

Vector Competence of *Aedes aegypti* and *Aedes vittatus* (Diptera: Culicidae) from Senegal and Cape Verde Archipelago for West African Lineages of Chikungunya Virus

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Abstract. To assess the risk of emergence of chikungunya virus (CHIKV) in West Africa, vector competence of wild-type, urban, and non-urban *Aedes aegypti* and *Ae. vittatus* from Senegal and Cape Verde for CHIKV was investigated. Mosquitoes were fed orally with CHIKV isolates from mosquitoes (ArD30237), bats (CS13-288), and humans (HD180738). After 5, 10, and 15 days of incubation following an infectious blood meal, presence of CHIKV RNA was determined in bodies, legs/wings, and saliva using real-time reverse transcription–polymerase chain reaction. *Aedes vittatus* showed high susceptibility (50–100%) and early dissemination and transmission of all CHIKV strains tested. *Aedes aegypti* exhibited infection rates ranging from 0% to 50%. *Aedes aegypti* from Cape Verde and Kedougou, but not those from Dakar, showed the potential to transmit CHIKV in saliva. Analysis of biology and competence showed relatively high infective survival rates for *Ae. vittatus* and *Ae. aegypti* from Cape Verde, suggesting their efficient vector capacity in West Africa.

INTRODUCTION

Chikungunya virus (CHIKV) belongs to the family *Togaviridae*, genus *Alphavirus*, and Semliki Forest antigenic complex. Seventy percent of human CHIKV infections result in a sudden onset of disease with high fever, severe arthralgia^{1,2} myalgia, nausea, vomiting, headaches, rash, nasal discharge, photophobia, and lymphadenopathy.³ The disease can cause severe morbidity and, during the epidemics in La Réunion in 2005, deaths were also associated with CHIKV infection.^{4,5}

This virus was first described in Tanganyika (now Tanzania) during a 1952–1953 epidemic of dengue-like illness.^{6,7} During the 1960s and 1990s, the virus was repeatedly isolated in central, southern, and western Africa, including Senegal, Côte d'Ivoire, Benin, Guinea, and Nigeria.^{7,8} Currently, chikungunya fever is endemic to almost 40 countries.⁸

Chikungunya virus is transmitted to humans and non-human primates by the bite of several mosquito species.¹ There are two epidemiologic transmission cycles: a sylvatic cycle, occurring primarily in Africa and mainly between wild primates and arboreal *Aedes* mosquitoes, where humans are accidental hosts, and an urban human-mosquito-human cycle.

In Senegal, the transmission cycles include a sylvatic cycle involving mainly *Aedes fuscifer*, *Ae. taylori*, and *Ae. luteocephalus* mosquitoes but also monkeys and humans. However, unlike dengue and yellow fever in Senegal, sylvatic CHIKV transmission may involve other vertebrate hosts, such as galagos (*Galago senegalensis*), palm squirrels (*Xerus erythropus*), or bats (*Scotophilus* sp.).⁹ Several amplifications of the sylvatic cycle have been detected in the Kedougou region, where CHIKV has been isolated from 13 mosquito species^{10,11} and 3 monkey species (patas, African green, and baboon).¹⁰ Beyond the three main vectors, CHIKV was isolated from *Ae. argenteopunctatus*, *Ae. dazieli*, *Ae. hirsutus*, *Ae. metallicus*, *Ae. neoafricanus*, *Ae. vittatus*, *Ae. irritans*, *Anopheles coustani*, *An. domicola*, *An. funestus*, *An. rufipes*, *An. gambiae* s.l., *Culex ethiopicus*, *Cx. poicilipes*, and *Mansonia uniformis*.

Chikungunya virus has also been isolated from *Ae. aegypti*, the major epidemic vector during outbreaks in Senegal.¹⁰ Several human outbreaks occurred in Senegal in 1966¹² and 1982¹³ near Dakar, during 1996–1997, at Niakhar and Kaffrine,¹⁴ and recently in Kedougou in 2004 among Peace Corps volunteers.¹⁵ In 2005, British soldiers became infected in the Saloum Islands and in 2006, outbreaks occurred in Bambey, near Dakar,¹⁶ and more recently in Kedougou in 2011 (Faye O and others, unpublished data). In Asia and the Indian Ocean basin, CHIKV circulation has caused major urban epidemics with *Ae. aegypti* and *Ae. albopictus* as the main vectors.

Phylogenetic analysis of viral sequences has identified three distinct CHIKV lineages: West African, Central/East African, and Asian,^{17,18} and has indicated that the Indian Ocean outbreaks resulted from the recent introduction of an African CHIKV strain.¹⁹ This strain was later introduced from Africa into southern Asia, and CHIKV has been detected in many humans travelers to Europe, North America, and South America.²⁰

There is evidence that several *Aedes* species, including *Ae. aegypti*, are susceptible to CHIKV infection, although their role in natural transmission has not been clearly elucidated in some regions.²¹ In addition, significant differences were reported for the vector competence caused by viral, mosquito species, and population or environmental factors.^{22–24} Thus, the 2005 outbreak in La Réunion was associated with an unusual vector, *Ae. albopictus*, after an adaptive mutation in the envelope protein (E1) gene of CHIKV^{19,24} and a subsequent, second-step, adaptive mutation in the E2 gene.²⁵

Despite the frequent isolation of CHIKV from mosquitoes in Senegal, no estimation of vector competence has been performed. Although CHIKV circulation has never been reported in Cape Verde, numerous travel and trade connections with continental Africa suggest a risk of its importation. The dengue 3 serotype epidemic, which concurrently affected Côte d'Ivoire, Cape Verde, and Senegal in 2009, suggests the potential for arbovirus exchanges between these countries. The risk for CHIKV epidemic emergence is also high because the epidemic vector *Ae. aegypti* is present and the human population is susceptible. To assess the risk for CHIKV to be

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TABLE 1
Mosquito species used in the study

Mosquito species	Habitat type	Collections				
		Breeding sites	Location	GPS coordinates	Date	Stage
<i>Aedes aegypti</i>	Forest gallery	Tree holes	Kedougou, Senegal	12°35'29"N, 12°13'37"W	July 2009	Eggs, larvae, pupae
	Urban	Artificial containers	Dakar, Senegal	14°39'20"N, 17°26'08"W	June 2010	Larvae, pupae
	Urban	Artificial containers	Praia, Cape Verde	15°03'31"N, 23°36'53"W	2009	Larvae, pupae
<i>Aedes vittatus</i>	Forest gallery	Rock holes	Kedougou, (Senegal)	12°35'29"N, 12°13'37"W	June 2009	Larvae, pupae

GPS = global positioning system.

established, a vector competence study of *Ae. aegypti* populations from Cape Verde was performed by using the A226V variant of CHIKV from La Réunion Island.²⁶ However the competence of the urban *Ae. aegypti* from Cape Verde to transmit West African CHIKV strains, which are most likely to be imported, has never been investigated. We report results of vector competence experiments with West African mosquitoes from Cape Verde and Senegal to disseminate and transmit different West African strains of CHIKV.

MATERIALS AND METHODS

Mosquito colonies. The mosquito species used include wild-type and urban populations of *Ae. aegypti* and a wild type population of *Ae. vittatus* reared from eggs, larvae, or pupae collected in the field (Table 1). To avoid selection of a single female oviposition, several breeding habitats were prospected and mixed in the laboratory. *Aedes aegypti* and *Ae. vittatus*, because of their abundance, anthropophagy, and proven association with CHIKV in nature, and presence in sylvatic and domestic environments, are good candidates for CHIKV enzootic and epidemic transmission. Females F₀ were fed several times on guinea pigs to obtain eggs. These eggs were hatched and the larvae reared to adults that were considered the F₁ generation used in this study. This F₁ generation were maintained exclusively with a 10% sucrose solution at a temperature of approximately 26°C, a relative humidity of 80%, and a light:dark photoperiod of 12:12 hours.

Viruses. Three CHIKV isolates from mosquitoes,¹³ a bat,²⁷ and a human¹⁵ were used for experimental infections. The viral strains used and their origin, genotype, and passage history are shown in Table 2. All virus strains had been passaged 5–6 times on *Ae. (Stegomyia) pseudoscutellaris* 61 cells (AP-61) and stored at –80°C before being used in this study.

Sequencing of CHIKV strains. Three CHIKV isolates were amplified with 40 others strains isolated over 43 years (1962–2005) from different hosts (human, mosquito, and non-human primates) and countries (Senegal, Côte d'Ivoire, and Central African Republic) by using reverse transcription–polymerase chain reaction (RT-PCR) with primers described.^{19,28,29} The sequences were aligned by using MUSCLE,²⁸ and a phylogenetic tree was constructed by using

the maximum-likelihood method implemented in MEGA version 5.2.1.²⁹

Oral infection of mosquitoes. Mosquito infections were performed according to procedures described.³⁰ Three- to five-day-old F₁ generations of females of each species that had never taken a blood meal were deprived of sucrose for 24 hours before being allowed to feed on a mixture of rabbit blood and CHIKV through a membrane feeder placed on top of each cage. The infectious meal consisted of 33% (v/v) rabbit erythrocytes washed with 1× phosphate-buffered saline, 33% (v/v) virus stock in Leibovitz 15 (L15) cell culture medium, 20% (v/v) fetal bovine serum, 1% (w/v) sucrose, and 5 mM ATP. The membrane feeder was maintained at 37°C and mosquitoes were allowed to feed for 60 minutes. After feeding, fully engorged mosquitoes were transferred to 1-liter cardboard cages (15–30 mosquitoes/cage) with a net on top and maintained with 10% sucrose at 27°C, a relative humidity of 80%, and a light:dark photoperiod of 16:8 hours. A sample of the blood meal was collected after mosquito feeding for virus titration.

Mosquitoes processing. At 5, 10, and 15 days post infection (dpi), samples of mosquitoes were collected randomly, cold anesthetized, and their legs and wings removed and transferred individually into separate tubes. The proboscis of each mosquito was then inserted into a capillary tube containing 1 µL of fetal bovine serum for salivation for up to 30 minutes. After salivation, each mosquito body and saliva sample was placed in a separate tube. Bodies and legs/wings were triturated in 500 µL of L15 medium. Only one experiment was performed for each mosquito species and with each virus strain.

Virus detection. Real-time RT-PCR was used to detect CHIKV from mosquito bodies, legs/wings, and saliva. RNA was extracted by using the QIAamp RNA Viral Kit (QIAGEN, Heiden, Germany) according to the manufacturer's recommendations. Amplification was performed using the QuantiTect Probe Kit (QIAGEN). The 25-µL reaction volume contained 5 µL of extracted RNA, 10 µL of buffer (2× QuantiTect Probe), 6.8 µL of sterile water, 1.25 µL of each primer, 0.5 µL of probe, and 0.2 µL of RT-PCR Master Mix Quantitect. The specific primers and probe sequences for CHIKV used have been described.¹¹ The thermal profile included reverse transcription for 10 minutes at 50°C, reverse

TABLE 2
Chikungunya virus strains used in this study

CHIKV strains	Isolation				Passage history*	Lineage
	Source	Year	Location			
ArD30237	Mosquito (<i>Aedes neoafricanus</i>)	1979	Kedougou 12°35'29"N, 12°13'37"W		5	West Africa I
CS13-288	Bat (<i>Scotophilus</i> sp.)	1962	Gagnik, 14°08'51"N, 16°05'48"W		Unknown	West Africa II
HD180738	Human	2005	Sine Saloum, 14°06'05"N, 16°29'33"W		6	West Africa I

*Passages were conducted with *Aedes (Stegomyia) pseudoscutellaris* 61 cells (AP61).

transcriptase inactivation and DNA polymerase activation for 15 minutes at 95°C, followed by 40 amplification cycles for 15 seconds at 95°C and for 1 minute at 60°C (annealing-extension step).

Data analysis. Three parameters describing vector competence were determined: infection (number of infected mosquito bodies per 100 mosquitoes tested), dissemination (number of mosquitoes with positive legs/wings per 100 mosquitoes infected) and transmission rates (number of mosquitoes with positive saliva per 100 mosquitoes with disseminated infection). These rates were compared for each species according to the extrinsic incubation periods (EIPs) tested, as well as between species and virus strains.

Fisher's exact tests were performed for comparison of infection, dissemination and transmission rates using R statistical software (R Foundation for Statistical Computing, Vienna, Austria). Differences were considered statistically significant at $P < 0.05$.

RESULTS

Sequence alignments showed that all the CHIKV strains analyzed had an alanine residue at E1 glycoprotein position 226 (Figure 1). Phylogenetic analysis of viral strains showed that there are two groups of CHIKV circulating in West Africa. The first, known as West Africa I (WAF I), is specific to West Africa and included strains collected during 1966–2005 in Senegal and Côte d'Ivoire. The ArD30237 and HD180738 used for mosquito infections belong to this lineage I. The other group, known as West Africa II (WAF II), including the CS13-288 isolate used in this study, is closely related to the East Central and South African (ECSA) lineage with old strains (1962 and 1963) and new strains (1999, 2001, 2004) from Senegal, Côte d'Ivoire, and Central African Republic (Supplemental Figure S1).

Infection, dissemination, and transmission rates of CHIKV are shown in Table 3. These rates are shown for mosquitoes tested after different EIPs, as well as the virus titer of each virus strain after exposure to mosquitoes. *Aedes vittatus* exhibited high infection rates between 50% and 100%, which increased significantly from 5 dpi to 15 dpi for the CS13-288 strain ($P = 0.015$); the other strains reached maximum infection rates at 10 dpi but without significant differences between incubation periods. The infection rate of HD180738 was significantly higher than that of ArD30237 at 15 dpi ($P = 0.026$) and that of CS13-288 at 10 dpi ($P = 0.023$). The dissemination rates ranged from 18.2% to 100% and increased with the EIP. However, the only significant differences were observed with the HD180738 strain between 5 dpi and 15 dpi ($P < 0.000005$) and 10 dpi and 15 dpi ($P < 0.002$). The CS13-288 exhibited a lower dissemination rate at 5 dpi than the other strains ($P < 0.017$). All virus strains reached the saliva, indicating transmission potential, with rates ranging from 20% to 58%. However, only the ArD30237 strain was present in saliva at 5 dpi. No significant differences were observed among potential transmission rates recorded based on viral strains or EIP.

Our results indicated a low susceptibility of *Ae. aegypti* populations from Dakar (infection rate $\leq 30\%$) for all CHIKV strains tested and with all EIP. The infection rates were comparable except those obtained between 5 dpi and 15 dpi for HD180738 and CS13-288, respectively ($P < 0.04$). Only two mosquitoes were able to disseminate the HD180738 and

ArD30237 virus strains by 15 dpi. No significant differences were observed among the dissemination rates regardless of viral strain or EIP considered, and no CHIKV reached the saliva of Dakar *Ae. aegypti*.

The same trend of low susceptibility was also observed in *Ae. aegypti* from Cape Verde for all CHIKV strains tested; infection rates ranged between 12% and 38%, and dissemination rates ranged from 0% to 100%. Only the ArD30237 strain was found in the saliva; rates ranged from 33% and 100%. No significant differences were observed among infection, dissemination, and transmission rates regardless of the viral strain and EIP.

The *Ae. aegypti* population from Kedougou exhibited infection rates ranging from 0% to 50%, which were comparable among all EIP for the CS13-288 and HD180738 strains, as well as between 10 dpi and 15 dpi ($P = 0.128$) for strain ArD30237. Dissemination rates ranged from 0% to 50%; CS13-288 began to disseminate at 5 dpi and the other strains began to disseminate at 10 dpi. No significant differences were observed among dissemination rates regardless of viral strain except the significantly higher rate at 15 dpi compared with 5 dpi for HD180738 ($P < 0.01$) and EIP except those obtained at 5 dpi and 15 dpi for HD180738 and CS13-288 strains, respectively ($P < 0.04$). Only the CS13-288 and HD180738 strains were potentially transmitted by this population from 10 dpi, without significant differences based on the EIP.

A comparative analysis of results obtained with different species at different EIP showed significant variations among the infection, dissemination, and potential transmission rates. With ArD30237, only infection rates showed significant differences by mosquito strain at 10 dpi ($P < 0.0003$) and 15 dpi ($P < 0.0000001$). Specifically, *Ae. vittatus* infection rates were significantly higher than all populations of *Ae. aegypti* tested ($P < 0.003$). For CS13-288, *Ae. vittatus* and *Ae. aegypti* from Kedougou exhibited different infection rates at 15 dpi ($P < 0.001$). For HD180738, only *Ae. vittatus* infection rates were significantly higher compared with those obtained from the *Ae. aegypti* populations tested at different EIP ($P < 0.01$). The dissemination rates were comparable among the mosquito species, except those obtained at 15 dpi for *Ae. vittatus* and *Ae. aegypti* from Kedougou ($P < 0.00001$). It was noteworthy that only these latter populations were able to potentially transmit this strain at comparable rates.

DISCUSSION

Three distinct CHIKV phylogenetic lineages, the West African, the Asian and ECSA lineages, were identified by using complete genome sequences from virus strains collected in different geographic areas.^{17,19} Our phylogenetic analysis showed the circulation of one lineage specific to West Africa, WAF I, and a second group present within the ECSA lineage, West Africa II. The presence of the WAF II within the ECSA lineage, which contain the oldest CHIKV strains,¹⁸ suggests that the WAF II lineage we sampled from mosquitoes and bats in Senegal may be ancestral to WAF I. The introduction of CHIKV into West Africa from the ECSA lineage could have occurred via movement of viremic persons or animals as reported for yellow fever and dengue viruses introduced into South America from Africa and Asia, respectively.^{31,32}

	A226V!						
'A67 '		KDVYANTQLV	LQRPAGTVH	VPYSQAPSGF	KYWLKERGAS	LQHTAPFGCQ	IATNPFVRAVN
'ArD30143SN79A.dalzieli '	
<u>'CS13-288SN62Scotophilus'</u>	M.
'SH2833SN66Human	
'SH3010SN65Human '	
'SH3012SN66Human '	
'SH3013SN66Human '	
'SH2834SN66Human '	
'SH2976SN65Human '	
'SH2893SN66Human '	
'SH3009SN66Human '	
'A301SN63Chauve-souris (Rao) '	V.....M.
'SH2892SN66Human '	
'ArD30022SN79 '	
<u>'ArD30237SN79Ae.luteocephalus '</u>	
'ArA2657 '	
'ArB16678 '	
'HB78RCA78Human ' a	
'ArD93229SN92Ae.dalzieli '	
'ArD120940SN96Ae.luteocephalus '	I.
'ArD128438SN97Ae.dalzieli '	
'HD131124SN98Human '	I.
'HA001-99CIV99Human '	M.
'ArD156076SN2001Ae.argenteopunctatus'	M.
'ArD175374SN2004Ae.taylori '	M.
'ArD175381SN2004Ae.luteocephalus '	M.
'ArD175393SN2004Ae.furcifer '	M.
'HD180738SN2005Human '	I.
'HD180761SN2005Humain '	I.
'AF192906RCA '	
'AY549583-RDC96 '	A.....
'AF192903-AFSUD76 '	M.
'AF192905-TAN53 '	M.
'AY549584-RDC2000 '	G.....C.....
'AF192896-Thailand88 '		E.....S.....M.
'AF192900-Thailand96 '		E.....S.....M.
'AF192892-AOF '	
'AM258991-IR2005 '	
'AM258992-IR2005 '	V.....
'AM258993-IR2005 '	V.....
'AM258994-IR2005 '	V.....
'EF051584-CAM2006 '	G.....V.....
'ArD122544SN96Ae.aegypti '	I.
'ArA30565CI96Culex.weschei '	
'ArA30548CI93Ae.africanus '	
'ArA30543CI93Ae.opok '	
'HD103091SN93Human '	
'HD103090SN93Human '	
'ArD92409SN94Ae.furcifer '	
'ArD93311SN93Ae.taylori '	
'SH2898SN66Human '	
'SH3014SN66Human '	
'HD180739SN2005Human '	I.
'HD180760SN2005Human '	I.

FIGURE 1. Amino acid sequences of the envelope 1 (E1) protein of chikungunya virus strains. Strains used for experimental mosquito infections are underlined, and gray shading indicates epidemic chikungunya virus of the East Central and South Africa lineage containing the A226V E1 substitution.

None of the CHIKV strains analyzed in this study had a valine substitution at position 226 in the E1 protein, which has been associated with major recent epidemics in Asia and the Indian Ocean.³³ This finding suggests genetic stability and low rates of molecular evolution of the enzootic strains circulating in West Africa. However, the presence of WAF II in the ECSA lineage suggests the epidemic potential of this subgroup. Furthermore, previous study showed that the E2-

L210Q mutation increase the ability of CHIKV to infect and disseminate the virus in *Ae. albopictus*.²⁵ Additional studies based on complete genomes of the WAF II strains are needed for a better understanding of the emergence of CHIKV in West Africa.

We have demonstrated the susceptibility and transmission potential of mosquitoes from Senegal and Cape Verde to transmit various strains of CHIKV circulating in the sub-region.

TABLE 3
Infection, dissemination, and transmission of mosquito species orally exposed to different CHIKV strains at 5, 10, and 15 days post-infection

Mosquito species	Geographic origin	CHIKV strains	Virus titer (PFU/mL)	Infection rate* (%)			Dissemination rate† (%)			Transmission Rate‡ (%)		
				5	10	15	5	10	15	5	10	15
<i>Ae. vittatus</i>	Kedougou	ArD30237	3.5 × 10 ⁶	3/6 (50)	6/6 (100)	9/11 (81.2)	2/3 (66.7)	5/6 (83.3)	9/9 (100)	1/2 (50)	1/5 (20)	4/9 (44.4)
		CS13-288	8.2 × 10 ⁶	11/20 (55)	15/20 (75)	18/19 (94.7)	2/11 (18.2)	6/15 (40)	12/18 (66.7)	0/2 (0)	3/6 (50)	7/12 (58.3)
		HD180738	9.4 × 10 ⁶	17/19 (89.5)	20/20 (100)	54/54 (100)	8/17 (47.05)	11/20 (55)	51/54 (94.9)	0/8 (0)	0/11 (0)	19/51 (37.2)
<i>Ae. aegypti</i>	Kedougou	ArD30237	10 ⁷	3/10 (30)	2/10 (20)	0/17 (0)	0/3 (0)	1/2 (50)	-	-	0/1 (0)	-
		CS13-288	5.0 × 10 ⁶	6/12 (50)	6/12 (50)	9/20 (45)	1/6 (16.7)	2/6 (33.3)	4/9 (44.4)	0/1 (0)	1/2 (50)	2/4 (50)
		HD180738	3.37 × 10 ⁶	9/18 (50)	6/20 (30)	18/48 (37.5)	0/9 (0)	2/6 (33.3)	8/18 (44.4)	-	1/2 (50)	4/8 (50)
Dakar	ArD30237	7.8 × 10 ⁶	0/10 (0)	1/10 (10)	1/16 (6.2)	-	0/1 (0)	1/1 (100)	-	-	0/1 (0)	
	CS13-288	6.9 × 10 ⁶	0/10 (0)	0/10 (0)	0/17 (0)	-	-	1/1 (100)	-	-	-	
	HD180738	9.3 × 10 ⁶	3/10 (30)	1/10 (10)	1/10 (10)	0/3 (0)	0/1 (0)	1/1 (100)	0/1 (0)	1/3 (33.3)	0/1 (0)	
Cape Verde	ArD30237	6.8 × 10 ⁶	3/8 (37.5)	1/10 (10)	1/16 (6.2)	3/3 (100)	1/1 (100)	0/1 (0)	1/3 (33.3)	1/1 (100)	-	
	CS13-288	2.1 × 10 ⁷	1/8 (12.5)	0/10 (0)	0/4 (0)	0/1 (0)	-	-	-	-	-	
	HD180738	4.2 × 10 ⁷	1/8 (12.5)	2/9 (22.2)	NT	0/1 (0)	1/2 (50)	-	-	0/1 (0)	-	

CHIKV = chikungunya virus; PFU = plaque-forming units; NT = not tested.
 * No. mosquitoes with infected bodies.
 † No. mosquitoes with positive legs and wings/no. mosquitoes with infected bodies.
 ‡ No. positive saliva/no. mosquitoes with positive legs and wings.

Although previous studies tested the vector competence of *Ae. aegypti* populations from Cape Verde, the CHIKV isolate used was from La Réunion Island, including A226V substitution in the envelope protein E1.²⁶ For mosquito populations from Senegal, despite their frequent associations with CHIKV in nature, vector competence studies have never been reported.

Only RT-PCR was used to detect CHIKV for two reasons. First, the purpose of this study was to show the competence of the vector and we have been focused on the detection of CHIKV in the different compartments of the mosquitoes and in the saliva. Because we have shown that the virus reached the saliva, it implies that the vector is competent. Second, in our experience with other viruses such as West Nile virus (Sall AA and others, unpublished data) and Usutu virus,³⁴ we have observed that RT-PCR and infectious viral particles are generally consistent and concordant in their conclusions and trends. Such a trend has also been confirmed on C6/36 cells for CHIKV in a recent study.³⁵ In this study, the ratio of RNA genomes: infectious viral particles is approximately 100:1. Although the ratio may vary depending on the cells type or multiplicity of infection, we believe such a ratio can be considered a reasonable estimate for CHIKV infection in our experiment.

Our results indicated a high susceptibility of *Ae. vittatus* populations from Kedougou and disseminated infections for all viral strains, regardless of the EIP. Transmission potential has also been demonstrated with all CHIKV strains for this species. The infection rates we obtained were much higher than those obtained with populations of *Ae. vittatus* from India,³⁶ which did not exceed 50%. These observations suggest a high degree of CHIKV adaptation to *Ae. vittatus* populations in Senegal, which was more pronounced with the ArD30237 strain that disseminated and reached the saliva at high rates at 5 dpi. Conversely, the other CHIKV strains disseminated more gradually regardless of the EIP and exhibited transmission potential only after 10 dpi and 15 dpi, respectively, for strains HD180738 and CS13-288. Early dissemination observed in *Ae. vittatus* within 5 dpi at a relatively high rates indicated high susceptibility for this species. This early dissemination, as well as early transmission potential, has been observed in previous studies.^{36,37}

The infection rates we observed were relatively low in all of the *Ae. aegypti* populations we tested compared with populations from other geographic regions. Studies of *Ae. aegypti* from Queensland, Australia, showed that after exposure to CHIKV at a titer of 10⁴ 50% cell culture infection doses/mosquito, 92% became infected and disseminated. Among the disseminated mosquitoes, 70% were able to transmit the virus.²¹

We believe that the low infection rates we obtained were not caused by virus titers used in our experiment for several reasons. First, it has been demonstrated that CHIKV viremia in patients ranges from 10^{3.9} to 10^{6.8} plaque-forming units (PFU)/mL.²⁰ Second, with the same virus titers and in the same experimental conditions, we obtained high infection rates with *Ae. vittatus*. Third, in New Zealand, it has been shown that the highest infection rates were obtained with an infectious blood meal at 10^{6.2} PFU/mL unlike other species exhibiting low infection with a blood meal titers ranging from 10^{7.8}–10^{10.5} PFU/mL.³⁸ Based on these considerations, the low infection rates we observed for *Ae. aegypti* populations from Senegal and Cape Verde probably reflect low susceptibility. The use of the CHIKV variant E1-226V may explain the high infection obtained in previous studies that tested populations

of *Ae. aegypti* from Cape Verde,²⁶ and central Africa.³⁹ The three strains used in this study all had the *Ae. albopictus*-adaptive alanine at E1 position 226, consistent with enzootic strains rather than recent epidemic strains of the La Réunion.^{23,24} The low infection rates we obtained were also similar to those observed in *Ae. aegypti* populations from North America, which were < 20%.⁴⁰

Our results show vector competence for CHIKV of *Ae. aegypti* populations from Cape Verde and Kedougou and vector incompetence of *Ae. aegypti* from Dakar. They also show differences in the oral receptivity of *Ae. aegypti* populations to the CHIKV lineage used according to their domestic or wild origin. The strains belonging to the West Africa I lineage were the only ones disseminated by the domestic population of *Ae. aegypti* from Dakar and transmitted by those from Cape Verde. *Aedes aegypti* from Dakar did not transmit any of the three CHIKV strains; no infection with CS13-288 was recorded. However, despite relatively low susceptibility, the Cape Verde *Ae. aegypti* exhibited efficient dissemination, especially with the ArD30237 strain (100% when dissemination occurred). This strain was the only one potentially transmitted at 5 dpi with rates ranging from 33% to 100%. The same trend was shown in a previous study²⁶; Cape Verde *Ae. aegypti* dissemination rates of an ECSA CHIKV strain were 91–100% depending on the EIP.

The sylvatic *Ae. aegypti* population from Kedougou was susceptible to both CHIKV lineages tested, including transmission potential. All three strains disseminated and reached the saliva, although the CS13-288 strain exhibited earlier dissemination.

From this study we can conclude that *Ae. aegypti* from Kedougou and Cape Verde, as well as *Ae. vittatus*, are capable of transmitting CHIKV. However, *Ae. vittatus* and *Ae. aegypti* from Cape Verde exhibited a minimum EIP of 5 days, and *Ae. aegypti* from Kedougou required 10 days for potential transmission. When considered in the context of their parity rates determined,^{11,41} and estimated survival rates of 0.8,⁵ 0.99,⁵ and 0.72,¹⁰ respectively, 32.8%, 95%, and 3.96% of the respective mosquito populations would be expected to survive long enough for transmission to occur. These findings suggest a relatively high transmission potential for *Ae. vittatus* from Kedougou and *Ae. aegypti* from Cape Verde. However, *Ae. aegypti* from Kedougou, despite its low survival rate, could be involved in maintaining the sylvatic cycle of CHIKV in this region.

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