

1 **High vector competence of *Aedes aegypti* and *Aedes albopictus* from ten American**
2 **countries as a crucial factor of the spread of Chikungunya**

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26 **ABSTRACT**

27 Chikungunya virus (CHIKV) causes a major public health problem. In 2004, CHIKV began
28 an unprecedented global expansion and has been responsible for epidemics in Africa, Asia,
29 islands in the Indian Ocean region, and surprisingly, in temperate regions such as Europe.
30 Intriguingly, no local transmission of chikungunya virus (CHIKV) has been reported in the
31 Americas until recently despite the presence of vectors and annually-reported imported cases.
32 Here, we assessed the vector competence of 35 American *Ae. aegypti* and *Ae. albopictus*
33 populations to three CHIKV genotypes. We also compared the number of viral particles of
34 different CHIKV strains in mosquito saliva at two different times post-infection. Primarily,
35 viral dissemination rates were high for all mosquito populations irrespective of the tested
36 CHIKV isolate. In contrast, differences in transmission efficiency (TE) were underlined in
37 populations of both species through the Americas suggesting the role of salivary glands in
38 selecting CHIKV for highly efficient transmission. Nonetheless, both mosquito species were
39 capable to transmit all three CHIKV genotypes, and TE reached alarming rates as high as
40 83.3% and 96.7% in *Ae. aegypti* and *Ae. albopictus* populations, respectively. *Ae. albopictus*
41 better transmitted the epidemic mutant strain CHIKV_0621 of the East-Central-South African
42 (ECSA) genotype than did *Ae. aegypti*, whereas this latter species was more capable of
43 transmitting the original ECSA CHIKV_115 strain and also the Asian genotype CHIKV_NC.
44 Therefore, a high risk of establishment and spread of CHIKV throughout the tropical,
45 subtropical and even temperate regions of the Americas is more real than ever.
46

47 **IMPORTANCE**

48 Until recently, the Americas have never reported chikungunya (CHIK) autochthonous
49 transmission despite its global expansion beginning in 2004. Large regions of the continent
50 are highly infested with *Ae. aegypti* and *Ae. albopictus* and millions of dengue (DEN) cases
51 are annually recorded. Indeed, DEN and CHIK viruses share the same vectors. Due to a recent
52 CHIK outbreak affecting Caribbean islands, the need for a Pan-American evaluation of vector
53 competence was compelling as a key parameter in assessing the epidemic risk. We
54 demonstrated for the first time that *Ae. aegypti* and *Ae. albopictus* populations throughout the
55 continent are highly competent to transmit CHIK irrespective to the viral genotypes tested.
56 The risk of CHIK spreading throughout the tropical, subtropical and even temperate regions
57 of the Americas is more than ever a reality. In light of our results, local authorities should
58 immediately pursue and reinforce epidemiological and entomological surveillance to avoid a
59 severe epidemic.

60

61 **Key words:** Americas, *Aedes aegypti*, *Aedes albopictus*, chikungunya virus, emergence.

62

63

64 **INTRODUCTION**

65 Chikungunya virus (CHIKV) is an alphavirus in the family *Togaviridae* that is transmitted by
66 mosquitoes, mainly *Aedes aegypti* and *Aedes albopictus* within an urban cycle. Since 2004,
67 CHIKV has reemerged in the Indian Ocean Islands and has caused severe epidemics in
68 several countries in tropical and subtropical regions in Africa and Asia, as well as in
69 temperate Mediterranean areas in Europe (1).

70 *Aedes aegypti* is widespread in the Americas, where it is the only confirmed natural
71 dengue virus (DENV) vector (2). Although its geographical distribution is more limited, *Ae.*
72 *albopictus* is considered a potential vector in the Americas due to the high vector competence
73 of local populations to DENV (3,4). More than two millions dengue cases are annually
74 reported in the American continent each year (5). The most critical epidemiological situation
75 is that described for South America, which reported more than 1.5 million dengue cases in
76 2013, with an incidence rate of more than 650 cases/100,000 inhabitants in the South Cone
77 alone (6). Such an epidemiological scenario points to the weakness of mosquito control
78 activities and the high receptivity to introduction and spread of other arboviroses transmitted
79 by both mosquito species like CHIKV in other parts of the continent (1,7,8). In fact, as
80 CHIKV and DENV share the same mosquito vector species, epidemic waves caused by both
81 viruses affect the same regions and human co-infections may occur (9,10). Moreover, the
82 intensification of intercontinental travels with recurrent returns of dozens of viremic CHIKV
83 cases from affected areas -that may bypass the surveillance systems due to the clinical
84 similarities with other viruses circulating in the Americas- exemplifies the vulnerability of
85 this continent to CHIKV epidemics (11,12). Indeed, Brazil, Canada, USA, French Guiana,
86 and the French West Indies (Guadeloupe and Martinique) have reported several imported
87 CHIKV cases since its re-emergence in 2004 (6,13).

88 Intriguingly, until December 2013, autochthonous CHIKV transmission has never
89 been reported in the Americas, a continent where all the conditions are apparently suitable for
90 its establishment: (i) it is a virgin continent for CHIKV, (ii) the main mosquito vectors of
91 CHIKV, *Ae. aegypti* and *Ae. albopictus*, are present with high densities in most areas, (iii)
92 imported cases are annually reported in periods of high mosquito density and activity, and (iv)
93 temperature and environmental conditions of large tropical and subtropical zones are
94 favorable to mosquito development and activity as well as to viral replication in the vector
95 (11,14). In the early December 2013, two laboratory-confirmed autochthonous CHIKV cases
96 were reported in the French territory of Saint-Martin Island in the Caribbean (6). Very rapidly,
97 an epidemic was established on the island with almost 2030 clinical cases and more than 765
98 confirmed cases today, and subsequently, some CHIKV cases were detected in Martinique,
99 Guadeloupe, Saint-Barthelemy, and also French Guyana (15). Therefore, CHIKV is
100 progressively spreading putting at a high epidemic risk the vastly-infested *Ae. aegypti* and *Ae.*
101 *albopictus* areas of the Americas.

102 To achieve efficient transmission, numerous factors regarding the invertebrate and the
103 vertebrate hosts, the virus, and the environmental conditions must ideally converge (16).
104 Concerning the mosquito host, vector competence is considered to be unique and
105 characteristic for each virus-vector pair. Indeed differences of vector competence can be
106 found between different populations belonging to a single insect vector species (17). Vector
107 competence is a quantitative phenotypic parameter controlled by genetic characteristics of
108 both vector and virus, which in turn is influenced by environmental conditions (18-20).
109 Mosquito vector competence to CHIKV and DENV seems to be determined by genotype-by-
110 genotype interactions, in which successful transmission depends on some specific
111 combination of mosquito and viral genetic characteristics (21-26). CHIKV has four major
112 lineages: East-Central-South Africa (ECSA), West Africa, Asian, and the Indian Ocean, a

113 monophyletic lineage descendant from the ECSA group (27). The CHIKV lineages have
114 displayed distinct transmission efficiencies in mosquito vector species and populations (25,28,
115 29). Throughout the 2005-2006 CHIKV epidemic in the Indian Ocean region, a CHIKV
116 lineage strain harboring a substitution of an alanine to valine at position 226 of the E1
117 envelope glycoprotein (E1-A226V) was better transmitted by *Ae. albopictus* (22,25,30). It
118 was later shown that other positions in the E2 glycoprotein exert epistatic effects on the
119 position E1-226V (23,24) and some substitutions can block the adaptation of the E1-226V to
120 *Ae. albopictus*. These epistatic interactions are lineage specific.

121 Determining vector competence of mosquito populations is a key parameter in
122 evaluating the risk of CHIKV transmission and spread. Given the alarming epidemiological
123 situation due to the very recent chikungunya outbreak affecting the Caribbean islands, the
124 need for evaluating the vector competence of American mosquito populations is compelling.
125 Until now, studies were only limited to mosquitoes from the USA and the French Caribbean
126 (31-34). With the aim of understanding the factors that may influence CHIKV emergence in
127 the Americas and the risk of CHIKV epidemic spreading throughout the continent, we carried
128 out a comprehensive Pan-American evaluation of vector competence of 35 *Ae. aegypti* and *Ae.*
129 *albopictus* populations from 10 countries towards three CHIKV isolates belonging to two
130 distinct lineages.

131

132

133 **MATERIALS AND METHODS**

134 **Ethics Statement.** The Institut Pasteur animal facility has received accreditation from the
135 French Ministry of Agriculture to perform experiments on live animals [see permit numbers
136 at http://webcampus.pasteur.fr/jcms/c_97619/agrements-des-animaleries] in appliance of the
137 French and European regulations on care and protection of the Laboratory Animals. This

138 study was approved by the Institutional Animal Care and Use Committee (IACUC) at the
139 Institut Pasteur. No specific permits were required for the described field studies in locations
140 which are not protected in any way and did not involve endangered or protected species.

141

142 **Mosquitoes.** Thirty five mosquito populations collected in 10 countries from North, Central
143 and South Americas were used: 22 populations of *Ae. aegypti* and 13 of *Ae. albopictus*
144 (Figure 1, Table 1). The mosquitoes were field-collected in 2012 with ovitraps (10-58 per
145 collection site). The mosquito collection sites were strategically chosen in order to essentially
146 represent the diverse climates, environments, ecotopes and dengue epidemiological history
147 across the American continent. The field collected eggs were immersed in water for hatching;
148 larvae were split by 100-150 individuals per pan and fed with yeast tablets. Emerging adults
149 were maintained in cages at $28^{\circ}\pm 1^{\circ}\text{C}$ with a 14h:10h light:dark cycle, 80% relative humidity,
150 and supplied with a 10% sucrose solution. The F1 generation was used for all infection assays.

151

152 **Viral Strains.** Three CHIKV isolates belonging to two distinct lineages were used: two
153 CHIKV isolates from La Réunion and one from New Caledonia. The isolates from La
154 Réunion were the strains (i) CHIKV 05.115 (CHIKV_115) and (ii) CHIKV 06.21
155 (CHIKV_0621), both isolated in 2005 (35) and provided by the French National Reference
156 Center for Arboviruses at the Institut Pasteur in Paris. The amino-acid consensus sequence of
157 these strains differed only by a single substitution: CHIKV_115 has an alanine at position 226
158 of the E1 envelope glycoprotein (E1-226A), whereas CHIKV_0621 harbors a valine at the
159 same position (E1-226V). It has been shown the E1-A226V substitution is located in a region
160 known to be involved in viral entry via fusion with endosomal membranes (36). Both strains
161 have an alanine at position 98 of the E1 glycoprotein (E1-98A) that has been shown to exert
162 no negative epistatic effects on the position E1-226; the position E1-98 is located at the base

163 of the fusion loop and presumably modulates the kinetics of the pH-dependent conformational
164 changes and fusion reaction in the endosomal compartment (37). Viral titer estimated by
165 serial 10-fold dilutions on Vero cells was 10^9 plaque forming units (pfu)/mL for both
166 CHIKV_115 and CHIKV_0621. Both strains were isolated on *Ae. albopictus* C6/36 cells
167 from human serum or viral stocks and were produced following three passages on *Ae.*
168 *albopictus* C6/36 cells then harvested and stored at -80°C until use for the mosquito
169 experimental infection assays. The New Caledonia CHIKV strain referenced as NC/2011-568
170 (CHIKV_NC), was isolated in 2011 (28, 37) and provided by the Institut Pasteur of New
171 Caledonia. Phylogenetic analysis using the complete CHIKV_NC genome nucleotide
172 sequence demonstrated that CHIKV_NC belongs to the Asian lineage, displaying 98.1%
173 nucleotide identity with other isolates of the Asian cluster of CHIKV phylogeny. CHIKV_NC
174 strain has an alanine at position E1-226 (E1-226A) and a threonine at position E1-98 (E1-
175 98T). It has been shown that in contrast with the ECSA genotype, the substitution E1-98T
176 exerts a negative epistatic interaction leading to block the ability of Asian CHIKV strains to
177 adapt to *Ae. albopictus* via the E1-A226V substitution (24). The whole genome sequence of
178 CHIKV_NC is available on GenBank under accession no. HE806461. CHIKV_NC 2nd
179 passage was used for the experimental infections of mosquitoes. The titer of CHIKV_NC
180 stocks was $10^{8.1}$ pfu/mL.

181

182 **Mosquito Oral Infections.** Five to seven day-old females were fed on an infectious blood-
183 meal containing 2 mL of washed rabbit erythrocytes, 1 mL of viral suspension supplemented
184 with a phagostimulant (ATP) at a final concentration of 5 mM. The titer of all performed
185 infectious blood-meals was $10^{7.5}$ pfu/mL. Mosquito feeding was limited to 50 min. After the
186 infectious blood-meal, non-engorged females were discarded. Fully engorged females were
187 transferred in cardboard containers and maintained with 10% sucrose at $28^{\circ}\pm 1^{\circ}\text{C}$. All 35

188 mosquito populations were challenged with the CHIKV_0621 strain (13 *Ae. albopictus* and
189 22 *Ae. aegypti* populations) whereas 22 populations (9 *Ae. albopictus* and 13 *Ae. aegypti*)
190 were challenged with the CHIKV_115 strain and 6 populations (3 *Ae. albopictus* and 3 *Ae.*
191 *aegypti*) with CHIKV_NC. Mosquito populations from the same location were
192 simultaneously tested with the CHIKV_0621 and CHIKV_115 strains.

193

194 **Dissemination and Transmission Analysis.** Batches of ~30 mosquitoes of each combination
195 of mosquito population-virus strain were analyzed at days 7 and 10 post-infection (pi) for all
196 the CHIKV strains tested. Days pi were defined according to the kinetics of CHIKV
197 dissemination and transmission efficiencies in *Ae. albopictus* from Paquetá, Rio de Janeiro,
198 Brazil (maximum at day 7 pi and slight decrease by day 10; see Figure 2). To estimate viral
199 dissemination, heads were removed from mosquitoes and ground in 250 μ L of Leibovitz L15
200 medium (Invitrogen) supplemented with 2% Fetal Bovine Serum (FBS) for further
201 inoculation onto cell C6/36 *Ae. albopictus* cell culture in 96-well plates. After incubation at
202 28°C for 3 days, plates were stained using hyper-immune ascetic fluid specific to CHIKV as
203 primary antibody. Alexa Fluor® 488 goat anti-mouse IgG was used as the second antibody
204 (Life technologies™).

205 To estimate viral transmission, saliva was collected from individual mosquitoes as described
206 in (38). For that, wings and legs were removed from each mosquito and the proboscis was
207 inserted into a 20 μ L tip containing 5 μ L of FBS. After 45 min of salivation, FBS containing
208 saliva was expelled into 45 μ L of Leibovitz L15 medium for titration. One limitation of this
209 technique is that the volume of saliva delivered by females could not be estimated.

210 Dissemination efficiency corresponds to the proportion of mosquitoes with virus detected in
211 heads among tested ones (i.e., engorged mosquitoes which have survived until the day of
212 examination). Transmission efficiency corresponds to the proportion of mosquitoes with virus

213 in the saliva among tested ones (i.e., surviving females including females unable to
214 disseminate the virus and those able to disseminate). The number of infectious particles per
215 saliva was estimated by titration using focus fluorescent assay on C6/36 *Ae. albopictus* cells.
216 Samples were serially diluted and inoculated onto C6/36 cells in 96-well plates, following
217 incubation at 28°C for 3 days. Then, plates were stained as explained above.

218

219 **Statistical Analysis.** Statistical analyses were performed with STATISTICA 8 software
220 (Statsoft Inc, USA). The numbers of infectious particles in saliva were compared using the
221 Kruskal-Wallis test. Dissemination and transmission efficiencies were compared using Chi-
222 square test. Kruskal-Wallis Z multiple comparison test was used to compare more than 5
223 dissemination and transmission efficiency rates.

224

225

226 **RESULTS**

227 **Dissemination efficiency.** To measure the ability of American *Ae. aegypti* and *Ae. albopictus*
228 to allow CHIKV to overcome the midgut barrier, dissemination efficiency (DE) was assessed
229 for each pairing mosquito population-virus strain at days 7 and 10 pi (Tables 2 and 3).

230 All *Ae. aegypti* and *Ae. albopictus* populations showed similar DE values at days 7 and 10 pi
231 for the three CHIKV isolates (Chi-square test: $p>0.05$). For CHIKV_0621, DE at day 7 pi
232 ranged from 60% to 100% for *Ae. albopictus* and from 93.3% to 100% for *Ae. aegypti*. For
233 CHIKV_115, DE at day 7 varied from 66.7% to 96.9% for *Ae. albopictus* and from 96.6% to
234 100% for *Ae. aegypti*, while for CHIKV_NC, DE ranged from 90% to 96.7% for *Ae.*
235 *albopictus* and from 96.9% to 100% for *Ae. aegypti*. *Ae. aegypti* tested populations displayed
236 similar DE values around 100% for the three CHIKV isolates (Chi-square test: $p>0.05$).
237 Likewise, DE obtained for *Ae. albopictus* were extensively high although rates were

238 significantly heterogeneous for CHIKV_0621 (Chi-square test: $p < 0.05$) and CHIKV_115
239 (Chi-square test: $p < 0.05$). Thus, when comparing DE values for a given virus between the two
240 mosquito species sampled in a same location, no significant difference was found except for
241 MXC in Mexico when infected with CHIKV_0621 (Chi-square test: $p < 0.05$) and
242 CHIKV_115 (Chi-square test: $p < 0.05$), and for VRB in United States when infected with
243 CHIKV_115 (Chi-square test: $p < 0.05$). In these three last cases, *Ae. aegypti* exhibited a
244 higher DE than *Ae. albopictus* collected in the same site whatever the viral strain. In addition,
245 no difference was observed in DE values between the three *Ae. aegypti* and *Ae. albopictus*
246 populations challenged with the CHIKV_NC isolate (Chi-square test: $p > 0.05$).

247

248 **Transmission efficiency.** In order to determine the ability of American *Ae. aegypti* and *Ae.*
249 *albopictus* to sustain CHIKV transmission, we assessed the transmission efficiency (TE) at
250 days 7 and 10 pi. only TE values at day 7 pi were presented in Figures 3 and 4 (see Table S1
251 for TE values at day 10 pi). The TE values obtained for *Ae. aegypti* and *Ae. albopictus* were
252 highly heterogeneous and lower than DE values.

253 When mosquitoes were exposed to CHIKV_0621, TE values ranged from 13.3% to 96.7 % at
254 day 7 pi and 6.7% - 85.2 % at day 10 pi. *Ae. albopictus* better transmitted CHIKV_0621 than
255 *Ae. aegypti* at day 7 pi (mean \pm CI: 44.7 ± 7.8 for *Ae. aegypti* and 55.8 ± 12.3 for *Ae.*
256 *albopictus*) and at day 10 pi (mean \pm CI: 33.1 ± 6.2 for *Ae. aegypti* and 55.5 ± 12.0 for *Ae.*
257 *albopictus*). Within a same mosquito species, TE values were significantly different (Chi-
258 square test: $p < 0.05$) at days 7 and 10 pi. When considering each of the 10 populations where
259 the two species co-exist (VRB, MXC, PAN, MAN, PNM, JRB, PAQ, VAZ, BEL, SAN), *Ae.*
260 *albopictus* exhibited a higher TE than *Ae. aegypti* when infected with CHIKV_0621 except
261 for the VRB population from Florida, United States (Figures 3 and 4, Table S1).

262 When mosquitoes were infected with CHIKV_115, TE values were comprised between
263 11.1% and 82.1% at day 7 pi and 10% - 76.7% at day 10 pi. *Ae. aegypti* better transmitted
264 CHIKV_115 than *Ae. albopictus* at day 7 pi (mean \pm CI: 49.5 ± 10.3 for *Ae. aegypti* and 49.5
265 ± 13.6 for *Ae. albopictus*). Within a same mosquito species, TE values were significantly
266 different (Chi-square test: $p < 0.05$) at days 7 and 10 pi. When considering each of the four
267 populations where the two species co-exist (VRB, MXC, PAN, and PAQ), one species did not
268 present a clear-cut advantage on the other to transmit CHIKV_115 (Figures 3 and 4, Table
269 S1).

270 Interestingly, among the eight *Ae. albopictus* populations simultaneously challenged with
271 CHIKV_0621 and CHIKV_115, four showed unexpected lower TE for CHIKV_115 and one
272 displayed equal rates (Figure 3, Table S1). Remarkably, TE rates were heterogeneous even
273 between *Ae. albopictus* populations geographically close, i.e. from Rio de Janeiro, Brazil
274 (JRB, PAQ, BEL, VAZ) when exposed to the same CHIKV_0621 isolate (Figures 3 and 4).

275 Lastly, when mosquitoes were exposed to the CHIKV_NC, TE values varied from 30% to
276 83.3% at day 7 pi, and 26.7%-53.3% at day 10 pi. *Ae. aegypti* better transmitted CHIKV_NC
277 than *Ae. albopictus* at day 7 pi (mean \pm CI: 64.5 ± 20.7 for *Ae. aegypti* and 48.9 ± 25.1 for *Ae.*
278 *albopictus*). Within a same mosquito species, TE values were significantly different (Chi-
279 square test: $p < 0.05$) at day 7 and not at day 10 pi (Chi-square test: $p > 0.05$) (see Table S1).

280 We also found that 23% - 56% mosquitoes collected in temperate regions, *Ae. albopictus* TYS
281 (Tyson, United States), *Ae. aegypti* SAL (Salto, Uruguay) and BUE (Buenos Aires,
282 Argentina) were able to efficiently transmit CHIKV_0621. Moreover, *Ae. aegypti* from the
283 last two sites of the Southern Cone were also competent to efficiently transmit CHIKV_0115
284 and CHIKV_NC at day 7 pi, respectively (SAL = 70% for CHIKV_115; BUE = 48.3% for
285 CHIKV_115 and 63.6% for CHIKV_NC).

286

287 **Intensity of transmission.** The intensity of viral transmission can be calculated by estimating
288 the viral load in saliva collected from mosquitoes. When infected with CHIKV_0621 isolate,
289 the number of viral particles in saliva ranged from 0.4 to 4.4 log₁₀ particles for *Ae. albopictus*
290 and from 0.4 to 5.1 log₁₀ for *Ae. aegypti*. Concerning mosquitoes infected with CHIKV_115
291 isolate, the number of viral infectious particles varied from 0.4 to 4.7 log₁₀ for *Ae. albopictus*
292 and from 0.4 to 5.0 log₁₀ for *Ae. aegypti*. For mosquitoes exposed to CHIKV_NC, the viral
293 load in saliva ranged from 0.4 to 2.9 log₁₀ for *Ae. albopictus* and from 0.4 to 4.2 log₁₀
294 particles for *Ae. aegypti* (Figure 5). Viral loads of the three tested CHIKV strains were
295 equivalent in *Ae. aegypti* populations, whereas *Ae. albopictus* displayed a slightly lower titer
296 when challenged with CHIKV_NC in comparison to CHIKV_0621 and CHIKV_115, both at
297 day 7 pi. Viral loads were highly heterogeneous between individuals belonging to the same
298 population and infected with a given viral strain, but the mean calculated for each mosquito
299 population was roughly similar overall. Indeed, when comparing viral load in saliva between
300 mosquito strains for a given virus at day 7 and 10 pi (Figures 5 and S1), no significant
301 differences were found either for *Ae. aegypti* or *Ae. albopictus* (Kruskal-Wallis test: p>0.05),
302 except for *Ae. albopictus* challenged with CHIKV_115.

303

304

305 **DISCUSSION**

306 All 35 populations of *Ae. aegypti* and *Ae. albopictus* collected throughout the Americas were
307 susceptible to CHIKV infection by all three tested genotypes. Thus, temperate as well as
308 tropical and subtropical Northern, Central and Southern American *Aedes* mosquitoes are
309 efficient CHIKV vectors. *Ae. albopictus* better transmitted the epidemic CHIKV_0621 strain
310 isolated on La Réunion Island in 2006 (35) than *Ae. aegypti*, whereas this latter species was
311 more capable to transmit the original strain CHIKV_115, both belonging to the ECSA

312 genotype (39). The Asian genotype represented by the CHIKV_NC strain (28) was better
313 transmitted by *Ae. aegypti*, although it was also efficiently transmitted by *Ae. albopictus*.

314

315 **Most American *Aedes* mosquitoes are highly susceptible to CHIKV**

316 More than 60% of mosquitoes per population were able to disseminate CHIKV after crossing
317 the midgut barrier (i.e., entry in epithelial cells, viral replication and release of virions from
318 the midgut basal lamina). Thus after being ingested with a blood-meal provided at a titer of
319 $10^{7.5}$ pfu/mL, CHIKV succeeded in disseminating within the mosquito hemocele which is an
320 essential prerequisite for transmission. It has been shown that a titer of $\sim 10^4$ pfu/mL in
321 monkeys was sufficient enough to infect mosquitoes (40). CHIKV transmission was highly
322 heterogeneous in American mosquitoes, ranging from 11.1% to 96.7% at day 7 pi when
323 considering all CHIKV strains. It should be underlined that we are not able to provide a
324 control of salivation and we hypothesize that a CHIKV-negative saliva did not correspond to
325 mosquitoes unable to salivate but to mosquitoes delivering a non-infected saliva. As expected
326 from previous studies (22,25,41,30), *Ae. albopictus* better transmitted the epidemic strain
327 CHIKV_0621 of the ECSA genotype than *Ae. aegypti*, even in cases where both mosquito
328 species cohabit. *Ae. aegypti* transmitted preferentially CHIKV_115 and also, the Asian
329 genotype CHIKV_NC in accordance with previous findings (28). CHIKV Asian strains have
330 a particular E1-98T substitution which constrains CHIKV adaptation to *Ae. albopictus* via E1-
331 A226V mutation (24). *Ae. aegypti* are more abundant in the Americas than *Ae. albopictus*
332 mosquitoes and the E1-98T substitution of CHIKV viral strains does not have a negative
333 effect on CHIKV interaction with *Ae. aegypti*. Thus, CHIKV Asian strains together with the
334 CHIKV ECSA strains, represent a real danger to the Americas. Intriguingly, the CHIKV
335 strain isolated during the last outbreak in the Caribbean also belongs to the Asian genotype
336 (42) primarily transmitted in the past by *Ae. aegypti*. Although the intensity of transmission is

337 highly variable between mosquitoes, the mean number of viral particles delivered by
338 mosquitoes was quite similar for each combination mosquito strain and viral strain.
339 Mosquitoes collected in tropical Latin America, Panama, Venezuela, Brazil, Bolivia,
340 Paraguay, Argentina and Uruguay showed the highest transmission efficiency with up to
341 10,000 viral particles detected in mosquito saliva. Interestingly, mosquitoes from the main
342 Brazilian city of Rio de Janeiro showed high transmission efficiencies. For example, 96.7% of
343 *Ae. albopictus* JRB were able to transmit CHIKV_0621 (see Table S1). Moreover, the
344 extrinsic incubation period of CHIKV, i.e. the time necessary for the virus to be detected in
345 saliva ready for transmission after being ingested with the blood-meal (43), in both mosquito
346 species is quite short (38). Indeed, an *Ae. albopictus* population from Rio de Janeiro (PAQ)
347 was able to transmit infectious viral particles as rapidly as 2 days pi (Figure 2). Therefore, the
348 risk of CHIKV establishment in densely populated cities such as Rio de Janeiro hosting more
349 than 6 million people and infested by anthropophilic *Aedes* mosquitoes should be considered
350 very high.

351

352 **Mosquitoes from temperate Americas are potentially capable of sustaining CHIKV**
353 **transmission**

354 The ability of CHIKV to extend its natural range of distribution to include temperate regions
355 was exemplified by the Italian outbreak in 2007 and the French local/autochthonous cases in
356 2010 (44,45). In the Americas, more than one hundred imported CHIKV cases were detected
357 in the United States between 1995 and 2009 (11). Some of them developed a viremia high
358 enough to infect mosquitoes. We found that 56.7% of *Ae. albopictus* TYS from Tyson
359 (United States) and 83.3% of *Ae. aegypti* SAL from Salto (Uruguay) were able to transmit
360 CHIKV_0621 at day 7 pi (see Table S1). Transmission efficiencies were lower for *Ae. aegypti*
361 BUE from Buenos Aires (Argentina) (i.e.,23.3%, see Figure 3, Table S1) but higher when

362 infected with the CHIKV_NC Asian genotype (i.e., 63.6%, see Figure 3, Table S1).
363 Therefore, the establishment of CHIKV in temperate American countries is not simply a
364 fiction even if less than 30% of both mosquito species collected in the South of United States
365 (VRB, Florida) were able to transmit CHIKV_0621. It has been found that *Ae. albopictus*
366 from Florida are more competent vectors of CHIKV than *Ae. aegypti* (31-33). Outbreaks of
367 DENV, also transmitted by *Aedes* mosquitoes, have occurred in Texas and Florida in the past
368 years (46), reinforcing the risk of epidemics due to imported arboviruses in the United States.
369 Local transmission of CHIKV could be maintained if the virus is introduced in the right place
370 at the right time. Taken together, these findings underline the high variation of susceptibility
371 to CHIKV of American mosquitoes, calling for including other factors (biological and
372 environmental) in assessing transmission potential risk (47). Moreover, mosquito genetic
373 structure should be promptly investigated. Phylogenetic analysis of both mosquito species
374 should bring additional information on colonization history of *Ae. aegypti* and *Ae. albopictus*
375 in the different countries of Americas (48,49). *Ae. aegypti* was most likely introduced in
376 North America during the slave trade (50) while *Ae. albopictus* was established in 1985 in the
377 United States (51) probably introduced in shipments of used tires from Japan (52), and in
378 Brazil in 1986 (53) probably arriving from tropical Asia (52).

379

380 **The fear becomes a reality**

381 Still absent until very recently, CHIKV has been detected for the first time in the Americas in
382 late December 2013. Currently, among the 2030 suspected CHIKV cases from the island of
383 Saint-Martin in the Caribbean, more than 765 were confirmed positive to CHIKV by serology
384 (15). The virus then spread to neighboring islands: Saint-Barthelemy with 380 cases,
385 Martinique with 3940 cases, Guadeloupe with 1460 cases. Until now, 10 autochthonous cases
386 have been reported in French Guiana which maintains a daily air link with the two other

387 French Overseas territories of Guadeloupe and Martinique. We previously showed that *Ae.*
388 *aegypti* from French Guiana and French West Indies were highly competent to disseminate
389 CHIKV and mosquito populations collected in dense housing environments exhibited the
390 highest susceptibility (34). Thus, the risk of CHIKV spread and establishment is real and
391 should concern all areas in the Americas where the vector mosquitoes are present.

392

393 **Co-circulation of CHIKV and DENV could have great implication on human health**

394 Interestingly, DENV is still circulating in the Caribbean together with CHIKV. Cases of co-
395 infection DENV-CHIKV in patients have been first reported in 1967 (54) and since the
396 emergence of CHIKV, reports of co-infections are increasing (10,55-63). Both viruses are
397 transmitted by the same mosquito vectors, *Ae. aegypti* and *Ae. albopictus*. Co-infection of a
398 mosquito vector by two viruses can occur after two successive infectious blood-meals taken
399 on two different viremic hosts or after a single blood-meal taken on a co-infected host. It has
400 been shown that CHIKV and DENV can be delivered together in one mosquito bite (64). As
401 co-infections were a quite common phenomenon, consequences on the clinical presentation of
402 the disease are expected.

403 Finally, the assessment of vector competence should be considered as a prerequisite to better
404 evaluate the potential risk of CHIKV outbreaks once the virus is introduced from endemic
405 regions. The numerous imported CHIKV viremic cases presaged the potential importance of
406 this emerging arbovirus for the Americas where both mosquito species are well established. In
407 light of epidemics now starting in the Caribbean, it remains imperative to pursue and
408 reinforce epidemiological and entomological surveillance actions and control against the
409 mosquitoes, *Ae. aegypti* and *Ae. albopictus*.

410

411

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432

433 **Competing interests**

434 The authors declare that they have neither competing interests nor conflict of interest related
 435 to this article.

436 **Authors' contribution**

437 RLO and ABF conceived the study. RLO, AVR and KZ carried out experimental infections of
438 mosquitos and performed titration assays. AVR, RLO and ABF drafted the manuscript. KZ
439 and RG helped to draft and to revise the manuscript. All authors read and approved the final
440 version of the manuscript.

441

442

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- 640

641 **Figure Legends**

642

643 **FIG 1** Mosquito populations tested. Color-code indicates localities where only *Ae. aegypti*
644 (red), only *Ae. albopictus* (blue) and both *Ae. aegypti* and *Ae. albopictus* were collected
645 (green). **TYS** Tyson (United States), **VRB** Vero Beach (United States), **MXC** Chiapas
646 (Mexico), **PAN** Panamá (Panama), **DEL** Delta Amacuro (Venezuela), **TUM** Tumbes (Peru),
647 **PUM** Punchana (Peru), **MAN** Manaus (Brazil), **STR** Santarém (Brazil), **PNM** Parnamirim
648 (Brazil), **CAB** Campos Belos (Brazil), **CPG** Campo Grande (Brazil), **JRB** Jurujuba (Brazil),
649 **PAQ** Paquetá (Brazil), **VAZ** Vaz Lobo (Brazil), **BEL** Belford Roxo (Brazil), **SAN** Santos
650 (Brazil), **BMA** Monteagudo (Bolivia), **SDG** Salto del Guairá (Paraguay), **ASU** Asuncion
651 (Paraguay), **SAL** Salto (Uruguay), **MIA** Misiones (Argentina), **ACO** Corrientes (Argentina),
652 **BUE** Buenos Aires (Argentina).

653

654 **FIG 2** Dissemination (A) and transmission efficiencies (B) of two CHIKV isolates and two
655 clones of the respective viral isolates in *Ae. albopictus* from Paquetá, Rio de Janeiro (Brazil).
656 At days 1, 2, 3, 7 and 10 after an infectious blood meal, mosquitoes were sacrificed and heads
657 and saliva were collected for determination of their infectious status. Mosquito heads were
658 individually ground in 250 μ L Leibovitz L15 medium supplemented with 4%, following
659 inoculation onto C6/36 *Ae. albopictus* cell monolayer in 96-well plates and incubation at 28°C
660 for 3 days. Plates were fixed with 3.6% formaldehyde, washed three times with PBS and
661 analyzed by indirect immunofluorescence assay (IFA). For saliva collection, each mosquito
662 had wings and legs removed and the proboscis inserted into a 20 μ L tip containing 5 μ L of
663 FBS. After 45 min of salivation, FBS containing saliva was expelled into 45 μ L of Leibovitz
664 L15 medium and inoculated onto C6/36 *Ae. albopictus* cell monolayer in 96-well plates.
665 Plates were incubated and stained (IFA) as described in Materials and Methods.

666 Dissemination efficiency corresponds to the proportion of mosquito females with
 667 disseminated virus in head among the tested ones. Transmission efficiency corresponds to the
 668 proportion of mosquitoes with infectious saliva among the tested ones. CHIKV_0621: strain
 669 isolated from La Réunion (E1-226V substitution); CHIKV_115: strain isolated from La
 670 Réunion (E1-226A); CHIKV_0621 (V): clone corresponding to a single virus isolated from
 671 CHIKV_0621; CHIKV_115 (A): clone corresponding to a single virus isolated from
 672 CHIKV_115. Clones were provided by Arias-Goeta C.

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674 **FIG 3** Transmission efficiency of three CHIKV isolates in 35 *Ae. albopictus* and *Ae. aegypti*
 675 populations from 10 American countries at day 7 post-infection. After an infectious blood-
 676 meal, mosquitoes were sacrificed and saliva was collected from individual mosquitoes and
 677 titrated by focus fluorescent assay on C6/36 *Ae. albopictus* cells to determine infectious status.
 678 Transmission efficiency corresponds to the proportion of mosquitoes with infectious saliva
 679 among tested ones. Viral strains: CHIKV_0621 isolated from La Réunion (ECSA genotype,
 680 E1-226V and E1-98A substitution), CHIKV_115 isolated from La Réunion (ECSA genotype,
 681 E1-226A and E1-98A substitution) and CHIKV_NC isolated from New Caledonia (Asian
 682 genotype, E1-226A and E1-98T substitution). Mosquito populations (from North to South):
 683 **TYS** Tyson (United States), **VRB** Vero Beach (United States), **MXC** Chiapas (Mexico), **PAN**
 684 Panamá (Panama), **DEL** Delta Amacuro (Venezuela), **TUM** Tumbes (Peru), **PUM** Punchana
 685 (Peru), **MAN** Manaus (Brazil), **STR** Santarém (Brazil), **PNM** Parnamirim (Brazil), **CAB**
 686 Campos Belos (Brazil), **CPG** Campo Grande (Brazil), **JRB** Jurujuba (Brazil), **PAQ** Paquetá
 687 (Brazil), **VAZ** Vaz Lobo (Brazil), **BEL** Belford Roxo (Brazil), **SAN** Santos (Brazil), **BMA**
 688 Monteagudo (Bolivia), **SDG** Salto del Guairá (Paraguay), **ASU** Asuncion (Paraguay), **SAL**
 689 Salto (Uruguay), **MIA** Misiones (Argentina), **ACO** Corrientes (Argentina), **BUE** Buenos
 690 Aires (Argentina). Error bars show the confidence intervals (95%).

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692 **FIG 4** Transmission efficiency of CHIKV_0621 and CHIKV_115 isolates in 35 *Ae. aegypti*
693 and *Ae. albopictus* populations from 10 American countries at day 7 post-infection.
694 Transmission efficiency corresponds to the proportion of mosquitoes with infectious saliva
695 among tested ones. Color-code indicates different degrees of transmission efficiency (TE):
696 yellow, mosquito strains with $TE \leq 30\%$ (low TE); pale-orange, strains with $30\% < TE < 70\%$
697 (moderated TE); red, strains with $TE \geq 70\%$ (high TE). Viral strains: CHIKV_0621 isolated
698 from La Réunion (ECSA genotype, E1-226V substitution) and CHIKV_115 isolated from La
699 Réunion (ECSA genotype, E1-226A substitution. Mosquito populations (from North to
700 South): **TYS** Tyson (United States), **VRB** Vero Beach (United States), **MXC** Chiapas
701 (Mexico), **PAN** Panamá (Panama), **DEL** Delta Amacuro (Venezuela), **TUM** Tumbes (Peru),
702 **PUM** Punchana (Peru), **MAN** Manaus (Brazil), **STR** Santarém (Brazil), **PNM** Parnamirim
703 (Brazil), **CAB** Campos Belos (Brazil), **CPG** Campo Grande (Brazil), **JRB** Jurujuba (Brazil),
704 **PAQ** Paquetá (Brazil), **VAZ** Vaz Lobo (Brazil), **BEL** Belford Roxo (Brazil), **SAN** Santos
705 (Brazil), **BMA** Monteagudo (Bolivia), **SDG** Salto del Guairá (Paraguay), **ASU** Asuncion
706 (Paraguay), **SAL** Salto (Uruguay), **MIA** Misiones (Argentina), **ACO** Corrientes (Argentina),
707 **BUE** Buenos Aires (Argentina).

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709 **FIG 5** Viral loads of three CHIKV isolates in saliva of *Ae. albopictus* and *Ae. aegypti*
710 mosquitoes from 35 populations from the Americas at day 7 post-infection. At day 7 after an
711 infectious blood-meal, mosquitoes were sacrificed and saliva was collected from individual
712 mosquitoes and titrated by focus fluorescent assay on C6/36 *Ae. albopictus* cells. Viral
713 strains: CHIKV_0621 isolated from La Réunion (ECSA genotype, E1-226V and E1-98A
714 substitution), CHIKV_115 isolated from La Réunion (ECSA genotype, E1-226A and E1-98A
715 substitution) and CHIKV_NC isolated from New Caledonia (Asian genotype, E1-226A and

716 E1-98T substitution). Mosquito populations (from North to South): **TYS** Tyson (United
717 States), **VRB** Vero Beach (United States), **MXC** Chiapas (Mexico), **PAN** Panamá (Panama),
718 **DEL** Delta Amacuro (Venezuela), **TUM** Tumbes (Peru), **PUM** Punchana (Peru), **MAN**
719 Manaus (Brazil), **STR** Santarém (Brazil), **PNM** Parnamirim (Brazil), **CAB** Campos Belos
720 (Brazil), **CPG** Campo Grande (Brazil), **JRB** Jurujuba (Brazil), **PAQ** Paquetá (Brazil), **VAZ**
721 Vaz Lobo (Brazil), **BEL** Belford Roxo (Brazil), **SAN** Santos (Brazil), **BMA** Monteagudo
722 (Bolivia), **SDG** Salto del Guairá (Paraguay), **ASU** Asuncion (Paraguay), **SAL** Salto
723 (Uruguay), **MIA** Misiones (Argentina), **ACO** Corrientes (Argentina), **BUE** Buenos Aires
724 (Argentina). Error bars refer to the standard error of mean titer for each pairing mosquito
725 population-virus strain.
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TABLE 1 Mosquito populations used. Populations are listed according to their country of collection, from North to South.

Mosquito population	Collection site	Country	Coordinates	Mosqui to species used	Climate	Dominant vegetation	Environm ent	History of Dengue incidenc e
TYS	Tyson Missouri	United States	38°31'N 90°33'W	AL	Temperate	Temperate grassland	Suburban	F
VRB	Vero Beach Florida	United States	27°35'N 80°22'W	AE/AL	Humid subtropical	Subtropical evergreen forest	Suburban	F
MXC	Tapachula	Mexico	14°53'N 92°15'W	AE/AL	Tropical wet and dry	Tropical deciduous forest	Suburban	M
PAN	Panamá/Colo n	Panama	08°59'N 79°30'W/ 09°21' N 79°53'W	AE/AL	Tropical wet and dry	Savana	Urban/ Suburban	L
DEL	Delta Amacuro Tucupita	Venezuela	09°03'N 62°02'W	AE	Tropical wet and dry	Savana	Suburban	L
PUM	Punchana Iquitos	Peru	03°43'S 73°15'W	AE	Tropical wet and dry	Amazon forest	Urban	H
TUM	Tumbes Huaquillas	Peru	03°29'S 80°15'W	AE	Arid	Desert	Suburban	L
MAN	Manaus	Brazil	03°06'S 60°03'W	AE/AL	Tropical wet	Amazon forest	Suburban	H
STR	Santarém	Brazil	02°25'S 54°42'W	AE/AL	Tropical wet	Amazon forest	Suburban	M
PNM	Parnamirim	Brazil	05°54'S 35°16'W	AE/AL	Semiarid	Transitional Tropical rainforest	Suburban	H
CAB	Campos Belos	Brazil	13°02'S 46°46'W	AE	Tropical wet and dry	Savana	Urban	L
BEL	Belford Roxo Rio de Janeiro	Brazil	22°45'S 43°24'W	AE/AL	Tropical wet and dry	Atlantic rain forest	Suburban	H
VAZ	Vaz Lobo Rio de Janeiro	Brazil	22°51'S 43°19'W	AE/AL	Tropical wet and dry	Atlantic rain forest	Urban	H
JRB	Jurujuba Rio de Janeiro	Brazil	22°55'S 43°07'W	AE/AL	Tropical wet and dry	Atlantic rain forest	Suburban	L
PAQ	Paquetá Rio de Janeiro	Brazil	22° 45'S 43°06'W	AE/AL	Tropical wet and dry	Atlantic rain forest	Suburban island	M
SAN	Santos	Brazil	23°57'S 46°20'W	AE/AL	Tropical wet and dry	Atlantic rain forest	Suburban	M
CPG	Campo Grande	Brazil	20°27'S 54°37'W	AE	Tropical wet and dry	Savana	Urban	H
BMA	Monteagudo	Bolivia	19°48'S 63°57'W	AE	Tropical wet and dry	Mountain Forest	Urban	L
ASU	Asunción	Paraguay	25°18'S 57°37'W	AE	Tropical wet and dry	Chaco	Urban	M
SDG	Salto del Guairá	Paraguay	24°03'S 54°18'W	AE	Humid subtropical	Savana	Suburban	L
MIA	Misiones	Argentina	25°36'S 54°34'W	AL	Humid subtropical	Paranaense forest	Rural	L
ACO	Corrientes	Argentina	27°28'S 58°50'W	AE	Humid subtropical	Humid Chaco	Urban	M
BUE	Buenos Aires	Argentina	34°35'S 58°22'W	AE	Temperate	Pampas	Urban	L
SAL	Salto	Uruguay	31°23'S 57°58'W	AE	Temperate	Pampa	Urban	F

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AE: *Ae. aegypti*; AL: *Ae. albopictus*; F: Free; L: Low; M: Medium; H: High

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Table 2. Dissemination efficiency of three CHIKV isolates in 22 *Ae. aegypti* and 13 *Ae. albopictus* populations from 10 American countries at day 7 post-infection.

Country	Mosquito population	CHIKV_0621		CHIKV_115		CHIKV_NC	
		AE	AL	AE	AL	AE	AL
United States	TYS	ND	96.7% (30)	ND	83.3%(30)	ND	ND
	VRB	100% (30)	93.3% (30)	100% (18)	73.3% (30)*	ND	ND
Mexico	MXC	96.7% (30)	73.3% (30)*	96.7% (30)	66.7% (30)*	ND	ND
Panama	PAN	96.7% (30)	96.7% (30)	96.7% (30)	93.3% (30)	100% (30)	96.7% (30)
Venezuela	DEL	100% (23)	ND	100% (28)	ND	ND	ND
Peru	TUM	100% (30)	ND	ND	ND	ND	ND
	PUM	100% (30)	ND	100% (29)	ND	ND	ND
Brazil	MAN	100% (30)	96.7% (30)	ND	90.3% (31)	100% (30)	90% (30)
	STR	100%(30)	100% (30)	ND	88.4% (26)	ND	ND
	PNM	100% (30)	93.3% (30)	ND	ND	ND	ND
	CAB	100% (30)	ND	ND	ND	ND	ND
	CPG	100% (30)	ND	100% (30)	ND	ND	ND
	JRB	100% (30)	100% (30)	100% (30)	ND	ND	ND
	PAQ	100% (30)	87.1% (31)	100% (30)	96.9% (29)	ND	ND
	VAZ	100% (30)	91.3% (23)	ND	ND	ND	ND
	BEL	100% (30)	90.9%(22)	ND	ND	ND	ND
	SAN	93.3% (30)	100% (30)	ND	87.5% (8)	ND	ND
Bolivia	BMA	100% (30)	ND	100% (30)	ND	ND	ND
Paraguay	SDG	100% (30)	ND	ND	ND	ND	ND
	ASU	100% (30)	ND	96.7% (30)	ND	ND	ND
Uruguay	SAL	100% (30)	ND	100% (30)	ND	ND	ND
	MIA	ND	60% (30)	ND	66.7% (26)	ND	93.3% (30)
Argentina	ACO	100% (30)	ND	100% (30)	ND	ND	ND
	BUE	100% (30)	ND	96.6% (29)	ND	96.9% (33)	ND

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Dissemination efficiency corresponds to the proportion of mosquitoes with disseminated virus in heads among tested ones. Numbers of analyzed mosquitoes are shown in parenthesis. The titer of infectious blood-meals was 10^{7.5} pfu/mL.

AE: *Aedes aegypti*; **AL:** *Aedes albopictus*; Viral strains: CHIKV_0621 isolated from La Réunion (ECSA genotype, E1-226V and E1-98A substitutions), CHIKV_115 isolated from La Réunion (ECSA genotype, E1-226A and E1-98A substitutions) and CHIKV_NC isolated from New Caledonia (Asian genotype, E1-226A and E1-98T substitutions). Mosquito populations (from North to South): **TYS** Tyson (United States), **VRB** Vero Beach (United States), **MXC** Chiapas (Mexico), **PAN** Panamá (Panama), **DEL** Delta Amacuro (Venezuela), **TUM** Tumbes (Peru), **PUM** Punchana (Peru), **MAN** Manaus (Brazil), **STR** Santarém (Brazil), **PNM** Parnamirim (Brazil), **CAB** Campos Belos (Brazil), **CPG** Campo Grande (Brazil), **JRB** Jurujuba (Brazil), **PAQ** Paquetá (Brazil), **VAZ** Vaz Lobo (Brazil), **BEL** Belford Roxo (Brazil), **SAN** Santos (Brazil), **BMA** Monteagudo (Bolivia), **SDG** Salto del Guairá (Paraguay), **ASU** Asuncion (Paraguay), **SAL** Salto (Uruguay), **MIA** Misiones (Argentina), **ACO** Corrientes (Argentina), **BUE** Buenos Aires (Argentina). ND: Not determined.

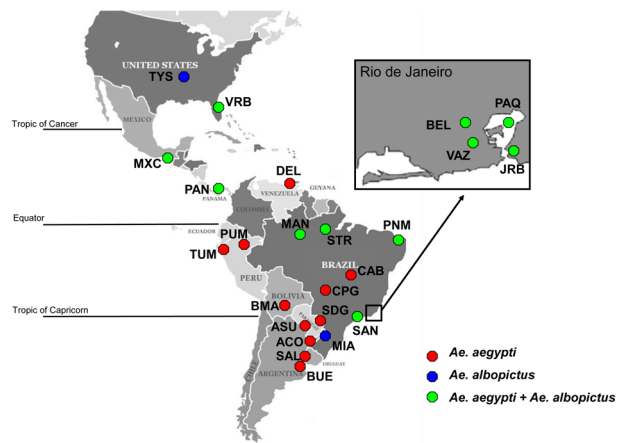
* Statistical differences of DE between the two mosquito species for a given virus (P<0.05)

749 **Table 3.** Dissemination efficiency of three CHIKV isolates in 22 *Ae. aegypti* and 13 *Ae. albopictus* populations
 750 from 10 American countries at day 10 post-infection.

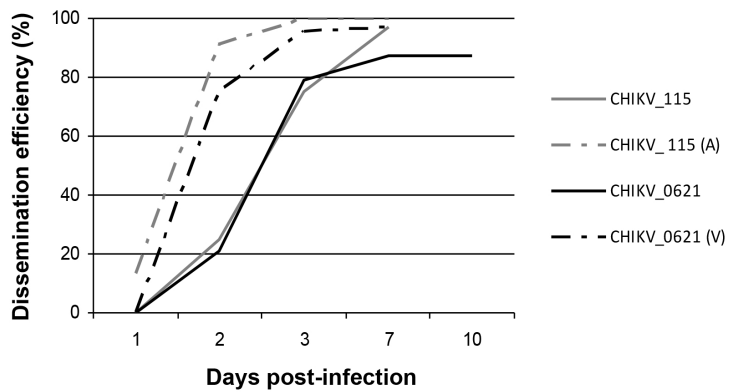
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Country	Mosquito population	CHIKV_0621		CHIKV_115		CHIKV_NC	
		AE	AL	AE	AL	AE	AL
United States	TYS	ND	93.3% (30)	ND	63.6%(11)	ND	ND
	VRB	100% (30)	85.7% (7)*	ND	96.7% (30)	ND	ND
Mexico	MXC	93.3% (30)	70.0% (30)*	100% (30)	53.3% (30)***	ND	ND
Panama	PAN	100% (30)	96.7% (30)	96.7% (30)	83.3% (30)	100% (30)	96.7% (30)
Venezuela	DEL	100% (10)	ND	100% (15)	ND	ND	ND
Peru	TUM	100% (30)	ND	ND	ND	ND	ND
	PUM	100% (29)	ND	100% (30)	ND	ND	ND
Brazil	MAN	100% (30)	100% (36)	ND	97.1% (34)	100% (30)	93.3% (30)
	STR	100%(30)	100% (20)	ND	ND	ND	ND
	PNM	100% (30)	90% (30)	ND	ND	ND	ND
	CAB	100% (30)	ND	ND	ND	ND	ND
	CPG	100% (30)	ND	100% (29)	ND	ND	ND
	JRB	100% (30)	100% (30)	100% (30)	ND	ND	ND
	PAQ	100% (30)	87.5% (32)*	100% (30)	ND	ND	ND
	VAZ	96.7% (30)	100% (32)	ND	ND	ND	ND
	BEL	100% (30)	88.9%(27)	ND	ND	ND	ND
	SAN	100% (29)	100% (30)	ND	ND	ND	ND
Bolivia	BMA	100% (30)	ND	100% (30)	ND	ND	ND
Paraguay	SDG	100% (30)	ND	ND	ND	ND	ND
	ASU	100% (30)	ND	93.3% (30)	ND	ND	ND
Uruguay	SAL	100% (30)	ND	100% (30)	ND	ND	ND
	MIA	ND	93.3% (30)	ND	80% (30)	ND	96.7% (30)
Argentina	ACO	100% (30)	ND	96.7% (30)	ND	ND	ND
	BUE	96.7% (30)	ND	100% (30)	ND	90% (30)	ND

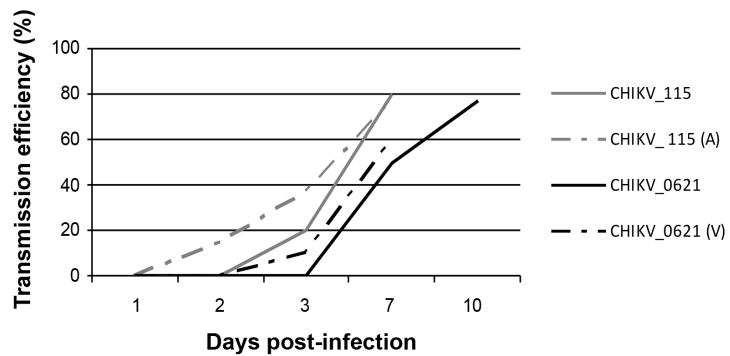
752 Dissemination efficiency corresponds to the proportion of mosquitoes with disseminated virus in heads among tested ones. Numbers
 753 of analyzed mosquitoes are shown in parenthesis. **AE:** *Aedes aegypti*; **AL:** *Aedes albopictus*; Viral strains: CHIKV_0621 isolated from
 754 La Réunion (ECSA genotype, E1-226V and E1-98A substitutions), CHIKV_115 isolated from La Réunion (ECSA genotype, E1-226A
 755 and E1-98A substitutions) and CHIKV_NC isolated from New Caledonia (Asian genotype, E1-226A and E1-98T substitutions).
 756 Mosquito populations (from North to South): **TYS** Tyson (United States), **VRB** Vero Beach (United States), **MXC** Chiapas (Mexico),
 757 **PAN** Panamá (Panama), **DEL** Delta Amacuro (Venezuela), **TUM** Tumbes (Peru), **PUM** Punchana (Peru), **MAN** Manaus (Brazil),
 758 **STR** Santarém (Brazil), **PNM** Parnamirim (Brazil), **CAB** Campos Belos (Brazil), **CPG** Campo Grande (Brazil), **JRB** Jurujuba
 759 (Brazil), **PAQ** Paquetá (Brazil), **VAZ** Vaz Lobo (Brazil), **BEL** Belford Roxo (Brazil), **SAN** Santos (Brazil), **BMA** Monteagudo
 760 (Bolivia), **SDG** Salto del Guairá (Paraguay), **ASU** Asuncion (Paraguay), **SAL** Salto (Uruguay), **MIA** Misiones (Argentina), **ACO**
 761 Corrientes (Argentina), **BUE** Buenos Aires (Argentina). ND: Not determined.
 762 * Statistical differences of DE between the two mosquito species for a given virus: * (P<0.05); *** (P<0.001).

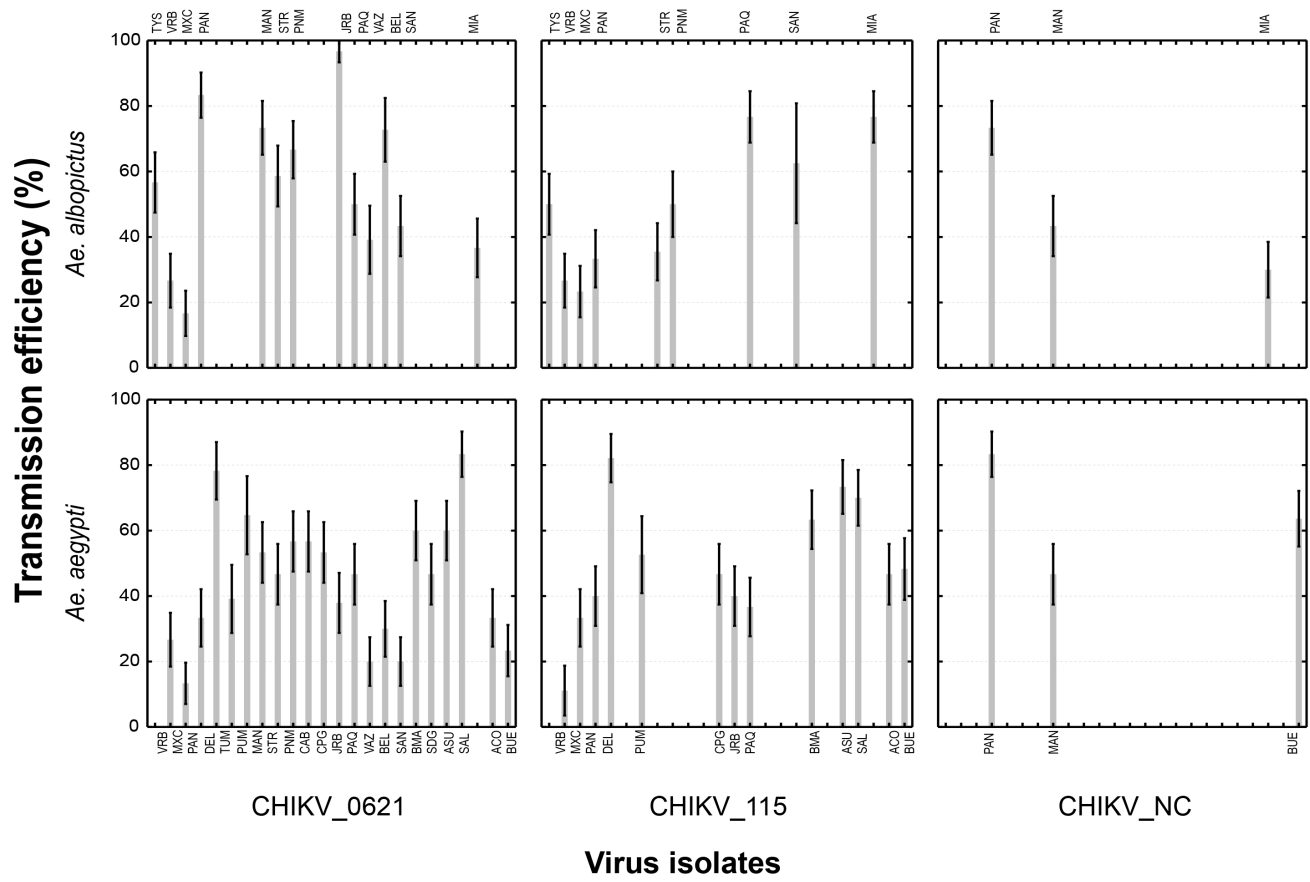


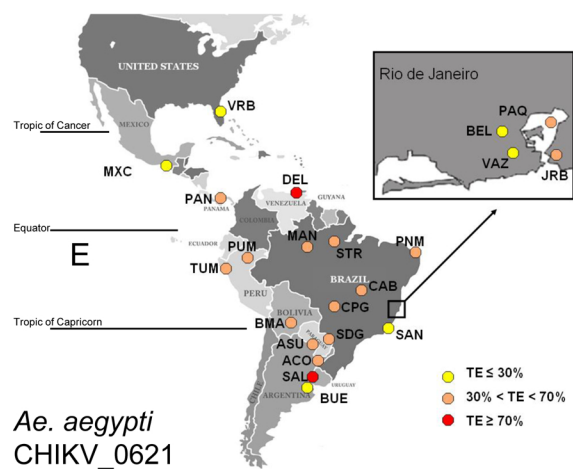
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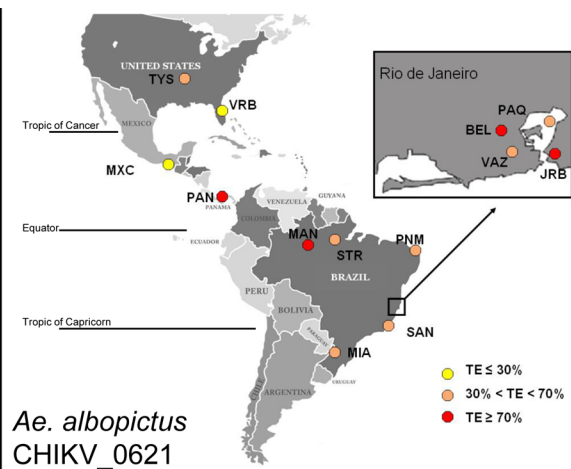
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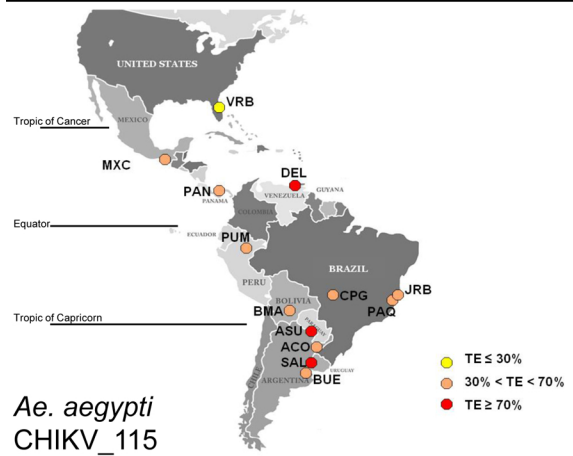




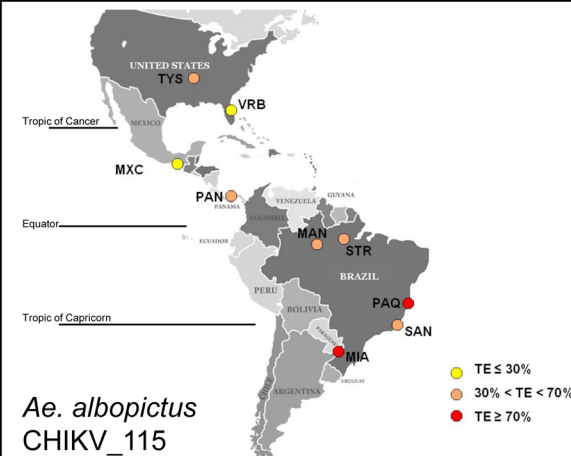
(A)



(B)



(C)



(D)

