Chapter 5

Dengue and Chikungunya Coinfection – The Emergence of an Underestimated Threat

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Additional information is available at the end of the chapter

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Abstract

Both Dengue (DENV) and Chikungunya (CHIKV) viruses can be transmitted by *Aedes* mosquito species and the diseases that they cause have several clinical symptoms in common. Co-circulation of DENV and CHIKV is increasing around the world and must therefore be considered as an emerging threat with an important public health concern. At present, very little is known about the clinical manifestations and biological consequences of coinfection by both viruses. Thus, numerous questions such as clinical severity and dynamics of viral replication of DENV and CHIKV coinfections, as well as vectorial competence, have yet to be addressed in this important and challenging research area. The ensuring knowledge will enhance the clinical surveillance and the development of diagnostic tools able to differentiate DENV and CHIKV in order to early detect virus invasion and local transmission, as well as to improve patient care and timely control measures. In this review, we highlight the current knowledge on DENV and CHIKV coinfections. We also discuss research perspectives and challenges in order to further understand the ecology and biology of this phenomenon.

Keywords: Chikungunya, Dengue, coinfection

1. Introduction

Arthropod-borne viruses represent a global threat for public health as they can be transmitted to humans by hematophagous arthropods that are rapidly spreading worldwide. These viruses belong to four major families, *Flaviviridae*, *Togaviridae*, *Rhabdoviridae*, *Reoviridae*, and *Bunyaviridae*, and are the etiologic agents of severe pathologies, such as yellow fever, dengue, and chikungunya diseases.

Dengue virus (DENV) is perhaps the most relevant arbovirus in terms of morbidity, mortality, and socioeconomic impact, threatening more than 2.5 billion individuals worldwide [1]. It belongs to the *Flaviviridae* family, genus *Flavivirus*, and is composed by four closely related serotypes (DENV-1, -2, -3, -4), all of which share the same icosahedral and enveloped structure with an average diameter of 50 nm. Its genome is composed of a single-stranded positive sense RNA molecule of approximately 11 kb that codifies three structural (Capsid, Membrane, and Envelope) and seven nonstructural proteins (NS1/NS2A/NS2B/NS3/NS4A/NS4B/NS5), flanked by two untranslated regions (UTRs) [2]. DENV is transmitted to humans by blood-feeding females of *Aedes* mosquitoes and, although the large majority of infections remain asymptomatic, some of them can cause a spectrum of illnesses, ranging from a flu-like disease of mild severity known as dengue fever (DF), to more severe clinical manifestations such as dengue hemorrhagic fever (DHF) that can progress to dengue shock syndrome (DSS) and death [3, 4].

Chikungunya virus (CHIKV), on the other hand, belongs to the *Togaviridae* family, genus *Alphavirus*. Like DENV, it is a small icosahedral-shaped enveloped virus approximately 70 nm in diameter. Its genome is a single-stranded positive sense RNA molecule of approximately 12 kb that contains two open reading frames (ORFs): the N-terminal ORF encodes four nonstructural proteins (nsP1 to nsP4), while its C-terminal counterpart encodes the five structural proteins C, E1, E2, E3, and 6K [5]. CHIKV is also transmitted to humans by the bite of *Aedes* mosquitoes. Clinically, infection by CHIKV is characterized by fever, headache, fatigue, rash and intense, invalidating and often persistent arthralgia that can last for years in 30–40% of infected individuals. Although rare, neurologic complications can be observed, particularly among infected neonates. The rate of mortality has been estimated to be 1 in 1000 [6, 7]. Since its first documented epidemic in 1952 in Africa, sporadic CHIKV outbreaks were reported in numerous African and Asian countries, until the virus dramatically emerged during the last decade [8]. Since then, the virus has been continuously expanding and in 2013 it reached South America and the Caribbean basin causing more than 440,000 cases of disease in more than 20 countries by mid-2014 [9, 10].

Since *Aedes* mosquitoes can be vectors of both DENV and CHIKV and as the endemic areas of these two viruses often overlap, cocirculation of DENV and CHIKV has been reported in various geographic areas, including Southeast Asia and intertropical Africa. However, despite increasing evidence showing that coinfection of humans by DENV and CHIKV is likely to be an emerging trend, very little is known about the clinical manifestations and biological consequences of this phenomenon. This represents an exciting area of research as several scientific questions remain to be answered. Are the cases of coinfection linked to the propagation of a specific species of *Aedes* mosquitoes? Can these coinfections increase the incidence of severe forms of dengue and chikungunya diseases? What are the dynamics of viral replication when both viruses infect the same cells? What are the cellular pathways that are altered upon coinfection and how do they contribute to the pathophysiology of the diseases? The current chapter will try to shed light on these interrogations by reviewing all the available data on DENV-CHIKV coinfections.

2. Epidemiology

Although similar in some aspects, the history traits and epidemiology of DENV and CHIKV have followed different patterns, both intrinsically linked to the ecology of the mosquito vectors. These topics will be reviewed individually for each virus and the cases of coinfection will be finally analyzed.

2.1. Dengue virus

2.1.1. History and epidemiology

The name dengue seems to derive from the Swahili *ki-dinga pepo*, which was employed to describe a disease characterized by cramp-like seizures. The word was introduced in the Caribbean by the slave trade from East Africa during the 1800s and progressively changed to "dengue" [11]. Dengue-associated symptoms are almost indistinguishable from those caused by other viral agents such as CHIKV, but it is generally assumed that the first reports of dengue-like illness were described in China between 269 and 992 BC. The first detailed clinical descriptions were made in the late eighteenth century by Benjamin Rush and David Bylon after epidemic episodes in Philadelphia and Indonesia, respectively [12, 13]. These cases were associated to flying insects developing in water reservoirs, but it was not until the beginning of the twentieth century that *Aedes* mosquitoes were identified as the main transmitting vectors of the virus [14, 15]. During World War II, increasing cases of dengue among the troops deployed in Africa and the Pacific led to substantial efforts to isolate the virus. Not surprisingly, the first two serotypes of DENV (DENV-1 and DENV-2) were isolated during this period in the Pacific [16]. DENV-3 and DENV-4 were discovered in the 1950s in Southeast Asia in the Philippines and Thailand [17].

Although the virus was initially thought to have originated in Africa, serological and phylogenetic studies rather point toward an Asiatic origin with a subsequent propagation to the African continent and to the Americas [18]. By analyzing the substitution rate of the Envelope (*E*) gene from DENV, it has been estimated that the origin of the virus is likely to date back to 1000 years ago and that it used primates as a reservoir [19]. The four serotypes of DENV seem to have evolved in the rainforests of Southeast Asia and cross-species transmission to humans have occurred independently in all four serotypes between 125 and 320 years ago for DENV-1 and DENV-2, respectively [3, 19, 20]. According to the results from sequence analysis of the junction of the *E* and *NS1* genes, the DENV-1 serotype has been further divided in five genotypes (I–V), while the same analysis of the *E* and *M* genes classifies DENV-3 isolates into four genotypes. DENV-2 and DENV-4 are conformed by five and three genotypes, respectively [18].

It is believed that the sylvatic forms of DENV have caused sporadic and accidental outbreaks in humans, essentially among rural communities. The burden of dengue disease seems to be linked to the widespread colonization of the tropics by *Aedes aegypti*, a species that is highly permissive to DENV and exhibits an anthropophilic behavior, thereby mediating an efficient interhuman transmission. Originally from West Africa, where it acquired its urban preference, *Ae. aegypti* may have been introduced in the Americas and Asia by sailing ships, creating the ideal conditions for the spread of the disease worldwide [21]. Indeed, until the 1970s, less than 100,000 cases of dengue, diagnosed by febrile illness and hemorrhagic manifestations were registered yearly, and DHF was documented only in a dozen of countries. Furthermore, concomitant circulation of more than one serotype of DENV in a geographic region, known as hyperendemicity, was restrained to Central America, Southeast Asia, and West Africa. Thirty years later, DENV had become hyperendemic in all continents, except Antarctica, and was responsible for more than 500,000 cases of DHF and DSS reported in almost 60 countries [22]. It has been estimated that 2.5 billion individuals are at risk of infection, especially in the Americas and Asia. Recent investigations estimate to 390 million the number of DENV infections per year worldwide, leading to 96 million symptomatic dengue cases [1]. Several factors may explain the worldwide emergence of DENV. The most important one is the demographic burden observed after World War II that led to the occupation of ecological niches where the virus was circulating [18]. Unplanned urbanization with inadequate waste management and water distribution systems have facilitated the development of *Ae. aegypti* mosquitoes in densely populated areas. The increased circulation of people and merchandise has also allowed the concomitant spread of both the virus and its vectors to new geographical areas [22, 23]. Furthermore, the lack of continuity in programs aimed to eradicate the mosquitoes by massive fumigation, allowed the resurgence of *Aedes* populations in areas that were almost freed from them, particularly in South America [24].

2.1.2. Transmission and vector competence

Two main cycles of transmission have been described for DENV (Figure 1). The primitive sylvatic enzootic transmission, in Asia and Africa, involves *Aedes* spp. as vectors and lower primates as reservoirs. Occasionally, blood-feeding females of *Aedes* mosquitoes may transmit DENV to rural human communities, but these are considered as accidental contacts [3]. The urban cycle is the most relevant and challenging type of transmission, being responsible for the emergence of dengue during the twentieth century. Indeed, DENV can be maintained in a mosquito-human-mosquito cycle in urban areas, having lost the dependency on an enzootic cycle for transmission [22]. The urban cycle involves essentially *Ae. aegypti* as a vector, since this mosquito has been shown to be highly anthropophilic. This species feeds almost exclusively on human blood as a protein source for egg development [25–27]. Furthermore, *Ae. aegypti* prefers to lay its eggs in artificial water containers such as used tires, cisternae and flower pots that surround human habitats, thereby transmitting the virus transovarially to its progeny [28, 29]. It also feeds on multiple human hosts during a single gonotrophic cycle, resting indoors after the blood meal. This behavior ideally contributes to sustain the urban cyle of transmission as it increases the probability of becoming infected and transmitting the virus to multiple hosts [27].

Other species, such as *Ae. polynesiensis* and *Ae. albopictus* may also account, although as yet to a lesser extent, for the DENV urban cycle of transmission. In that sense, an increasing attention has been paid to the role of *Ae. albopictus* in the spread of DENV worldwide. This species is currently the most invasive mosquito in the world [30] and several vector competence experiments performed under laboratory conditions have shown that *Ae. albopictus* mosquitoes are more susceptible to DENV than *Ae. aegypti* [31–34], raising concerns over the possibility that the expansion of this vector will increase the risk of DENV spreading to new geographical areas. However, these experiments remain controversial, because conflicting results have been obtained showing that *Ae. aegypti* is more, or equally, susceptible to DENV infection than its counterpart *Ae. albopictus* [35–37]. In a very interesting paper, Lambrechts et al. [38] analyzed the relative public health importance of *Ae. albopictus* for DENV transmission, by performing a meta-analysis of reported studies that compared the oral susceptibility of *Ae. aegypti* and *Ae. albopictus* to DENV. The results revealed that although *Ae. albopictus* was more susceptible to infection than *Ae. aegypti*, as measured by midgut infection, the rate of virus dissemination to other tissues, as measured by the presence of the virus in the mosquito's head, was lower for *Ae. albopictus* [38]. Thus, according to these laboratory experiments, *Ae. albopictus* would not a represent a serious concern for DENV spread, as compared to *Ae. aegypti*, because of its lower capacity to become infectious and to act as an efficient vector. As pointed out by the authors, vector competence experiments are only one component of the natural and more complex vectorial capacity of a mosquito, which depends on other factors such as the ecology, the behavior, and the genetics of the vector and the virus. For example, it has been shown that vector competence can vary significantly among the vector subspecies: the *Ae. aegypti aegypti* subspecies, which is more anthropophilic than the *Ae. aegypti formosus* subspecies, is also more susceptible to DENV infection [39]. In addition, the DENV genotype is determinant, since *Ae. aegypti* is less susceptible to American DENV-2 genotypes than to Asian genotypes [40, 41]. Therefore, laboratory experiments of vector competence should be carefully interpreted and should be validated by entomological and ecological studies in the field. In that sense, several ecological observations indicate that the contribution of *Ae. albopictus* to the emergence of DENV should not be underestimated: (i) *Ae. albopcitus* was responsible of DENV outbreaks in areas where *Ae. aegypti* was absent or rare [26], such as Macao (China) [42] and Hawaii in 2001 [43], La Réunion Island in 2004 [44], Mauritius in 2009 [45], and Europe in 2010 when the first autochthonous dengue cases were reported in France [46] and Croatia [47]. (ii) The vector is massively and actively spreading worldwide and is, as mentioned above, considered to be one of the most invasive mosquito species in the world. Since 1979 it has colonized large areas of North, Central, and South America, Africa, Australia, and more than 20 countries in Europe [30, 48], where it has been mainly introduced through the trade of used tires [48, 49]. (iii) In contrast to *Ae. aegypti*, *Ae. albopictus* has the potential to adapt to low temperatures, allowing to colonize temperate climates with cold winters such as those found in Europe and North America. Indeed, it has been shown that immature forms of *Ae. albopictus* can develop in temperatures as low as 10ºC [50], and that some populations have a diapausing egg state, allowing them to resist cold winters with average temperatures below 0ºC [51, 52]. This capacity to resist low and adverse temperatures is linked to an increased efficiency to synthesize lipids in cold temperatures, as compared to *Ae. aegypti*, restricting this latter species to tropical and subtropical areas [53, 54]. (iv) Although it has generally been assumed that the feeding behavior of *Ae. albopictus* is opportunistic and zoophilic, mainly ingesting blood from nonhuman mammals, some studies have shown that *Ae. albopictus* mosquitoes caught in the wild preferentially feed on humans in Cameroon [55], Thailand [25], North Carolina [56], and the Andaman and Nicobar archipelago in India [26]. These results have been confirmed by laboratory host preference experiments with *Ae. albopictus* specimens from La Réunion Island [57]. This suggests that the feeding behavior of *Ae. albopictus* may be changing and switching to humans as a main source of blood, thus increasing the risk of human-to-human transmission mediated by this mosquito. (v) *Ae. albopictus* and *Ae. aegypti* are sympatric in numerous areas of the world, often sharing breeding sites and larval habitats [58–61]. This may lead to competitive interactions between two species eventually leading to the decline of one of the two. As suggested by field experiments performed in the United States [60, 62] and Brazil [63], when larvae from *Ae. albopictus* and *Ae. aegypti* compete for resources, it is *Ae. albopictus* that has a competitive advantage, giving a possible explanation to the local decline and extinction of indigenous *Ae. aegypti* populations following the introduction of *Ae. albopictus*. Altogether, these data emphasize the potential of *Ae. albopictus* to substitute *Ae. aegypti* and become the main vector of DENV.

2.2. Chikungunya virus

2.2.1. History and epidemiology

The name Chikungunya, meaning "the disease that bends up the joints" comes from the Makonde people in Tanzania, where the virus was first recognized in 1952 [64, 65]. Although arthralgia is one of the characteristic symptoms of chikungunya disease, most of the clinical manifestations are almost indistinguishable from those of Dengue. Thus, it is difficult to trace back the first epidemics of CHIKV in the literature and historical records. Nonetheless, it is generally assumed that the virus has been responsible for episodic outbreaks in Africa for several centuries before being imported to Asia and America by sailing ships during the eighteenth and nineteenth centuries [66–68].

On the basis of the phylogenetic analysis of the open reading frame of several CHIKV strains, the virus has been divided into three clades: West African (Waf), Asian, and East/Central/South African (ECSA), [69]. According to this study, the current CHIKV strains derived from a common ancestor that existed around 500 years ago. The divergence between the ECSA and the Asian clades occurred during the end of the nineteenth and the beginning of the twentieth centuries. Interestingly, despite their close geographic proximity, the ECSA and West African strains are highly divergent for reasons that are not yet completely understood. The recent Indian Ocean monophyletic lineage (IOL) originated from the ECSA group at the beginning of the twentieth century [69].

It is assumed that CHIKV originated in Africa, where it circulated in an enzootic cycle responsible for sporadic human epidemic outbreaks during the twentieth century in Tanzania in 1952 [64], Uganda in 1958 [70], South Africa in 1976 [71], Sudan in 1988 [72], and Senegal in 1996 [73], all arising from rural communities in close proximity to forested areas. However, more recent CHIKV outbreaks linked to indigenous ECSA strains have arisen in urban centers, as observed in Congo, Cameroon, and Gabon during 2000–2010 [74–76].

In Asia, the virus was first isolated in Thailand in 1958 [77] and was responsible for large epidemics affecting millions of people in Sri Lanka and India between 1963 and 1973, when the last CHIKV epidemic was recorded in 2005 [78–80]. This year marks the reemergence of CHIKV on the Indian subcontinent with the introduction of the IOL coming from islands in the Indian Ocean [80–82]. Indeed, after its initial detection in Kenya in 2004 [83], IOL subsequently spread to these islands, among which Mauritius, Comoros, Mayotte, Seychelles, La Réunion, and Madagascar, during 2005–2006 [84, 85]. The extent of the epidemics by this new strain is reflected by the example of La Réunion, where 266,000 individuals, a third of the island population, became infected, which resulted in around 260 deaths, most of them elderly people [86, 87]. After its introduction in India and Sri Lanka, the IOL CHIKV strains spread quickly throughout Southeast Asia, being responsible of outbreaks in Malaysia [88], Singapore [89], and Thailand [90] in 2008, China in 2010 [91], Cambodia in 2011 [92], and Bhutan in 2012 [93]. Overall, it is estimated that CHIKV has caused more than two million cases since 2004 in Africa and Asia [94]. IOL strains also have become a concern in Europe, where they were imported by infected travelers returning from India and were responsible for outbreaks in Italy in 2007 [95] and France in 2010 [96] and 2014 [97], both likely transmitted by resident populations of *Ae. albopictus*.

In America, the presence of CHIKV has formally been identified in 2013 in Saint Martin Island during a large and ongoing epidemic in the Caribbean basin [98], although it is suspected to be responsible for several epidemics since the nineteenth century. Since then, CHIKV has spread to the other Antilles islands where *Ae. aegypti* was the only known vector present [98]. The virus then reached Central, South, and North America, where 11 cases of local CHIKV transmission were recorded in 2014 [9]. Overall, the CHIKV burden in the Americas caused more than a 1.7 million suspected cases, with almost 60.000 confirmed cases and more than 200 deaths in the 2013–2015 period [99]. Interestingly, this epidemic burden was not initiated by the highly invasive IOL, but rather by Asian CHIKV strains [98]. These strains have maintained their endemic circulation in Asia alongside the IOL burden, provoking recent outbreaks in Indonesia and the Philippines [100, 101] as well as in the Pacific [102].

2.2.2. Transmission and vector competence

Similar to DENV, two modes of transmission have been described for CHIKV that rely on the same *Aedes* vectors (Figure 1). In Africa, CHIKV has been circulating in an enzootic cycle between forest-dwelling *Aedes* spp. mosquitoes and nonhuman primates as a reservoir [103]. This mode of transmission is believed to be the source of the sporadic and remote African Chikungunya outbreaks recorded during the twentieth century. However, increasing urbanization and the establishment of anthropophilic and peridomestic *Ae. aegypti* and *Ae. albopictus* populations, seem to be changing the dynamics of CHIKV spread in Africa, provoking larger epidemics associated with an urban cycle of transmission that relies on humans as a reservoir. This urban transmission is most likely responsible for the recent Chikungunya outbreaks in Western Africa [75, 76].

In Asia, CHIKV has traditionally circulated in an urban cycle associated with the presence of *Ae. aegypti* and *albopictu*s mosquitoes. As a consequence, Asian CHIKV epidemics have been larger and have spread more rapidly than those in Africa.

Vector competence studies, (reviewed in [103]), have shown that both *Ae. aegypti* and *Ae. albopictus* are highly susceptible to CHIKV infection and are both able to transmit the virus to humans. However, the Indian Ocean epidemics have provided a very interesting case of viral adaptation to a specific vector. Indeed, during the second half of 2005, a genetic change (alanine to valine substitution) occurred at position 226 of the E1 membrane fusion glycoprotein in the viral IOL strains that were circulating in the area. This mutation was absent in the initial strains and became prevalent after its introduction, being present in more than 90% of the isolates obtained in La Réunion during 2005–2006 [104]. Further laboratory experiments showed that this mutation was directly responsible for an increase in CHIKV infectivity for *Aedes albopictus*, improving viral dissemination and the transmission to suckling mice without affecting viral fitness in *Ae. aegypti* [105, 106]. Interestingly, this mutation was acquired independently in several distinct geographical locations (India and West Africa) where, similarly to La Réunion, *Aedes albopictus* is widely present and is actively displacing indigenous *Ae. aegypti* populations. Therefore, it has been suggested that the A226V mutation is a clear example of convergent evolution, allowing CHIKV viral strains to adapt to the prevalent mosquito vector [107]. The molecular explanation for the increased fitness in *Aedes albopictus* conferred by A226V mutations has not been completely unraveled. It was initially suggested that this mutation increased the dependency on cholesterol during the virus-host cell fusion step, based on the observation that mutated CHIKV isolates showed an attenuated viral growth in C6/36 mosquito cells devoid of cholesterol, as compared to original, nonmutated, strains [6, 105]. Therefore, it was suspected that this differential phenotype was responsible for the preferential replication in *Aedes albopictus* mosquitoes. However, further studies revealed that there is no a clear correlation between the dependence on cholesterol and capacity of the virus to infect *Ae. albopictus*, suggesting that these are two independent phenotypic effects of the E1 226 mutation [108]. Interestingly, second-step adaptative mutations have been described in A226V CHIKV strains that further potentiate viral replication in *Ae. albopictus*. One of these consists in a leucine for glutamine substitution in position 210 (L210Q) of the E2 protein that mediates viral binding. This mutation, characterized in viral isolates from Kerala in Southwest India [109], facilitates infection of midgut epithelial mosquito cells, thereby increasing viral dissemination and transmission by *Aedes albopictus* without a significant effect on *Ae. aegypti* [110]. Altogether, these data demonstrate that some CHIKV strains are rapidly evolving to exploit *Aedes albopictus* as a major vector in areas where it is abundant, raising concern about the epidemic potential of these strains in the Europe and North America where the mosquito is rapidly spreading.

Another interesting observation is that A226V mutation appeared in ECSA CHIKV strains and not in the Asian strains circulating in areas where *Aedes albopictus* is common [111]. This phenomenon has been attributed to evolutionary constraints imposed by epistatic interactions between residues 226 and 98 of the E1 glycoprotein. Indeed, all endemic Asian strains have a threonine in position 98 that is absent in both IOL and ECSA strains and that limits the adaptative effect of the A226V mutation in *Aedes albopictus* [111]. This constraint is likely to guarantee that the ongoing American CHIKV epidemics, caused by Asian strains, will be sustained by *Ae. aegypti* instead of *Ae. albopictus* in areas where they are sympatric [103]. However, this dynamic may change if introduced *Ae. albopictus*-fitted ECSA and IOL strains settle in the area.

In addition, a recent experimental study conducted by Stapleford et al. [112] showed the emergence of two new mutations V80I and 129V on E1 glycoprotein of the CHIKV A226V strain. Positive selection of these mutations appears to improve the stability and fusogenic activity of these variants. This study offers an interesting predictive approach to guide the monitoring of CHIKV strains involved in future outbreaks [112].

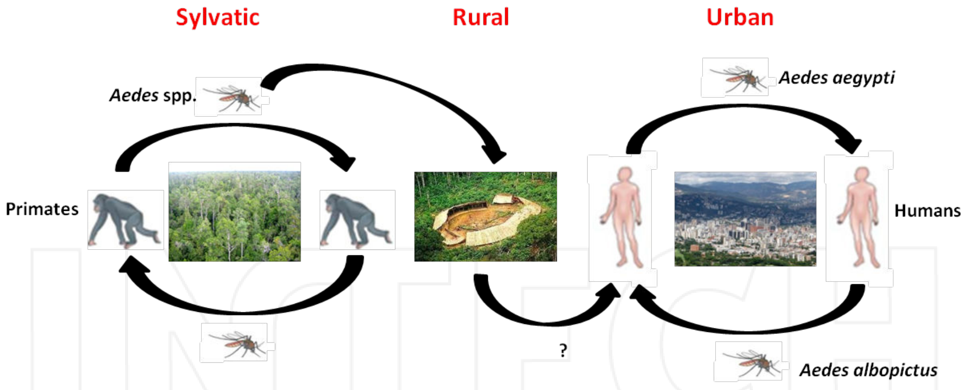

Figure 1. Transmission cycles of DENV and CHIKV. In the sylvatic cycle, primate hosts and several species of *Aedes* mosquitoes sustain DENV and CHIKV transmission. Occasionally, rural communities surrounding forests can become infected. The urban cycle of transmission may have been initiated by the migration of infected individuals from those rural communities to the cities. The urban cycle involves essentially *Ae. aegypti and Aedes albopictus* as vectors and humans as reservoirs. Adapted from [113–115].

2.3. DENV-CHIKV coinfections

2.3.1. History of reported cases

To date, the number of diagnosed cases of DENV-CHIKV coinfections is surprisingly small and available information is often incomplete, making it difficult to establish epidemiological trends. However, it is noteworthy that the number of reported cases has increased considerably during the past 10 years (Table 1, Figure 2), indicating that the phenomenon is becoming a concern among the scientific community because of its potential impact on human health and economy. Indeed, although the first documented cases of DENV-CHIKV coinfections date back to the 1960s in Vellore, South India, when 14 cases were reported during a CHIKV epidemic outbreak [116, 117], and in Thailand [118], where nine cases were documented, it was not until 2006 that the diagnosis of concomitant infections experienced a real interest, possibly due to the burden of cases of chikungunya infection in the Indian Ocean's island and Southeast Asia where DENV is endemic.

In 2006, two cases of coinfection corresponding to two female patients were described in Malaysia, and 20 more were recorded during the CHIKV outbreak in La Réunion the same year. More cases of coinfection were reported in Madagascar and Sri Lanka in 2006–2007 and in Gabon, India, Nigeria and Singapore during 2007–2010, coinciding with the epidemics of CHIKV caused by IOL strains during this period in the area. The most recent cases were diagnosed in South America, India and Nigeria in 2013–2014. Of note, two of these cases corresponded to infected travelers returning to Portugal and Germany after being infected in Angola and India, respectively [119,120], raising concern about the possible spread of coinfection cases in Europe where *Aedes albopictus* is present.

Number of cases	Location	Year	DENV serotype	CHIKV clade			Severe symptoms	Vector	Reference
				Asian	ECSA	Waf			
9	Thailand	1962-1964	NS[a]		NS		NS	NS	[118]
14	Vellore, India	1964	DENV-2		NS		Absence	NS	[116, 117]
20	La Réunion	2005-2006	NS		X		NS	NS	[121]
2	Kinta District, Malaysia	2006	DENV-1		X		1 case of DHF	NS	[122]
10	Toamasina, Madagascar	2006	DENV-1		NS		Absence	*Ae. albopictus*	[123]
3	Kandy, Sri Lanka	2006-2007	NS		NS		1 case with Guillain barré syndrome	NS	[82]
37	Gabon	2007-2010	DENV-2		X		Absence	*Ae. albopictus*	[124, 125]
1	Chennai, India	2008	NS	NS			Absence	NS	[119]
63	Nigeria	2008	NS				Absence	NS	[126]
6	Delhi, India	2009	DENV-3 DENV-4 DENV-3/4 DENV-1/4		X		2 DHF, 1 dead	NS	[127]
1	Singapore	2009	DENV-2		X		Absence	NS	[128]
43	Maharashtra and Odisha, India	2013	DENV-2 DENV-1 DENV-3		X		3 cases of DHF	NS	[129]
16	Saint Martin	2013-2014	DENV-1 DENV-2 DENV-4	X			Absence	*Ae. aegypti*	[130]
1	Nigeria	2014	NS	NS			Absence	NS	[131]
1	Luanda, Angola	2014	DENV-4		X		Absence	NS	[120]
2	India	NS	NS	NS			Absence	NS	[132]

Table 1. Reported cases of DENV/CHIKV coinfections. [a] NS: Not specified.

As shown in Table 1, the four serotypes of DENV can be found in association with both the Asian and ECSA CHIKV clades, depending of the strain of CHIKV that cocirculates with

DENV in a particular area, pertaining to Asian CHIKV strains in America and ECSA strains in Asia and Africa. However, it would be interesting to study if some particular associations of DENV and CHIKV genotypes are favored in nature. For example, is the circulation of some virulent DENV strains associated with the simultaneous presence of specific CHIKV genotypes? Such preferential associations could provide insight into viral coevolution and allow to define strategies to limit the morbidity associated with certain highly pathogenic viral strains.

2.3.2. Transmission and vector competence

Some of the studies reported in Table 1 provide interesting information about the relative importance of *Aedes albopictus* and *Ae. aegypti* in the spread of coinfection cases in natural conditions. Perhaps the most complete and documented cases of coinfection from an epidemiological perspective have been provided by a large clinical, virological, and entomological study performed in Gabon between 2007 and 2010 [124, 125]. During this period, an active surveillance of acute febrile symptoms was implemented in the healthcare centers of Libreville and all the major towns of the country. Blood was sampled from patients who met the case definition for diagnosis of CHIKV and DENV infection, as determined by quantitative PCR [124, 125]. A total of 4287 patients were tested among which 1567 (36.6% of the individuals) were CHIKV-positive, 376 (8.3%) were DENV-2 positive (no other serotype was reported) and 37 (0.9%) were coinfected with both viruses. All cases occurred in densely populated areas during the rainy season, when conditions are ideal for mosquito breeding. Two large epidemic outbreaks were observed, during 2007 and 2010, with sporadic cases in between. In 2007, the vast majority of CHIKV and DENV-2 infections were reported around the capital Libreville, in the Northwest, with nine cases of coinfection. During 2008 and 2009, the viruses moved to Lambaréné, Ndjolé, and Lastourville in the center and the south of the country, respectively, however no cases of coinfection were reported. Finally, the 2010 outbreak occurred in the southeast of the country and was centered around Franceville, close to the Congo border where 28 cases of coinfected patients were recorded. The phylogenetic study, based on the isolates recovered from monoinfected and coinfected patients not only showed that CHIKV belonged to the ECSA lineage, but also that the Gabonese strains from 2010 derived from those reported in 2007, which were in turn closely related to the CHIKV strains isolated in 2006 from an outbreak in Cameroon [74]. These findings suggest that CHIKV is spreading rapidly in Western Central Africa with a north to south dynamic, a trend that seems to be confirmed by the identification of the virus in Southern Congo in 2011 [133] and Angola in 2014 [120]. On the other hand, DENV-2 isolates from 2010 also derived from those of 2007 and were found to cluster in the cosmopolitan genotype, which gathers strains isolated in diverse areas of the world such as India, China, Australia, and Saudi Arabia. The most likely explanation for these observations is that the DENV-2 Gabonese strains were imported by infected travelers coming from the latter areas or by infected mosquitoes introduced in Gabon along with imported products [124, 125].

During this study, mosquitoes were captured around the coinfected patient's homes. After identification of the species, viral presence was determined by quantitative PCR from pooled mosquitoes abdomen. In total, 661 *Aedes* mosquitoes were analyzed. A large majority of the

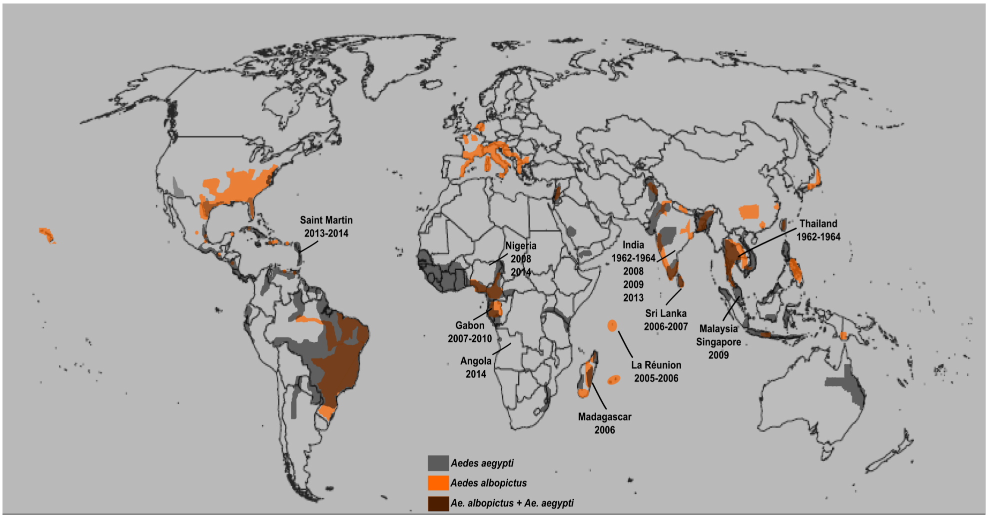

Figure 2. Geographical distribution of *Aedes albopictus* and *Aedes aegypti* populations and locations where DENV/CHIKV coinfections have been described. Adapted from [134].

mosquitoes was constituted by *Ae. albopictus* (571 specimens, 86% of the caught specimens), followed by *Ae. simpsoni* and *Ae. aegypti* (52 and 38 specimens, respectively). From the 46 pooled abdomens of *Ae. albopictus* 11 (23.9% of total) tested positive for CHIKV, 18 (39.1%) for DENV-2 and three (6.5%) for both viruses. This was the first report of a concomitant infection of mosquitoes by CHIKV and DENV in nature. In the case of *Ae. aegypti*, one out of the three pools (33.3%) tested positive for CHIKV, whereas DENV-2 was not detected. All three *Ae. simpsoni* pools tested negative for both viruses. Although the lack of detection of both viruses in *Ae. aegypti* specimens could be attributed to the large predominance of *Ae. albopictus*, which increases the probability of the latter vector to be coinfected, another study comparing the roles of the two species in the emergence of DENV and CHIKV in central Africa confirmed that the only species naturally infected by DENV and CHIKV was *Ae. albopictus* [135]. This is not surprising with respect to CHIKV, because the viral isolates circulating in the area have acquired the A226V substitution and are therefore particularly adapted to grow in *Ae. albopictus* [107, 125]. The absence of *Ae. aegypti*, naturally infected with DENV, has been attributed to the poor susceptibility of this mosquito to DENV in this region, explaining the lack of DENV outbreaks in West and Central Africa until the turn of the century with the introduction of *Ae. albopictus* [39]. This notion has been confirmed by laboratory experiments showing that *Ae. aegypti* specimens collected from Cameroon are less susceptible to DENV infection than mosquitoes of Asia and South America [135].

Besides its greater susceptibility to CHIKV and DENV, *Ae. albopictus* may be more efficient in the maintenance of DENV and CHIKV transmission cycles because of its aggressive feeding behavior, with a human biting rate that is significantly higher than the one observed in *Ae. aegypti* populations in suburban areas of Central Africa, according to a study led by Paupy et al. [135]. This may increase the probability of *Ae. albopictus* mosquitoes of becoming infected by DENV and CHIKV and transmitting the viruses to human hosts.

These data indicate that *Ae. albopictus* is more fitted to transmit both viruses and to act as the primary vector of DENV and CHIKV in Gabon which should raise concerns about the spread of CHIKV/DENV coinfections to the rest of Africa in view of the continuing progression of *Ae. albopictus* on this continent.

The disproportion in the abundance of *Aedes* species in Gabon is striking as *Ae. albopictus* was first reported in the country in 2006 [136, 137], suggesting that the species is rapidly proliferating and actively displacing endemic populations of *Ae. aegypti*. This trend has been confirmed by a subsequent entomological study carried in different locations of Gabon, demonstrating that *Ae. albopictus* largely outnumbered the endemic *Ae. aegypti* populations in suburban areas where patches of vegetation are likely to be present, as well as in small towns particularly affected by the 2007–2010 DENV/CHIKV epidemics such as Cocobeach, Oyem, and Lastourville [135].

This trend and the role of *Ae. albopictus* as the main vector of CHIKV/DENV coinfections were confirmed by Ratsitorahina et al. who conducted a virologic and entomologic study in the city of Toamasina, located in the eastern coast of Madagascar, following an outbreak of dengue-like symptoms (DLS) [123]. CHIKV was suspected as the etiologic agent, because the virus was previously detected in La Réunion, the Seychelles, and Comoros archipelagos during the IOL CHIKV epidemic. Blood samples were taken from 55 febrile patients manifesting headache, myalgia, arthralgia, retroorbital pain, or rash. Molecular and serological diagnostics identified CHIKV (IO lineage, highly adapted to *Ae. albopictus*) and DENV-1 (closely related to strains isolated in La Réunion in 2004) in 38 of the 55 patients, among which 10 cases corresponded to coinfections. An entomologic study was performed in five neighborhoods in which DLS were reported, by catching mosquitoes larvae and adults in potential breeding sites. *Ae. albopictus*, a species that has been circulating in Madagascar since the 1970s [58], was the only urban vector of DENV and CHIKV and no other species were identified. Among all the mosquito pools tested for the presence of CHIKV and DENV, 21.7% tested positive for CHIKV. Data for DENV was not given. The identification of the breeding sites revealed that populations of *Ae. albopictus* from Toamasina exploit diverse artificial peridomestic containers such as tires, coconut shells, discarded cans, pots, etc., which are traditionally associated with *Ae. aegypti* populations. This highlights the notion that *Ae. albopictus* is able to adapt to the ecological niches found in urban areas and therefore could replace *Ae. aegypti* as the main vector of the urban transmission cycle of DENV and CHIKV. Accordingly, a large entomologic survey carried in 15 sites across Madagascar during the 2007–2009 period, revealed that *Ae. albopictus* has extended its geographical distribution on the island and that its population density has become consequently higher than that of *Ae. aegypti*, a species that is becoming rare. This contrasting result to what was previously reported in the 1970s–1980s, can be explained by the environmental plasticity of this species that exhibits a greater capacity to adapt to different climatic conditions and to anthropogenic changes in the natural habitats, as compared to *Ae. aegypti* [58].

Vector competence studies performed in laboratory conditions have shown that *Ae. albopictus* specimens from La Réunion are able to disseminate DENV and CHIKV and to deliver both infectious particles concomitantly in its saliva after being orally exposed to DENV and CHIKV strains, circulating in the island during 2004–2006 [138]. Another study revealed that *Ae. aegypti*, orally fed with CHIKV and DENV, is unable to sustain dual infection [139]. Although these results should be interpreted with caution since vector susceptibility is highly dependent on the origin of *Aedes* specimens, as well as the type of CHIKV and DENV strains involved in the infection, they corroborate what has already been shown in natural conditions and furthermore confirm the potential of *Ae. albopictus* to transmit both viruses more efficiently than *Ae. aegypti*.

Taken together, these results reveal an important and threatening role of *Ae. albopictus* through its ability to concomitantly transmit DENV and CHIKV in areas where it circulates sympatrically with *Ae. aegypti*. It is to be noted however, that in locations where *Ae. albopictus* is still absent, *Ae. aegypti* can sustain the concomitant transmission of both viruses, as recently reported during the Caribbean CHIKV outbreak. This epidemic that initiated in Saint Martin then spread to the French Antilles, causing a total 570 confirmed cases of infection with the Asian strain of CHIKV, 65 with DENV and 16 cases of coinfection (Table 1) [130]. *Ae. aegypti* was identified as the unique potential vector of this outbreak [98]. Nonetheless, because of the rapid propagation of *Ae. albopictus* in South America, this dynamic may change in a close future.

Another important topic related to CHIKV/DENV coinfections, is the mode by which these viruses can be transmitted to humans. Two main possibilities could be envisaged: an individual transmission of each virus by different monoinfected mosquitoes or concomitant transmission by a coinfected vector. In that sense, the study performed by Caron et al. [124] gives several interesting clues based on the analysis of viral loads detected in coinfected Gabonese patients. The results revealed the presence of two distinctive groups of patients, based on the presence of viral RNA-derived complementary DNA (cDNA): one group with a high DENV-2 cDNA load and low CHIKV cDNA load and the other with high cDNA levels of both viruses. According to this pattern of infection, the authors suggested two different modes of transmission. In patients with the highest DENV-2 cDNA, the blood samples were most likely taken during the acute phase of DENV infection and the early or late stage of CHIKV infection, suggesting that the viruses were more likely to have been transmitted by the bite of two different mosquitoes each infected with one virus, although with several days of interval, which might explain the gap between the replication kinetics of either virus. However this interpretation should be taken with caution, as several other possibilities may exist. For example, both viruses could have been transmitted by the same mosquito and DENV-2 might have replicated more efficiently than CHIKV due to genetic factors intrinsic to the human host, thus establishing a competitive state in which CHIKV could have been disadvantaged. Alternatively, DENV-2 viral load in the coinfected mosquito salivary glands may have largely exceeded that of CHIKV. As a result, the number of DENV viral particles transmitted to the human host during the mosquito bite may have been higher than for CHIKV, consequently explaining the difference in the observed cDNA loads. Another possibility, noted by Caron et al. [124], is that the immune response against DENV may have limited the replication of CHIKV. In the second group of patients with high cDNA loads for both viruses, indicative of a blood sample taken during the acute phase of both CHIKV and DENV-2 infections, the dual infection may have resulted from two rapidly succeeding bites of different mosquitoes, each infected by one virus or from the bite of a single coinfected mosquito [124].

3. Pathogenesis

From a public health perspective, the concern about coinfections is their possible impact on the pathogenesis and the outcome of dengue and chikungunya diseases. Is there a correlation between the cases of coinfection and the severity of symptoms? Because, in terms of morbidity, severity and mortality DENV has a higher impact on human health than CHIKV, the major preoccupation is that CHIKV/DENV coinfection could increase the incidence of DHF and DSS.

DHF symptoms appear around the time of defervescence, 3–7 days after the first symptoms of DF. It is characterized by an increase in capillary permeability with a loss of plasma volume that is preceded by thrombocytopenia and leukopenia. Hamorrhagic symptoms include petechiae, ecchymoses, and purpuric lesions. If a critical volume of plasma is lost through leakage, DSS may follow. This phase is characterized by a narrow pulse pressure that can be underestimated as most of the patients remain conscious and lucid. Prolonged hypotensive shock and hypoxia may result in organ failure, acidosis, intravascular coagulation, and death if not corrected in time [3, 4].

Although the pathogenesis of DENV infection is not well understood, several risk factors may increase the severity of the disease: the viral genotype (the Asian genotype of DENV-2 is considered to be a virulent strain), the age (children are less able to compensate plasma leakage than adults), the ethnicity (Caucasian are more susceptible to develop severe forms of the disease), chronic diseases (individuals with allergies, asthma, and diabetes are at higher risk than healthy people) and secondary infection with a new DENV serotype [140–143]. The latter issue has received particular attention, because it may be a major determinant for the development of severe cases of dengue. Indeed, when preexisting antibodies from a primary DENV infection bind to an infecting DENV particle during a subsequent infection with a different dengue serotype, the antibodies from the primary infection cannot neutralize the virus. Instead, the resulting antibody-virus complexes attach to Fc receptors at the surface of monocytes, macrophages, and dendritic cells (DCs), resulting in increased infection [113, 144, 145]. This phenomenon, known as antibody-dependent enhancement of infection (ADE), may explain the higher viremia and levels of circulating antigens detected in patients with DHF as compared to patients with DF [146, 147]. ADE accounts for the particular propensity of populations living in DENV hyperendemic regions to develop severe forms of dengue. ADE may also contribute to increased capillary permeability and to a “cytokine storm” that could aggravate the disease [148–150]. Another phenomenon increasing the risk of severe disease during secondary infections with DENV is the original antigenic sin or Hoskins effect. This effect refers to the tendency of the immune system to respond to a secondary infection through the activation of memory B and T cells induced by the primary infection. These cells show a decreased affinity for secondary antigens and are less effective in the control of the infection [151]. In particular, it has been shown that during the secondary infection by a different strain of dengue virus, the cytotoxic T lymphocytes release cytokines, rather than causing the lysis of infected cells, thereby increasing vascular permeability and exacerbating the damage of endothelial cells [152]. Taken together these data indicate that secondary heterotypic infections with DENV are an important factor in the aggravation of dengue disease.

However, despite the identification of risk factors, little attention has been paid to the potential effect of the simultaneous presence of CHIKV on the propensity to develop DHF. To date, the scarcely available clinical data about coinfections impedes to establish clear conclusions. The large majority of the studies analyzing the clinical symptoms of CHIKV/DENV coinfected patients failed to identify a particular predisposition to develop DHF, as no severe symptoms were observed (Table 1). Furthermore, two studies that compared the biological and clinical symptoms between monoinfected and coinfected patients did not observe more severe manifestations or biological disorders in patients with a mixed infection, suggesting that the two viruses do not exert additive effects [124, 153].

The rare cases of DHF in coinfected patients were observed in one of the two patients coinfected in Malaysia [122] and in India in 2009 [127]. The latter case deserves further attention: during this episode of DENV/CHIKV coinfections in Delhi, 69 blood samples were taken from patients with acute fever. Forty-eight were DENV-positive, eleven tested positive for ECSA lineage and six were positive for both viruses. From these six samples, three were positive for DENV-3, one for DENV-4, one for DENV-3/DENV-4 and one for DENV-1/DENV-4, constituting the first cases of concomitant infections with multiple DENV serotypes in CHIKV/DENV infected patients. Two of the six patients manifested severe hamorrhagic symptoms with central nervous system involvement and one died. It was not specified whether the severe cases corresponded to patients infected with a single DENV serotype or with two different serotypes, making it difficult to link the severity of the disease to the concomitant presence of CHIKV or to the presence of two different DENV serotypes. However, the particular high incidence of severe symptoms following superinfection by CHIKV and several DENV serotypes highlights the potential threat of CHIKV infection to human health in areas where DENV is hyperendemic [127].

Overall, these results do not establish a clear association between the severity of dengue and chikungunya diseases and the concomitant presence of both viruses. However, the number of CHIKV/DENV coinfections reported to the date is too small to draw firm conclusions. Further studies need to be undertaken with large cohorts of infected patients to gain better insight in this process, particularly taking into account that many severe cases associated with coinfections may have passed unnoticed, as the diagnosis of both viruses has not systematically been undertaken in the past. Moreover, the increase in coinfections with both viruses could lead to a rapid viral evolution, potentially resulting in the appearance of highly infective and pathogenic CHIKV and DENV strains.

4. Cell biology of CHIKV/DENV coinfections

Very little is known about the interactions that are established by the viruses and their host cells during coinfections. The fact that viral RNA of both viruses has been detected in *Aedes* abdomens strongly suggests that they can disseminate and coexist simultaneously in these mosquitoes. Accordingly, both viruses are able to concomitantly infect *Ae. aegypti* midgut and upregulate the expression of proteins involved in the oxidative stress, energy production, and carbohydrate/lipid metabolism. This shows that CHIKV and DENV are able to simultaneously circumvent the physical barrier established by the midgut to propagate to other *Aedes* organs and tissues [154]. However, almost nothing has been described at the cellular level: to date, only one work addressed the concomitant effect of CHIKV/DENV infection in mosquito cells but no information is able regarding vertebrate cells.

In this study, Potiwat et al. [155] infected *Ae. albopictus* C6/36 cells with different multiplicities of infection (MOIs) of CHIKV (ECSA strain) and DENV-3 isolates from Southern Thailand, detecting the presence of viral RNA in cell culture medium by RT-PCR. They observed that both viruses were able to replicate and generate viral progeny when cells were challenged simultaneously with a mixed viral preparation in which the viruses were added at the same MOI. However, when the proportion of viral input was changed and the titer of DENV largely exceeded the one of CHIKV, DENV was able to suppress CHIKV replication. The reciprocal (larger titer of CHIKV than DENV) did not exert any effect on DENV replication. When infection by each virus was performed sequentially at the same titer (superinfection conditions in which one virus was added 1 hour before the other), viral progeny was detected for the two viruses independently of the order of infection. Although these experiments were not validated with other viral strains and mosquito cells, they provide the first cell biology evidence that both viruses can replicate actively in the same cells when these are challenged simultaneously or sequentially. This supports the two modes of mosquito coinfection that have been suggested to occur in natural conditions: a mosquito could get coinfected by ingesting its blood meal from a viremic individual carrying both viruses, or sequentially by ingesting the blood from two different individuals each infected by a single virus. When the blood meal is taken from a single individual, the successful replication of both viruses in mosquitoes may require some conditions to be fulfilled, such as the presence of enough infectious CHIKV/DENV particles in a proportion that falls within a certain range. In other words, the quantity of one of the viral species should not overwhelmingly exceed the other one to avoid any competitive suppression. In that sense, a very recent study performed by Nuckols et al. [156] seems to confirm *in vivo* what is observed at the cellular level. In this work, *Ae. aegypti* and *Ae. albopictus* mosquitoes were challenged either simultaneously or sequentially with CHIKV and DENV-2 mixed in blood meals. After mosquito sacrifice, viral dissemination and transmission potential were assessed by detecting CHIKV and DENV RNA in the mosquito's head and saliva, respectively. The results show that both *Aedes* species exhibited a dual disseminated infection when viruses were administered at the same time or sequentially. However, CHIKV and DENV were only detected concomitantly in mosquito's saliva from specimens exposed to each virus sequentially and not simultaneously. Thus, this laboratory experiments suggest that *Aedes* mosquitoes are able to transmit both viruses to vertebrate hosts when they acquire

CHIKV and DENV with a time interval and that simultaneous acquisition of both viruses may generate competitive interactions that decrease their potential transmission. Although in this study the viral titers of DENV (3.2×10^6 focus forming units/mL) and CHIKV (1.5×10^5 plaque forming units) used to infect the mosquitoes were not comparable, it would have been interesting to assess if the competitive exclusion was due to the excess of one viral species over the other one or if it could be seen independently of the viral input.

In the work of Potiwat et al. [155], competitive suppression was only observed when the amount of viral particles from DENV largely exceeded the one from CHIKV and not the reciprocal. Both viruses are able to exploit similar cell surface receptors for attachment, such as prohibitin and heat shock proteins that can be found in mosquito cells [157–160], leading to possible competitive interactions between both viruses for attachment and viral entry. However, it is highly unlikely that this is the reason for the suppression of CHIKV replication by DENV, as no inhibition of DENV replication was observed when CHIKV particles outnumbered DENV particles, as it would be expected if the viruses rely on the same receptors for infectious entry. An alternative, is that the viruses are able to exploit different receptors on the same cell, and that an excess in DENV particles attached to the cell surface sterically interfere with CHIKV-receptors interactions. Another possibility is that the excessive entry of infectious DENV particles leads to the hijacking of cellular components necessary for CHIKV replication, or to the production of viral components that inhibit CHIKV infection.

There is no information about the cellular biology of CHIKV/DENV coinfection in mammalian cells and we can only speculate about the possible mechanisms involved in viral replication. As summarized in Table 2, CHIKV and DENV share similar mechanisms of entry, which could lead to suppressive competition between the viruses in the early steps of infection. For example, they are able to exploit similar cellular receptors for attachment, they are internalized mainly by clathrin-mediated endocytosis and their fusion occurs in the endosomal system. The cellular tropism is also similar, although it seems to be larger in the case of DENV, a phenomenon that could be explained by the longer and more frequent circulation of the virus among human beings, allowing it to adapt and exploit a more diverse range of cellular targets. However, the viral RNA of both viruses can be detected in the blood of coinfected humans, suggesting that they are both able to concomitantly invade, replicate and spread in different organs to establish a systemic infection resulting in viremia. Thus, these viruses seem to have adopted different replicative strategies to overcome the potential competition for cellular resources when they infect the same mammalian cells, and/or have established cooperative interactions to guarantee their survival and propagation in human hosts. For example, during the cellular attachment step, the viruses may use different not yet characterized receptors or use an abundant cell surface molecule to limit competition. Also, although both viruses enter cells by clathrin-mediated endocytosis, some differences exist in the pathways and molecular partners involved in the process between CHIKV and DENV. Indeed, the depletion of the fuzzy homologue (FUZ), a cytoplasmic effector protein involved in planar cell polarity, ciliogenesis, and mammalian embryonic development, strongly inhibits clathrin-mediated endocytosis of CHIKV and other alphaviruses without affecting DENV entry [161]. This suggests that both viruses exploit parallel clathrin pathways involving different effector proteins. Furthermore, DENV and CHIKV membrane fusion, a step necessary for the release of the viral genome in the cell, takes place in distinct cellular compartments: the first one occurs in Rab7^{+} late endosomes, while the second one takes place preferentially in Rab5^{+} early endosomes [162–164].The explanation for the selective use of these compartments could be linked to the lipidic composition of the endosomes: fusion of flaviviruses seems to require the presence of anionic lipids such as phosphatidylserine and bis(monoacylglycero)phosphate that are present in the late endosomes [165], while alphaviruses may have distinct requirements.

		DENV	CHIKV	Reference
Tropism in humans (main cell targets)	Keratinocytes	+	-	[166–172]
	Fibroblasts	+	+	
	Dendritic cells	+	-	
	Monocytes	+	-	
	Macrophages	+	+	
	Epithelial cells	+	+	
	Endothelial cells	+	+	
Attachment receptors in mammalian cells (name of the specific receptors)	Glycosaminoglycans	+	+	Reviewed in [173, 174]
	Heat Shock Proteins	+ GRP78 HSP70 HSP90	+ HSP60	
	Laminin Receptor	+	NI	
	Prohibitin	Used in mosquitoes	+	
	C-type lectins	+ DC-SIGN L-SIGN CLEC5A Mannose receptor	NI	
	Phosphatidylserine receptors	+ TIM TAM	+ TIM TAM	
	Integrins	+ Integrin $\alpha v\beta 3$	+ ITGAV ITGB1	
	Scavenger receptors	+	NI	

		DENV	CHIKV	Reference
	Claudin-1	+	NI	
	Nkp44	+	NI	
Main internalization pathway		Clathrin-dependent endocytosis	Clathrin-dependent endocytosis	[161, 164, 170, 174–179]
Compartment of viral fusion		Late endosomes	Early endosomes	[162–164]
Strategy for viral replication		Synthesis of a single polyprotein cleaved by viral and cellular proteases to generate individual viral proteins; replication of viral genome in RCs associated to ER-derived membranes	Inferred from other Alphaviruses: synthesis of two polyproteins from two ORFs autoproteolytically cleaved to generate individual viral proteins; replication of viral genome in RCs associated to endosomes/lysosomes-derived membranes	[5,180–190]
Place of assembly		ER membrane	Plasma membrane	[191–194]
Mechanism of release		Secretion	Budding at the plasma membrane	

+: positive tropism and positive interaction with the indicated receptor. -: negative tropism and negative interaction with the indicated receptor. NI: no information available.

Table 2. Comparison of CHIKV and DENV tropism's in humans and of the replicative strategies developed by these viruses in mammalian cells.

Also, fusion of alphaviruses seems to depend on the activity of the TSPAN9 tetraspanin protein, as depletion of the protein selectively blocks the fusion of Semliki Forest Virus (SFV) without altering the one of DENV. TSPAN9 may control the correct routing of the viruses to the early endosomes and maintain these compartments in a permissive state for alphaviruses fusion but not for flaviviruses [161].

There are also differences in the mechanisms involved in CHIKV and DENV synthesis of viral proteins, genome replication and assembly of the viral components to form mature infectious virions (Table 2). In the case of CHIKV, almost all the information about these processes has been inferred from studies performed with related alphaviruses such as SFV and Sindbis Virus (SINV). Once the viral genome is released into the cytoplasm, it is translated from two different open reading frames to generate the nonstructural (nsP1234) and structural (C-pE2-6K-E1) polyproteins [5, 180]. The nonstructural polyprotein is cleaved by the nsP2 viral protease to generate the individual nonstructural proteins that are going to form replication complexes

(RCs) in charge of the viral genome replication [195]. These RCs are associated to virus-induced membranous cytoplasmic structures that are derived from the endosomes and lysosomes [181–184]. The structural polyprotein is cleaved autoproteolitically by the C protein which is released in the cytoplasm. The rest of the polyprotein (pE2-6K-E1) is translocated to the endoplasmic reticulum (ER) where it is further processed by the host cell signal peptidase to generate the individual PE2, 6K, and E1 proteins [196]. These proteins are then routed to the plasma membrane through the Golgi network where the furin-like protease cleaves the pE2 to generate the E2 and E3 mature proteins. At the plasma membrane, all the structural proteins gather together along with the genomic viral RNA, and the interaction between the C and E2 proteins drives the budding process, giving rise to enveloped virions that are released to external medium [191, 192].

In the case of flaviviruses, upon release of the viral genome into the cytoplasm, the nonstructural (NS) and structural proteins are translated from a single ORF to generate a large polyprotein that translocates to the membrane of the ER. There, the viral NS2B-NS3 protease and the host cell signalase cleave the polyprotein to generate the individual nonstructural proteins and the C, pre-Membrane (prM) and E proteins [185–188]. The nonstructural proteins form RCs associated to virus-induced membranes derived from the ER, known as vesicle packets, and drive the replication of the viral genome [189, 190]. Flavivirus assembly results from the association of C proteins with the genomic RNA into ER-derived membranes where all the structural proteins are displayed. The assembly generates immature viral particles that acquire their lipid envelope by budding into the lumen of the ER. These particles are routed through the Golgi network, and final maturation occurs at the trans-Golgi where the furin cleaves the prM to generate the mature M protein. These mature virions are then secreted to the external medium [193, 194].

Thus, the different replicative strategies, assembly compartments and release mechanisms used by flaviviruses and alphaviruses, may allow CHIKV and DENV to replicate simultaneously without a substantial overlap in their cellular requirements.

Another tempting possibility is that both viruses contribute to shut-off the antiviral cellular mechanisms, creating a favorable environment for viral replication. For example, the type I interferon response (IFN I) represents an important antiviral response against DENV and CHIKV. Accordingly, treatment with either IFN-α or IFN-β suppresses the replication of both viruses in cell culture [170, 197, 198]. Therefore, CHIKV and DENV have developed strategies to counteract the cellular defense system. In the case of DENV, almost all the nonstructural proteins are able to alter the IFN I response. Indeed, NS2A and NS4B inhibit the interferon α/β response by blocking the activation and translocation of the signal transducer and activator of transcription 1 (STAT1) to the nucleus and the subsequent transcription of antiviral genes [199]. Furthermore, DENV NS2B/NS3 proteolytic activity has been involved in the inhibition of type I IFN response by degrading human stimulator of interferon gene (STING) protein in dendritic cells, which are known to be a primary target of DENV [200, 201]. STING is an adaptor protein that senses nucleic acids of incoming pathogens and triggers signaling pathways that activate the expression of IFN I and proinflammatory cytokines [202, 203]. DENV NS5 protein is also able to interact with STAT2 and bridge the protein to cellular ubiquitin ligases, thereby promoting the STAT2 proteasome-dependent degradation [204, 205].

In the case of CHIKV, it has been established that nsP2 is a potent inhibitor of the type I and II IFN-stimulated JAK-STAT signaling by blocking the phosphorylation of STAT-1 and its translocation to the nucleus [206]. Therefore, a scenario could be envisioned in which the IFN response inhibitory effects of CHIKV and DENV nonstructural proteins are added to create a more potent shut-off of the antiviral cellular response that would be beneficial for both viruses.

5. Perspectives and challenges

Very little is known about the ecology and biology of CHIKV and DENV coinfections. Since a decade, the increasing number of reported cases in Asia, Africa, and America shows that it is a generalized phenomenon that has been underestimated. Both *Ae. aegypti* and *Ae. albopictus* mosquito vectors are able to transmit these viruses and have been directly involved in some of the coinfection cases described to the date. A particular attention has been paid to *Ae. albopictus*, as: (i) it is the only species that has been shown to sustain concomitant infection by both viruses in natural conditions: (ii) it is an aggressive species that is spreading worldwide and displacing resident populations of *Ae. aegypti*; (iii) it is installed in the northern hemisphere contrary to its *Ae. aegypti* counterpart, representing a threat for the transmission of CHIKV and DENV among nonimmune populations in Europe and North America. However, further studies are required to evaluate the precise impact of each *Aedes* species on the transmission of DENV and CHIKV taking into account that infection susceptibility, vector ecology and interactions among sympatric populations of *Aedes* mosquitoes can deeply vary between geographical regions. A better understanding of these dynamics at the local level may allow to adapt vector control measures to each situation according to the results obtained from the field.

The clinical consequences of CHIKV/DENV coinfections remain largely unknown. Indeed, the available data is not enough to conclude if the concomitant infection by both viruses is able to aggravate the clinical symptoms caused by DENV and CHIKV monoinfections. A systematic and larger clinical survey should be done to assess if coinfections are associated to severe forms of dengue and chikungunya diseases. This is particularly important, as clinical studies may justify further research on the pathogenesis of CHIKV/DENV coinfections to understand the immunological events that are triggered. This information could be useful to design and improve prophylactic vaccines against each virus.

Finally, the almost complete absence of information on the cell biology of CHIKV and DENV coinfections open a large range of research opportunities. In that sense, the mechanisms by which the viruses avoid competition or find cooperative mechanisms to replicate simultaneously are two major axes of research that should be addressed more deeply. By identifying common cellular targets of both viruses, antiviral drugs may be designed to treat coinfected patients or even to produce vaccines that are able to concomitantly immunize against both viruses.

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