Molecular epidemiology of yellow fever virus

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ABSTRACT

Despite a safe and effective vaccine, there are still approximately 200,000 cases, including 30,000 deaths, caused by yellow fever virus (YFV). The last 25 years has seen the classic studies on YFV extended by the use of molecular techniques. A total of seven genotypes of the virus have been identified, five in Africa and two in South America. Extensive genetic studies have been used to refine the epidemiology of YFV and been applied to investigations of outbreaks and epidemics. Overall, these studies have greatly aided public health and will continue to make important contributions in the future.

Key words: yellow fever, flaviviruses, molecular epidemiology, genetic variation

1. Yellow fever virus

Yellow fever virus (YFV) is the prototype virus of the family Flaviviridae that takes its name from the latin for yellow (flavus). The virus is a member of the genus Flavivirus that contains 67 human and animal viruses. Initially, the genus was divided, on the basis of plaque reduction neutralization tests, into eight serocomplexes and 17 viruses, including YF virus, that were antigenically distinct from other members of the genus (1) and subsequent nucleotide sequencing of a region of the nonstructural protein NS5 gene of most flaviviruses has resulted in identification of genetic relationships that closely follow those of serological relationships (2). YFV is closely related to nine other flaviviruses (Banzí, Boubouí, Edge Hill, Jugra, Saboí, Potiskum, Sepik, Uganda S and Wesselsbron). Interestingly, none of these nine viruses are found in South America and Sepik virus, found in New Guinea, is the most closely related to YFV.

YFV was first isolated in 1927 by French and American researchers in Africa, who isolated the strains French viscerotropic and Asibi, respect-
tively. Strain Asibi was isolated in Ghana, West Africa while the French viscerotropic virus was isolated from a Syrian man, Françoise Mayali, in Senegal. During the 1930s both of these wild-type strains were used to empirically derive live attenuated vaccines known as the French neurotropic vaccine and 17D, respectively. The first South American strain, JSS, was isolated in Brazil in 1935.

Despite a very effective live attenuated 17D vaccine, YFV remains a major public health threat. The World Health Organization estimates approximately 200,000 cases occur each year, with 30,000 deaths. However, only 10% of these cases occur in South America, in part due to extensive vaccination coverage, yet case fatality rates from South America have been reported as high as 50% (3), but these values were based exclusively on hospitalized cases.

2. Transmission cycles

Three types of mosquito vectored transmission cycles are recognized for YFV: sylvatic or “jungle” cycle [monkey-mosquito-monkey]; intermediate cycle [monkey-mosquito-human]; and urban cycle [human-mosquito-human]. Only the jungle and urban cycles have been described in South America.

The “Urban” cycle involves transmission of YFV between humans by *Ae. aegypti*, a domestic vector, which breeds close to human habitation in water and scrap containers including used tires, in urban areas or dry savannah areas. The last large epidemic of urban YF in South America was in 1928 in Rio de Janeiro {Soper, 1977 560 /id}. Significantly, *Ae. aegypti* has reinfested all tropical South American countries in the last 30 years resulting in large scale epidemics of dengue but few documented cases of urban YF. Initially, *Ae. aegypti* was considered the only mosquito vector of YFV. The jungle transmission cycle involving sylvatic mosquitoes and non-human primates was first identified in Vale do Canaã, Brazil, in 1932. Subsequently other foci of disease were identified in localities with no *Ae. aegypti* present. During a 1938 outbreak near Rio de Janeiro, YFV was isolated from *Ae. leucocelaenus* (later redesignated as *Haemagogus leucocelaenus*), *H. capricorni*, and an unidentified sabethine species but subsequent studies implicated *H. janthinomys* and *S. chloropterus* as important vectors in South America (reviewed in 4).

Unfortunately, knowledge of vector competence of YFV is limited. Many of the vectors associated with transmission of YFV cannot be colonized (e.g. *H. janthinomys*) while there have been detailed studies on *Ae. aegypti* due to the ability to colonize this mosquito. Furthermore, many studies have utilized artificial blood meals and a limited number of strains (mostly West African plus a few South American strains). Although we have a basic understanding of vector competence, it is unlikely that studies of a few virus strains in colonized *Ae. aegypti* will be representative of all YFV-mosquito interactions. First, multiple virus genotypes have been identified (see below) and the vector has been poorly studied in a number of South American countries and it is often assumed, but not proven, to be *H. janthinomys*. Clearly, there is a need to revisit vector competence of YF virus, particularly in South America.

3. Epidemiology

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 ten-fold greater than the reported number of cases. However, YF often 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. The last case in Panama was in 1974 while, in 2009, Argentina and Paraguay had their first YF cases for over 40 years, and, in 1998, the first cases of YF were reported in French Guiana since 1902. 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.

Studies conducted by the Rockefeller Foundation in South American countries (Brazil, Trinidad, and Colombia) in the 1940s and 1950s 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 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. 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. Phylogenetic studies have been used to investigate the epidemiology of YF in Brazil and Peru. Studies by Vasconcelos et al. (3,5) 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. Further, Vasconcelos et al. (6) have recently reported evidence implicating human movement as a mechanism to move YFV across large distances in short periods of time. Concurrent studies of 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 (7). 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 (8).

4. YFV Genome

The flavivirus genome is a single-stranded, positive-sense RNA, 10,500-11,000 nucleotides in length. The first flavivirus genome to be sequenced was the YF 17D-204 vaccine virus by Rice et al. (9). The genome was 10,862 nucleotides in length and is arranged into a 118 nucleotide 5’ non-coding region (5’NCR), followed by a single open reading frame consisting of 10,233 nucleotides that encodes a single polyprotein of 3411 amino acids, which is co- and post-translationally cleaved by host and virus encoded proteases the structural genes capsid (C), premembrane/membrane (prM/M) and envelope (E), and nonstructural genes NS1, NS2A, NS2B, NS3, NS4A, 2K, NS4B and NS5 respectively, and a 3’ non-coding region (3’NCR) of 511 nucleotides.
5. Genetic variation

Initial genetic studies of YFV strains used T1 oligonucleotide fingerprinting, a technique where virion RNA is digested with T1 ribonuclease that cuts RNA after G residues. Radiolabelled digested RNA is separated by 2-dimensional electrophoresis based on size and charge of fragments resulting in a characteristic “fingerprint” for each virus. Subsequent studies have sequenced small regions of the genome based on either the prM and E protein genes or the NS5/3’NCR using either direct sequencing of viral RNA using primer extension sequencing or RT-PCR.

The initial studies by Deubel et al. using oligonucleotide fingerprinting of the genomic RNA, described three variants of YF virus, of which two were found in Africa (10). Strains from Ivory Coast and Burkina Faso were very similar, but a different variant was described in the Central African Republic (10). This was the first demonstration of genetic variation between West African and East & Central African strains of YF virus. Based on an analysis of three strains (1337 [Ecuador, 1979], 788379 [Trinidad, 1979] and 614819 [Panama, 1974]), a third variant of YF virus was described in South America indicating that South American YF strains were genetically differentiated from the African strains. The different variants were designated “topotypes”, due to the geographic differences in origins of strains, and homology among the different topotypes was 70 –75%. Strains isolated in the same geographical region (Senegal and Gambia) between 1976 and 1983 were very similar (92 to 100% homology), and had 82% - 88% similarity to those isolated earlier in 1965 (11). These studies showed that geographically separate and epidemiologically unrelated YF virus strains were genetically distinct, and that YF strains evolved slowly. Furthermore, YF strains of the same topotype were identified in large areas, despite climatic variation within the areas, indicating genetic stability of the YF topotypes over time and space.

Following the publication of the YF 17D-204 vaccine virus genome, Ballinger-Crabtree and Miller (12) published the structural protein and NS1 protein gene region of the Peruvian strain 1899 isolated in 1981 and showed the sequence was related to 17D-204, but differed by 14.7%. Subsequent studies by Lepineic et al. (13) based on the partial nucleotide sequence of the E protein gene described four genetic variants (genotypes) of YF virus, of which three were found in Africa and the other was the South American strain from Peru that had been sequenced by Ballinger-Crabtree and Miller. Similar to the previous work by Deubel et al. (10), the genotypes were found in distinct geographic regions; two of the genotypes were found in West Africa and one in East/Central Africa. The YF genotypes were identified on the basis of phylogenetic analyses, and variation within partial fragments of the E gene nucleotide sequence (13). Similar to the fingerprinting studies above (10), these studies indicated very little sequence variation between YF strains isolated many years apart, further supporting the genetic stability of the genotypes over time. In addition to demonstrating genetic variation between genotypes, and genetic stability of YF strains within the genotypes, the geographic distribution pattern of the genotypes was elucidated. The West Africa Genotype I was found from eastern Ivory Coast and Burkina Faso to Cameroon including Nigeria, while West Africa Genotype II was found in western Ivory Coast and Mali to Senegal. The third genotype was found in Eastern and Central Africa, including Central African Republic, Ethiopia and the Democratic Republic of Congo (formerly Zaire).

Subsequent studies using different regions of the YF genome (14-16) confirmed the genotype classification proposed by Deubel et al. (10) and Lepiniec et al. (13). In addition, these studies showed that phylogenetic analyses based on different portions of the YF genome gave consistent results suggesting that different regions of the genome can be used to represent the entire genome of YF virus. For example, studies by Wang et al. (15) used the 5’ 1320 nucleotides of the genome,
a region from the NS4A and NS4B genes and the 3’NCR, and observed very similar phylogenetic relationships among YF strains. Mutebi et al. (16) analyzed a 670-nucleotide fragment from the prM/M &E region that included part of the prM/M gene and part of E gene. A large number of YF strains (38) from Africa were included in these studies and two additional genotypes were described, one in East Africa (East African Genotype) and the other in Angola (Angola genotype), indicating that YF virus in Africa was genetically more heterogeneous than originally reported (16). Currently, five genotypes of YF virus are recognized in Africa, each genotype circulating in a different geographic region.

The studies of Wang et al (15) were very important for understanding the molecular epidemiology of YFV. First, two genotypes of YFV were identified in South America. Strains from Ecuador [1337] and Trinidad [788379] represented South American genotype I while three strains from Peru [149, 153 and 1899] represented South American genotype II. Second, examination of the 3’NCR showed that only West African strains had a 3’NCR of 511 nucleotides while strains from Central/East Africa and South America had shorter 3’NCRs (443-469 nucleotides) due to the absence of YF specific repeat sequences (RYFs). Central/East African strains have two RYFs and West African strains have three RYFs while South American strains only have one copy of the RYF. The function of the RYF motif is not known. It was speculated that duplication of the RYF took place in West Africa subsequent to the introduction of YF virus into South America. Overall, the nucleotide sequencing studies described above identified a total of seven genotypes based on ≥9% nucleotide variation with the Angola genotype the most divergent (17-23% nucleotide sequence variation) from all other African genotypes suggesting that it diverged from other East and Central African genotypes of YF virus a long time ago and has subsequently evolved independently. Although there is extensive nucleotide variation between genotypes, there is no more than 7.6% amino acid divergence between them.

The last decade has seen great strides made in our understanding of the molecular epidemiology of YFV in South America. De Filippis et al. (17) showed that South American genotype I was found in Brazil. An analysis of 79 YF isolates collected in Brazil from the first isolate (JSS) in 1935 through to 2001 showed a single genotype (South America I) circulating in the country, with the exception of a single strain from Rondonia, which represented South America genotype II. Two clades were identified; an older clade that appears to have become extinct and another, which has become the dominant lineage in recent years. There was significant nucleotide diversity between strains (up to 7.4%), while amino acid divergence ranged up to 4.6% (6). Subsequently, a recent phylogenetic analysis showed that sequences generated from strains from 2004 and 2008 formed a new subclade within the clade 1 of the South American genotype I (18).

Overall, to date two genotypes have been 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 and Bolivia, plus some isolates from Brazil and Trinidad (8).

Examination of Genbank shows an apparent large number of genomic sequences for YFV but most are examples of the three vaccine substrains (17D-204, 17D-213 or 17DD) or isolates from cases of severe adverse events following immunization. Thus, at present there are seven genomes of wild-type isolates from Africa plus two from Trinidad. This includes strain 788379 (1979) from Trinidad (19) and strain TVP11767 from Trinidad (2009)(20) (Table 1). The latter Trinidadian strain was shown to be a member of South American genotype I while 788379 is an unusual isolate. Genomic sequencing shows that it clusters with West African strains, while T1 oligonucleotide fingerprinting (10) and partial sequencing (15) show
that it clusters with South American strains. This paradox has not been resolved but the genomic sequence is very similar to the French neurotropic vaccine strain. Thus, at the present time there is no published genomic sequence for a South American genotype II strain of YFV.

6. 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 support this hypothesis. Studies to date indicate that YFV is genetically more heterogeneous in Africa than in South America (13,16), suggesting an African origin for YF. Furthermore, West African strains are phylogenetically closer to South American strains than to East or central African strains (3,13,14), suggesting that South American strains evolved from West African strains. 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. The evolutionary history of YFV was elucidated using a phylogenetic analysis of a large YFV data set, representing 133 isolates sampled from 22 countries over a period of 76 years (21). This study estimated that the currently circulating strains of YFV arose in Africa within the last 1,500 years, emerged in the Americas following the slave trade approximately 300-400 years ago, and then spread westwards across the South American continent. These results have been supported by a recent study looking at a Bayesian phylogeographic approach that suggest a Brazilian origin for YFV in the Americas and an overall dispersal rate of 182 km/yr (95% HPD 52 - 462), with Brazil as the major source population for surrounding countries (20). Finally, a recent study based predominantly on studies of wild-type isolates in Africa and a few from the Americas has shown that YFV exhibits markedly lower rates
of evolutionary change compared to the dengue viruses, despite genetic relatedness, the same mosquito vector, and vertebrate hosts (22).

7. Concluding remarks

Following the pioneering work of Rice and colleagues who determined the genomic sequence of the first flavivirus, YFV, we have used genetic information to investigate and understand the epidemiology of YFV. Specifically, great strides have been made in using molecular techniques to investigate outbreaks and epidemics, and this has greatly aided public health. However, there is still much to be done; in particular, our understanding of South American genotype II is very limited. Finally, there is an urgent need to use our molecular tools to study virus pathogenesis. While it is clear that there are at least seven genotypes of YFV, we have no knowledge of any biological differences between genotypes. For example, differences in virulence for vertebrate hosts or vector competence, or do all genotypes use the same mosquito vectors? It is clear that there are geographic differences in mosquito hosts in Africa but is the same true in South America? Much remains to be investigated to further our knowledge of this important public health problem.

REFERENCES


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