GloPID-R report on chikungunya, o'nyong-nyong and Mayaro virus, part 5: Entomological aspects

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A B S T R A C T

The GloPID-R (Global Research Collaboration for Infectious Disease Preparedness) chikungunya (CHIKV), o'nyong-nyong (ONNV) and Mayaro virus (MAYV) Working Group has been established to investigate natural history, epidemiology and clinical aspects of infection by these viruses. Here, we present a report dedicated to entomological aspects of CHIKV, ONNV and MAYV. Recent global expansion of chikungunya virus has been possible because CHIKV established a transmission cycle in urban settings using anthropophilic vectors such as *Aedes albopictus* and *Aedes aegypti*. MAYV and ONNV have a more limited geographic distribution, being confined to Africa (ONNV) and central-southern America (MAYV). ONNV is probably maintained through an enzootic cycle that has not been characterized yet, with *Anopheles* species as main vectors and humans as amplification hosts during epidemics. MAYV is transmitted by *Haemagogus* species in an enzootic cycle using non-human primates as the main amplification and maintenance hosts, and humans becoming sporadically infected when venturing in or nearby forest habitats. Here, we focused on the transmission cycle and natural vectors that sustain circulation of these viruses in their respective locations. The knowledge of the natural ecology of transmission and the capacity of different vectors to transmit these viruses is crucial to understand CHIKV emergence, and to assess the risk that MAYV and ONNV will expand on wide scale using anthropophilic mosquito species not normally considered primary vectors. Finally, the experts identified knowledge gaps and provided adapted recommendations, in order to address future entomological investigations in the right direction.

1. Introduction

Chikungunya (CHIKV), Mayaro (MAYV) and o'nyong-nyong virus (ONNV) are mosquito-borne alphaviruses (family *Togaviridae*). After its first isolation in Tanzania in 1952, CHIKV has been sporadically detected in Africa and Asia and, since 2004, has extended its geographic range causing outbreaks in the Indian Ocean, south-eastern Asia, Europe and the Americas. This global expansion has been possible because CHIKV established a transmission cycle in urban settings using anthropophilic vectors such as *Aedes albopictus* and *Aedes aegypti* (Coffey et al., 2014). MAYV and ONNV have a more limited geographic distribution, being confined to Africa (ONNV) and central-southern America (MAYV) (Rezza et al., 2017; Mackay and Arden, 2016). ONNV is probably maintained through an enzootic cycle that has not been characterized yet, with *Anopheles* species as main vectors and humans as amplification hosts during epidemics (Rezza et al., 2017). MAYV is transmitted by *Haemagogus* species in an enzootic cycle using non-human primates (NHPs) as the main amplification and maintenance hosts, and humans becoming sporadically infected when venturing in or nearby forest habitats (Mackay and Arden, 2016).

The knowledge of the natural ecology of transmission and the capacity of different vectors to transmit these viruses is crucial to understanding CHIKV emergence, and to assess the risk of a large-scale circulation of MAYV and ONNV. For this reason, the GloPID-R (Global Research Collaboration for Infectious Disease Preparedness) chikungunya (CHIKV), o'nyong-nyong (ONNV) and Mayaro virus (MAYV) Working Group presents here a report dedicated to entomological aspects of these pathogens. The experts of GloPID-R have performed a systematic review of English-written literature on entomological aspects of the three viruses present on PubMed until September 2018. A part of this reviewed literature derives from Spanish and Portuguese-written publications and annual reports (*i.e.* from Instituto Evandro Chagas (IEC), Oswaldo Cruz Foundation (Fiocruz)). In particular, we focused on the transmission cycle and natural vectors that sustain circulation of these viruses in their respective locations. Moreover, we assessed the possibility that MAYV and ONNV will expand on wide scale using anthropophilic mosquito species not normally considered primary vectors. Finally, the experts identified knowledge gaps and provided adapted recommendations, in order to address future entomological investigations in the right direction.

2. Chikungunya virus

2.1. Africa

2.1.1. Natural vectors

The main vectors of CHIKV in Africa are *Aedes* ssp mosquitoes of the subgenera *Diceromyia*, *Stegomyia*, and *Aedimorphus* (Jupp and McIntosh, 1988; Diallo et al., 1999, 2012).

In West Africa, CHIKV has been detected in over 30 mosquito species, including *Ae. (Diceromyia) furcifer, Ae. (Stegomyia) luteocephalus, Ae. (Stegomyia) africanus, Ae. (Aedimorphus) dalzieli, Ae. (Stegomyia) aegypti, Ae. (Diceromyia) taylori, Ma. (Mansonioides) africana*, and *An. (Cellia) gambiae* between 1966 and 2015 (Diallo et al., 1999, 2012; Robert et al., 1993; Boorman and Draper, 1968; Moore et al., 1974). The detection of CHIKV from male *Ae. furcifer* in Senegal and Cote d'Ivoire suggests vertical transmission (Diallo et al., 2012).

In Central Africa, CHIKV was detected in *Ae. aegypti* and *Ae. albopictus* in Brazzaville (the Republic of Congo) in 2011 (Mombouli et al., 2013) and from pools of six mosquito species collected throughout the Central African Republic between 1968 and 1991 (Institut Pasteur. Institu, 2018; Saluzzo et al., 1980). In South Africa, CHIKV was isolated from 16 pools of the *Ae. furcifer/taylori* group (mainly *Ae. furcifer*) in April 1976 (McIntosh BM, 1977). In 1970–1971, only one CHIKV strain was isolated in Angola from *Ae. aegypti* (Filipe et al., 1973). In Uganda, CHIKV was isolated in the Zika forest from *Ae. africanus* in 1956 and from *Ma. africana* and *Coquillettidia fuscopennata* in 1961 (Weinbren et al., 1958; McCrae et al., 1971). An entomological study conducted in the Kyala district of Tanzania in 2015 detected CHIKV from pools of *Ae. africanus* and *Ae. aegypti* (Bisimwa, 1880).

In the Indian Ocean, *Ae. albopictus* and *Cx. quinquefasciatus* were found to be naturally infected by CHIKV on Reunion island (Bessaud et al., 2006), while only *Ae. albopictus* was found to be naturally infected in Madagascar (Ratsitorahina et al., 2008).

While numerous mosquito species have been shown to be infected with CHIKV in nature, *Ae. aegypti* and *Ae. albopictus* are the two main epidemic vectors. Indeed, in the urban human transmission cycle, *Ae. aegypti* was shown to be the main vector of transmission of CHIK epidemics in western and central Senegal, Tanzania, Angola, Mozambique, Kenya, and Comoros while *Ae. albopictus* was the main vector of transmission in La Reunion Island, Seychelles, Mauritius, Madagascar, Gabon, and Cameroon.

CHIKV has also been occasionally isolated in other mosquito species of the genera *Aedes* (*Ae. vittatus, Ae. neoafricanus, Ae. hirsutus, Ae. fulgens, Ae. argenteopunctatus, Ae. dalzieli, Ae. vigilax,* and *Ae. camptorhynchites*), *Culex* (*Cx. poicilipes, Cx. ethiopicus,* and *Cx. quinquefasciatus*), *Mansonia* (*Ma. Africana* and *Ma. uniformis*), and *Anopheles* (*An. coustani, An. funestus, An. rufipes,* and *An. domicola*) (Diallo et al., 1999, 2012; Bessaud et al., 2006; Jupp et al., 1981; Jupp and McIntosh, 1990). In many instances (in particular regarding *Culex* and *Anopheles* mosquitoes) this may reflect the capability of the mosquitoes to bite infected animals or humans but does not imply that they play a significant role in the natural cycle and epidemiology of CHIKV.

2.1.2. Enzootic cycle

In the enzootic cycle, CHIKV is transmitted between arboreal *Aedes*

spp. vectors, and mainly non-human primate amplification hosts, as further outlined below. In this cycle, humans are considered incidental hosts and are infected when they enter the forest or by infected vectors (*Ae. furcifer* in West and South Africa, and *Ae. africanus* in East and Central Africa) spilling over villages located near forests (Jupp and McIntosh, 1988; Diallo et al., 1999, 2012). CHIKV or anti-CHIKV antibodies were detected in animals in several countries and localities in Africa. In south-eastern Senegal, CHIKV was isolated from three NHP species (*Cercopithecus aethiops*, *Papio papio*, and *Erythrocebus patas*) and other wild animal species including bats (*Scotophillus*), palm squirrels (*Xerus erythropus*) and bushbabies (*Galago senegalensis*) (Diallo et al., 1999). CHIKV has also been isolated from several NHPs, including bushbabies (*Galago senegalensis*), vervet monkeys (*Chlorocebus pygerythrus)*, and baboons (*Papio papio*) and the golden sparrow (*Auripasser luteus*) in Nigeria (Moore et al., 1974). Anti-CHIKV antibodies have been detected in wild chimpanzees in the DRC, vervets (*Ceropithecus aethiops pygerythrus*), baboons (*Papio ursius*, *P. d. dogueri*) and colobus monkeys (*Colobus a. abyssinicus*) in Senegal, Ethiopia, the DRC, Kwazulu Natal and Uganda. Anti-CHIKV antibodies have been detected also in several others species including birds and reptiles in Zimbabwe and Senegal, elephants from Zambia and the DRC, buffalo from the DRC, and domestic animals including horses in Nigeria, and bovines in Guinea and South Africa (Osterrieth et al., 1961; Renaudet et al., 1978; Konstantinov, 1990; Cornet et al., 1968; Mcintosh et al., 1964; Andral et al., 1968; Olaleye et al., 1989; Adesina and Odelola, 1991; Dickinson et al., 1965).

2.2. Americas

2.2.1. Natural vectors

The main natural vectors associated with CHIKV transmission in the Americas are *Ae. aegypti* and *Ae. albopictus* mosquitoes. The incrimination of these two mosquito species in the Americas involved a series of comprehensive vector competence studies performed with mosquito populations from various locations in the region (Girod et al., 2011; Vega-Rúa et al., 2015a; Honório et al., 2018), and by virus detection in field-collected mosquitoes (White et al., 2018; Costa-da-Silva et al., 2017; Cevallos et al., 2018; Farraudière et al., 2017; Díaz-González et al., 2015). Even if *Ae. albopictus* is less widely distributed in the Americas when compared to *Ae. aegypti* (Kraemer et al., 2015), it has been well established in the region since at least 1985 (Moore, 1999). In the Caribbean region, *Ae. albopictus* is present in Barbados, Cuba, the Dominican Republic, Haiti, Cayman Islands, Trinidad & Tobago, while in continental America, the species is present in the United States, Mexico, Guatemala, Salvador, Belize, Honduras, Nicaragua, Panama, Costa Rica, Colombia, Brazil, Venezuela, Paraguay and Argentina (Carvalho et al., 2014). The two CHIKV lineages currently circulating in the Americas (Asian and East/Central/South African–ECSA) are not predicted to be capable of adaptation for more efficient transmission by *Ae. albopictus*, differently from Indian Ocean Lineage strains; this is due to epistatic constraints in the Asian and ECSA lineages that were introduced in 2013 and 2014, respectively (Tsetsarkin et al., 2016).

In 2016, a pool of *Cx. quinquefasciatus* was reported to be infected with a CHIKV strain from the ECSA lineage in Haiti (White et al., 2018). However, a vector competence study conducted in Florida with a colony from Indian River County (F10 generation) from this latter species revealed that even when mosquitoes become orally infected, they were not able to disseminate nor transmit (Richards et al., 2010). Taken together, the results suggest that, while *Cx. quinquefasciatus* is undoubtedly able to bite humans infected by CHIKV, it is not a competent vector of CHIKV transmission in urban settings in the Americas. Regarding sylvatic mosquitoes, experimental data from Brazil suggested that *Ae. terrens* and *Haemagogus leucocelaenus* mosquitoes were highly competent for CHIKV, which highlights the potential of CHIKV vectors to establish an enzootic transmission cycle in the continent (Lourenço-de-Oliveira and Failloux, 2017).

2.2.2. Enzootic cycle

The potential of a sylvatic transmission cycle maintaining CHIKV in the Americas has been poorly investigated. Old World NHPs (the Catarrhini) comprise the superfamilies *Hominoidea*, including humans, and *Cercopithecoidea* (Springer et al., 2012). Evidence for the ability of CHIKV to infect representatives of both superfamilies may imply a relatively broad host range of these emerging arboviruses within Old World primates. Because New World NPHs (the Platyrrhini) arose from Old World ancestors about 36 million years ago (Bond et al., 2015), susceptibility to CHIKV may be a broadly conserved trait. However, differential susceptibility of New World NHPs to yellow fever virus (YFV) illustrates that individual assessments will be required to identify candidate NHP species potentially maintaining CHIKV in the Americas.

With regards to vectors, *Aedes* mosquitoes may be among the prime suspects for potential sylvatic transmission cycles. CHIKV may be able to explore sylvatic cycles in Latin America based on the high number of mosquito and NHP species and their large population sizes in Latin America, as well as the relatively close contact between NHPs and humans (Bueno et al., 2016; Althouse et al., 2016). However, recent serosurveys of new world NHPs collected in urban and peri-urban regions identified low seropositivity rates. Although some CHIKV infections occur, this raises doubts as to whether NHPs have the potential to serve as reservoirs of CHIKV in the Americas (Moreira-Soto et al., 2018).

Little is known regarding the potential implication of other animals in sylvatic transmission CHIKV cycles in the Americas. No evidence was found from the experimental infections of several species of North American mammals including ungulates, rodents, lagomorphs, bats, carnivores and birds (Bosco-Lauth et al., 2016). Relatively high viremia in experimental infection of ectothermic vertebrates such as snakes and toads have been observed but the potential role in CHIKV maintenance remains speculative (Bosco-Lauth et al., 2018).

2.3. Asia

2.3.1. Natural vectors

In Asia, CHIKV transmission occurs with both *Ae. aegypti* and *Ae. albopictus* mosquito species (Gratz, 2004). Although *Ae. aegypti* was previously the only recognised major urban vector of CHIKV, today it is widely accepted that both *Ae. aegypti* and *Ae. albopictus* are the two main vectors of CHIKV transmission in Asia. Indeed, *Ae. aegypti* is a common mosquito species in Asia, found in high densities in urban areas because of the use of man-made containers used to store water as well as the presence of other larval breeding sites (*i.e.* tires, fish tanks). Since the first reported outbreak of chikungunya in 1958 in Asia, *Ae. aegypti* has been commonly incriminated. It was not until the re-emergence of chikungunya epidemics in the early 21st century that *Ae. albopictus* has also been shown to play a role in transmission (Tsetsarkin et al., 2007).

The role of these two vector species in the transmission of CHIKV in India, Southeast Asia (Malaysia, Thailand, Vietnam, Cambodia, Laos) and neighbouring countries (Singapore, Philippines, Micronesia) has been well documented (Zeller et al., 2016).

The transmission of CHIKV was demonstrated with prevalence studies of CHIKV in *Aedes* spp., *e.g.* in Thailand (Thavara et al., 2009), vector competence studies with field-caught mosquitoes, *e.g.* in India and Thailand (Tesh et al., 1976; Turell et al., 1992) as well as vertical transmission studies of *Ae. aegypti* following observations of CHIKV-infected male mosquitoes, *e.g.* in Thailand and India (Thavara et al., 2009; Agarwal et al., 2014). Interestingly, a recent study suggested that *Cx. gelidus* were able to experimentally transmit CHIKV (Sudeep et al., 2015), suggesting the potential of secondary vectors may play a role in the transmission of CHIKV. However, additional experimental studies will be required to establish wheter *Culex* species mosquitoes play any

role in the transmission of CHIKV.

Overall, the limited number of *Aedes* species involved in CHIKV transmission in Asia compared to Africa, despite a higher total number of *Aedes* species in Asia, may suggest our lack of knowledge and reiterate the need for further surveillance studies in order to elucidate the vector range of CHIKV transmission in the region.

2.3.2. Enzootic cycle

The urban CHIKV transmission cycle can be maintained by both *Ae. aegypti* and *Ae. albopictus* (Pulmanausahakul et al., 2011). Although, in Asia, no sylvatic cycle has been observed (Higgs and Vanlandingham, 2015), the first serological study was reported in 1993 by investigating 115 wild *Macaca sinica* monkeys in Sri Lanka (Peiris et al., 1993). Furthermore, in 2001, 40 wild orangutans, 31 semi-captive orangutans and 114 humans were sampled in Malaysian Borneo in order to detect arboviruses: the results showed no infection of wild or semi-captive orangutans by CHIKV (Wolfe et al., 2001). Collectivelly, these two limited in scope studies may suggest that CHIKV may have not been able to establish a sylvatic transmission cycle in Asian, despite a long history of urban transmission in the region (Halstead, 2015).

Two studies describe the presence of CHIKV in non-human primates. In 1999, in the Philippines, 54 *Macaca fascicularis* monkeys were sampled, with 14.8% IgM positive and 59.3% positive IgG, indicating exposure to CHIKV (Distribution of three arb, 2003). IgG rates were significantly higher in the oldest monkeys compared to the younger ones (65.9% *vs* 33.3%). These results may support the hypothesis of a continuous sylvatic transmission cyvle among monkeys.

In 2007–2008 in Malaysia, 105 sera of wild *Mac. fascularis* monkeys were analyzed to assess CHIKV exposure (Apandi et al., et al.). Serum samples were inoculated into different cell lines, RNA extracted and amplified by RT-PCR, followed by sequencing. Four samples were positive for CHIKV and the authors demonstrated that the sequences between human and non-human primates were similar, supporting the hypothesis of exchange and cross transmission or spillback from the urban cycle into NHPs (Apandi et al., et al.).

These two studies showed exposure of *Mac. fascularis* species to CHIKV in Malaysia and the Philippines. However, an enzootic transmission cycle can be proven when there evidences of (i) sustainable transmission in mosquitoes and monkeys, (ii) sufficient genetic divergence between urban ad sylvatic viruses, and (iii) epizootics (virus circulation far from transmission by peridomestic vectors). To date, it is not possible to demonstrate the existence of such a sylvatic transmisson cycle in Asia since studies remain limited for accurate depiction on the regular role of enzootic cycles in most of these countries.

2.4. Europe

2.4.1. Natural vectors

Ae. albopictus was first introduced in Europe in 1979 in Albania (Adhami and Reiter, 1998) and again, in Italy in 1990 (Sabatini et al., 1990; Dalla Pozza and Majori, 1992). The species is now well established in 20 European countries (Medlock et al., 2015) and since 2007, this mosquito has been responsible for local CHIKV outbreaks: Italy in 2007 (Rezza et al., 2007; Angelini et al., 2008) and 2017 (Venturi et al., 2017) and France in 2010 (Grandadam et al., 2011), 2014 (Delisle et al., 2014) and lastly 2017 (Calba et al., 2017). All European CHIKV strains belonged to the ECSA genotype, with strains from Italy-2007, France-2014 and France-2017 carrying the A226V mutation in the E1 gene, which has been involved in the increased transmissibility by *Ae. albopictus*.

Ae. albopictus had been incriminated in the autochthonous transmission of CHIKV in Europe. CHIKV RNA was detected in field-collected *Ae. albopictus* during the course of entomological investigations in Castiglione di Ravenna and Castiglione di Cervia in Italy in August 2007 (Bonilauri et al., 2008). Moreover, experimental mosquito infections confirmed that among other mosquito species, *Ae. albopictus* was the most competent vector to transmit CHIKV (Vazeille et al., 2008; Talbalaghi et al., 2010; Vega-Rua et al., 2013; Severini et al., 2018; Moutailler et al., 2009).

To date, *Ae. albopictus* has been established as the main CHIKV vector of transmission in Europe. Nevertheless *Ae. aegypti,* which was eradicated in Europe since the 1950s, has been detected again around the Black Sea in southern Russia, Abkhazia, and Georgia in 2004 and north-eastern Turkey in 2015 (Akiner et al., 2016). This species was responsible for the last dengue outbreaks in Europe, Greece in 1927–1928 (Rosen, 1986). In addition, a new invasive mosquito *Ae. koreicus* has established an authochthonous transmission in Europe since 2001 and is also able to experimentally transmit CHIKV (Ciocchetta et al., 2018). Its distribution overlaps that of *Ae. albopictus* (Baldacchino et al., 2017). These species could in the future become alternative vectors of transmission and will require sustainable surveillance.

2.4.2. Enzootic cycle

There is currently no evidence for an enzootic cycle in Europe.

2.5. Pacific area

2.5.1. Natural vectors

CHIKV was detected in the South Pacific Islands in New Caledonia (2011), Papua New Guinea (2012), Yap State (2013), French Polynesia (2013), Tonga (2014) and American Samoa, Samoa, and Tokelau in 2014 (Aubry et al., 2015; Roth et al., 2012). In the Pacific region, the main vectors of CHIKV are mosquitoes of the sub-genus *Stegomyia: Ae. aegypti, Ae. albopictus, Ae. polynesiensis* and *Ae. hensilli*, all four being anthropophilic. The establishment of *Ae. aegypti* in the Pacific islands coincides with the massive human migration during Second World War. *Ae. albopictus* invaded the Pacific region in the 1960s and is now well established in Papua New Guinea, the Torres Strait region of Australia, Fiji, Solomon Islands, Tonga and probably Vanuatu (Guillaumot et al., 2012). Contrary to the two others, *Ae. polynesiensis* is native to these islands and is widespread in the Eastern part of Oceania, including Fiji, Samoa Islands, French Polynesia, and Pitcairn horwood. *Ae. hensilli* is the most predominant *Aedes* mosquito in some islands of Western Pacific Ocean in Micronesia (Palau, Yap) (Savage et al., 2015).

New Caledonia was the first territory affected by CHIKV in 2011 (Dupont-Rouzeyrol et al., 2012). Despite a high vector competence of *Ae. aegypti* towards Asian and ECSA CHIKV genotypes, no outbreak was observed. In 2012–2013, Papua New-Guinea underwent the largest CHIKV epidemic in the region caused by the ECSA genotype (Roth et al., 2012). The Asian genotype was responsible for the outbreak in Yap State in 2013–14 where *Ae. hensilli* was suspected as the vector (Ledermann et al., 2014). From October 2014 to March 2015, French Polynesia experienced a CHIKV outbreak. Two mosquito species were implicated as vectors: *Ae. aegypti and Ae. polynesiensis*. Vector competence assays confirmed that both species are susceptible to the Asian genotype of CHIKV (Richard et al., 2016). *Opifex fuscus*, *Ae. antipodeus* and *Ae. notoscriptus,* active in New Zealand, were proved to be highly competent vectors of CHIKV, although no endemic activity in NZ is currently evident (Kramer et al., 2011).

2.5.2. Enzootic cycle

There is currently no evidence for an enzootic cycle in the Pacific area.

3. O'nyong-nyong virus

3.1. Africa

3.1.1. Natural vectors

ONNV was isolated for the first time in Africa from 39 pools of *An. funestus* and 15 pools of *An. gambiae* collected in Uganda and Kenya in

1959–60 (Williams et al., 1965a). *An. funestus* was considered to be the principal vector during this epidemic. Both species were able to transmit the virus experimentally. In 1978, a single ONNV strain was recovered from a pool of *An. funestus* mosquitoes collected from western Kenya, after a period of many years with no evidence of ONNV activity (Johnson et al., 1981a). The virus was also isolated in 1997 in south-central Uganda from 1 pool of *An. funestus* and *Mansonia uniformis*, respectively (Lutwama et al., 1999a). The isolation of ONNV from *Ma. uniformis* was the first association of this virus with Culicine spp. mosquitoes. *An. funestus* larvae are found in larger bodies of clear, permanent water, including lake shores and river margins and larvae of this species can be found year-round in some areas of Africa (Evans, 1938). *An. gambiae* larvae are found in ephemeral sunlit water bodies. Both *Anopheles* species are highly anthropophilic and adult females prefer to feed and rest inside human dwellings. *Mansonia uniformis* is a culicine mosquito that is very common and widely spread in most of sub-Saharan Africa. Its larvae are found in ponds and lakes containing water plants. This species is considered a large mammal feeder but also commonly feeds on birds and humans both inside and outside of houses, primarily at night (Laurence, 1960; Ba et al., 2006). Like *An. funestus* and *An. gambiae,* it is frequently found resting indoors.

3.1.2. Enzootic cycle

Little is known about the putative enzootic cycle of ONNV during inter-epidemic periods in Africa. However, the few available data may suggest the existence of this cycle. Indeed, in West Africa, ONNV was isolated from sentinel mice in Senegal (Lhuillier et al., 1988). Specific neutralizing antibodies against ONNV were detected in four species of duikers (*Cephalophus* and *Philantomba* spp.) in the DRC, forest buffaloes (*Syncerus caffer nanus*) in the DRC and Gabon, and mandrills (*Mandrillus sphinx)* in Gabon (Kading et al., 2013). These detections occurred during periods without known epidemic ONN activity. These large mammals may be involved as vertebrate reservoirs in the enzootic cycle of ONNV in Africa. Of note, these serological results should be put in perspective with the fact that discriminating between antibodies to ONNV and CHIKV is difficult, even using the gold standard of the neutralisation assay (Pezzi et al., 2019).

3.2. Americas, Asia, Europe, Pacific area

There is currently no evidence for ONNV circulation outside Africa.

4. Mayaro virus

4.1. Americas

4.1.1. Natural vectors

Mayaro virus (MAYV) circulates in an enzootic cycle in Central and South America between NHPs and arboreal mosquitoes.

Primatophilic forest mosquitoes belonging to the genus *Haemagogus* are considered the main MAYV vectors. Numerous natural infections by MAYV have been reported in *Hg. janthinomys* (Groot et al., 1961; Hoch et al., 1981; Azevedo et al., 2009), reinforcing the plausibility of its prominent role as primary vector in the enzootic cycle across the American continent.

MAYV has also been detected in other arboreal mosquito species belonging to the same genus as well as from *Sabethes* species (4). In contrast to *Haemagogus* species, *Sabethes* mosquitoes usually fly within or close the forest patches. Other arborel species, including *Psorophora ferox* (Galindo et al., 1966), as well as non-arboreal mosquitoes such as *Cx. vomerifer* (Galindo and Srihongse, 1967) and *Coquilettidia venezuelensis* (Aitken et al., 1960, 1969) have already been found naturally infected with MAYV and they are considered as secondary vectors of transmission.

Ae. serratus and *Ae. scapularis* can be experimentally infected with MAYV (Aitken and Anderson, 1959), and therefore should be considered as potential bridge vectors. Non-engorged urban mosquito species – *Ae. aegypti* and *Cx. quinquefasciatus* - were found naturally infected with MAYV in the city of Cuiaba, central region of Brazil (Serra et al., 2016a). Both species demonstrated no or poor infection and dissemination rates when orally challenged with artificial blood meals containing MAYV at titers similar to those usually found in infected humans (Brustolin et al., 2018; Long et al., 2011), which may limit chances to initiate an urban transmission cycle. However, *Ae. aegypti* could experimentally transmit MAYV when taking a blood meal with high viral load; therefore the hypothesis that it could contribute to urban transmission should not be totally neglected (Long et al., 2011). *Ae. aegypti* from Florida State (USA) (Wiggins et al., 2018a) and from Iquitos (Peru) (Long et al., 2011) were competent to transmit MAYV in laboratory conditions.

Concurrent detection of MAYV and dengue virus in a febrile child in Haiti, with no history of travel abroad, was recently reported (Lednicky et al., 2016). Few data are available about MAYV vectors in Haiti; however, the patient was living in a semi-rural area, a very different setting from the forested regions where most MAYV cases have been detected and where *Haemagogus* spp live. The fact that the child was co-infected with DENV may suggest a possible implication of *Aedes* mosquitoes, but the complete absence of additional information supporting epidemiological evidence for circulation of MAYV in Haiti should warn caution regarding this case.

Finally, *Ae. albopictus* from Brazil and from Florida (USA) (Wiggins et al., 2018a; Smith and Francy, 1991a; Mitchell, 1991) and anopheline species from Asia, Africa and North America (*An. stephensi, An. gambiae*, *An. freeborni* and *An. quadrimaculatus*) were shown to be competent to transmit MAYV in laboratory conditions (Brustolin et al., 2018). The actual implications of these experimental results remain to be further investigated.

4.1.2. Enzootic cycle

In nature, MAYV is transmitted in an enzootic cycle where NHPs appear to be the main amplifying vertebrate hosts and arboreal mosquitoes are the primary vectors. *Hg. janthinomys* more frequently bites NHPs in the tree canopies (Hoch et al., 1981; F) Ecologia de Haemagog, 1679). Humans can acquire the infection when entering the forest or in forest-urban transition zones (Hoch et al., 1981); however, females of *Hg. janthinomys* and other *Haemagogus* species may also disperse several kilometres from the forest (Causey et al., 1950) and this ability increases the risk of human biting and infections even outside the natural environment where the enzootic cycle occurs.

Despite its primatophilic behavior, *Hg. janthinomys* may feed on other animals such as cattle, birds, dogs, rodents and horses (Alencar et al., 2005). This habit may explain observations of MAYV isolation in other vertebrate hosts, besides NHPs and their seropositivity, although they likely are incidental hosts that may not play a role in the transmission (Mackay and Arden, 2016; de Thoisy et al., 2003; Calisher et al., 1974). Identification of human co-infections by dengue virus and MAYV has raised the question of an intermediate or peri-urban cycle where the transmission would be limited to anthropophilic mosquitoes and humans (Zuchi et al., 2014). This issue remains to be more completely investigated.

According to serological and virological surveys, at least five American NHP genera can be considered reservoirs of MAYV. Antibodies against MAYV and viral isolation were demonstrated in marmosets (*Callithrix argentata),* squirrel monkeys (*Saimiri spp.*), howler monkeys (*Alouatta belzebul)*, red howler monkeys (*Alouatta seniculus)*, black howler monkeys (*Alouatta villosa)*, white-faced saki (*Pithecia pithecia)* and golden hand tamarins (*Saguinus midas)*. Antibodies have also been detected in other mammals, such as sloths, armadillos, coatis, equids, opossums, rats and agoutis, which are considered potential reservoirs but whose viremia levels and ability to infect mosquitoes still require further investigation. It appears that MAYV can also infect lizards, such as *Tropidurus t. hispidus* and *Ameiva ameiva* (Mackay and

Arden, 2016; Hoch et al., 1981; de Thoisy et al., 2003; Hervé et al., 1986). Birds were also seropositive for MAYV, including doves, and the virus was isolated from the orchard oriole *Icterus spurius* (Calisher et al., 1974).

MAYV is currently limited to its enzootic cycle. However, increased human incursions into forests or in their vicinity of forests for agricultural or recreational activities will likely increase the probability of human MAYV infections, a trend that causes grave concern for public health authorities (Mackay and Arden, 2016).

A recent multimodel inference study suggested that overlapping MAYV transmission cycles may already occur in Brazilian Amazon settlements involving forest and domesticated synanthropic mosquitoes, which may play a critical role for the emergence of peri-urban and urban MAYV cycles (Abad-Franch et al., 2012). In fact, active MAYV circulation in Brazil has been suggested in seroprevalence survey that took place in Manaus (Amazonas State) in 2007–2008 (Mourão et al., 2011). More recently, MAYV IgM antibodies were detected in human samples in the outskirts of the city of Goiânia (*cerrado,* Goiás State) in 2014–2015 (Brunini et al., 2017).

Since the environmental factors which led to the increase of other arboviruses continue to prevail in the Americas, MAYV outbreaks in forest-urban transition zones around ever expanding urban centers are possible, as it is observed for YFV epidemics in Brazil (Possas et al., 2018).

4.2. Africa, Asia, Europe, Pacific area

There is currently no evidence for MAYV circulation outside America.

5. O'nyong-nyong and Mayaro virus: potential vectors other than *haemagogus* and *anopheles* spp.

5.1. Mayaro virus

The main vector implicated in MAYV transmission is the diurnal canopy-dwelling mosquito of the genus *Haemagogus*. During a concurrent MAYV-YFV outbreak that occurred in Belterra (Brazil) in 1978, only *Haemagogus* mosquitoes were found to be positive for MAYV, and 9 viral strains were isolated from *Hg. janthinomys* mosquitoes (Hoch et al., 1981). A MAYV isolate was also obtained from a pool of *Hg. janthinomys* during a 2008 outbreak near Belem, Brazil (Azevedo et al., 2009). *Haemagogus* spp prefer rural areas with proximity to forests and are responsible for maintaining MAYV in a sylvatic cycle involving zoophilic mosquitoes and vertebrate hosts other than humans. They rarely show anthropophilic behaviors, so that spillover events leading to human infections are sporadic and mostly involving rural communities living or working next to the forest (Mackay and Arden, 2016).

It is unclear at this stage whether and how an urban transmission cycle of MAYV is possible. In 2007–2008, a surveillance study of patients with febrile illness allowed to detect 33 patients MAYV positive by IgM detection and/or PCR (Mourão et al., 2011). This specific study took place in Manaus, a large city and capital of the Amazonas State, in Western Brazilian Amazon, with about 2 million people, infested by the urban mosquito *Ae. aegypti* and *Ae. albopictus*. Similarly, in 2011–2012, patients presenting with acute febrile illness were tested for MAYV in the state of Mato Grosso (Central-West Brazil) (Zuchi et al., 2014); in the context of a concomitant large dengue fever outbreak, 15 MAYV PCR-positive patients were identified, without any recent history of travel or access to rural or sylvatic areas (Zuchi et al., 2014). The following year, Serra et al. (2016b) reported isolation from *Ae. aegypti* in Cuiabá (state of Mato Grosso) of MAYV strains with 99–100% identity with viral sequences obtained from humans in 2011–2012. Altogether, these findings in Manaus and Mato Grosso do not provide definitive evidence for actual transmission by urban vectors, in particular because the fragmentation of urban patterns does not allow to exclude exposure to *Haemagogus* spp, but they raise concern about the possible urbanization of MAYV fever involving humans as reservoir (Tesh et al., 1999).

The vector competence of anthropophilic, urban-dwelling mosquito species for MAYV should be further evaluated. Several mosquito species, other than the main MAYV vector *Haemagogus* spp, have been found both naturally infected by MAYV in ecological studies or proven capable of hosting systemic replication and transmitting MAYV in experimental studies.

Mosquitoes from genus *Aedes* have some characteristics that make them more likely than any other mosquito species to establish and maintain an urban transmission cycle of MAYV. Among them, *Ae. aegypti* and other *Aedes* spp. are of particular interest because they have a world-wide distribution, are highly anthropophilic and extremely opportunistic (Ding et al., 2018; Carvalho and Moreira, 2017). They are already involved in arboviral transmission cycle of dengue virus 1–4 (DENV 1–4), Zika virus (ZIKV), yellow fever virus (YFV) and chikungunya virus (CHIKV) (Epelboin et al., 2017; Vega-Rua et al., 2014; Klitting et al., 2018; Higa, 2011). Pools of *Ae. aegypti* have been found to be positive in urban settings in Brazil (Cuiabá, Mato Grosso) (Serra et al., 2016b). To better assess their possible role in urbanization of MAYV transmission cycle, both *Ae. aegypti* and *Ae. albopictus* have been tested under laboratory conditions using vector populations representing different geographical regions. Long et al. demonstrated for the first time that *Ae. aegypti* mosquitoes from Peru were susceptible to a MAYV strain isolated from a febrile patient in the Loreto region of Peru (Long et al., 2011). On the contrary, Brustolin et al. suggested that *Ae. aegypti* are poor vectors for MAYV, given their very low transmission rates observed with both genotype D and L MAYV strains (Brustolin et al., 2018). More recently, experimental infection and transmission potential was evaluated in populations of *Ae. aegypti* and *Ae. albopictus* collected in Florida (Wiggins et al., 2018b). The study showed that *Ae. albopictus* had a significantly higher rate of susceptibility than *Ae. aegypti*, while rates of dissemination were generally higher in *Ae. aegypti* than in *Ae. albopictus*. Both mosquito species exhibited low rates of MAYV infection in saliva (Wiggins et al., 2018b). Previously tested *Ae. albopictus* from Missouri (USA) and Brazil suggested that these mosquitoes are susceptible to MAYV infection (Smith and Francy, 1991b; Kantor et al., 2019).

Overall, a limited number of experimental infections suggest that *Aedes* spp. mosquitoes could serve as possible vectors of MAYV. The efficiency of transmission varied reflecting differences of susceptibilities among various mosquito populations and the viral strains used and suggesting an interplay between host genotype and pathogen genotype (G x G) (Lambrechts et al., 2006). Kantor et al., for example, observed two different infection patterns in *Ae. aegypti* using two strains of MAYV, both belonging to D genotype, with different transmission potential (Kantor et al., 2019). Moreover, different laboratory environments and techniques used by the working groups can explain the divergence of results.

The potential for MAYV to be transmitted by these vector species depends also on the duration and the levels of human viremia, but little information is available for MAYV infection kinetics in humans. Viremia levels have been detected in the range of 2.7–5.3$\log_{10}$ PFU equivalents/mL from 22 acute sera of febrile patients (Long et al., 2011). Viral transmission by *Aedes* spp have been observed in a study where infectious blood meals for mosquitoes had higher viral titers than those observed in infected humans (Wiggins et al., 2018b). This obviously limits our comprehension of viremia level that is necessary for the mosquito to become infected after feeding on the viremic host. Moreover, the virus seems to have a short viremic window (Pezzi et al., 2019), during which a small percentage of mosquitoes can potentially become infectious. Taken together, short viremic time in humans and high virus titers needed to infect mosquitoes may limit MAYV transmission by *Aedes* mosquitoes.

Some factors can actually balance the low efficiency observed in viral transmission by *Aedes* spp, among them (i) *Aedes* spp abundancy,

(ii) increased susceptibility of *Aedes* spp to MAYV after genetic changes and (iii) increased viremic titers and/or duration of viremias in hosts.

(i) It is possible to assume that if *Aedes* spp. are present in large numbers, these mosquitoes can probably expand transmission cycle of MAYV, by acting as secondary vectors in the context of an outbreak, or as bridging vectors between transmission cycles in distinct ecosystems.
(ii) The risk of genetic changes leading to increased efficiency of vector infection should not be underestimated. CHIKV, an alphavirus with well-described history of urbanization and global spread after a single amino acid substitution, taught us that few mutations can result in a virus better adapted to replicate and be transmitted by *Ae. albopictus* (Schuffenecker et al., 2006). MAYV homology with CHIKV raises concerns about the impact of microevolutionary changes can produce on viral transmission via anthropophilic mosquitoes; in that case, the risk of establishment of an urban cycle and further spread of MAYV into non-sylvatic areas would rise.
(iii) Increased viremic titers in hosts are another possible factor improving indirectly viral transmission. Venezuela equine encephalitis virus (VEEV) provides an example of how a genetic change can increase titers of viremia in equine hosts: a single mutation transformed an enzootic avirulent strain into an epidemic and virulent strain, capable of generating sufficient equine viremia for amplification (Anishchenko et al., 2006). If a similar mutation tended to be fixed in MAYV, *Aedes* spp could get easily infected with an increased possibility of sustaining human transmission.

Anopheles spp. have been investigated through a vector competence study (Brustolin et al., 2018) because their wide geographic distribution and their opportunistic and anthropophilic behavior make them a potential vector for MAYV transmission (Sinka et al., 2012). The four species used (*An. freeborni, An. gambiae, An. quadrimaculatus and An. stephensi*) represent mosquitoes dispersed worldwide: North America, Africa and Southeast Asia. They all showed to be laboratory competent vectors for MAYV and, except for *An. freeborni*, they transmitted both MAYV genotype D and L strains (Brustolin et al., 2018). The short extrinsic incubation period (EIP) of MAYV observed in this study, with *Anopheles* spp able to transmit the virus 7 days post infection, is a factor that might increase vectorial capacity, as well as their tendency to bite several times during a single gonotrophic cycle. *Anopheles* spp are often underestimated as potential vector for arboviruses and until now, they are known to transmit just two alphaviruses: ONNV (Rezza et al., 2017) and, under laboratory conditions, CHIKV (Yadav et al., 2003). Further detailed studies are needed to understand the vector competence of these mosquitoes for MAYV; this characterization could provide valuable information about the potential of this virus to emerge where *Anopheles* spp. are present.

Culex spp., like *Aedes* spp., raise concern for a possible urbanization of MAYV transmission because of their anthropophilic habits and their wide geographic distribution. A single study reported natural infection in Cuiabá, the large capital of the State of Mato Grosso, Brazil in 2013: 12 out of 403 *Cx. quinquefasciatus* tested positive for MAYV by PCR (Serra et al., 2016b). However, when tested under laboratory conditions, *Cx. quinquefasciatus* mosquitoes were refractory to infection with MAYV genotype D, and weakly susceptible to genotype L, but not able to transmit the virus (Brustolin et al., 2018). This vector competence study highlights the fact that the positivity of one species in field-collected specimens does not allow alone to classify it as a vector. Poor or null infection and transmission rates displayed by *Cx. quinquefasciatus* suggest that these mosquitoes are not competent MAYV vectors and they probably would not favor viral transmission in urban context.

Following the path of CHIKV, YFV and ZIKV, MAYV has the potential to establish an epidemic scenario. The switch from a sylvatic into an urban cycle could be facilitated by several factors such as abundant availability of urban vectors and possible viral changes increasing vector susceptibility to the arbovirus. Understanding the genetic or ecologic barriers that currently limit MAYV to a mainly enzootic cycle is necessary; in particular, the role of anthropophilic vectors as possible secondary or bridging vectors should be better assessed. Evidences that urban mosquitoes (in particular those from genus *Aedes)* could transmit MAYV exist, and this circumstance may contribute to the urbanization of MAYV transmission, using susceptible population as amplifying hosts. More studies are needed to understand if these candidates are efficient vectors, and which combination of virus and vector strains can be a better source for the initiation of a large-scale transmission in urban settings.

5.2. *O'nyong-nyong virus*

Unlike all other alphaviruses, ONNV is unique in its transmission pattern. In fact, the main vectors are known to be night-feeding anopheline mosquitoes, rather than culicines, typically *Anopheles funestus* and *An. gambiae*. During 1959–1962 outbreak involving both Eastern and Western Africa, unexpectedly, entomological investigations led to isolate ONNV from 39/144 pools of *An. funestus* and 15/206 pools of *An. gambiae* (Williams et al., 1965b). Field-collected mosquitoes maintained ONNV for at least 20 (*An. funestus*) and 13 days (*An. gambiae*), and viral transmission was observed using laboratory-infected mosquitoes of each species (Williams et al., 1965b). These findings corroborated the idea that *An. funestus* was the main vector of ONNV, and that *An. gambiae* was also involved. Larvae of anopheline mosquitoes are typically found in larger bodies of clear, permanent water, such as lake shores, river margins, and swamps. Their features such as, anthropophily, endophily and flight-range made it possible to cause another wide-range epidemic in 1996–1997 in Uganda (Lutwama et al., 1999b). The role of anopheline mosquitoes in ONNV transmission was confirmed from the isolation of a viral strain from a pool of *An. funestus* mosquitoes (Lutwama et al., 1999b). The small number of *An. gambiae* collected during this outbreak did not allow to detect positive specimens, so the role of these mosquitoes in viral transmission could not be assessed (Lutwama et al., 1999b).

ONNV ability to infect *An. gambiae* has been recently evaluated both *in vitro* and *in vivo* (Vanlandingham et al., 2005). Three ONNV strains (SG650, Gulu, and Igbo Ora) were able to replicate in cell lines derived from *An. gambiae. In vivo*, significantly higher rates of infection were observed with ONNV SG650 in *An. gambiae* when compared with the Gulu and Igbo Ora strains; the dissemination rate of Gulu was significantly lower than for the other two ONNV strains. Variations of viral strains in their ability to infect mosquitoes could be explained, at least in part, by the fact that there were differences in passage history among ONNV strains (Vanlandingham et al., 2005). Vanlandingham et al. investigated the determinants of vector specificity of ONNV in *An. gambiae* and *Ae. aegypti* mosquitoes using chimeric viruses constructed with genes from both ONNV and CHIKV. All the chimeras were able to infect *Ae. aegypti*, suggesting that ONNV has the potential to spread using this vector; differently, all the ONNV viral structural proteins are necessary to infect *An. gambiae* mosquitoes, so that the potential of CHIKV to infect *Anopheles* mosquitoes seems to be limited (Vanlandingham et al., 2006). A second study provided conflicting results, since nonstructural protein 3 (nsP3) was identified as the primary molecular determinant of ONNV vector specificity for *An. gambiae* (Saxton-Shaw et al., 2013). Further research is needed to investigate the molecular basis of virus-vector relationships, that could help to predict the risk of host range expansion.

During inter epidemic periods, anopheline mosquitoes seem able to sustain viral circulation, as demonstrated by the isolation of one ONNV strain from a pool of *An. funestus* in 1978 in Western Kenya (Johnson et al., 1981b). Recently, *Anopheles* mosquitoes were found to be naturally infected with ONNV in Democratic Republic of Congo in 2014 (Mbanzulu et al., 2017). If anopheline mosquitoes are recognised as main vectors for ONNV, virus ability to be transmitted by other

mosquito species has been poorly evaluated.

Few competence studies assessed the possible relationship between ONNV and *Aedes* mosquitoes. Buckley observed that ONNV replicated in *Ae. albopictus* cell lines (Buckley and Weiss, 1971); similarly, SG650, Gulu, and Igbo Ora strains used by Vanlandingham et al. showed to be capable of replication in C6/36 cells (Andral et al., 1968). The same three strains were also infectious to *Ae. aegypti* mosquitoes, with some strain variations, even if dissemination rates between the three strains were not found to be significantly different. Similar results were observed by another study group, with ONNV strains able to replicate to sufficient titers to produce infection and to disseminate in this mosquito species (Vanlandingham et al., 2006). However, no replication in cell lines from *Ae. aegypti* has been observed in a previous study (Buckley and Weiss, 1971).

The lack of naturally ONNV-infected *Aedes* vectors, together with contradictory results from competence studies about ONNV transmission in *Ae. aegypti in vitro vs in vivo*, deserves to be clarified.

During 1996–1997 outbreak in Uganda, ONNV was isolated from a pool of *Ma. uniformis* mosquitoes (Lutwama et al., 1999b). This species is very common in Sub-Saharan Africa and feeds on humans both inside and outside houses, primarily at night. The detection of a ONNV-positive pool of *Ma. funestus* has obviously implications for viral spread because of its large geographic distribution and its anthropophilic behavior; however, the vector competence of this species for ONNV is unknown and needs to be investigated.

No other competence or ecological studies about possible ONNV transmission by other vectors could be found in literature. Consequently, it is extremely difficult to estimate the risk of ONNV circulation on large scale using vectors other than *Anopheles* spp. The unusual capability of ONNV to infect both anopheline and culicine mosquitoes deserves to be better assessed, because this factor may lead to a potential epidemic situation.

In order to suggest research priorities, experts identified gaps of knowledge presented in Paragraph 6, and provided adapted recommendations, summarized in Paragraph 7. A more detailed version of expert recommendations is available in Supplementary data.

6. Gaps of knowledge

6.1. All three viruses

6.1.1. Vector competence studies

Protocols for experimental infections of mosquitoes with arbovirus are currently poorly standardized, making it difficult to compare results obtained across different laboratories.

6.1.2. Experimental evolution studies

Investigating virus adaptation to different vectors is necessary to predict future variants with high epidemic potential.

6.1.3. Molecular determinants of transmission

This has been analyzed in depth in the case of CHIKV but remains to be examined for ONNV and MAYV.

6.2. Vector control

Insecticide treatments are far from the panacea considering the insecticide resistance of most mosquito populations. Accumulated knowledge on vector ecology, behaviour, dynamics, and vector competence can open new opportunities for alternative control strategies.

6.3. Chikungunya virus

6.3.1. Vector competence studies – Europe (i)

In Europe, *Ae. albopictus* is well established in 20 countries and since 2007, this mosquito has been responsible for local CHIKV and DENV cases (Ding et al., 2018). The vector competence of several populations from European countries has been assessed using CHIKV ECSA and Asian genotypes (Vega-Rúa et al., 2015b); the data show that mosquitoes are highly susceptible to CHIKV and environmental temperatures also affect transmission which strongly depends on the three-way combination of mosquito population, virus strain and temperature by a genotype-by-genotype-by-environment (G x G x E) interaction. However, effect of environmental temperatures on vector susceptibility has been evaluated in this study only.

6.3.2. Vector competence studies – Europe (ii)

Other invasive species could be involved as CHIKV vector. *Ae. koreicus,* a human-biting mosquito colonizing domestic habitats, is capable of transmitting CHIKV (Ciocchetta et al., 2018). *Ae. aegypti*, present in Madeira island (Portugal) and also in regions close to Europe, Georgia and Turkey, is highly susceptible to CHIKV (Aedes aegypti - current k, 2019). *Aedes vexans* from Italy are also susceptible to CHIKV infection, so were other *Aedes* species, *Aedes caspius* and *Aedes detritus* (Talbalaghi et al., 2010). Information are lacking for these species, as well as for *Ae. japonicus* which proliferates in urban settings and feed on a wide range of hosts, and that is susceptible to several arboviruses (West Nile virus-WNV, Japanese encephalitis-JEV, La Crosse virus-LACV) (Schaffner et al., 2011).

6.3.3. Vector competence studies – Africa (i)

CHIKV vector competence studies with zoophilic *Aedes* mosquitoes are far from complete. It has been demonstrated that *Ae. vittatus*, a savannah species predominant in African forest galleries and *Ae. bromeliae*, a more opportunistic species (breeding in rural, *domestic* and peridomestic areas) are laboratory competent vectors of CHIKV (Mulwa et al., 2018). Vector competence of other local *Aedes* species needs to be further investigated. Moreover, since most information concern vector competence of domestic *Aedes aegypti aegypti*, the specific role of Ae. *aegypti formosus* and Ae. *albopictus* is not clear.

6.3.4. Vector competence studies – Africa (ii)

Entomological studies on the two forms of the *Aedes aegypti* complex, the sylvatic form in continental Africa, *Ae. ae. formosus*, and the domestic *Ae. ae. aegypti*, have been mainly focused in regions where entomological teams are well established (e.g. Senegal, Cameroon, Central African Republic, Kenya) and do not cover large part of African continent.

6.3.5. Vector competence studies – Pacific region

The Pacific region hosts a multitude of endemic mosquito species including *Ae. polynesiensis* and *Ae. hensilii*. Experimental infections showed that both were susceptible to CHIKV and that they can intervene as secondary vectors after the invasive species *Ae. aegypti* and *Ae. albopictus* (Ledermann et al., 2014; Richard et al., 2016). Since they are both anthropophilic species and colonize domestic and peri-domestic environments, the role of these species requires more attention. More studies should be promoted by local research agencies to better design control strategies. Likewise, funding sources can be dedicated to more knowledge on their genome sequences, helping in a better understanding of their evolutionary biology in these fragmented islands, considered as hot spots of endemism.

6.3.6. Vector competence studies – Americas

Vector competence data of anthropophilic mosquitoes *Ae. aegypti* and *Ae. albopictus* are relatively complete and variations were described according to geographical populations and CHIKV genotypes tested (Richards et al., 2010; Vega-Rua et al., 2014). However, testing zoophilic mosquitoes are at its infancy.

6.3.7. Co-infections in mosquitoes

Vector microbiota (bacteria, endosymbionts, and insect specific

viruses) affect vector biology and physiology including interactions with exogenous pathogens. With the development of mass sequencing technologies, access to the small RNA and bacterial worlds has opened new avenues in understanding the role of vector virome/bacteriome in transmission of arboviruses. However, these technologies remain costly for low-income countries and therefore, very limited, so that the role of microbiome in mosquitoes' ability to transmit co-infecting pathogens is difficult to assess.

6.3.8. Enzootic cycle in Asia and Americas

Little is known about the risk of CHIKV spillback into an enzootic cycle; recurrent human cases at the fringe of the jungle as well as repeated isolations of CHIKV in sylvatic mosquitoes are clues corroborating this risk.

6.4. O'nyong-Nyong virus

6.4.1. Vector competence studies

Culicine mosquitoes (*Aedes* and *Culex*) are the main vectors of arboviruses (*Flaviviridae, Togaviridae, Phenuiviridae*) but not of human malaria. *Anopheles* mosquitoes transmit malaria parasites and ONNV as a unique arbovirus. While *Aedes* and *Anopheles* mosquitoes are strongly human-biting in nature and then exposed to both types of pathogens, no data are compelling enough to explain this segregation of duties so far.

6.4.2. Enzootic cycle

ONNV has not yet been detected outside of Africa but the potential exists for emergence because *Anopheles* mosquitoes are widespread, including the same human-biting mosquitoes that transmit human malaria. Studies on potential vectors of ONNV including enzootic mosquitoes remain scarce.

6.5. Mayaro virus

6.5.1. Vector competence studies

Human cases have been limited to Central and South Americas, particularly to regions in and around the Amazon basin. Spillovers are repeatedly observed underlining the possible role of arboreal mosquitoes *Haemagogus, Sabethes* and anthropophilic *Aedes* spp. in MAYV transmission, that needs to be better defined.

SUMMARY OF EXPERT RECOMMENDATIONS (see Supplementary data for more detailed recommendations).

6.6. All three viruses

6.6.1. Vector competence studies

To allow comparison between results from different laboratories, protocols for vector competence studies should be standardized.

6.6.2. Experimental evolution studies

Experimental selection with multiple passages of viruses in mosquitoes may prove useful. Examination of viral populations at each passage will allow detecting genetically fixed variants.

6.6.3. Surveillance of sylvatic reservoirs and emergence events

Changes in mosquito distribution often lead to circulation of pathogens they transmit. The circulation of MAYV in endemic or enzootic regions in America, and CHIKV and ONNV in different regions in Africa should be monitored, in order to assess the risk of emergence in human and/or animal populations.

6.6.4. Vector control

A major effort is necessary to design adapted vector control strategies. The use of endosymbionts such as *Wolbachia*, genetically modified mosquitoes with refractory phenotype to arboviruses, sterile mosquitoes are as much possibilities to envisage on the field.

6.6.5. Molecular determinants of replication in vectors and transmission

To investigate more deeply interactions between mosquitoes and arboviruses, development of molecular tools, reverse genetics methods, genome sequencing of more mosquito species and improving mosquito reference genomes are urgently required.

6.7. Chikungunya virus

6.7.1. Vector competence studies

The fact that that the environment can strongly modify adaptive properties of genotypes should encourage to develop more experimental assessments of vector susceptibility using field-collecting mosquitoes submitted to different environmental temperatures.

6.7.2. Co-infections in mosquitoes

Improving our understanding of the role of virome/microbiome in mosquitoes' ability to transmit co-infecting pathogens is necessary. Collaborations to obtain financing for the local teams and capacity-building research activities, access and development of research facilities for experimental infections, should be encouraged by national and international research funding agencies. Moreover, since co-infections are likely in vectors (as DENV-CHIKV co-infection reported in Aedes mosquitoes (Caron et al., 2012)), mass viral screening should be facilitated with the development of multiplex approaches and high-throughput systems. These technologies will provide an exhaustive inventory of arboviruses carried by mosquitoes using the same batches of field collected mosquitoes for nearly 100 viruses screened.

6.8. O'nyong-nyong virus

6.8.1. Vector competence studies

To investigate ONNV competence of *Anopheles* and *Aedes* species, knowledge of antiviral barriers in *Anopheles* and viral persistence in *Aedes* should be investigated. Development of molecular tools (reverse genetics studies for infectious/chimeric clones), experimental evolutionary studies may help to identify and dissect the molecular mechanisms that permit virus infection in *Aedes* and virus exclusion in *Anopheles*.

6.8.2. Enzootic cycle

Ecological and epidemiological studies to identify and characterize the putative ONNV enzootic cycle should be encouraged, in order to be prepared for future emergences in the image of what happened for ZIKV qualified as harmless before the 2015 pandemic.

6.9. Mayaro virus

6.9.1. Vector competence studies (I)

To better understand the role of *Haemagogus, Sabethes* and *Aedes* species in MAYV transmission, viral isolations from field-collected mosquitoes and vector competence studies should be promoted. This would help to assess the potential of MAYV to cause millions human cases like CHIKV and ZIKV did in a recent past. Likewise, molecular tools (e.g. infectious clones) to examine viral molecular determinants for mosquito susceptibility are advisable.

6.9.2. Vector competence studies (II)

Vector competence of mosquitoes from African continent should be assessed for MAYV, in order to evaluate the risk of possible wider spread.

6.9.3. Potential urban cycle

Contrary to CHIKV, MAYV makes attempts to jump outside the enzootic cycle in the Amazon basin with a growing number of human cases reported. Change of vector host range should be tracked.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.antiviral.2019.104670.

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