, early diagnostic methods that enable surveillance for YF. The establishment of laboratory-based surveillance linked to reactive control measures has been in place for many years in some countries [90]. The next decade should see improved diagnostic methods come into wider use in endemic regions, and it is likely that large scale epidemics can thereby be averted by early intervention. Continued progress in vaccinating populations in endemic areas of Africa will be made, including integration of YF 17D vaccine into programs of routine infant immunization in an expanding number of countries in Africa [91]. These efforts, which have included prioritization, based on risk modeling [92] and surveillance for adverse events, and confirmed a good safety profile in Africa [93], will diminish the likelihood of epidemic disease. Because YF is a zoonosis the effort must be sustained. Finally, whereas long-range field studies on YF ecology, an area of great interest from the 1930s to the 1980s, have virtually ceased, directed studies are now being performed under the guidance of WHO to elucidate the risk of YF in areas of epidemiologic uncertainty; examples include recent studies of YF risk in the Central African Republic, Cameroun, and Rwanda.

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Competing interests

There are no competing interests

Ethical approval

No approvals were required.

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