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Fever versus Fever: the role of host and vector susceptibility and interspecific competition in shaping the current and future distributions of the sylvatic cycles of dengue virus and yellow fever virus

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Abstract

Two different species of flaviviruses, dengue virus (DENV) and yellow fever virus (YFV), that originated in sylvatic cycles maintained in non-human primates and forest-dwelling mosquitoes have emerged repeatedly into sustained human-to-human transmission by *Aedes aegypti* mosquitoes. Sylvatic cycles of both viruses remain active, and where the two viruses overlap in West Africa they utilize similar suites of monkeys and *Aedes* mosquitoes. These extensive similarities render the differences in the biogeography and epidemiology of the two viruses all the more striking. First, the sylvatic cycle of YFV originated in Africa and was introduced into the New World, probably as a result of the slave trade, but is absent in Asia; in contrast, sylvatic DENV likely originated in Asia and has spread to Africa but not to the New World. Second, while sylvatic YFV can emerge into extensive urban outbreaks in humans, these invariably die out, whereas four different types of DENV have established human transmission cycles that are ecologically and evolutionarily distinct from their sylvatic ancestors. Finally, transmission of YFV among humans has been documented only in Africa and the Americas, whereas DENV is transmitted among humans across most of the range of competent *Aedes* vectors, which in the last decade has included every continent save Antarctica. This review summarizes current understanding of sylvatic transmission cycles of YFV and DENV, considers possible explanations for their disjunct distributions, and speculates on the potential consequences of future establishment of a sylvatic cycle of DENV in the Americas.

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Introduction : Dengue and Yellow Fever Viruses

Dengue virus (DENV) and yellow fever virus (YFV) are closely related and remarkably similar in some aspects of their natural history. Both belong to the genus *Flavivirus*, family *Flaviviridae*. All of the approximately 53 recognized species of flaviviruses (Grard et al., 2010) share a 10.6 kb, single-stranded, positive sense RNA genome comprising three structural genes and seven non-structural genes; the former make up the virion and most of the latter participate in genome replication and/or opposition of host immune defenses (Harris et al., 2006). Species in this genus cluster into one of four major clades by the taxonomy of their host as well as their mode of transmission (Cook and Holmes, 2006): (i) transmitted between vertebrate hosts by mosquitoes, (ii) transmitted among vertebrate hosts by ticks, (iii) transmitted between vertebrates without any known vector (likely by direct transmission) and (iv) directly transmitted between arthropods. Both DENV and YFV cluster with the mosquito-transmitted clade and belong to a subgroup primarily transmitted by A*edes* mosquitoes. As discussed below, both DENV and YFV originated in sylvatic cycles, in Asia and Africa respectively, maintained in non-human primates and forest-dwelling *Aedes* mosquitoes, and both have a history of successful emergence into sustained transmission among humans by *Aedes aegypti*.

These extensive similarities render the differences in the biogeography and epidemiology of the two viruses all the more striking. First, YFV has established a sylvatic cycle in the Americas but remains absent in Asia; whereas sylvatic DENV has spread to Africa but has not been documented in the New World (Vasilakis et al., 2011). Second, sylvatic YFV has a long history of emerging from the sylvatic cycle into urban transmission cycles among humans, but these invariably died out (Barrett and Monath, 2003). In contrast four different types of DENV have established human transmission cycles that are ecologically and evolutionarily distinct from their sylvatic ancestors (Vasilakis and Weaver, 2008). Finally, interhuman transmission of YFV has been detected only in Africa and the Americas, whereas DENV transmission among humans has been documented on every continent save Antarctica (Gubler, 2012b). Here we review the current state of knowledge about the evolution and ecology of sylvatic YFV and DENV and the factors known to influence emergence and spread of these viruses among humans. We present possible explanations for their disjunct distributions ; in particular we consider whether the current distribution of sylvatic YFV and DENV reflect vagaries of trade and travel, constraints imposed by host immunity or vector competence, or indirect or direct interactions between the two viruses themselves. Finally we speculate on the likelihood and potential consequences of establishment of a sylvatic YFV cycle in Asia as well as a sylvatic DENV cycle in the Americas.

Transmission cycles and evolutionary dynamics of arthropod-borne viruses

Both DENV and YFV are arthropod-borne viruses (arboviruses), a group of viruses that are transmitted among vertebrate hosts by arthropod vectors and must replicate in both vertebrate and vector to perpetuate transmission (Figure 1). This cycle of alternating infection of vertebrates and arthropods imposes substantial constraints on arbovirus evolution (Weaver, 2006). Although all arboviruses possess an RNA genome and therefore have the potential for extraordinarily rapid evolution, rates of nucleotide substitution among

arthropod-transmitted viruses are often lower than those of their directly transmitted counterparts (Jenkins et al., 2002). One explanation for this paradox is the trade-off hypothesis, which proposes that mutations that enhance fitness in hosts decrease fitness in vectors and vice versa, leading to intermediate fitness phenotypes and slow rates of evolution.

If the trade-off hypothesis is correct, then release from host alternation via serial infections of a single host species should accelerate genomic evolution and lead to increased fitness the passaged host and loss of fitness in the bypassed host. To date, numerous studies have tested this prediction, using serial passage of a variety of arboviruses in vertebrate hosts and arthropod vectors in cultured cell systems and *in vivo* [reviewed in (Ciota and Kramer, 2010)]. While these studies have offered some support for the trade-off hypothesis, the results have not uniformly conformed to predictions. Moreover, phylogenetic analyses have shown that rates of evolution can differ between arboviruses that utilize different classes of vectors; for example tick-borne viruses of the genus *Flavivirus* evolve about 2.5 times more slowly than their mosquito-borne congeners (Gould et al., 2003; Zanotto et al., 1996). Indeed, even arboviruses that utilize the same hosts and vectors can show significant differences in rates of evolution; particularly salient to this review, dengue virus has an approximately five-fold faster rate of nucleotide substitution than yellow fever virus (Sall et al., 2010). Clearly, host alternation has an impact on rates of evolution in arboviruses but host alternation alone is insufficient to explain all of the variation in these rates.

If host alternation in a single cycle is evolutionarily tricky for arboviruses, emergence into a novel transmission cycle may be doubly so. Parrish et al. (Parrish et al., 2008) have pointed out that vector transmission may enhance the potential for pathogen emergence if vectors feed broadly across host taxa. On the other hand, if vectors are highly host species-specific, then opportunities to jump into new hosts will be rare. Moreover, most such spillover events will terminate in dead-end infections of a single vector or host. Thus infection of the novel host will only occur in physical proximity to vectors that feed on the ancestral, reservoir host. Onward transmission in this cycle will require a four-way balancing act among fitness in the ancestral suite of hosts and vectors and the novel host and vector system.

Despite these obstacles, it is clear that arboviruses do regularly emerge into new transmission cycles. Notable among these are YFV, well-known for its ability to move from a jungle cycle into a devastating, albeit transient, urban cycle in humans, and DENV which has emerged from an enzootic cycle on four separate occasions to establish ecologically distinct human transmission cycles. Yet for all this apparent ecological flexibility, the ancestral, sylvatic cycles of both YFV and DENV are constrained to a subset of the geographic regions where potential hosts and vectors occur. YFV does not occur in Asia and sylvatic DENV does not occur in the New World (Figure 2).

Evolutionary origins of yellow fever and dengue virus

Despite the importance of DENV for human disease, many aspects of its origin and evolution remain unclear. In particular, phylogenetic analysis of available DENV gene sequence data has only been able to resolve some aspects of DENV evolutionary history (Chen and Vasilakis, 2011). These phylogenies clearly support the hypothesis that DENV jumped from a non-human primate reservoir to humans, and that this process of cross-species transmission resulted in four sustained transmission chains in humans, creating the DENV-1 to DENV-4 serotypes that circulate in human populations today. Multiple sylvatic strains have been isolated for only two of the four serotypes, DENV-2 and DENV-4; in both of these serotypes sylvatic strains comprise genetically-distinct sister-groups to human viruses, providing strong evidence that they constitute distinct, ancestral, transmission cycles

(Vasilakis et al., 2011; Vasilakis et al., 2010b). Although humans have undoubtedly been exposed to sylvatic DENV many times, the descendants of only four cross-species transmission events remain extant.

What is less clear is where and when the cross-species transmission events that led to the emergence of human DENV took place. Although some of the earliest and best descriptions of dengue disease are from the Americas, notably that of the Philadelphia epidemic of 1780 documented by Benjamin Rush (Rush, 1789), epidemiological records indicate that these DENV likely originated in Africa and were imported into the Americas at the time of slave trade [reviewed in (Vasilakis and Weaver, 2008)]. However, the following lines of evidence suggest that the cross-species transmission events that led to the emergence of DENV took place in the forests of Southeast Asia. First, sylvatic strains of three of the four DENV serotypes, DENV-1, 2 and 4, have been isolated from primates resident in Southeast Asia, and there is serologic evidence of DENV-3 circulation in Southeast Asia (Rudnick, 1965; Rudnick, 1986). In contrast, only sylvatic DENV-2 has been detected in West Africa (Carey et al., 1971; Cornet et al., 1984; Monlun et al., 1992; Saluzzo et al., 1986b; Vasilakis et al., 2008b; Zeller et al., 1992). Second, emergence of sylvatic DENV occurred in congruence with the establishment of urban populations sufficiently large to sustain DENV transmission in Asia (Vasilakis et al., 2010b; Wang et al., 2000). Third, the incidence of dengue disease is enormous in Southeast Asia but apparently is relatively low in Africa. Fourth, serologic studies from the 1950's suggest that both monkeys and humans living within the forests of peninsular Malaysia in isolation from *Ae. aegypti* had been exposed to DENV (Smith, 1956). In this area *Ae. albopictus* may have served as a bridge vector for human infection. Although this vector is considered to play a minor role in the global transmission of DENV (Lambrechts et al., 2010), experimental studies with laboratory-based colonies indicate that it is highly susceptible to DENV (Gubler and Rosen 1976). High susceptibility to DENV has also been demonstrated for several other *Aedes* spp distributed throughout Oceania [(Rosen et al., 1985) and reviewed in (Rodhain and Rosen, 1997)]. Finally, experimental studies have revealed that *Ae. aegypti formosus*, the ancestral progenitor to *Ae. aegypti aegypti*, is relatively insusceptible to DENV and plays a minor role in the sylvatic DENV-2 transmission cycle in West Africa (Gubler et al. 1979; Bosio et al., 1998; Diallo et al., 2008; Diallo et al., 2005). Collectively, these lines of evidence support the hypothesis of an Asian origin of DENV.

The evolutionary origins of YFV are better understood than those of DENV. Comparison of nucleotide sequences of YFV strains indicates that the virus arose at a relatively early stage in flavivirus evolution, at least thousands of years ago (Bryant et al., 2007; Zanotto et al., 1996). The origins of YFV (like its domestic inter-human vector, *Ae. aegypti*) appear to be in Africa. There was evolutionary radiation of related flaviviruses in the YFV group in Africa (e.g. Uganda S, Banzi, Jugra, Wesslesbron) as well as spread of this phylogenetic group to Asia and Australia (Sepik, Edge Hill viruses) (Macdonald et al., 2010), but no members of the YFV phylogenetic group, other than the cognate virus, are known from the New World. This is important, since it suggests that YFV was likely introduced into the New World relatively recently; we discuss current understanding of the journey of YFV to the America below. The fact that only two genotypes of YF virus occur in the Americas (de Souza et al., 2010; Vasconcelos et al., 2004)), whereas at least 5 genotypes are described in Africa (Mutebi et al., 2004; Mutebi et al., 2001) also points to Africa as the evolutionary cradle of YFV (see the section on yellow fever virus in Africa, below).

Sylvatic arbovirus cycles in the Old World

Sylvatic yellow fever virus in Africa

In the great rainforests of central Africa and extending outward along riverine forests, *Aedes africanus* mosquitoes are the primary vectors of sylvatic YFV (Figure 3). Other mosquito species that have similar larval habitats and biting preferences are also involved in YFV transmission, including *Ae. furcifer-taylori, Ae. vittatus, Ae. luteocephalus, Ae. opok, Ae. metallicus*, and *Ae. simpsoni sensu latu* (including *Ae. bromeliae*) (Cordellier, 1991; Ellis and Barrett, 2008; Monath 1989). These species have in common the use of natural collections of rainwater as breeding sites, primatophilic feeding habits, and, (for many) host seeking behavior in the forest canopy. Occasional isolations of YF virus have been made from insect species that have different ecological relationships, including non-*Stegomyia* mosquitoes (*Mansonia spp.*, *Ae. dentatus*, and *Eretmopodites chrysogaster*) and even phlebotimine flies (Smithburn et al., 1949); these likely reflect opportunistic diversions from the primary cycle. Although YFV is primarily a mosquito-borne virus, *Amblyomma variegatum* ticks have been found naturally infected with the virus in central Africa (Germain et al., 1979) and are experimentally competent vectors. The significance of ticks in the ecology of YFV is unclear, but infection of ticks could represent a mechanism for persistence in nature. Moreover this finding suggests how flaviviruses that share mosquito and tick vectors could evolve to favor an alternative transmission cycle.

Most sylvatic vectors involved in YFV transmission have defied colonization in the laboratory, and consequently little is known about their vectorial competence. Our knowledge about their role in the ecology of transmission is generally limited to virus isolations made from field-collected mosquitoes. Important questions that bear on the process of spillover from the sylvatic to human cycle, such as the threshold for oral infection following a blood meal, the extrinsic incubation period prior to transmission, and the rate of vertical transmission, are largely unknown for sylvatic vector species. Adding to the complexity of vector-host relationships is the existence of multiple genotypes of YFV. While some of these genotypes appear to have geographic ranges that correspond to the predominant role of a particular mosquito vector species (for example *Ae. africanus* for the East Central YFV genotype and *Ae. simpsoni* for the East African genotype), there is no clear relationship since multiple vector species may be engaged in natural transmission cycles of each genotype. Again, the inability to perform experimental studies of sylvatic species limits our understanding of their susceptibility to and transmission of different YFV genotypes.

Interestingly, *Ae. albopictus* is not an efficient vector of YFV (Miller et al., 1989a). This stands in contrast to DENV, for which *Ae. albopictus* serves an important role as a bridging vector and occasionally as an epidemic vector when *Ae. aegypti* is absent. This failure to participate in the YFV cycle may reflect the fact that *Ae. albopictus* only invaded YFV territory (tropical America and Africa) within the last 25 years, whereas it has been sympatric with DENV in Asia during the span of dengue evolution.

Only non-human primates have been clearly implicated as vertebrate hosts in the sylvatic transmission cycle of YFV (Figure 3). This restriction of host range is probably a significant factor in constraining the genetic diversity of YFV. In general, viremias are of short duration in monkeys and apes (2–5 days), although longer viremias have been documented experimentally, e.g. 9 days in *Colobus abyssinicus* (Woodall et al., 1968). African monkeys and apes develop viremias sufficient to infect mosquitoes, but do not show overt signs of illness or succumb to YF disease. There is considerable debate about whether humans of African origin also have evolved some genetic resistance to YFV (Watts, 2001), particularly as there is some evidence that humans of African origin may be protected from severe

dengue disease following infection (Halstead et al., 2001; de la C Sierra et al. 2007). However this is a subject that cannot be resolved from any current lines of evidence. *Cercopithecus* spp. are the most important hosts in forest and savanna habitats in Africa, while in East Africa, *Colobus* spp. appear to play an important role. However, many monkey species are involved in amplification and maintenance cycles, and the virus is opportunistic with respect to its nonhuman primate hosts. Interestingly, *Galago senegalensis*, a prosimian (suborder *Lemurioidea*), appears to play a role in YF transmission in parts of East (but not West) Africa and, unlike other non-human primates, may develop fat alhepatitis following YF infection. The role of galagos in Africa appears to be limited to dry savanna regions, and may reflect a relatively recent expansion of virus activity, explaining the high level of pathogenicity in this host (Haddow, 1952).

Sylvatic dengue virus in Asia

Although human dengue disease has been recognized for millenia (discussed below), the first evidence of sylvatic DENV cycles came from studies performed by Smith and Rudnick in the 1950s and 1960s in Asia. Smith, working in Penang, Malaya, first detected DENV antibodies in arboreal monkeys; however, in the same locations, very few ground-dwelling animals were seropositive, suggesting DENV circulation in the canopy (Smith, 1956). Later, Rudnick and colleagues working in Malaysian forest reserves, distant from human habitations and without *Ae. aegypti*, detected widespread DENV-neutralizing antibodies in wild monkeys (*Macaca nemestrina, M. fascicularis, Presbytis cristata* and *P. melaphos*) (Rudnick, 1965) (Figure 3). Serosurveys of the aborigine Orang Asli tribe (forest-dwelling people with limited contact with urban populations where *Ae. aegypti*-borne dengue occurred) suggested 82% exposure to DENV, although no clinical dengue was reported in this group (Rudnick and Lim, 1986). Earlier, similar studies of aboriginal populations in areas of the Philippines outsde the distribution of *Ae. aegypti* revealed similarly high rates of DENV seroprevalence (Hammon et al., 1958), consistent with sylvatic transmission.

Subsequently, the placement of sentinel monkeys (*M. fascicularis* and *P. obscura*) in the forest canopy resulted in the isolation of sylvatic DENV-1, -2 and -4 (Rudnick and Lim, 1986). Although DENV-3 was not isolated from these sentinels, DENV-3 seroconversions suggested the concurrent circulation of all four DENV serotypes (Rudnick and Lim, 1986). Additionally, intensive mosquito sampling yielded a single DENV-4 isolate from *Aedes* (*Finlaya*) *niveus s. l.*, canopy-dwelling mosquitoes that also bite primates at ground level (Rudnick, 1986) (Figure 3). Eventually, partial genomic sequences from several of the mosquito and monkey isolates from Malaysia revealed that each occupied a basal position within its respective DENV serotype clade, consistent with the hypothesis that they represent ancestral lineages that gave rise independently to endemic/epidemic lineages represented by human and *Ae. aegypti* DENV isolates (Wang et al., 2000).

After the pioneering field studies of Rudnick and colleagues ended in 1975, little effort was made to continue studies of sylvatic dengue in Asia. However, in 2008 a Malaysian man visiting an area not far from Rudnick's field site developed dengue hemorrhagic fever (DHF) with thrombocytopenia and an elevated hematocrit. Serum samples from this patient yielded a DENV-2 isolate that grouped phylogenetically into the basal, sylvatic DENV-2 lineage along with Rudnick's 1970 monkey isolate (Cardosa et al., 2009). This was the first evidence from Asia that sylvatic DENV strains can cause human disease and the first unambiguous evidence that sylvatic DENV can cause DHF, suggesting that they can readily emerge into an urban transmission cycle with little or no adaptation to humans.

Sylvatic dengue virus in Africa

The first evidence of a sylvatic DENV cycle in Africa was discovered in the 1970s, when DENV-2 antibodies were detected in non-human primates inhabiting both gallery and lowland forests in Nigeria (Fagbami et al., 1977). Direct evidence first came in 1974, when DENV-2 was isolated from arboreal *Ae. luteocephalus* mosquitoes in eastern Senegal, in a relatively remote collection site far from human habitations (Robin et al., 1980). Later, this and other human DENV-2 isolates from West Africa associated with dengue fever were shown to be phylogenetically distinct from strains isolated from the human - *Ae. aegypti*, cycle, again occupying a basal position within the DENV-2 group (Rico-Hesse, 1990). Further evidence that non-human primates in West Africa serve as amplification hosts of sylvatic DENV was provided from serosurveys of non-human primates in eastern Senegal as well as isolation of sylvatic DENV-2 from a patas monkey (*Erythrocebus patas*) in 1981. Together these findings suggested the occurrence of successive sylvatic DENV-2 epizootics in 1974 and 1981, in the absence of detectable disease in humans residing near forest habitats (Saluzzo et al., 1986a). The 1981 epizootic was also evidenced by over 100 DENV-2 isolates from arboreal and primatophilic *Ae. taylori, Ae. furcifer, Ae. opok, Ae. luteocephalus* and *Ae. africanus* in the West African nations of Guinea, Côte d'Ivoire, Senegal and Burkina Faso (Cordellier et al., 1983; Diallo et al., 2003; Hervy et al., 1984; Roche et al., 1983; Rodhain, 1991) (Figure 3). Subsequent epizootic amplifications have been identified at roughly 8-year intervals (1974, 1980–1982–1989–1990–1999–2000 and 2008 in Senegal), punctuating silent periods in which no virus was isolated despite sampling. To date, mathematical models suggest that this periodicity in virus amplification is driven by extinctions followed, after approximately eight years, by reintroductions (Althouse et al., 2012, in press).

Phylogenenetic analysis of DENV isolates from West African monkeys and arboreal mosquitoes indicate that all occupy a basal DENV-2 clade that groups with the Asian, sylvatic DENV-2 clade (Kershner et al. 1986, Wang et al. 2000). Given that sylvatic DENV most likely originated in Asia, the West African sylvatic DENV-2 strains must be the descendants of a strain transported from Asia at least hundreds of years ago, presumably on sailing ships in the course of trade.

Although a few human infections with West African sylvatic DENV-2 strains have been identified serendipitously in eastern Senegal, no major outbreaks of disease have been associated with these viruses aside from a 1966 epidemic in Nigeria, from which several isolates of sylvatic viruses were made from humans (Carey et al., 1971). Although the vector(s) responsible for transmission during the Nigerian outbreak were not identified, susceptibility studies (Diallo et al., 2005) as well as natural virus isolations from *Ae. furcifer* (Diallo et al., 2003) and ecological studies (Diallo et al., 2012) suggest that this species is an important sylvatic vector that also serves as a bridge vector to humans in nearby villages. *Ae. aegypti* does not appear to be an important vector in eastern Senegal because, as discussed above, the ancestral forest form, *Ae. aegypti formosus*, is relatively refractory to infection (Diallo et al., 2005) and the more susceptible domesticated form, *Ae. aegypti aegypti*, does not occur in this area.

Emergence and circulation of dengue and yellow fever viruses into human transmission cycles

Emergence of Dengue Virus

Dengue virus spillover in zones of emergence—Max Germain (Germain et al., 1976) coined the term 'zone of emergence' in 1976 to describe the forest-savannah transition belt most conducive for spillover of YFV from non-human primates into humans.

Germain's description of this transition zone in Central African Republic (CAR) shares many similarities with the moist savannah habitats that surround forests in rural areas of West Africa and Southeast Asia where human infections with sylvatic DENV has been documented (Cardosa et al., 2009; Carey et al., 1971; Franco et al., 2010; Franco et al., 2011b; Monlun et al., 1992; Robin et al., 1980; Rudnick, 1986; Saluzzo et al., 1986a; Saluzzo et al., 1986b; Smith, 1956; Vasilakis et al., 2008b), as well as comparable habitats in West Africa and South America where spillover of YFV has been documented (Baudon et al., 1986; Carey et al., 1972; Germain et al., 1980; Vasconcelos et al., 2001; Vasconcelos et al., 1997). Unlike YFV spillover infections, where clinical disease is prominent, sylvatic DENV infections, as well as DENV infections in the human cycle, may often be missed due to the absence of clinical disease. In the 1981–1982 epizootic in Senegal, serological studies indicated that approximately 11% of children in the region had probably been infected by sylvatic DENV-2, yet no clinical disease was reported (Saluzzo et al., 1986a). Similarly, as described above in *Sylvatic Cycles in the Old World*, Hammon in the Philippines and Smith and Rudnick in Malaysia reported serological evidence of high levels of DENV infection in populations without contact with urban areas or *Ae. aegypti,* and without apparent dengue disease.

Nonetheless, sylvatic DENV infections that do cause disease may be misclassified as human DENV, due to erroneous assumptions about the absence of such spillover and the impossibility, at present, of distinguishing human and sylvatic lineages of the same DENV serotype with antibiody-based assays. This problem is illustrated by events that occurred in the mid-1960's in Nigeria. Previous serologic surveillance studies of febrile patients residing within the Ibadan city limits, carried under the auspices of the Rockefeller Foundation, had demonstrated that DENV circulation was endemic in the region, as demonstrated by the isolation of DENV-1 and -2 strains, which were all classified as strains from the human transmission cycle (Anonymous, 1968, 1969, 1971–1972). However, closer examination by phylogenetic analyses of 3 available strains out of the 10 strains that were isolated in 1966 (Carey et al., 1971), classified these strains as sylvatic DENV (Vasilakis et al., 2008b). Additionally, a handful of serendipitous investigations of febrile illness in Senegal that led to virus isolation and characterization and coincided with concurrent enzootic amplifications (Monlun et al., 1992; Robin et al., 1980; Saluzzo et al., 1986b), enhanced our understanding of human illness due to sylvatic DENV infection. These cases documented that clinical illness due to sylvatic DENV infection is indistinguishable from classic dengue fever (DF) due to infection with the ecologically and genetically distinct DENV from the human transmission cycle.

Two recent human cases from West Africa and Southeast Asia expand the paradigm of human clinical presentation following sylvatic DENV infection. The first report, of a Malaysian man who developed DHF due to infection with sylvatic DENV-2, was discussed above in the section on Sylvatic Arboviruses in the Old World (Cardosa et al., 2009). A year later, a similar clinical illness was observed in a 27-year old male resident in the region of Canchungo, Guinea-Bisau, coinciding with the 2008–2009 sylvatic DENV amplification in West Africa (Franco et al., 2011a). The implications of these cases are significant because they represent the first indications that sylvatic DENV human infections can present with severe manifestations of dengue disease.

Collectively, these examples provide epidemiological support that spillover epidemics of sylvatic DENV are possible in both rural and urban areas, however, such events may not be recognized either by incomplete surveillance or attribution of disease to strains from the human transmission cycle. Therefore, it is evident that comprehensive ecological and epidemiological studies are needed to assess the degree and routes of ecological contact between humans and sylvatic DENV.

Early dengue epidemics—The earliest description of dengue disease in the historical record date back to the Chin Dynasty [Common Era (CE) 265–420] as well as the Tang Dynasty (CE 610) and Northern Sung Dynasty (CE 992) (Gubler, 1997). These early records described a disease called 'water poison,' due to its association with water-associated flying insects and whose clinical manifestations indicate a dengue-like illness that included fever, rash, arthralgia, myalgia and hemorrhagic manifestations. Reports of a similar illness appeared 700 years later in the 17th Century New World (French West Indies and Panama) describing an acute illness with prolonged convalescence (Gubler, 1997). Possible evidence of the first global pandemic of dengue is provided a century later when several reports from Indonesia, Egypt, the USA and Spain describe an illness with similar clinical presentations to dengue (Bylon, 1780; Christie, 1881; Hirsch, 1883; Pepper, 1941; Rush, 1789). The significance of these reports lies in the timeline of the epidemics, starting in Batavia, present day Jakarta in 1779, gaining widespread global distribution and reaching pandemic proportions by 1788 when Benjamin Rush, provided the first formal description of dengue disease in Philadephia (Rush, 1789). DENV took approximately 10 years to circle the globe during a time of increasing global commerce aided by sailing ships.

DENV was introduced and established in the Caribbean basin via the flourishing slave trade out of Africa sometime in the early 1800s. Steadman's vivid accounts from St. Thomas refer to the disease as '*Dandy fever*' and '*the Dandy*' describing the severe joint and muscle pain associated with the disease (Steadman, 1828). As the ships sailed to neighboring ports of call so did the disease, affecting primarily the Caucasian population leaving the African population mostly unaffected, suggesting previous exposure or genetic resistance to disease. When dengue finally arrived in Cuba, its moniker had changed to '*dunga,*' which was later transformed into '*dengue*', from the Spanish '*andar en dengue*' (Christie, 1881). However it was Leichtenstern who first recognized dengue as a disease of seaports and coastal regions that would also spread inland along rivers, like the Mississippi in the United States (Leichtenstern, 1896). Furthermore, the invasion of the neotropics by the African *Ae. aegypti aegypti* mosquito vector, most likely due to the movement of people (i.e. slave trade, commerce, migration) and their water storage containers by sailing ships, facilitated the increased incidence of dengue disease (Daniels, 1908; Smith, 1956; Stanton, 1920; Steadman, 1828; White, 1934).

While commerce and invasion of a new vector dramatically altered dengue epidemiology from abrupt seasonal onset of epidemics to endemicity in the neotropics, nonetheless DENV epidemics continued to occur intermittently, but with great intensity. For example the 1922 dengue epidemic that began in Galveston, Texas spread throughout the Gulf of Mexico region, the Southern Atlantic states, and the Caribbean, and close to 2 million people were infected (Rice, 1922; Vasilakis and Weaver, 2008). World War II catalyzed DENV transmission due to the enormity of the ecologic, demographic, and epidemiologic changes that occurred. The war led to changes in water storage practices and rapid transportation of susceptible humans and vectors over long distances. These changes resulted in significant expansion of the geographic distribution of DENV and its vectors, but also brought increased awareness of the disease prompting the establishment of scientific commissions to study dengue and its etiologic agent (Hota, 1952; Sabin, 1952; Sabin and Schlesinger, 1945). While the privations experienced by Asia in the aftermath of World War II (uncontrolled urbanization and resulting inadequacies inhousing, water distribution systems, sewer and waste management) led to explosive epidemics (Gubler, 1997), no epidemics were reported in the Americas for the 20 years following the conclusion of WWII. This quiescence was attributable to the initiation in 1947 of the *Ae. aegypti* eradication program under the auspices of the Pan American Health Organization (PAHO), which was primarily undertaken to prevent urban epidemics of yellow fever, as described in the next section.

Impact of the *Aedes aegypti* eradication campaign—Experiments performed by Walter Reed and colleagues showing that YFV was an arbovirus immediately triggered efforts to control the disease via the eradication of its vector, *Aedes aegypti*. These mosquitoes are container breeders, so initial control attempts focused on larvicides to control the aquatic life stage of the mosquito (Severo, 1955). Later, fumigation with DDT was added to kill adult mosquitoes. Due to its low cost and long-range effectiveness, DDT extended the reach of the eradication campaign (Severo, 1955), resulting in a rapid and widespread depopulation of the mosquito across the Americas (Figure 4). These efforts were sustained (Camargo, 1967; Soper, 1967) for several decades, and at the height of the eradication campaign in the early 1960's, 21 countries within Central and South America and several islands in the Caribbean (Cayman Islands and Bermuda) reported *Aedes aegypti* eradication by the standards established by the Pan American Health Organization (PAHO) (Brathwaite Dick et al., 2012; Soper, 1963; http://www.scielosp.org/scielo.php?script=sci_arttext&pid=S1020-49891997000100023). The only countries where eradication efforts were not undertaken were the United States of America, Venezuela, Guyana, French Guiana, most of the Caribbean Islands and Suriname (reviewed in (Gubler, 1997)).

However the progress made toward eradication in the early part of the century began to backslide during the 1960s. The United States only initiated its own eradication campaign in 1964, which was in any case doomed to fail because of inadequate funding allocated by Congress. The US, as well as Venezuela and the Antilles, were sources of reinfestations elswhere and other countries lost enthusiasm for the program. Campaigns elsewhere lost political popularity as apathy towards vector control efforts grew (Brathwaite Dick et al., 2012; Camargo, 1967). Decreased surveillance resulted in local and controllable mosquito reinfestations, which led to unchecked territorial spread. This was compounded by evolution of resistance of *Ae. aegypti* to DDT (Camargo, 1967). Consequently, most eradication programs had been terminated on the national level by the 1970's. The failure to eradicate *Ae. aegypti* from the entire region allowed the re-infestation of regions that had achieved eradication only a few years previously (Gubler, 1989). This process continued into the 21st century, together with increasing resistance to permethrin insecticides. In present day the geographic range of *Ae. aegypti* has expanded to areas where it had never previously occurred (Duenas et al., 2009; Grech et al., 2012; Llinas and Gardenal, 2011) (Figure 4).

Dengue epidemiology in the 21st century—Reinfestation of the Americas by *Ae. aegypti* created a dramatic increase in DENV activity as well as the introduction of new DENV genotypes and serotypes (reviewed in (Araujo et al., 2009; Chen and Vasilakis, 2011; Nogueira et al., 2001; Vasilakis and Weaver, 2008)). As a result of hyperendemicity (circulation of multiple serotypes at any given time within the region), incidence of severe dengue disease (DHF and DSS) skyrocketed (Carrington et al., 2005). Currently, autochthonous DENV transmission has been reported throughout the Americas with the exception of Canada, Uruguay and continental Chile, resulting in a 4.5-fold increase in the number of total dengue cases since the early 1980s (San Martin et al., 2010). These events mirror DENV epidemiology in Southeast Asia in the 1950s and 1960s and are in part attributable to the introduction of a Southeast Asian strain of DENV-2 into Cuba, probably from Vietnam in 1981 (Kouri et al., 1983; Rico-Hesse, 1990). Rico-Hesse and colleagues have suggested that because of its increased fitness this lineage, now designated as the Southeast Asian/American genotype, may have displaced the American genotype of DENV-2 from the Americas (Cologna et al., 2005). However, there is evidence that strains of American DENV-2 may still circulate in localities of Central (Foster et al., 2004) and South America (Watts et al., 1999). Moreover, although this American genotype is considered of low epidemiological impact because of its association with mild DEN, there is evidence that several outbreaks attributed to this genotype were associated with severe

dengue disease [(Barnes and Rosen, 1974, Loison et al. 1973, Lopez-Correa et al. 1978, Moreau et al. 1973, Steel et al., 2010) ; reviewed in (Chen and Vasilakis, 2011)].

The initial occurrence of severe dengue disease in the Americas and Southeast Asia showed significant epidemiological differences. Whereas in Asia severe dengue disease occurred primarily young children, in the Americas it most commonly affect older age groups (Guilarde et al., 2008; Kalayanarooj and Nimmannitya, 2003; Koh et al., 2008; Kongsomboon et al., 2004; Lee et al., 2008; San Martin et al., 2010; Siqueira et al., 2005; Witayathawornwong, 2005). However in the past decade, a trend toward infections in younger age groups (De Rivera et al., 2008; Hammond et al., 2005) and a downward shift in the age of severe dengue disease cases (Nunes-Araujo et al., 2003; Rodriguez-Barraquer et al., 2011; Teixeira et al., 2008; Teixeira et al., 2009) has been reported for the Americas. The overall burden of dengue disease in the Americas is currently estimated to be 99 – 1,300 disability adjusted life years (DALYs) per million, depending on the spatiotemporal attributes of epidemics (Guzman et al., 2010; Luz et al., 2009; Mathers et al., 2007; Torres and Castro, 2007; Wettstein et al., 2012), imposing enormous economic burdens on national economies and on patients for whom the costs associated with DENV infection significantly exceed average monthly income [(Anez et al., 2006; Armien et al., 2008; Luz et al., 2011; Shepard et al., 2011; Suaya et al., 2009; Wettstein et al., 2012) and reviewed in (Beatty et al., 2011; Gubler, 2012b)].

Emergence of Yellow Fever Virus

Spillover in Africa—As described in detail above, YFV likely evolved at least thousands of years ago in Central African rainforests where it was transmitted between sylvatic mosquito vectors and nonhuman primates. During the rainy season in Africa, vectors such as *Ae. furcifer-taylori, Ae. luteocephalus, Ae. simpsoni,* and *Ae. vittatus* can reach high densities in gallery forests and moist savanna regions. Such sylvatic vectors can be responsible for rapid virus amplification, spillover into humans and inter-human transmission. Once the virus is introduced to humans, inter-human transmission can be sustained and amplified by the domesticated vector *Ae. aegypti*. Transmission by *Ae. aegypti* enables the virus to spread into dry habitats and urban areas where water is stored in containers. One of the clearest examples of the transition from sylvatic to the *Ae. aegypti* cycle was documented during an epidemic in the Gambia (1978–79) (Germain et al., 1980).

The forest-savanna ecotone of Africa has been called the Zone of Emergence (of YFV) by Germain and colleagues (Germain et al., 1981). In this area humans replace monkeys as primary hosts in the YFV cycle, and epidemics with a high force of infection can originate. Once the virus has been introduced to humans and *Ae. aegypti* vectors, the prevalence of human infection can reach as high as 30% of the population (Monath et al., 1980). Such outbreaks are probably relatively recent occurrences in evolutionary terms mirroring rising human population densities in rural Africa.

In contrast to the sylvatic vectors of YFV, *Ae. aegypti* is readily colonized and thus more is known about its vector competence. Interestingly, domestic *Ae. aegypti aegypti,* responsible for widespread epidemic spread of YFV in West Africa was found experimentally to be a relatively inefficient vector of the virus (Miller et al., 1989b). However, the anthropophilic nature of this species combined with its very high densities in urban areas overcomes its low vectorial capacity. The more primitive, dark form of *Ae aegypti, Ae. aegypti formosus*, is a generalist that breeds in natural containers. *Ae. aegypti formosus* feeds on animals as well as humans but can opportunistically invade the human environment. It is an even less competent vector of YFV than *Ae. aegypti aegypti* (Brown et al., 2011).

The relatively low vector competence of *Ae. aegypti* (both domestic and sylvatic forms) in West Africa may have constituted a biological filter for selection of strains of YFV with increased virulence (capacity to produce viremia) for humans. Since African monkeys had evolved resistance to clinical disease during a long period of adaptation in the sylvatic cycle, selection for viremogenic strains would not have unbalanced the natural transmission cycle. Thus the involvement of humans in YF transmission, the domestication of *Ae aegypti* vectors, and the evolution of highly virulent virus strains were probably synchronous events in the evolution of YF in Africa.

Yellow Fever Virus in the New World—Could YFV have occurred first in a sylvatic cycle in the Americas as it did in Africa? This has always appeared unlikely. The break up of Pangaea and the separation of the African and South American continents occurred about 150 million years ago during the Cretaceous period, and probably long before the evolution of YF (or dengue) viruses. The potential for introduction of virus by movement of nonhuman primate hosts or by mosquito vectors that would survive windborne spread across the Atlantic appears remote. Moreover, the first recognition of YF in the New World was in 1647–48.

The hypothesis of a sylvatic origin of New World YFV was laid to rest by Bryant et al. (Bryant et al., 2007) whose study of rates of nucleotide substitution and divergence of clades offered convincing evidence that YFV was introduced into the Americas about 400 years ago from West Africa. This genetic record is highly consistent with the historical course of the slave trade. Around the time YF was first described in the early 17th Century, the Portuguese and other European countries had begun importing a vast number of slaves from Africa to continental South America and the Caribbean to work on sugar plantations. *Ae. aegypti* vectors would likely have been introduced to the New World at about this time as well. The actual means of introduction of YFV could have been by viremic humans or by *Ae. aegypti* vectors, since YFV transmission on board sailing vessels was not uncommon. Alternatively, the virus could have been introduced on artificial containers or imported vegetation, by means of dessicated eggs laid by infected *Ae. aegypti* or sylvatic vectors.

Relatively soon after its introduction to the Americas via human agency, YFV spilled back into non-human primates to establish a New World sylvatic cycle. YFV transmission appears to be highly efficient in the sylvatic cycle, as evidenced by high viremias and rapid spread through monkey populations, whereas transmission the ‘urban’ cycle may be constrained by vector density and lower viremias in humans. For this reason, early efforts by Gorgas to remove the threat of urban YF through sanitation and *Ae. aegypti* control were highly successful. However YFV readily entered an American sylvatic cycle comprised of entirely new species of hosts and vectors. This jump is particularly intriguing because, as with West Nile virus, it was accomplished in complete geographic separation from the ancestral sylvatic cycle. Reluga et al. (Reluga et al., 2007) have proposed that repeated contact between novel hosts and reservoir hosts enhances the likelihood of pathogen emergence from the reservoir into the novel hosts, but clearly this sustained contact was not necessary for the emergence of sylvatic YFV in the Americas.

In South America, as in Africa, YFV infected a wide variety of monkey species. However, unlike African monkey species, many common neotropical species, notably howler monkeys (*Alouatta*), squirrel monkeys (*Saimiri*), spider monkeys (*Ateles*), and owl monkeys (*Aotus*), are highly susceptible to YFV infection and develop clinical disease (Bugher, 1951). Indeed, epizootics involving deaths of monkeys provide a signal of the danger of YF to humans. The susceptibility of neotropical monkeys to clinically overt YFV infection likely reflects the recent introduction of YFV into the Americas; over sufficient time the virus-host relationship may evolve to a state similar to that in the Old World. When another Old World

flavivirus, West Nile, was introduced into the Americas, it also caused illness and death in avian species that it encountered for the first time. In contrast DENV, for which there is no evidence that a sylvatic cycle ever has ever been established in the New World, does not cause clinical illness in most New World primates following experimental infection (Table 1); recent reports of clinical illness in marmosets infected with DENV strains may represent an exception to this rule (Omatsu et al., 2011, 2012).

A variety of New World mosquitoes (Figure 5) participate in the sylvatic YFV transmission cycle, including *Haemagogus albomaculatus, Hg. spegazzini, Hg. janthinomys, Sabethes·chloropterus, Sa. albipivus, Sa. glaucodaemon, Sa. soperi,* and *Sa. cyaneus*, and the virus has also been detected in *Psorophora ferox* and *Ae. serratus* in Brazil (Cardoso Jda et al., 2010; Monath 1989). In South America, the population density and biting rates of sylvatic vectors are far less than those in Africa, and the incidence of human infection with YFV (the result of infection of humans by vectors that acquired virus from monkeys) is low; the number of yellow fever cases reported from South America usually ranges from 100 to 500 per year (http://www.paho.org/english/ad/fch/im/fieldguide_yellowfever.pdf). No clear instance of inter-human transmission by *Haemagogus* spp. is recorded.

Yellow fever epidemiology in the 21st century—Today, spillover to interhuman spread by *Ae. aegypti* in South America is a rare event; the last episode involved only a handful of cases in Paraguay in 2008. There is no clear explanation why *Ae. aegypti*-borne transmission, commonplace through the 1930s has not reappeared, given the re-infestation of South America due to the senescence of vector control efforts, the growth of urban populations, the absence of YF vaccination in densely populated coastal areas, the juxtaposition of the sylvatic cycle, and the many opportunities for movement of viremic persons. The reasons are complex and probably multifactorial, including a barrier of YF vaccination in areas where sylvatic YF occurs and possible cross-protection afforded by dengue immunity.

Geographical Conundrums : Why are sylvatic yellow fever and sylvatic dengue cycles ‘missing’ from Asia and the Americas, respectively ?

Several hypotheses, reviewed below, have been advanced to explain the apparent absence of sylvatic DENV from the Americas and the well-established absence of sylvatic and human YFV from Asia.

Failure of surveillance ?

Is the absence of documented sylvatic YFV and DENV cycles in Asia and the Americas, respectively, truly evidence of an absence or simply the absence of evidence ? In the case of YFV, it is exceedingly unlikely that the virus could go unnoticed were it circulating in Asia due to the striking symptoms of human disease and the high penetrance of clinical illness in both humans and novel primate hosts. Thus we can confidently assert that YFV is absent from Asia. DENV infection, in contrast, results in a high proportion of subclinical infections in humans and the signs of infection in novel primate hosts are extremely mild (see Susceptibility of Non-Human Primate Hosts, below). Moreover there have been several studies that have suggested the presence of sylvatic DENV in the Americas (Roberts et al. 1984 ; de Thoisy et al., 2004; de Thoisy et al., 2009). However these studies are open to alternative interpretations (see section below on Potential dengue virus hosts in the America), and are counterbalanced by other studies in DENV-endemic regions of South America that have not found evidence of a sylvatic DENV cycle [(Rosen, 1958) and Watts, personal communication]. Thus additional surveillance for sylvatic DENV in both non-human primates and mosquito vectors in South America should be a high priority for dengue

researchers. Indeed, surveillance should be expanded in Africa and Asia as well, as Figure 2 is unlikely to represent the complete range of sylvatic DENV on these continents.

Accidents of history?

The process of species invasion is highly stochastic, as illustrated by the fact that West Nile virus has jumped into the New World in the past 14 years but chikungunya virus has not despite widespread availability of susceptible hosts and competent vectors (Pesko et al., 2009; Richards et al., 2010). Prior to the modern era no major trade routes connected West Africa and Southeast Asia (http://people.hofstra.edu/geotrans/eng/ch5en/conc5en/tradeflows14001800.html), perhaps explaining the failure of the strains of West African YFV strains to move into Asia despite their ready colonization of the Americas. Moreover, for reasons that are unclear, YFV has never been documented in coastal areas of East Africa, the most likely launching pad for export to Asia. While the slave trade resulted in frequent movement of .humans and cargo from West Africa to the Americas, the cyclic dynamics of sylvatic DENV transmission (discussed above) coupled with infrequent infection of humans in this area may have prevented introduction of sylvatic DENV to the New World. If these hypotheses are correct then it is only a matter of time before each of these viruses fills in the ‘gaps’ in their distributions.

Vectorial capacity of mosquitoes

Potential yellow fever virus vectors in Asia—*Aedes aegypti* is widespread in Asia, however a common hypothesis for the absence of YFV on the continent is that these Asian populations of *Ae. aegypti*, as well as other *Aedes* species endemic to Asia, lack the ability to transmit the virus (Amaku et al., 2011). A key experiment to assess this hypothesis was conducted by Gubler et al. (Gubler et al. 1982), who infected a monkey wtih a West African strain of YFV and fed populations of *Aedes aegypti* from West Africa, East Africa, Asia and the Caribbean on the animal. Viremia at the time of feeding was $10^{8.3}$ MID_{50}/ml. Rates of infection ranged from 42.1 to 96.4%, with American strains of *Aedes* showing the highest susceptibility and African strains showing the lowest. Asian strains were intermediate but nonetheless showed high levels of infection and, for one Sri Lankan strain, transmission of YFV. Thus there is no reason to believe that Asian *Aedes aegypti* would not be competent vectors for YFV.

Potential dengue virus vectors in the Americas—DENV would be excluded from establishing a sylvatic cycle in the Americas if mosquitoes that feed on potential primate hosts are not competent vectors for the virus. Little is known about the ability of New World mosquito species to be infected and/or transmit either human or sylvatic DENV (Table 2). In the early 1900s, *Culex quinquefasciatus* was implicated as a vector of human dengue by Ashburn and Craig (Ashburn and Craig, 1907), who observed a possible case of transmission by this species. However this claim was questioned by Cleland (Cleland and Bradley, 1918) who identified *Ae. aegypti* as the primary vector of DENV. Experiments by Siler et al. (Siler et al., 1926) also supported *Ae. aegypti* as the primary vector of DENV, while finding no evidence for transmission by *Cx. quinquefasciatus.* Chinese researchers have reported that *Culex* may be responsible for dengue outbreaks in China (Luo, 1993; YanDe et al., 2000), but there is little evidence for transmission of DENV by New World *Culex* species (Table 2). Parenteral injection of 10^2 MID_{50} of DENV failed to infect *Cx. quinquefasciatus* (Huang et al., 1992) as did oral feeding on an artificial bloodmeal that infected 100% of *Ae. aegypti* females (Vazeille-Falcoz et al., 1999). In the latter study *Cx. quinquefasciatus* were infected with DENV only when inoculated with very high titers of virus intrathoracically. Similarly Hanley et al. (Hanley et al., 2005) found minimal midgut infection and no evidence of dissemination of DENV in orally infected *Cx. tarsalis*.

In contrast, other New World mosquitoes, particularly *Aedes* species, do appear to be capable vectors of DENV. *Ae. mediovittatus* was deemed a likely vector of DENV-2 in Puerto Rico by Gubler et al. (Gubler et al., 1985) because under experimental conditions it was infected at higher rates then *Ae. aegypti* (27.9–74.2% vs. 3.2–43.4%) with three different strains of DENV-2. Gubler et al. (Gubler et al., 1985) also reported preliminary findings that supported transmission of DENV-1 to mice by *Ae. mediovittatus*, as well as transovarial transmission. Freier and Rosen (1988) reported remarkably high rates (≥ 20%) of vertical transmission of DENV by *Ae. mediovittatus. Oc. japonicus,* a species that originated in Asia but has invaded North America, showed high rates of infection after feeding on bloodmeals containing DENV (Schaffner et al., 2011). Freir and Grimstad (Freier and Grimstad, 1983) reported that the Texas treehole mosquitoes *Ae. triseriatus, Ae. brelandi, Aed. hendersoni,* and *Ae. zoosophus* were susceptible to DENV-1, and that *Oc. triseriatus* was capable of transmitting the virus to rabbit blood in a feeding apparatus. De Souza and Freier (de Souza and Freier, 1991) found that Central American *Hg. equinus* could be infected by DENV-1 parenterally and transmit the virus to their offspring. Finally, DENV-1 has been detected in a pool of wild *Hg. leucocelaenus* collected in Brazil, implicating this species as a potential DENV vector.

In sum, these studies suggest that the absence of sylvatic DENV from the New World is not attributable to a lack of competent vectors. In particular, *Hg. leucocelanus,* a widely-distributed (Figure 5), primatophilic mosquito, is a known vector of sylvatic YFV and seems custom-made to sustain sylvatic DENV. Clearly, however, much more experimental work is needed to fully characterize the DENV competency of vectors implicated to date, particularly in the genus *Haemagogus*, and other potential vectors such as *Sabethes* spp.

Susceptibility of non-human primate hosts

YFV and DENV would not be able to establish sylvatic cycles if no susceptible, non-human hosts were available in Asia and the Americas, respectively. However, as discussed below, this is not the case.

Potential yellow fever virus hosts in Asia

In 1928, Stokes and colleagues first demonstrated that rhesus macaques, an Asian primate species, can be infected with YFV via the bite of an infected *Ae. aegypti*, develop disease similar to yellow fever in humans, and can transmit the virus back to *Ae. aegypti* (Stokes et al., 2001). Rhesus and cynomolgus macaques have since been utilized extensively for testing the safety of YFV live-attenuated vaccines (Levenbook et al., 1987), to which they are highly susceptible. These findings strongly suggest that free-living Asian monkeys would serve as highly susceptible hosts for spill-back of YFV from humans, as in the establishment of the American sylvatic cycle of YFV, or for transmission from imported primates or mosquitoes infected via the sylvatic YFV cycle. Moreover, monkey mortality during the initial spread of sylvatic YFV in Asia would likely be high, as was the case in the Americas.

Potential dengue virus hosts in the Americas

Because non-human primates are the gold standard for evaluation of DENV vaccines and drugs (Cassetti et al., 2010), the replication dynamics and immunogenicity of a wide variety of DENV strains of each of the four serotypes have been evaluated in a number of different monkey species as well as two ape species (Table 1). Importantly, all of the DENV strains tested so far derive from the human transmission cycle, and the infection dynamics of sylvatic DENV in non-human primates has not been assessed. However given the similarity in the replication profiles of sylvatic and human DENV in various culture models for human replication (Rossi et al., 2012; Vasilakis et al., 2010a; Vasilakis et al., 2008a; Vasilakis et

al., 2007) and the ability of sylvatic DENV to generate severe dengue disease in humans (Cardosa et al., 2009; Franco et al., 2011a), it is reasonable to suppose that human and sylvatic DENV will show similar infection dynamics in non-human primates. Table 1 summarizes the pattern of viral replication, immune response, and clinical signs of infection in non-human primates experimentally infected with human DENV. We identified studies via Pubmed using the search terms «dengue and primate» and traced additional studies via the reference sections of these papers. Only studies that presented data in tabular or textual form, rather than as graphs or summary statistics, were included. Table 1 includes all such studies of New World primates, a convenience sample of studies in macaques, and all studies of other Old World primates.

In a sharp contrast to YFV, clinical signs of DENV infection were extremely rare in both Old World and New World primates, and, when detected, very mild. Viremia produced by all four DENV serotypes of was brief duration (< 7 days), similar to YFV in Old World primates. However DENV viremia was also of uniformly low titer ($< 3 \times 10^3$ pfu/ml), whereas YFV can reach extremely high titers (10^8 pfu/ml) in susceptible monkey species (Schlesinger et al., 1986). Overall, no striking differences in DENV infection dynamics between New and Old World primates were apparent. Despite the low level of DENV replication, all species of primates marshalled a robust neutralizing antibody response to infection, with most 50% plaque reduction neutralization titers ($PRNT_{50}$) >100. While at first glance low viremia produced in monkeys might seem to pose an obstacle for transmission, it is important to note that two studies have shown that monkeys infected with human DENV are capable of transmitting the virus to mosquitoes even when viremia is undetectable (Scott et al., 1980; Watts et al., 1987). Thus, there is no reason to suppose that DENV titers in the range observed in Table 1 (10–1000 pfu/ml) are inadequate for transmission.

Based on these data, it seems highly likely that if sylvatic DENV were introduced into the Americas from Asia or Africa, the virus would readily infect New World primates. Unlike YFV, sylvatic DENV would not trigger a wave of deaths as it spread among New World primates. Additionally, the similarity of susceptibility to human DENV among all primates suggests that human DENV might spill back into non-human primates in areas where no sylvatic cycle exists to establish a derived sylvatic cycle, as YFV did in the Americas.

Even if sylvatic DENV did face an adaptive barrier to infection of New World primates, there are a number of free-living populations of Old World primates that have become established in the New World that could serve as gateway hosts for the virus (Figure 6). In particular, African green monkeys (*Chlorocebus sabaeus*) have become highly abundant on various Caribbean islands. At present there are approximately 40,000 African green monkeys living on St. Kitts, similar to the number of human inhabitants of the island, and concerns have been raised about their role as reservoirs of disease (Whitehouse et al., 2010).

Finally, although there is no experimental evidence to date for non-primate hosts of DENV, it is not impossible that competent hosts exist outside the order Primates. De Thoisy and colleagues (de Thoisy et al., 2004; de Thoisy et al., 2009) reported evidence, including virus isolation, of extensive DENV infection of a variety of mammals, including bats, marsupials and rodents, in French Guiana. Phylogenetic analysis grouped these viruses closely with human isolates, suggesting a potential spillback event. However the taxonomic diversity of hosts in this study is unprecedented. Thus further investigation is required to understand the implication of the studies in French Guiana.

Competition between dengue virus and yellow fever virus

Competition between arboviruses may occur indirectly, mediated by cross-neutralization, or directly during concurrent infection. Since only vertebrates generate immunological memory, they are the host in which cross-neutralization may play a role. Conversely, since infection of invertebrate vectors is lifelong whereas infection of vertebrate hosts is usually transient, direct competition is more likely to occur in the vector. Such competition could potentially result in competitive exclusion of a virus from a particular geographic area where its competitor is established, particularly the exclusion of YFV from Asia (Sabin, 1952), where DENV has been endemic for centuries.

Indirect competition between yellow fever virus and dengue virus

A seminal study by Theiler and Anderson (Theiler and Anderson, 1975) showed that, relative to control monkeys, monkeys previously exposed to DENV showed diminished viremia and decreased mortality from a YFV infection, suggesting cross-protection against YFV by anti-DENV immunity. They concluded such cross-protection might be sufficient to block the entry of YFV into dengue-endemic regions of Asia. However a similar study by Sabin (Sabin, 1952) concluded that immunity to DENV conferred no significant protection against YFV. Moreover recent studies of live-attenuated YFV and DENV vaccines have generally found no evidence for cross-neutralization (Durbin and Whitehead, 2010; Mansfield et al., 2011), although some studies have reported atypical (Kanesa-Thasan et al., 2003) or enhanced immune responses (Eckels et al., 1985; Qiao et al., 2011) to live-attenuated DENV vaccine strains in YFV-immune individuals. Thus, although there are tantalizing hints of cross-protectione among flaviviruses in the literature, evidence to date weighs against the hypothesis that DENV excludes YFV from Asia via this mechanism.

Direct competition between yellow fever virus and dengue virus

While DENV and YFV do sometimes co-circulate among humans in Africa, e.g. (Phoutrides et al., 2011), a search of Pubmed using the terms « yellow fever, dengue, coinfection/co-infection » yielded no results, suggesting that such infections occur in regions with inadequate surveillance/reporting, that concurrent surveillance for both viruses is rare, or that co-infections themselves are rare. Sabin experimentally co-infected humans with DENV and the 17D vaccine strain of YFV (Sabin, 1952). He reported that when infection of the two viruses occurred in close temporal proximity (DENV infection concurrent with or three days following YFV infection), replication of DENV was delayed by 3–6 days and disease was attenuated If the interval between YFV and DENV infection was 1 week, DENV replication was unaffected but disease was milder ; if the interval was 5 weeks then neither DENV replication nor disease were affected. Unfortunately the dynamics of YFV 17D in these experiments was not reported.

Sabin also conducted the converse experiment in rhesus macaques, infecting them first with wild type YFV, which was lethal for control monkeys, and then with DENV. If the interval between YFV and DENV infection was less than four days, the majority of monkeys survived, when it was 4–7 days the time to death increased though the monkeys did eventually die.

Lastly Sabin tested potential competition between the viruses in *Ae. aegypti* (Sabin, 1952). He found that mosquitoes that had previously fed on DENV-viremic humans were less likely to become infected with YFV either when fed on YFV-viremic monkeys or on artificial bloodmeals spiked with YFV. He proposed that such interference might be sufficient to prevent the establishment of YFV into areas where a high proportion of mosquitoes are infected with DENV. Potential for such interference will depend on the

proportion of susceptible mosquitoes that are infected by each virus. Such a percentage has been difficult to quantify because although the absolute percentage of infected mosquitoes in a given population has frequently been measured, vector competence of that same population has rarely been tested concurrently.

What will the future bring ?

A sylvatic yellow fever virus cycle in Asia ?

Given the long history of trade between Africa and Asia, as well as the pace and volume of passenger travel by air and freight shipments by sea in the present day (Reiter, 2010), it is difficult to believe that YFV has not been introduced repeatedly to Asia, only to be 'beaten back,' possibly by indirect or direct competition from established flaviviruses such as DENV. Several lines of investigation could shed light on the mechanism for this possible competitive exclusion. The first is additional evaluation of the competence of potential Asian vectors to transmit YFV. The second is carefully-designed experimental studies of competition between YFV and DENV in the mosquito vector as well as immunologically mediated cross protection in human and other primate hosts. Vector studies will be challenging because they must rely on wild type YFV, as the 17D vaccine strain is not capable of infecting mosquitoes (McGee et al., 2008). They should utilize multiple strains of each virus as well as multiple populations of *Ae. aegypti*. Finally, intensive surveillance of human populations in which both viruses circulate may reveal immune-mediated interactions between the two, though the effects of YFV vaccination will undoubtedly complicate such studies. Immunologically mediated interference between DENV and YFV is especially worthy of study, because of the paucity of experimental data (Theiler and Anderson, 1975), the historical observations suggesting that immigrants from India (likely dengue-immune) were protected from YF disease (Monath, 1989).

A sylvatic dengue virus cycle in the Americas ?

Sylvatic DENV could be transported to the Americas in the body of an infected monkey, a vector mosquito, vertically infected mosquito ova, or a human. International transportation of non-human primates is prohibited in most countries for most species, and though smuggling certainly does occur, introduction via a non-human primate seems unlikely. The vectors of sylvatic DENV are container-breeding mosquitoes, the group of vectors with the highest likelihood of inadvertant, human-mediated introduction (Lounibos, 2002). However the most recently detected case of sylvatic DENV infection in a human was acquired in Senegal but detected in Spain (Franco et al., 2011a), so movement of the virus into the Americas in the same fashion is also quite possible. Alternatively, a sylvatic DENV cycle could be established by spillback of human DENV, as occurred with YFV.

The evidence reviewed here suggests that a newly arrived sylvatic DENV or a spillback strain would find susceptible Old World and New World primate hosts and possibly competent sylvatic vectors in the Americas, obviating the need for an extensive period of adaptation to the new transmission cycle. If we extrapolate from the effects of human DENV, sylvatic strains are unlikely to cause detectable disease in in most neotropical monkey species. Thus, unlike sylvatic YFV, whose arrival in the New World was heralded by a silencing of howler monkeys reminiscent of Rachel Carson's vision of a *Silent Spring*, establishment of sylvatic DENV would likely go unnoticed until inexplicable human infections were detected in non-endemic areas or areas lacking *Ae. aegypti* and *Ae. albopictus*. Moreover once entrenched, a sylvatic DENV cycle would be impossible to eradicate. Our best hope for preventing the establishment of sylvatic DENV in the Americas is a combination of rigorous arbovirus surveillance in populations of both Old and New World primates in areas where human and monkey activity intersects, coupled with

experimental studies of the infection dynamics and clinical manifestations of sylvatic DENV in both lineages of primates. When a DENV vaccine becomes available, vaccination could also eventually reduce the probability of sylvatic spillback. We have previously demonstrated that antibody raised in response to vaccination with live-attenuated DENV vaccine viruses in humans neutralizes sylvatic strains of the same serotype in vitro (Vasilakis et al. 2008c).

We end this review by noting that we believe that future establishment of a sylvatic DENV cycle in the Americas is a real possibility. We therefore advocate that researchers conduct the studies described above before sylvatic DENV has the chance to gain a foothold in the Americas.

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- Dengue virus (DENV) and yellow fever virus (YFV) originated in sylvatic cycles that are maintained in non-human primates and forest-dwelling mosquitoes.
- The sylvatic cycle of YFV originated in Africa and was introduced into the New World, probably as a result of the slave trade, but is absent in Asia
- The four different types of DENV have established human transmission cycles that are ecologically and evolutionarily distinct from their sylvatic ancestors
- Transmission of YFV among humans has occurred in Africa, Europe and the Americas, whereas DENV is transmitted among humans across most of the range of competent *Aedes* vectors
- There is no evidence of sylvatic DENV transmission is the Americas
- Since there is a low or no adaptive barrier for emergence of sylvatic DENV into the human transmission cycle, it is quite possible that a newly arrived sylvatic DENV or a spillback strain would find susceptible New World primate hosts and possibly competent sylvatic vectors to establish a sylvatic transmission cycle in the Americas

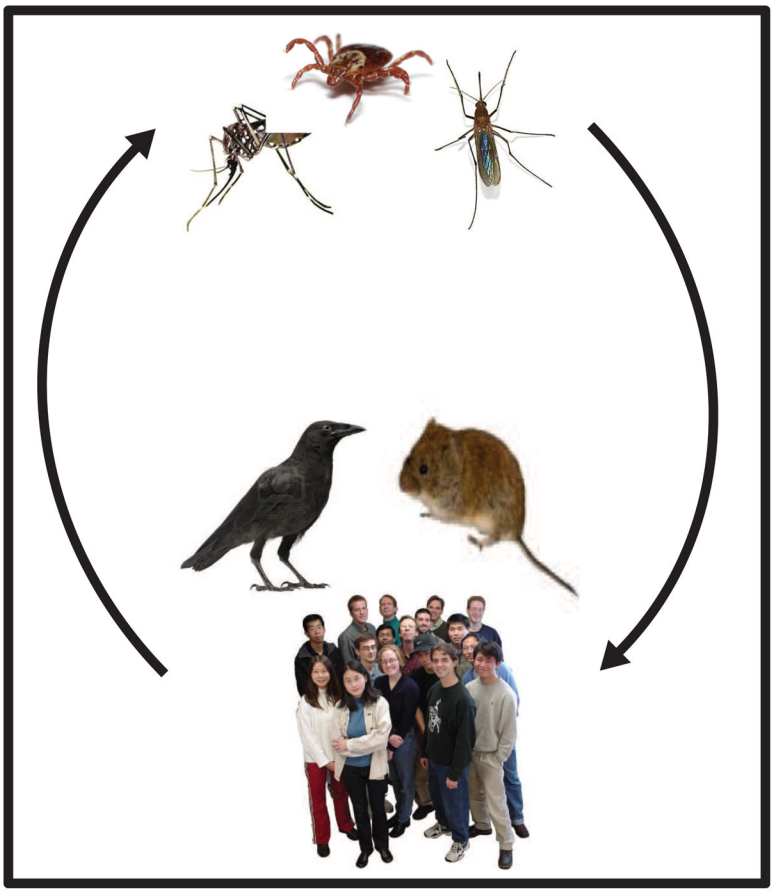

Figure 1.
The arbovirus life cycle

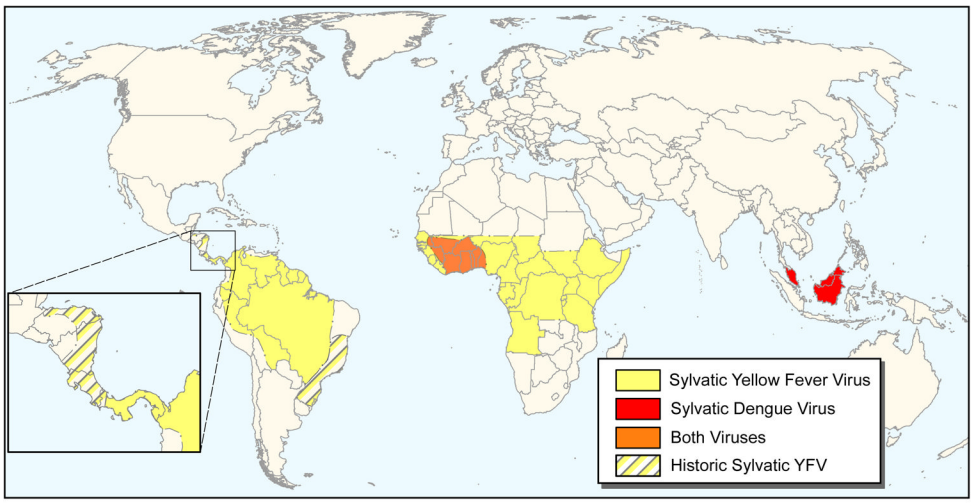

Figure 2.
Worldwide distribution of documented, contempory foci of circulation of sylvatic dengue virus and sylvatic yellow fever virus and historic foci of sylvatic yellow fever virus (From the maps of (Clements, 2012; Lepiniec et al., 1994; Vasilakis et al., 2011) and http://www.who.int/csr/resources/publications/yellowfev/CSR_ISR_2000_1/en/)).

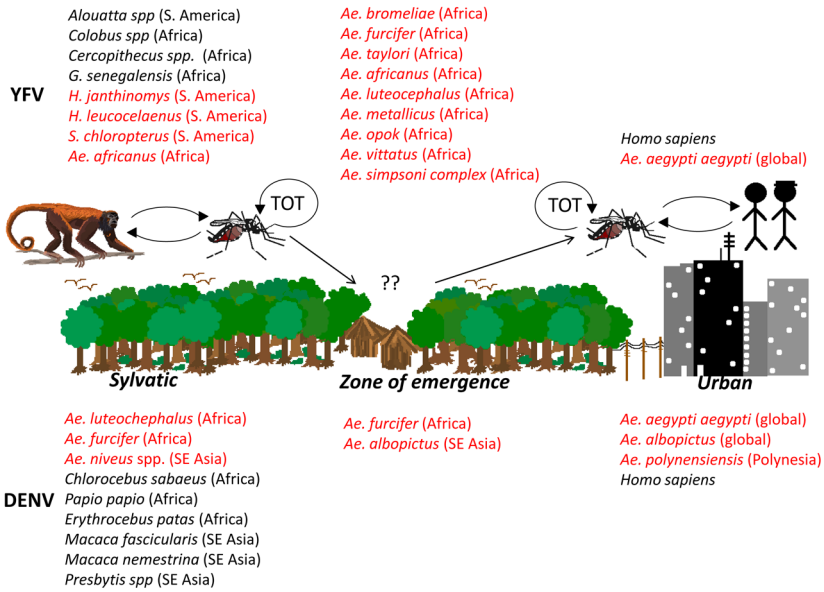

Figure 3.

Comparison of the major mosquito vectors (in red text) and primate hosts (in black text) involved in sylvatic transmission, spillover and urban transmission of yellow fever virus (top) and dengue virus (bottom).

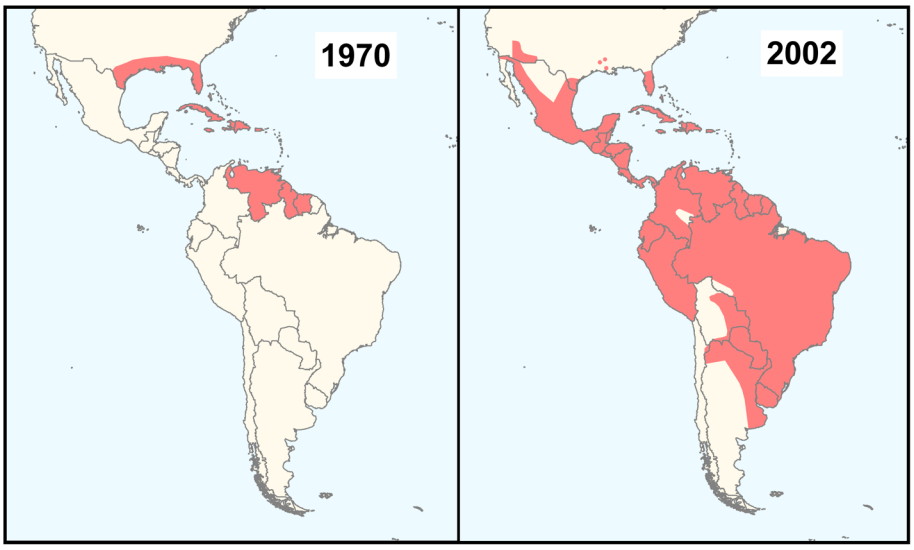

Figure 4.
Distribution of *Aedes aegypti* in the Americas in 1970, immediately following the *Ae. aegypti* eradication campaign, and in 2002, three decades after the cessation of the campaign.

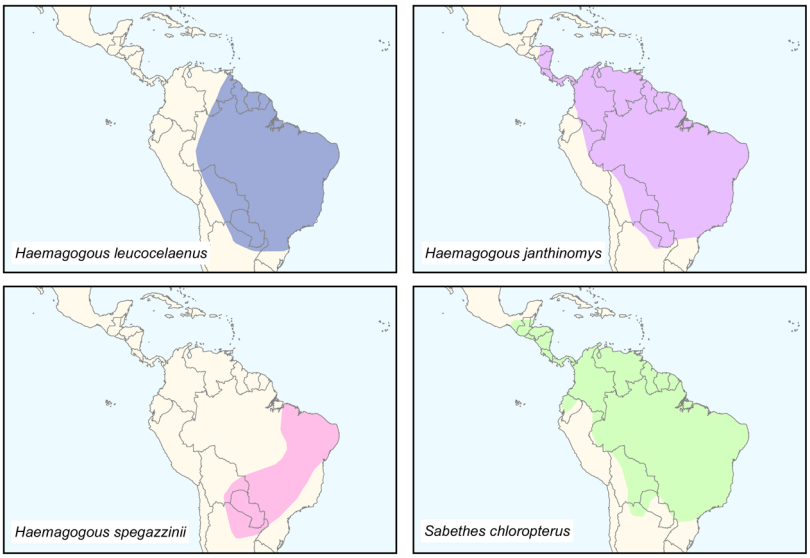

Figure 5.
Distribution of known mosquito vectors of sylvatic yellow fever virus in the New World. Data taken from http://apps.who.int/iris/handle/10665/60575

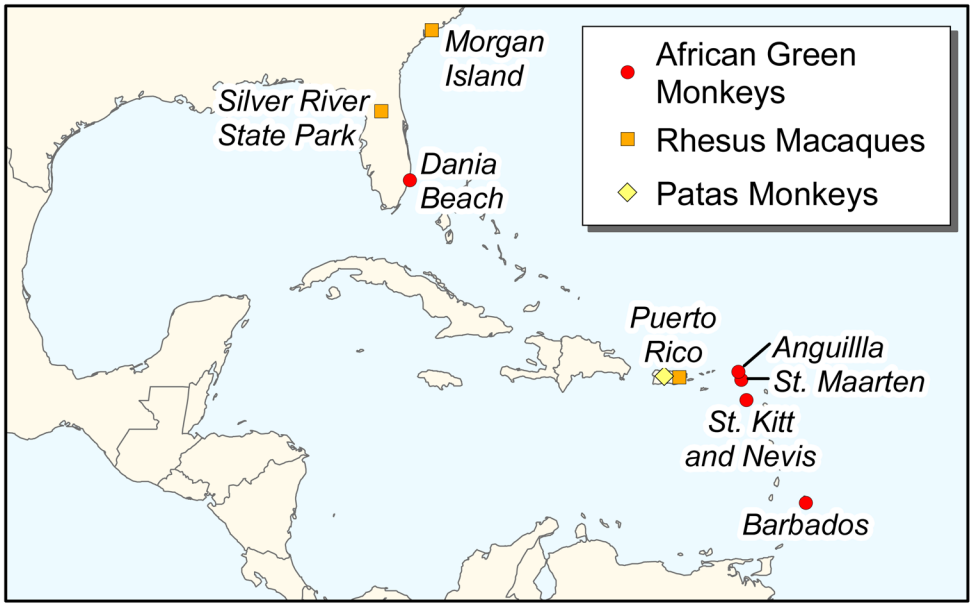

Figure 6.

Documented locations of free-living colonies of Old World non-human primates, some which represent potential hosts for sylvatic DENV, in the New World. Data taken from (Gonzalez-Martinez, 2004; Hill, 1966; Taub & Mehlman, 1989; Wolfe, 2002) and http://www.sptimes.com/2004/01/27/State/Development_evolves_t.shtml

Table 1

Replication profiles and immunogenicity of wild type dengue virus in dengue virus seronegative, non-human primates of the New and Old World.

	Species	Common name	Virus	Inoculum titer (PFU)[d]	Virus detection	% viremic	Mean no. days viremic	Max virus titer	% seroconversion [e]	Geometric mean $PRNT_{50}$ (Day PI)	Signs/Symptoms associated with infection	Ref
New World	*Aotus nancymae*	Owl monkey	DENV-1 West Pac 74	1.25×10^4	IFA	100	4.0	nr	100	453 (28)	nr	(Kochel et al., 2000)
	Aotus nancymae	Owl monkey	DENV-1 West Pac 74	1.1×10^4	IFA	70	3.7	nr	100	640 (28)	nr	(Maves et al., 2011)
	Aotus nancymae	Owl monkey	DENV-1 West Pac 74	2.0×10^4	IFA	100	3.4	nr	nr	nr	nr	(Raviprakash et al., 2003)
	Aotus nancymae	Owl monkey	DENV-1 West Pac 74	2.0×10^4	IFA	100	3.8	nr	100	nr	Extreme lethargy, loss of appetite, lymphandenopathy, splenomegaly	(Schiavetta et al., 2003)
	Aotus nancymae	Owl monkey	DENV-1 IQT6152	1.0×10^4	IFA	100	4.33	nr	100	4599.5 (21)	nr	(Kochel et al., 2005)
	Aotus nancymae	Owl monkey	DENV-2 S16803	2.0×10^4	IFA	75	1.0	nr	nr	100	Extreme lethargy, loss of appetite, lymphandenopathy, splenomegaly	(Schiavetta et al., 2003)
	Aotus nancymae	Owl monkey	DENV-3 Asian	1.0×10^4	RT-PCR	100	3.6	nr	80	73.6 (24)	nr	(Blair et al., 2006)
	Aotus nancymae	Owl monkey	DENV-3 CH53489	2.0×10^4	IFA	75	1.3	nr	nr	100	lymphandenopathy, splenomegaly	(Schiavetta et al., 2003)
	Aotus nancymae	Owl monkey	DENV-4 341750	2.0×10^4	IFA	100	1.3	nr	nr	100	lymphandenopathy, splenomegaly	(Schiavetta et al., 2003)
	Callithrix jaccus	Common marmoset	DENV-1 02-17/1	3.5×10^7	RT-PCR	100	3	5.0×10^5 vRNA copies/ml	100	nr	nr	(Omatsu et al., 2011)
	Callithrix jaccus	*Common marmoset*	*DENV-2 DHF0663*	*$3.5–4.4 \times 10^7$*	*RT-PCR*	*100*	*1.3*	*1.6×10^7 vRNA copies/ml*	*100*	*113.1 (21)*	*nr[f]*	(Omatsu et al., 2011)
	Callithrix jaccus	*Common marmoset*	*DENV-2 DHF0663*	*1.8×10^5*	*RT-PCR*	*100*	*3*	*9.5×10^6 vRNA copies/ml*	*100*	*113.1 (21)*	*nr[f]*	(Omatsu et al., 2011)
	Callithrix jaccus	*Common marmoset*	*DENV-2 DHF0663*	*1.8×10^4*	*RT-PCR*	*100*	*5*	*2.0×10^6 vRNA copies/ml*	*100*	*nr*	*nr[f]*	(Omatsu et al., 2011)
	Callithrix jaccus	*Common marmoset*	*DENV-2 DHF0663*	*1.8×10^3 pfu*	*RT-PCR*	*100*	*5*	*6.9×10^5 vRNA copies/ml*	*100*	*nr*	*nr[f]*	(Omatsu et al., 2011)

	Species	Common name	Virus	Inoculum titer (PFU)[d]	Virus detection	% viremic	Mean no. days viremic	Max virus titer	% seroconversion [e]	Geometric mean $PRNT_{50}$ (Day PI)	Signs/Symptoms associated with infection	Ref
	Callithrix jaccus	Common marmoset	DENV-2-JAM/77/07	1.2×10^5	RT-PCR	100	5	2.8×10^6 vRNA copies/ml	100	nr	*nr*[f]	(Omatsu et al., 2011)
	Callithrix jaccus	Common marmoset	DENV2-MAL/77/08	1.9×10^5	RT-PCR	100	5	9.6×10^6 vRNA copies/ml	100	nr	*nr*[f]	(Omatsu et al., 2011)
	Callithrix jaccus	Common marmoset	DENV-3 DSS1403	4.5×10^6	RT-PCR	100	1	5.5×10^4 vRNA copies/ml	100	nr	nr	(Omatsu et al., 2011)
	Callithrix jaccus	Common marmoset	DENV4-05-40/1	1.5×10^6	RT-PCR	100	1	2.5×10^4 vRNA copies/ml	100	nr	nr	(Omatsu et al., 2011)
	Saguinus midas and Saguinus labiatus	Red-handed and white lipped tamarins	DENV-2-DHF0663	6.0×10^7	RT-PCR	100	6	2.0×10^7 vRNA copies/ml	nr	nr	nr	(Yoshida et al., 2012)
Old World	*Chloro cebus aethiops* [a]	African green monkey	DENV-1 16007	1.0×10^5	Plaque assay	100	2	≤ 100 pfu/ml	nr	nr	nr	(Halsted et al., 1973)
	Chlorocebus aethiops	African green monkey	DENV-2 16681	1.0×10^5	Plaque assay	100	2	≤ 100 pfu/ml	nr	nr	nr	(Halsted et al., 1973)
	Chlorocebus aethiops	*African green monkey*	*DENV-2 SB8553*	*1.0×10^6*	*ELISA and Flow Cytometry*	*100*	*1.7*	*nr*	*100*	*16–21 (30)*	*none*	(Martin et al., 2009a)
	Chlorocebus aethiops	*African green monkey*	*DENV-2 SB8553*	*1.0×10^4*	*ELISA and Flow Cytometry*	*100*	*5.7*	*nr*	*100*	*22–46 (30)*	*none*	(Martin et al., 2009a)
	Chlorocebus aethiops	African green monkey	DENV-2 CS85.3	1.0×10^5	Plaque assay	75	1.3	nr	100	114 (30)	none	(Martin et al., 2009b)
	Chlorocebus aethiops	African green monkey	SB8540	1.0×10^5	Plaque assay	75	1	nr	100	247 (30)	none	(Martin et al., 2009b)
	Chlorocebus aethiops	African green monkey	SB8553	1.0×10^5	Plaque assay	100	3	nr	100	476 (30)	none	(Martin et al., 2009b)
	Chlorocebus aethiops	African green monkey	DENV-3 16562	$1.0 \times 10^{4.5}$	Plaque assay	100	1	≤ 1×10^2 pfu/ml	nr	nr	nr	(Halsted et al., 1973)
	Erythrocebus patas	Patas monkey	DENV-1 16007	nr	Plaque assay	100	1	“low”	100	nr	nr	(Halsted et al., 1973)
	Erythrocebus patas	Patas monkey	DENV-2 16681	nr	Plaque assay	100	1	“low”	100	nr	nr	(Halsted et al., 1973)

	Species	Common name	Virus	Inoculum titer (PFU)[d]	Virus detection	% viremic	Mean no. days viremic	Max virus titer	% seroconversion [e]	Geometric mean $PRNT_{50}$ (Day PI)	Signs/Symptoms associated with infection	Ref
	Erythrocebus patas	Patas monkey	DENV-3 16562	3.0×10^4	Plaque assay	0	na	na	100	nr	nr	(Halsted et al., 1973)
	Erythrocebus patas	Patas monkey	DENV-4-4328S	2.0×10^3	Plaque assay	0	na	na	100	nr	nr	(Halsted et al., 1973)
	Hylobateslar[b]	White-handed gibbon	DENV-1-BKM72S-67 or DENV-1-BKM117967	8.0×10^2	Plaque assay	100	3.0	nr	100	nr	no clinical illness	(Whitehead et al., 1970)
	Hylobateslar[b]	White-handed gibbon	DENV-1-BKM 72S-67 or DENV-1-BKM117967	3.5×10^1	Plaque assay	100	3.6	nr	100	nr	no clinical illness	(Whitehead et al., 1970)
	Hylobateslar[b]	White-handed gibbon	DENV-2-BKM1749	1.6×10^3	Plaque assay	100	5.7	nr	100	nr	no clinical illness	(Whitehead et al., 1970)
	Hylobateslar[b]	White-handed gibbon	DENV-3-24969	$5.0–6.6 \times 10^2$	Plaque assay	100	3.8	nr	100	nr	no clinical illness	(Whitehead et al., 1970)
	Hylobateslar[b]	*White-handed gibbon*	*DENV-4 KS16868*	*$3.3–5.0 \times 10^3$*	*Plaque assay*	*100*	*4.0*	nr	100	nr	no clinical illness	(Whitehead et al., 1970)
	Hylobateslar[1]	*White-handed gibbon*	*DENV-4 KS16868*	*6.6×10^2*	*Plaque assay*	*100*	*4.3*	nr	100	nr	no clinical illness	(Whitehead et al., 1970)
	Macaca fascicularis	Cynomolgus macaque	DENV-1 WP	1.0×10^5	Plaque assay	100	5.0	4.0×10^3 pfu/ml	100	905 (14)	nr	(Osorio et al., 2011)
	Macaca fascicularis[c]	Cynomolgus macaque	DENV-2 NGC	1.0×10^5	Plaque assay	100	4.5	8.0×10^3 pfu/ml	100	10,240 (14)	nr	(Osorio et al., 2011)
	Macaca fascicularis	Cynomolgus macaque	DENV-3 Sleman/78	1.0×10^5	Plaque assay	100	3.0	4.0×10^3 pfu/ml	100	905 (14)	nr	(Osorio et al., 2011)
	Macaca fascicularis	Cynomolgus macaque	DENV-4 814669	1.0×10^5	Plaque assay	100	2	2.0×10^2 pfu/ml	100	1810 (18)	nr	(Osorio et al., 2011)
	Macaca fascicularis	Cynomolgus macaque	DENV4 4328-S (Leahi)	1.0×10^5	Plaque assay	100	3.4	$>3.0 \times 10^2$ pfu/ml	100	nr	nr	(Price et al., 1974)
	Macaca mulatta	Rhesus macaque	DENV-1 16007	$5.0 \times 10^3–5.0 \times 10^5$	Plaque assay	100	4.98	nr	100	nr	Enlargement of lymph nodes, hemorrhage at inoculation site	(Halsted et al., 1973)
	Macaca mulatta	Rhesus macaque	DENV-1 16007	$1.0 \times 10^{0.9} – 5.0 \times 10^1$	Plaque assay	100	nr	nr	100	nr	nr	(Halsted et al., 1973)
	Macaca mulatta	*Rhesus macaque*	*DENV-1 WP*	*1.0×10^5*	*RT-PCR*	*100*	*5*	*4.2 (PFU eq/ml $\times 10^3$)*	*100*	*254 (30)*	*nr*	(Markoff et al., 2002)
	Macaca mulatta	*Rhesus macaque*	*DENV-1 WP*	*1.0×10^4*	*RT-PCR*	*100*	*4*	*2.4 (PFU eq/ml $\times 10^3$)*	*100*	*254 (30)*	*nr*	(Markoff et al., 2002)
	Macaca mulatta	*Rhesus macaque*	*DENV-1 WP*	*1.0×10^3*	*RT-PCR*	*100*	*4.7*	*2.4 (PFU eq/ml $\times 10^3$)*	*100*	*508 (30)*	*nr*	(Markoff et al., 2002)

	Species	Common name	Virus	Inoculum titer (PFU)[d]	Virus detection	% viremic	Mean no. days viremic	Max virus titer	% seroconversion [e]	Geometric mean $PRNT_{50}$ (Day PI)	Signs/Symptoms associated with infection	Ref
	Macaca mulatta	Rhesus macaque	DENV-1 Puerto Rico/94	1.0×10^5	Plaque assay	100	2.8	1×10^2 pfu/ml	100	103 (28)	nr	(Blaney et al., 2007)
	Macaca mulatta	Rhesus macaque	DENV-2 16681	5.0×10^3–5.0×10^5	Plaque assay	100	3.95	nr	100	nr	Enlargement of lymph nodes, leucopenia, lymphocytosis	(Halsted et al., 1973)
	Macaca mulatta	Rhesus macaque	DENV-2 16681	$1.0 \times 10^{0.9} – 5.0 \times 10^1$	Plaque assay	100	nr	nr	100	nr	nr	(Halsted et al., 1973)
	Macaca mulatta	Rhesus macaque	DENV-2 16681	1.0×10^7	RT-PCR	100	13	~ 1.0×10^6 pfu/ml	100	nr	Hemorrhage, increased creatine phosphokinase (CPK), thrombocytopenia, neutropenia	(Onlamoon et al., 2010)
	Macaca mulatta	Rhesus macaque	DENV-2 PR 159	8.3×10^5	Plaque assay	100	3.8	6.4×10^3 pfu/ml	100	nr	no evidence of neurovirulence	(Harrison et al., 1977)
	Macaca mulatta	Rhesus macaque	DENV-2 PR 159	1.3×10^5	Plaque assay	100	4.2	$<6.5 \times 10^1$ pfu/ml	100	560 (30)	no clinical illness	(Scott et al., 1980)
	Macaca mulatta	Rhesus macaque	DENV-2 BM50-76	2.0×10^6	Plaque assay	100	4.5	$<6.5 \times 10^1$ pfu/ml	100	640 (30)	no clinical illness	(Scott et al., 1980)
	Macaca mulatta	Rhesus macaque	DENV-2 Tonga/74	1.0×10^5	Plaque assay	100	4.5	1.3×10^2 pfu/ml	100	311 (28)	nr	
	Macaca mulatta	Rhesus macaque	DENV-2 21868	3.4×10^5 pfu	Plaque assay	100	4.5	$<6.5 \times 10^1$ pfu/ml	100	nr	no clinical illness	(Scott et al., 1980)
	Macaca mulatta	Rhesus macaque	DENV-3 16562	5.0×10^3–5.0×10^5	Plaque assay	63	1.83	nr	100	nr	none	(Halsted et al., 1973)
	Macaca mulatta	Rhesus macaque	DENV-3 16562	$1.0 \times 10^{0.9} – 5.0 \times 10^1$	Plaque assay	100	nr	nr	100	nr	nr	(Halsted et al., 1973)
	Macaca mulatta	Rhesus macaque	DENV-3 Sleman/78	1.0×10^5	Plaque assay	100	3.5	6.0×10^1 pfu/ml	100	253 (28)	nr	(Blaney et al., 2008)
	Macaca mulatta	Rhesus macaque	DENV-4 4328S	5.0×10^3–5.0×10^6 fu	Plaque assay	92	3.64	nr	100	nr	Enlargement of lymph nodes; hemorrhage at inoculation site	(Halsted et al., 1973)
	Macaca mulatta	Rhesus macaque	DENV4-4328-S (Leahi)	1.0×10^5	Plaque assay	100	3.2	$>3.0 \times 10^2$ pfu/ml	100	nr	nr	(Price et al., 1974)
	Macaca mulatta	Rhesus macaque	DENV-4 814669	1.0×10^5	Plaque assay	100	3.6	6.0×10^1 pfu/ml	100	nr	532 (28)	(Durbin et al., 2001)
	Pan troglodytes	Chimpanzee	DENV-1 49313	1.3×10^3	Plaque assay and mosquito ID_{50}	100	5	3.4 $\log_{10}$ pfu/ml	100	113 (42)	none	(Scherer et al., 1978)
	Pan troglodytes	Chimpanzee	DENV-2 PR 159	3.0×10^6 pfu	Plaque assay	100	5.5	nr	100	nr	nr	(Harrison et al., 1977)

	Species	Common name	Virus	Inoculum titer (PFU)[d]	Virus detection	% viremic	Mean no. days viremic	Max virus titer	% seroconversion [e]	Geometric mean $PRNT_{50}$ (Day PI)	Signs/Symptoms associated with infection	Ref
	Pan troglodytes	Chimpanzee	DENV-2 NC38	4.0×10^3	Plaque assay and mosquito ID_{50}	100	5.4	2.0×10^3 pfu/ml	100	14 (42)	none	(Scherer et al., 1978)
	Pan troglodytes	Chimpanzee	DENV-3 49080	5.0×10^2	Plaque assay and mosquito ID_{50}	100	2.5	1.5×10^2 pfu/ml	100	160 (42)	none	(Scherer et al., 1978)
	Pan troglodytes	Chimpanzee	DENV-4 17111	6.0×10^2	Plaque assay and mosquito ID_{50}	100	5.0	1.5×10^2 pfu/ml	100	9 (42)	none	(Scherer et al., 1978)
	Pan troglodytes	Chimpanzee	DENV4-4328-S (Leahi)	1.0×10^5	Plaque assay	100	3.6	$>3.0 \times 10^2$ pfu/ml	100	nr	nr	(Price et al., 1974)

[a] Reported as *Cercopithecus aethiops*

[b] All *Hylobates lar* were splenectomized.

[c] Reported as *Macaca philippensis*

[d] Italics indicate matched, dose de-escalation studies

[e] By PRNT, hemagglutination inhibition, complement fixation IgG ELISA, or protection from homologous viral challenge

[f] A subsequent study in which marmosets were infected with the same DENV strains at similar doses (Omatsu et al., 2012) reported that thrombocytopenia, leucopenia, increases in alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen, and lactate dehydrogenase, fever and decreased activity were associated with infection.

Table 2

Experimental studies of the susceptibility of New World mosquito species to infection with dengue virus

Species	DENV Serotype	Strain	Method of Exposure	Dose[1]	% infected (N)[2]	Dissemination[3]	Transmission[4]	Titer	Reference
Aedes mediovittatus	DENV-2	New Guinea C	Peroral	1.0×10^7 MID_{50}/ml	34.7 (49)	Yes, % not reported	nr	nr	(Gubler et al., 1985)
		Puerto Rico		1.0×10^6 MID_{50}/ml	27.9 (43)	Yes, % not reported		nr	
		Puerto Rico		1.0×10^8 MID_{50}/ml	74.2 (31)	Yes, % not reported		nr	
Ochlerotatus (formerly *Aedes*) *japonicus*	DENV-2	Bangkok, Thailand (1974)	Peroral	10^7 FFU/mL	91 (11)	91%	9%	20 ffu/saliva (N=1)	(Schaffner et al., 2011)
Ochleratatus (formerly *Aedes*) *triseriatus*	DENV-1	Fiji 1975	Peroral	1.0×10^7 MID_{50}/ml	66.1 (230)	66%	Yes-% not reported	nr	(Freier and Grimstad, 1983)
Ochleratatus (formerly *Aedes*) *brelandi*	DENV-1	Fiji 1975	Peroral	1.0×10^7 MID_{50}/ml	46.6 (58)	47%	0%	nr	(Freier and Grimstad, 1983)
Ochleratatus (formerly *Aedes*) *hendersoni*	DENV-1	Fiji 1975	Peroral	1.0×10^7 MID_{50}/ml	25.0 (44)	25%	0%	nr	(Freier and Grimstad, 1983)
Ochleratatus (formerly *Aedes*) *zoosophus*	DENV-1	Fiji 1975	Peroral	1.0×10^7 MID_{50}/ml	8.0 (50)	8%	0%	nr	(Freier and Grimstad, 1983)
Culex pipens molestus	DENV-1	Oahu, Hawaii (1944)	Peroral	7.0×10^7 MID_{50}/ml	0 (14)	NA	NA	NA	(Rosen et al., 1985)
			Parenteral	nr	0 (7)	NA	NA	NA	(Rosen et al., 1985)
	DENV-2	New Guinea C	Peroral	1.0×10^7–1.5×10^9 MID_{50}/ml	0 (4)	NA	NA	NA	(Rosen et al., 1985)
	DENV-3	H85	Peroral	1.5×10^7 MID_{50}/ml	0 (8)	NA	NA	NA	(Rosen et al., 1985)
			Parenteral	nr	0 (8)	NA	NA	NA	(Rosen et al., 1985)

Species	DENV Serotype	Strain	Method of Exposure	Dose[1]	% infected (N)[2]	Dissemination[3]	Transmission[4]	Titer	Reference
				nr	0 (6)	NA	NA	NA	(Rosen et al., 1985)
	DENV-4	H241	Peroral	3.0×10^7 MID_{50}/ml	0 (10)	NA	NA	NA	(Rosen et al., 1985)
			Parenteral	nr	22.2 (9)	nr	nr	nr	(Rosen et al., 1985)
				nr	0 (4)	NA	NA	NA	(Rosen et al., 1985)
Culex quinquefasciatus	DENV-1	Hawaii	Parenteral	1.0×10^2 MID_{50}	0 (60)	NA	NA	NA	(Huang et al., 1992)
	DENV-2	New Guinea C	Parenteral	1.0×10^2 MID_{50}	0 (60)	NA	NA	NA	(Huang et al., 1992)
	DENV-3	H87	Parenteral	1.0×10^2 MID_{50}	0 (60)	NA	NA	NA	(Huang et al., 1992)
	DENV-4	H241	Parenteral	1.0×10^2 MID_{50}	0 (60)	NA	NA	NA	(Huang et al., 1992)
Culex tarsalis	DENV-4	Caribbean Strain 814669:	Peroral	2.6×10^7 PFU	12.5 (40)	0%	0%	NA	(Hanley et al., 2005)
Haemagogus equinus	DENV-1	Fiji 1975	Parenteral	nr	Yes, % not reported	100%	nr	nr	(de Souza and Freier, 1991)

MID50 : mosquito infectious dose 50 ; FFU : focus forming units ;PFU : plaque forming units

Infected : virus detected in any type of tissue; nr: not reported

Dissemination: % mosquitoes in which virus was detected in a tissue outside of the midgut (most commonly the head); NA: not applicable

Transmission: % mosquitoes in which virus was detected in saliva or material on which mosquito fed; NA: not applicable