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Global Spread and Persistence of Dengue

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Key Words

flavivirus, mosquito-borne, *Aedes*, emergence, genotype, RNA virus

Abstract

Dengue is a spectrum of disease caused by four serotypes of the most prevalent arthropod-borne virus affecting humans today, and its incidence has increased dramatically in the past 50 years. Due in part to population growth and uncontrolled urbanization in tropical and subtropical countries, breeding sites for the mosquitoes that transmit dengue virus have proliferated, and successful vector control has proven problematic. Dengue viruses have evolved rapidly as they have spread worldwide, and genotypes associated with increased virulence have expanded from South and Southeast Asia into the Pacific and the Americas. This review explores the human, mosquito, and viral factors that contribute to the global spread and persistence of dengue, as well as the interaction between the three spheres, in the context of ecological and climate changes. What is known, as well as gaps in knowledge, is emphasized in light of future prospects for control and prevention of this pandemic disease.

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INTRODUCTION

Globally, as many as 1 in 100 people are infected each year by one or more of four serotypes of dengue virus (DENV1–4), a mosquito-borne, positive-strand RNA virus in the genus *Flavivirus*, family *Flaviviridae*. Tens of millions of cases of dengue fever (DF) are estimated to occur annually, including up to 500,000 cases of the life-threatening dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) (156). Epidemic DHF/DSS emerged 50 years ago in Southeast Asia (60) but was first seen in the Americas only in 1981 (75) and in South Asia in 1989 (88). Since the 1950s, the incidence of DHF/DSS has increased over 500-fold, with more than 100 countries affected by outbreaks of dengue (156). DHF/DSS has become one of

the top ten causes of pediatric hospitalization in Southeast Asia (155), and the number of cases of DHF/DSS in the Americas alone has grown dramatically (98).

Dengue is associated with explosive urban epidemics and has become a major public health problem, with significant economic, political, and social impact (46). Some of the reasons for the dramatic increase in the geographic spread of dengue, including its more severe forms, are known with some certainty, whereas other reasons remain a subject of debate and speculation. This review highlights the epidemiological history of dengue and explores the various reasons for its dramatic spread and persistence during the past 50 years. To this end, aspects of human, mosquito, and virus biology, ecology, and evolution are explored, along with the interaction between these spheres (Figure 1).

THE HUMAN SPHERE

Introduction

DF is a self-limited though debilitating febrile illness characterized by headache, retro-orbital pain, myalgia, arthralgia, and rash. DHF is marked by increased vascular permeability (plasma leakage), thrombocytopenia, and hemorrhagic manifestations; DSS occurs when fluid leakage into the interstitial spaces results in

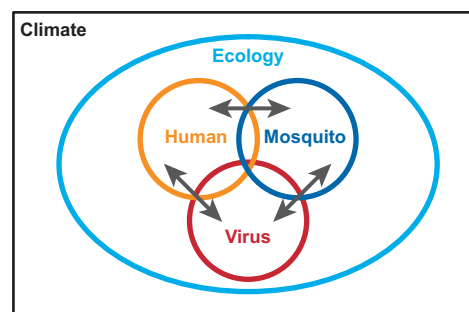


Figure 1

The interplay of human, mosquito, and virus biology contributes to the clinical spectrum and geographic distribution of dengue. Each sphere influences and affects the others, all in the context of ecology and against the backdrop of climate and climate change.

DENV: dengue virus

DF: dengue fever

DHF/DSS: dengue hemorrhagic fever/dengue shock syndrome

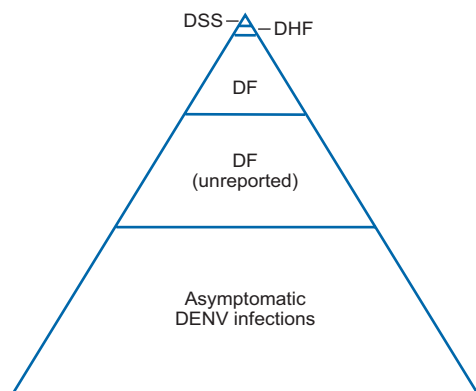


Figure 2

DENV infections and disease are represented by a pyramid. An estimated 100 million infections occur each year, 10%–50% of which are symptomatic, but only a fraction of DF cases are reported. Finally, severe disease due to DENV infection represents the tip of the pyramid. The most severe forms of dengue are DHF and DSS, which present with higher frequency in secondary than in primary DENV infections.

hypovolemic shock, which without appropriate treatment may lead to death (43, 56). Apparent disease due to dengue has been described as the tip of the iceberg (78), as less than 10% of symptomatic dengue cases are reported (156) and 50%–90% of all DENV infections are asymptomatic (10, 17, 29, 140) (**Figure 2**). The most severe disease, DHF/DSS, is found at the very tip of the pyramid, and its incidence varies significantly between primary and secondary DENV infections. A secondary DENV infection results when a person previously infected with one serotype is exposed to a different serotype, and it has been documented as the single most important risk factor for severe dengue (17, 29, 39, 53, 58, 125, 140). For example, Thai data of DENV infections in children under 15 years of age demonstrated that 0.18% of primary infections and 2% of secondary infections manifest as DHF/DSS (78). In this section, we describe dengue and review the reasons for the spread and persistence of epidemic DF and DHF/DSS and what is known about the risk for infection and severe disease.

History and Spread of Dengue

Clinical descriptions of a dengue-like syndrome were recorded as far back as A.D. 992 in China, although the first epidemics of well-documented cases of what are believed to be dengue occurred in 1779–1780 (42). The viral etiology of dengue was suggested experimentally a century ago (7), but it was not until World War II that technical advances enabled Japanese (67) and American (122) investigators to isolate DENV. The first two DENV serotypes were identified at this time, followed by the third and fourth serotypes when DHF/DSS emerged in urban centers in the Philippines and Thailand in 1954 (60, 103). It has been hypothesized that the movement of troops during World War II, together with destruction of the environment and human settlements, contributed to the spread of the viruses and, to a certain extent, their mosquito vectors throughout Southeast Asia and the Western Pacific (42, 114). Since then, Southeast Asia has remained hyperendemic for all four DENV serotypes. In the Americas, the decline and reemergence of epidemic dengue since the 1980s has been even more closely linked to the presence of its mosquito vectors, *Aedes aegypti* and *A. albopictus* (**Figure 3**).

Increases in human population, uncontrolled urbanization, and international travel can explain much of the spread and persistence of dengue in the twentieth and early twenty-first centuries (42, 114). It has been estimated that the minimum population size required to sustain dengue transmission is 10,000–1,000,000 (76), and early epidemics of dengue in Southeast Asia were linked to towns with populations over 10,000 (133). A mathematical model examining the spatiotemporal incidence of DHF over a 15-year period in Thailand estimated that epidemics of DHF originate in the capital city of Bangkok every 3 years and travel outward to the rest of the country at a rate of 148 km per month (23), stressing the role of cities in dengue transmission dynamics. In the tropical and subtropical areas where *A. aegypti* and *A. albopictus*

Secondary infection:

a subsequent infection with a heterotypic serotype of DENV, occurring months to years after the primary infection

Hyperendemic: the presence of numerous serotypes of dengue virus cocirculating in one location

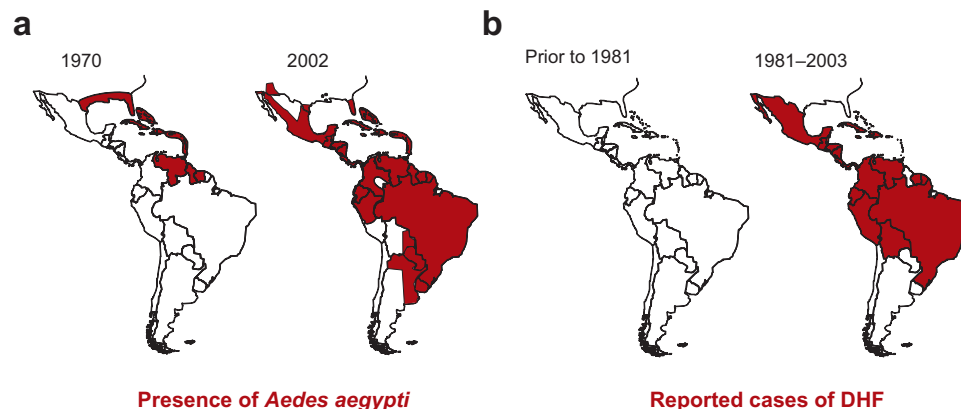


Figure 3

The spread of *Aedes aegypti* and DHF in the Americas. (a) The shaded areas represent the regions in the Americas where *A. aegypti* was present in 1970 (left) and in 2002 (right). (b) Shaded areas represent the countries that reported cases of DHF prior to 1981 (left) and between 1981 and 2003 (right). The increased distribution of DHF mirrors the dissemination of *A. aegypti*. Reprinted with permission from the U.S. Centers for Disease Control and Prevention.

mosquitoes are present, urban growth has not been accompanied by well-organized water and waste management programs because of the sheer numbers of people migrating from the countryside and because of the lack of resources available for infrastructure and public health measures in these regions (72, 119). In settings where the availability of water is intermittent and piped water supplies may be nonexistent, both indoor and outdoor containers used for water storage comprise key *Aedes* breeding sites (112). The indestructibility of discarded plastics and the increased numbers of unused tires, combined with poor garbage disposal systems, have led to the accumulation of numerous additional breeding sites. Commercial shipping has been linked to the spread of both *A. aegypti* and *A. albopictus* between regions (63, 133, 135), and plane travel has greatly increased the dissemination of dengue viruses via rapid transit of viremic individuals around the world (42). In other words, much of the increase in DENV infections in recent decades can be explained by an increase in both human population and mosquito breeding habitats, combined with the dissemination of both mosquitoes and dengue viruses to new geographic regions.

Risk for DENV Infection

An estimated 3.5 billion people, or half the world's population, are at risk for DENV infection in tropical and subtropical countries (12). Fundamentally, exposure to an infected *A. aegypti* mosquito determines an individual's risk for acquiring dengue. By avoiding the mosquito and eliminating breeding sites around the home and workplace, an individual can mitigate that risk to some extent, although there are factors beyond an individual's immediate control. In the past, epidemics of dengue have occurred in the United States as far north as Philadelphia (42), and *A. aegypti* as well as *A. albopictus* are present in South and Central United States today. Yet, most dengue cases in the United States are reported in travelers returning from endemic countries (110). This can be attributed to improved infrastructure, such as reliable sources of piped water that remove the need for *Aedes*-friendly water storage containers, air conditioning powered by an electric grid with few interruptions, and screens covering windows and enclosing patios. These variables greatly reduce the exposure of people to mosquitoes, so that if a viremic individual returns to the United States, the possibility of that

person contacting an appropriate vector is fairly low, and thus the potential of DENV transmission to the community is low. Even in the context of a region highly endemic for dengue, such as Puerto Rico, a higher incidence of disease is correlated with lower socioeconomic status and lack of window and door screens (149).

The risk of a dengue epidemic can be modeled mathematically by the basic reproductive rate of the virus (R_0), which corresponds to the number of subsequent infections that would be produced in a group of susceptible individuals given the introduction of one infected person (4). For vector-borne diseases, R_0 takes into account measures of transmission capacity, specifically the number of female mosquitoes per human host, the human-biting rate of the mosquito species, the proportion of bites that produce an infection, the average duration of infection in humans, the proportion of bites of viremic humans that result in infected mosquitoes, mosquito mortality rates, and latent periods of incubation in both hosts (4). When $R_0 < 1$, transmission is interrupted; $R_0 = 1$ results in endemicity; and $R_0 > 1$ results in an increase in cases (i.e., a potential epidemic). R_0 values for dengue in endemic regions are estimated to range between 1.33 and 11.6 (57). An additional factor to consider is herd immunity, or the number of immune individuals in the population, which can be represented as “p,” such that $(1-p)R_0$ determines the effective R_0 . This equation is useful in vaccination programs in terms of estimating the number of vaccinated individuals (p) needed to interrupt transmission ($R_0 < 1$) for a given disease (4, 37).

Risk Factors for DHF/DSS

Risk factors for developing DHF/DSS include pre-existing immunity from a previous DENV infection, time between infections, age, ethnicity and host genetic background, sequence of infecting serotypes, and viral genotype (43, 55). In response to a primary infection with DENV, protective immunity to the infecting

serotype is believed to last a lifetime. As evidence, serotype-specific immunity was protective for up to 18 months in human volunteers (121), and neutralizing antibodies and serotype-specific T cells have been found in patients in Cuba, Greece, and Japan 20–40 years after an isolated dengue epidemic (51, 68, 129). However, complete cross-protective immunity from a secondary infection was present in human volunteers for only 1–2 months after a primary DENV infection, with partial immunity present up to 9 months, resulting in a milder disease of shorter duration upon reinfection (121). After the emergence of DHF/DSS, studies seeking to explain the cause of this new and more severe manifestation of dengue identified a second, heterologous DENV infection as a risk factor for DHF/DSS (58). Prospective studies in Thailand, Burma, and Indonesia (17, 29, 39, 125, 140), as well as studies of sequential epidemics of dengue in Cuba (53), confirmed the association of secondary infection with more severe disease. Evidence from Cuba has suggested that increased time between infections may also increase disease severity (53). After an isolated DENV1 epidemic in Cuba in 1977, two separate DENV2 epidemics caused by closely related Southeast Asian DENV2 strains occurred in 1981 and 1997 on the island. Strikingly, death rates were almost 40 times greater when the interval between infections was 20 years, compared with 4 years.

The pathogenic mechanism of DHF/DSS is still poorly understood. A predominant theory regarding DHF/DSS pathogenesis attributes the higher incidence of DHF/DSS among secondary infections to the phenomenon of antibody-dependent enhancement (ADE) (56). The ADE theory postulates that after an initial period of cross-reactive protection, antibodies from a primary infection remain cross-reactive with other DENV serotypes but have waned to nonneutralizing levels. These nonneutralizing antibodies could then mediate an increased uptake of virus into monocyte/macrophage cells via the Fc receptor, leading to increased viral replication and immune activation

R_0 : basic reproductive rate

Herd immunity: the threshold level of collective immunity in a population, above which transmission of a particular pathogen will be disrupted and not be maintained

Neutralizing antibodies: antibodies capable of preventing infection of a cell/host by a pathogen

ADE: antibody-dependent enhancement

HLA: human
leukocyte antigen

accompanied by cytokine release (56). Field studies have found evidence of higher levels of viremia in DHF patients, which supports the assertion that increased viral replication is associated with more severe disease (8, 83, 93, 145). A different but complementary theory of immunopathology involves reactivation of cross-reactive memory T cells specific for the previous rather than the current DENV infection, resulting in delayed viral clearance and/or increased cytokine secretion along with increased apoptosis of both infected and uninfected bystander cells (118). There is immunological evidence that this phenomenon of “original antigenic sin” may occur during secondary DENV infections (91). In both theories, cytokines are believed to play a direct role in the immunopathogenesis of DENV, owing to their proinflammatory effects on vascular endothelial cells that presumably lead to leaky junctions and increased capillary permeability (118).

Most epidemiologic studies find that children under age 15 are at increased risk for DHF/DSS, independent of other risk factors (17, 52, 61, 125, 140), which may be related to increased capillary fragility and decreased tolerance for insult to microvascular integrity in this age group (38). A few studies have indicated that Africans and people of African descent may have genetic polymorphisms that confer partial protection against severe dengue (59, 130). Recent work has identified DENV epitopes that, in the context of specific human leukocyte antigen (HLA) types, may be associated with immune enhancement (92, 131, 159). Other studies have more broadly correlated certain HLA types with disease severity and/or protection from severe disease (128, 136). Defining the link between disease risk and HLA type, race, or DENV cellular receptor (123) and cytokine polymorphisms (32) has the potential not only to provide important information regarding the pathogenesis of secondary DENV infection, but also to serve as a potential prognostic tool to identify individuals at increased risk for severe disease.

THE MOSQUITO SPHERE

Introduction

The principal vector of DENV is the *A. aegypti* mosquito, an anthropophilic species that has adapted extremely well to the urban environment, which is found both indoors and outdoors in close proximity to human dwellings (112). *A. aegypti* is believed have originated in the jungles of Africa and was most likely spread throughout the rest of the world via slave and trading ships during the seventeenth to nineteenth centuries (112, 133). It was noted some time ago that epidemics of dengue seemed to correlate with the spread of *A. aegypti* in South and Southeast Asia, appearing first in port towns and moving inland over time along waterways (133). Now a fully domesticated mosquito, *A. aegypti* is an efficient vector of DENV because of its preference for laying its eggs in artificial containers, biting humans, and remaining indoors, where it has access to its favorite host (112).

A. albopictus is a secondary vector of DENV in Southeast Asia, the Western Pacific, and increasingly in Central and South America (40), but it has also been documented as the sole vector during certain dengue epidemics (3, 28). Prior to 1979, this species was found only in Asia and in the Western Pacific, but it has spread to much of the rest of the world in recent decades (40, 133). The invasion of North America by *A. albopictus* was first confirmed with its discovery in Houston, Texas, in 1985 (18), probably arriving in shipments of used tires from Japan (63, 70). The range of *A. albopictus* stretches farther north than that of *A. aegypti*, and its eggs are somewhat resistant to subfreezing temperatures (63), raising the possibility that *A. albopictus* could mediate a re-emergence of dengue in the United States or Europe. For example, *A. albopictus* can survive the winters in northern Italy (113) and was recently implicated in an outbreak of Chikungunya virus in Italy (117). In this section, we describe the mosquitoes that transmit dengue, the hypothesis of DENV emergence from the

jungles of Southeast Asia, the potential for re-emergence, variations in vector competence between *Aedes* strains, mechanisms of viral persistence in mosquitoes between epidemics, past vertical control programs for *A. aegypti*, and current community-based strategies for vector control.

The Sylvatic Cycle and Emergence/Re-Emergence of DENV

A number of forest-dwelling *Aedes* mosquitoes, known as tree-hole mosquitoes, have been identified as vectors of a sylvatic cycle of DENV in the jungles of Africa (DENV2) and Southeast Asia (DENV1–4), involving mainly nonhuman primates. The viruses in this sylvatic cycle are phylogenetically distinct from those in the urban cycle of dengue involving *A. aegypti* (147), though sylvatic strains of DENV may occasionally cause disease in humans (111, 124). It is thought that DENV emerged from the jungles of Southeast Asia, with *A. albopictus* or perhaps other *Aedes* species maintaining the virus in a sylvatic cycle involving nonhuman primates and humans living in the countryside. This hypothesis was reached in part on the basis of studies in the 1950s that documented high levels of anti-DENV antibodies in both nonhuman primates and rural inhabitants in the apparent absence of disease (120, 133, 134). Neutralizing antibodies against DENV1 and DENV2, the only serotypes known at the time, were present in about 50% of children up to 15 years of age in diverse rural communities in Malaysia, in contrast with much lower levels (3%–9%) in the cities of Singapore and Kuala Lumpur (134). In Southeast Asia in the 1950s, *A. aegypti* was still primarily found only in towns and cities, whereas *A. albopictus* was common in coastal and inland rural areas (133). Thus, the combined evidence argues for a rural source for the dengue viruses in Southeast Asia, possibly with *A. albopictus* as the primary vector.

In Africa, a nondomesticated, forest-dwelling subtype of *A. aegypti*, *A. aegypti formosus*, is present that demonstrates a low biting rate for humans; however, sylvatic DENV2

strains have been recovered primarily from other *Aedes* species in the jungles of West Africa, including *A. luteocephalus*, *A. furcifer*, *A. taylori*, and *A. vittatus* (25, 111). The potential for epidemic DENV strains to re-emerge has been addressed experimentally in several studies that suggested that viral adaptation to the vector was required for efficient transmission by *A. aegypti* and *A. albopictus* (90), but that adaptation to vertebrate hosts was not required for the emergence of DENV from a sylvatic cycle (144). Two sylvatic *Aedes* species from West Africa, *A. furcifer* and *A. luteocephalus*, were highly susceptible to both sylvatic and endemic DENV2, raising the possibility that adaptation of DENV to peridomestic mosquitoes does not necessarily result in loss of infectivity for some sylvatic *Aedes* species (26). However, both domestic and forest-dwelling *A. aegypti* from Senegal were poorly infected by sylvatic and endemic DENV strains, and another investigation found that populations of *A. aegypti formosus* from different parts of Africa were less susceptible to DENV2 than were *A. aegypti* from Southeast Asia, South America, and the South Pacific (30). These studies illustrate the complexity of the coevolution of DENV with its mosquito vectors.

Vector Competence

Variations in vector competence among strains of *A. aegypti* and between *A. aegypti* and *A. albopictus* have received a fair amount of attention. Early studies had shown that *A. aegypti*, though clearly correlated with epidemic dengue, was not as easily infected with DENV as *A. albopictus* (69, 115), leading to the hypothesis that the adaptation of DENV to *A. aegypti* had selected for viruses that caused higher viremia (112). However, other studies using field-caught mosquitoes, as opposed to laboratory strains, demonstrated comparable susceptibility between the two species (153) or a higher susceptibility by *A. aegypti* (89, 146). Although differences between the use of laboratory strains versus field-caught mosquitoes may explain some of these discrepant results

Vector competence:

the capacity of a vector to transmit a pathogen by virtue of being susceptible to infection and dissemination and subsequently capable of transmission to an appropriate host

Vertical**transmission:**

transfer of DENV from an infected female mosquito to her offspring, either by transovarial transmission or by infection of the egg at time of oviposition

Vector control**program (vertical versus horizontal):**

a vertical control program is a top-down approach in its design and implementation and is usually government led; whereas a horizontal or community-led (bottom-up) program may have institutional support, but focuses primarily on including community participation

House index (HI):

the percentage of houses with containers infested with mosquito larvae and/or pupae

(146), the question remains what might account for different vector competence among mosquitoes, and whether coevolution between dengue viruses and *A. aegypti* has occurred since DENV emerged from the jungle. Substantial variation in susceptibility exists between different strains of both *A. aegypti* (14, 47, 138) and *A. albopictus* (48, 85) mosquitoes from different locations. This suggests that genetic differences in the vector may be responsible for varying susceptibilities to DENV, and specific quantitative trait loci (QTLs) in *A. aegypti* have been linked to vector competence (13). Recent sequencing of the *A. aegypti* genome (94) will facilitate identification of genes linked to previously described QTLs associated, for example, with midgut and other barriers to infection. Comparative genomics analyses are also now possible with sequenced *Drosophila melanogaster* and *Anopheles gambiae* genomes (148).

Vertical Transmission of DENV in Mosquitoes

In most endemic countries, dengue displays a seasonal pattern related to temperature and rainfall (33, 57, 112). This has led many investigators over the years to question how the virus overwinters, or persists during dry or cold seasons. One possibility is that a population of infected mosquitoes could survive throughout the interim and introduce the virus during the next season. *Aedes* mosquitoes remain infected with DENV for life, and the longest lifespan recorded to date is 174 days, although a more typical survival rate is 1–2 weeks (112). A second possibility is passage of the virus to the next generation of mosquitoes via survival in an infected egg. Early studies had shown no evidence of vertical transmission of DENV in *Aedes* mosquitoes (112), but more recent studies have demonstrated that vertical transmission is possible both in the laboratory and in the wild (49). Some evidence exists that *A. albopictus* mosquitoes are more efficient at vertical transmission than *A. aegypti*, which would make them a candidate for maintaining DENV during interepidemic periods

(116). Thus, vertical transmission of DENV in mosquitoes is possible, whether or not the mechanism is truly transovarial or mediated by infection of the mature egg at the time of oviposition (112). Finally, given the high number of asymptomatic cases of dengue (10, 17, 29, 140), it is also possible that silent transmission in humans by a reduced number of vectors maintains DENV transmission between epidemics.

Past Experience with Vertical Vector Control Measures

Because *A. aegypti* facilitated the emergence of epidemic dengue in urban centers around the world and is still the primary vector of dengue today, most control efforts have focused on this species. Mosquito control measures are particularly important given the current lack of dengue-specific vaccines or therapeutics (66, 71, 154), and they play a central role worldwide. A fundamental distinction in the design of a vector control program is whether it takes a government-led, vertical (top-down) approach or a community-led, horizontal (bottom-up) approach (41).

A vertical, Pan-American Health Organization–led campaign focused on controlling urban yellow fever in the mid-twentieth century succeeded in eliminating *A. aegypti* from most countries in the Americas by 1965 (135) and had the additional benefit of reducing the incidence of dengue in the region. Nonetheless, the mosquito remained in the northern-most countries of South America and in some locations in the Caribbean, as well as in the United States (135), which discontinued its program in 1969 without having achieved the goal of eradication (132). This campaign established the use of larval source reduction as a means of controlling mosquito populations, as well as three indices used to monitor larval density that are still in use today (35, 155), in particular the house index (HI). The control program also included the use of outdoor insecticidal sprays (DDT and malathion) in and around all breeding sites (98). However, since the program was discontinued in the early 1970s, *A. aegypti* has

returned to almost all countries in the Americas (43).

Two other successful vertical control programs were undertaken by the governments of Singapore and Cuba. DHF was first reported in Singapore in 1960 (19), and beginning in 1968, the Vector Control Unit of the Ministry of Health established a program of entomologic surveillance, larval source reduction, public education, and law enforcement targeted to control both *A. aegypti* and *A. albopictus* (97). This program succeeded in bringing the HI down from almost 50% to approximately 2% by 1973, where it has remained until the present time. The seroprevalence of DENV infection in the general population declined to 43% in 1996, with only 6.7% of primary school children positive for anti-DENV antibodies, compared with 71% in some locations in Thailand (141), 69% in Yogyakarta, Indonesia (39), and 90% in urban Nicaragua (10). However, after 15 years of low incidence, Singapore has recently experienced a resurgence in dengue, without a concurrent change in HI values (97). This increase has been attributed in part to lowered herd immunity, increased virus transmission outside the home, and a shift in policy from vector surveillance to case detection. Another element contributing to this resurgence likely includes tens of millions of people who visit, transit through, and commute to work in Singapore every year, leading to a high potential for reintroduction by viremic individuals.

In the case of Cuba, a devastating epidemic of DHF/DSS in 1981, the first in the Americas, resulted in over 10,000 cases of severe illness and 158 deaths. The Cuban government initiated a vertical, systematic campaign aimed at eradicating the *A. aegypti* vector from the island, and *A. aegypti* was eliminated from 13 of Cuba's 14 provinces (75). Some 10,000 health workers remained committed to the control program, and for 15 years no dengue cases were reported in Cuba (74, 75). However, dengue (due to DENV2) re-emerged in Cuba in 1997, though it was detected early and confined to Santiago de Cuba. In 2000, DENV3 was isolated in Cuba for the first time, and it caused

an epidemic of DF/DHF in the city of Havana during 2001–2002. Once again, the Cuban government mobilized a major vector control campaign, and every house in Havana was inspected 10 times. Starting with an HI of 0.49% at the beginning of the epidemic, within three months the house index had been reduced to 0.01% (50). In 2006, another outbreak of DF/DHF was reported from 4 of 14 provinces (105); however, few details about this epidemic are publicly known. Unfortunately, even these vector control programs that maintained a HI of less than one percent were not able to prevent the recurrence of epidemic dengue, probably due to low herd immunity combined with constant reintroduction of DENV from international visitors and Cuban medical workers returning from endemic countries.

With their past successes, Singapore and Cuba had long been considered to have model dengue control programs, owing in part to their unique political and geographical situations. These two countries implemented consistent programs and policies that made possible the long-term control of dengue, rather than relying only on emergency responses to manage epidemics. However, both locales have faced reintroductions of dengue in spite of low reported vector indices, likely due in large part to the continued influx of people from endemic regions either as tourists, migrant workers, or recipients of cultural exchange, combined with a highly susceptible native population that, ironically, resulted from the success of vector control programs in these countries.

Community-Based Vector Control Programs

In Southeast Asia, the World Health Organization established an Aedes Research Unit in Bangkok, Thailand, in 1966 to investigate control measures for *A. aegypti* after it was identified as a vector of the newly emerging epidemics of hemorrhagic fever in the region (60, 84). These measures included a new method of pesticide application, ultralow-volume spraying, to reduce adult mosquito populations (84), as well as

scaling up the more labor-intensive, but highly effective, targeting of breeding sites in residences by health workers (11, 99). Originally intended as a measure to control or halt an ongoing epidemic by drastically reducing densities of adult mosquitoes in a short period of time (84), ultralow-volume spraying became widely used as a “preventive” measure in many parts of the world during the next two decades (41), despite accumulating evidence of its having little impact on reducing incidence of disease (95).

Various factors eroded the effectiveness of overreliance on both mass pesticide spraying and government-led vertical models for vector control, including increases in pesticide resistance, an awareness of the detrimental side effects of pesticide use, decreased government funding for public health services, and a push from the global health community to move toward horizontal programs integrating education and community participation (72). However, no alternative community-based models were available at the time for vector control programs. Early attempts to establish such a program in Thailand were unsustainable because the community was not involved in the program design and had no stake or understanding of the program and thus did not continue it in the absence of government support (44). Early community-based programs were designed with strong educational components; however, many of them were not successful in motivating community participation. The lesson of early control programs throughout the 1980s and early 1990s was that community-based programs need to incorporate a sense of ownership to be sustainable (44).

The key to dengue control is to close the motivational gap between community knowledge and sustainable practice in reducing mosquito breeding sites. New evidence-based methodologies focus on furnishing community members with key concepts and training so they can gather their own data, evaluate control programs, and generate and implement their own improved interventions based on successes and challenges encountered in their specific geographical and cultural set-

tings. Thus, theoretical education about dengue and its vectors is not enough; people are motivated to change behavior by informed dialogue based on their own evidence that forms the basis for their own decisions. Evidence-based approaches, such as Communication-for-Behavioural-Impact (COMBI) (100) and the SEPA (Socializing Evidence for Participatory Action) program based on CIET methods (5) (<http://www.ciet.org/en/>), are proving more successful in behavioral change and reduction of entomological indices and hold promise as community-based vector control programs, especially in conjunction with some degree of institutional support, though their long-term sustainability and impact on dengue incidence are still under evaluation (L. Lloyd, personal communication; E. Harris, J. Arostegui, J. Coloma & N. Andersson, unpublished results).

Equally important to a successful control program is the ability to effectively target and monitor *A. aegypti* populations as part of a source reduction strategy. A method of identifying highly productive breeding sites that facilitates targeted source reduction efforts has been developed to replace the more traditional larval indices, thus maximizing the effect of control measures (34, 35). This pupal/demographic survey method involves counting the numbers of pupae (the stage between larvae and adult mosquitoes) per container, thus identifying which container types are responsible for the largest output of adult mosquitoes, as well as relating the results to the density of local human populations. Input from the pupal/demographic survey can be combined with temperature and herd immunity values to create mathematical models of transmission thresholds [the container-inhabiting mosquito simulations model (CIMSIM) and the dengue simulation model (DENSIM)] that can provide target values of pupal densities to interrupt transmission for control programs (33, 34, 36). The hope is that these indices will provide a more precise ability to monitor and predict the potential for dengue epidemics than has the traditional HI, which does not necessarily correlate with dengue transmission (35).

Another promising avenue has been the development of biological control methods as alternatives to pesticides, including larvivorous fish, larvae-eating copepods (*Mesocyclops*), toxins, insect growth hormones, or viruses targeted to mosquito larvae (reviewed in Reference 86). The principal goal of all vector control programs is to minimize the populations of adult mosquitoes to interrupt or at least minimize the transmission threshold, R_0 . Even reducing the vector population without eliminating it can mitigate the impact of an epidemic (33).

THE VIRUS SPHERE

Introduction

The four dengue viruses fall into a distinct serogroup among the mosquito-borne flaviviruses, showing a phylogenetic relationship with the Japanese encephalitis virus group and more distantly with YFV (77, 82). The current DENV serogroup progenitor is estimated to have arisen approximately 1000 years ago using molecular clock techniques (142), and most phylogenies show that DENV4 is the most divergent serotype, followed by DENV2, and then DENV1 and DENV3 as the most closely related serotypes (82, 142, 157). Phylogenetic analysis of sylvatic and endemic/epidemic strains suggests that each serotype emerged separately from a sylvatic ancestor (147), and this emergence is estimated to have occurred about 125–320 years ago, varying by serotype (142).

Based on sequences of the complete envelope (E) gene or the E-NS1 boundary using a cut-off of 6% divergence (106), DENV1 is currently divided into—four to five genotypes, including a sylvatic clade (27, 158). DENV2 is divided into six subtypes, designated as Sylvatic, American, Cosmopolitan, Asian 1, Asian 2, and Asian-American (27, 64, 82, 147), although the last three subtypes have on occasion been collapsed into a single Asian genotype (108). DENV3 has been divided into four genotypes (I–IV) (64, 80, 87), sometimes including a genotype V (27). Finally, DENV4

is divided into two endemic genotypes (I–II) and one sylvatic genotype and shows the least genetic diversity among the serotypes, at least among available strains (27, 64, 79, 147). Overall, as further sequences become available, these genotypic structures are likely to be revised, possibly with the appearance of a new genotype or the collapse of two or more genotypes into one. In this section, we discuss the association of certain DENV genotypes or subtypes with disease severity or increased fitness in the context of host immunity.

Viral Genotypes and Virulence

With only 62%–67% homology based on amino acid sequences (152), the four dengue viruses could have been classified as separate viral groups but instead are treated as four DENV serotypes pertaining to a single group. Nonetheless, differences in severity associated with individual serotypes or particular sequences of serotypes in sequential infection have been observed, and it remains an open question whether some serotypes are inherently more pathogenic than others. DENV2 viruses have most commonly been associated with DHF/DSS (9, 17, 53, 96, 125, 140), along with DENV1 and DENV3 viruses (9, 39, 50, 62, 88); DENV4 appears to be the most clinically mild, although it too can cause severe disease (96). DENV2 and DENV4 have been associated with increased disease severity as a secondary infection, whereas DENV1 and DENV3 seem to cause more severe disease in primary infection than do the other two serotypes (9, 62, 96, 145).

In most studies, secondary infection by any of the four DENV serotypes remains the greatest risk factor for severe disease (56). Nonetheless, the association of some DENV genotypes with increased disease severity, whether or not in the context of secondary infection, has now been well documented, in particular involving certain genotypes of DENV2 and DENV3. In general, Southeast Asia appears to serve as a source for viral diversity, generating a multitude of strains, some of which are

UTR: untranslated region

inherently more virulent and perhaps more successful than others, as evidenced by their worldwide spread and possible displacement of earlier DENV strains. Compelling evidence from phylogenetic studies suggests that only DENV2 strains that originated in Southeast Asia are associated with DHF/DSS in the Americas, and not the native American strains that were originally imported from the South Pacific (27, 109). Subsequent functional analysis revealed that Thai DENV strains (Asian genotype) replicated to higher titers than American genotype DENV2 strains in human monocyte-derived macrophages and dendritic cells (21, 102). Full-length sequencing of Asian and American genotypes revealed several key nucleotide differences, particularly at position 390 in the E protein and in the 5' and 3' untranslated regions (UTRs) (81). Substitution of N390 found in the Asian genotype by the American genotype D390 reduced virus output from both human monocyte-derived macrophage and dendritic cell cultures (21, 102), and this reduction was enhanced by replacing the Asian genotype 5' and 3' UTRs with those from the American genotype (21). The Asian DENV2 strains also disseminated in a larger percentage of field-caught *A. aegypti* mosquitoes compared with American DENV2 strains (6), and when the mosquitoes were coinoculated with equal titers of Asian and American strains, the Asian strains were consistently recovered from a larger percentage of mosquitoes than were the American strains (20). Thus, it is possible that the success of the Southeast Asian DENV2 strains is due in part to more efficient replication in human target cells as well increased transmission by vector mosquitoes. Only one exception to this paradigm has been reported; Shurtleff et al. (127) described the association of DHF with an American genotype DENV2 from Venezuela, as determined by analysis of the 3' UTR.

Another recent example involves a clade replacement within the DENV2 Asian/American genotype identified by phylogenetic analysis of full-length genomes from Nicaraguan patients; interestingly, this correlates temporally

with a large increase in disease severity, and the new clade is significantly associated with severe disease (A. Balmaseda, T. Gomez, M. Henn, C. Rocha & E. Harris, unpublished results). The mechanism(s) responsible for the increased fitness and/or virulence of the new DENV2 clade is currently under investigation. Although an increase in viral virulence must be considered in the context of host immunity, the possibility exists that more virulent dengue viruses will continue to evolve in Southeast Asia and spread worldwide, displacing more benign genotypes in the years to come (107, 157).

The DENV3 serotype provides another convincing example of how increased viral diversity has led to the emergence or evolution of a clade of viruses strongly associated with DHF/DSS. DENV3 genotype III includes isolates from East Africa, South Asia, and Latin America, and has been associated with an increase in DHF/DSS in these regions (27, 87). Emergence of epidemic DHF/DSS in Sri Lanka in 1989 led investigators to question the reasons for this occurrence. After eliminating the possibility of a general increase in virus circulation or a change in serotype prevalence on the island (88), the decisive factor was identified as a clade replacement event (87). Both DENV3 III subtypes A and B were present in Sri Lanka in 1989, but only one subtype persisted after 1989 and was involved in all subsequent cases of DHF/DSS on the island. Additionally, DENV3 III subtype B has since spread to the Americas, where it has also been associated with epidemics of DHF/DSS (27, 50, 87). Other genotypes of DENV3 may also be associated with increased severity of disease; genotype I viruses reintroduced into islands of the Western Pacific have been associated with DHF/DSS, in contrast to past epidemics of DF associated with genotype IV (80). As viral strains with increased virulence are identified via the marriage of phylogenetic and epidemiologic analyses, the next challenge will be to define the molecular basis of this increased pathogenesis. With this information in hand, a combination of active surveillance for and rapid detection of viral genotypes with

potential for increased virulence could help identify at-risk populations and individuals, respectively.

Virus Evolution and Host Immunity

Intriguing evidence to explain differences in viral virulence in relation to pre-existing host immunity of native and introduced DENV2 genotypes derives from observations in Iquitos, Peru. After years of DENV1 circulation, a large number of secondary infections with DENV2 were documented in Iquitos in the complete absence of severe dengue (150), which was unexpected given the increased risk of DHF/DSS typically observed in secondary DENV infections. The DENV2 genotype present in Iquitos belonged to the native American genotype (150), in contrast to the DENV2 strains that have caused epidemics of DHF in the Caribbean and throughout Latin America (27, 61, 75, 109). The question arose whether the lack of DHF/DSS due to these secondary DENV2 infections with native American strains was caused by an inherent lack of virulence compared with Southeast Asian DENV2 strains, and/or whether anti-DENV1 antibodies present among the population of Iquitos neutralized or at least mitigated secondary "American" DENV2 infection by virtue of cross-reacting, neutralizing antibodies. In fact, sera from Iquitos residents characterized by a monotypic, anti-DENV1 response have higher cross-reactive neutralizing titers against American DENV2 strains than against Asian DENV2 strains (73). Antibodies arising from a DENV1 infection in Cuban patients also demonstrated higher neutralizing ability against the American DENV2 genotype than against the Asian DENV2 genotype (51), raising the possibility that the Asian DENV2 strains have epitopes divergent from those that may be shared between DENV1 and American DENV2 strains (73).

Several investigators have taken advantage of detailed information available from Bangkok, Thailand, which has remained hyperendemic for all four DENV serotypes since at least 1958

(60), to tease out the correlations between the periodicity of dengue epidemics and potential cross-protection between serotypes. Serotype- and severity-specific data collected between 1973 and 1999 showed that each serotype displays a somewhat different pattern of oscillation across this time period, and that together the four serotypes exhibit rather complex dynamics (96). Mathematical models have been designed to test the theory that the interaction between the periodicity of alternating epidemics due to different serotypes and host immunity can explain the patterns seen in Bangkok (2, 151). One model describes a scenario in which temporary cross-immunity between serotypes and seasonal fluctuations in vector populations explain serotype dynamics in Bangkok (151), and posits that ADE and differences in viral virulence are less important in shaping patterns of transmission (although it does not exclude both playing a key role in disease). Another model postulates that moderate cross-immunity alone can explain the oscillations and periodicity of individual serotypes (2), and that clade replacement events seen within each serotype are also associated with serotype-specific periodicity in combination with cross-reactive protection (2, 158). In particular, the authors propose that clade replacements within DENV1 serotypes in Thailand are best explained by a combination of mutations fixed by stochastic events plus cross-protective immunity to an incipient increase in DENV4 (158), as these two serotypes show a striking out-of-phase periodicity with one another.

The studies discussed above would suggest that viral evolution must then be considered in the context of cocirculation of different serotypes and the presence of cross-protective immunity. Alternatively, other theories have been proposed suggesting that the phenomenon of ADE could explain the periodicity and alternating epidemics caused by multiple serotypes in Mexico and Thailand (24, 31). Although most studies of DENV evolution have shown strong evidence for negative or purifying selection (157, 158), support for adaptive evolution has been reported

Extrinsic incubation period: the latent period in a vector mosquito before the virus has disseminated to the salivary glands, from where it can be transmitted to a vertebrate host as the mosquito takes a blood meal

(15, 16, 143), as well as some examples of recombination (1, 65). Even so, most studies suggest that positive selection and recombination have not played a decisive role in the overall evolution of DENV. To date, it appears that a combination of random genetic drift, rapid evolution, an ever-increasing number of infections, and perhaps the sporadic selectively driven replacement of viral clades characterizes DENV evolution, along with a complex interaction with serotype-specific host immunity that is only now beginning to be unraveled (2, 79, 114, 157). A better understanding of this last interaction is crucial in the face of imminent large-scale tetravalent dengue vaccine trials, because selection pressures due to host immunity will be greatly increased by trials and the eventual implementation of dengue vaccines.

OTHER FACTORS

Climate

The term climate change refers to multi-year, large-scale changes in climate patterns, including fluctuations in both rainfall and temperature; global warming refers to an increase in the average global temperature related to the greenhouse effect, whereby solar radiation is trapped beneath the earth's atmosphere. Changes in the composition of the atmosphere have been predicted to lead to a 2.0°C–4.5°C rise in global temperatures by the year 2100 (126), which could have an impact on vector-borne diseases (137). There has been a great deal of debate on the implications of global warming for human health (22, 139). Models that discuss the specific impact on dengue focus particularly on humidity (54) and temperature (101) in an attempt to predict the impact on the geographic range of mosquito vectors. Other perspectives highlight the overriding importance of infrastructure and socioeconomic differences that exist today and already prevent the transmission of vector-borne diseases, including dengue, even in the continued presence of their vectors (104). At the moment, there is

no consensus, but in the case of dengue it is important to note that even if global warming does not cause the mosquito vectors to expand their geographic range, there could still be a significant impact on transmission in endemic regions. For instance, a 2°C increase in temperature would simultaneously lengthen the lifespan of the mosquito and shorten the extrinsic incubation period of DENV, resulting in more infected mosquitoes for a longer period of time (33).

Public Policy

A great deal of work is currently directed to the development of tetravalent dengue vaccines and specific antivirals, which will hopefully provide additional tools for reducing the health impact of dengue in the near to mid-term future (66, 71, 154). At present, sustainable vector control programs that can maintain low mosquito densities, as well as good surveillance programs that can quickly identify incipient epidemics and thus trigger mobilization of emergency control measures, will continue to be our most important tools for controlling dengue for some time. Inevitably, these measures will face the challenge that much of public health faces—how to convince both policymakers and residents that only their vigilance now can prevent the need to cope with large epidemics in the future. Dengue will continue to be a challenge for public health officials and policymakers for the reasons discussed in this review, namely, increases in human population, urbanization, and international travel; the plethora of mosquito habitats due to daunting challenges in vector control; and the increasing occurrence of DF and DHF epidemics related in part to changes in viral virulence and to host immune status. Although we understand the general principles behind the spread and persistence of dengue, and further research questions remain to be explored, knowledge alone is not enough. The overriding question is, Can we take this knowledge and use it to contain or reverse the trend, or will the prevalence of dengue continue to increase in the years to come?

SUMMARY POINTS

1. The incidence of DF and of DHF/DSS has increased dramatically in the past 50 years, and key reasons for this increase include population growth, uncontrolled urbanization, spread of the mosquito vectors, and movement of the virus in conjunction with the rapid transit of people around the globe.
2. The risk for acquiring dengue relates foremost to the host's immune status and exposure to an infected mosquito. Risk factors for DHF/DSS include most importantly previous exposure to a heterotypic dengue virus, as well as the time between infections, age, ethnicity and genetic background, and the genotype and serotype of the infecting virus.
3. Originally found in the jungles and rural areas of Southeast Asia, dengue virus is now maintained primarily in an urban cycle involving humans and *A. aegypti* and *A. albopictus* mosquitoes, and the challenge of controlling urban breeding sites for these vectors has hindered progress in containing the dengue pandemic.
4. Dengue virus may be maintained between epidemic cycles by silent transmission in humans (asymptomatic infections) and/or vertical transmission or overwintering in the mosquito vectors.
5. Past vertical or government-led vector control programs have been somewhat successful using intensive source reduction techniques combined with targeted insecticide use. Ultimately, these programs are either unsustainable and/or they have been unable to prevent dengue transmission. New approaches that encompass both community participation and targeting of highly productive breeding containers via pupal/demographic surveys hold promise to minimize dengue epidemics in the future.
6. Some genotypes and subtypes of dengue virus appear to cause more severe disease; functional analysis in vitro confirms that certain strains may be inherently more virulent in both mammalian cells and mosquitoes. However, there is also clearly a role for host immunity in determining the fitness of dengue virus strains and thus influencing viral evolution.
7. Although development and evaluation of dengue-specific vaccines and therapeutics are currently underway, these tools will not be available for general use in the immediate future. Therefore, our best hope for confronting the continued spread of dengue at the moment is to use the knowledge we already have to design more effective control measures, while pursuing remaining research questions that will allow the design of more effective measures in the future through a better understanding of the complex interaction of human, mosquito, and viral biology.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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2. Showed that temporary cross-immunity can explain oscillations and periodicity of individual DENV serotypes in Thailand and linked clade replacement events to potential cross-reactive protection between serotypes.

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36. Developed weather-driven simulation model of urban dengue transmission that allows customization by input of location-specific parameters such as human demographics, herd immunity, and mosquito density.

42. Comprehensive review of the historical incidence of dengue and reasons for its dramatic expansion shortly after World War II.

58. One of the first studies to document enhanced disease during secondary DENV infection.

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