

1 **Title: Laboratory strains of *Aedes aegypti* are Competent to**

2 **Brazilian Zika virus**

3 **Short title: *Aedes aegypti* laboratory strains and Zika virus**

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17 **Keywords:** *Aedes aegypti*; Zika virus; ZIKV^{BR}; Rockefeller; Higgs white eyes; Rexville

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21 **Abstract**

22 Since the Zika outbreaks are unprecedented human threat in relation to congenital
23 malformations and neurological/autoimmune complications as well as its high potential
24 to spread in regions presenting the vectors, improvements in mosquito control is a top
25 priority. Thus, *Aedes aegypti* laboratory strains will be fundamental to support studies in
26 different research fields implicated on Zika-mosquito interactions which are the basis for
27 the development of innovative control methods. In this sense, we determined the main
28 infection aspects of the Brazilian Zika strain in reference *Aedes aegypti* laboratory
29 mosquitoes.

30 We orally exposed Rockefeller, Higgs and Rexville mosquitoes to a Brazilian ZIKV
31 (ZIKV^{BR}) and qRT-PCR was applied to determine the infection and dissemination rates,
32 and viral levels in mosquito tissues as well as in the saliva. The ZIKV^{BR} kinetics was
33 monitored during the infection in Rockefeller mosquitoes. Rockefeller strain was the
34 most susceptible at 7 days post-infection but all strains presented similar infection levels
35 at 14 days post-infection. Although variations in the saliva detection rates were
36 observed, we confirmed that ZIKV^{BR} was present in saliva from Rockefeller, Higgs and
37 Rexville females at detectable levels at 14 days post-infection. The ZIKV^{BR} kinetics in
38 Rockefeller mosquitoes showed that the virus could be detected in the heads at 4 days
39 post-infection but was more consistently detected late in infection. The viral levels
40 peaked at 11 days post-infection in the mosquito bodies, remaining stable until 14 days
41 post-infection, in contrast to the heads, where the mean viral levels only peaked at 14
42 days post-infection.

43 Our study presents the first evaluation on how Brazilian Zika virus behaves in reference
44 *Aedes aegypti* strains and shed light on how the infection evolves over time. Vector

45 competence and basic hallmarks of the ZIKV^{BR} development were revealed in
46 laboratory mosquitoes. This study provides additional information to accelerate studies
47 focusing on ZIKV-mosquito interactions.

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57 Introduction

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59 Currently, the world is facing a new outbreak of the emerging Zika virus (ZIKV)
60 [1]. Its association with neurological and autoimmune complications as well as infants
61 born with microcephaly [2,3] has caused a global healthcare crisis. Due to the severe
62 situation, it was launched a document containing an operation plan to help affected
63 countries to establish a strategy to control the disease and improvements in vector
64 control were highlighted as priorities [4].

65 The outcome of vector infection will rely on the specific interactions between the
66 mosquito and virus genotypes. Therefore, better understanding of the mosquito vectors-
67 ZIKV interactions is the basis to generate the development of innovative strategies that
68 can be added to the arsenal in the combat of ZIKV. Recently studies have reported
69 significant differences in susceptibility for ZIKV infection between wild mosquito
70 populations of *Aedes aegypti*, *Aedes albopictus* and other *Aedes* species [5–7]. These
71 vector competence studies focusing main vector species as well as variations in
72 susceptibility of different populations from the same species are primordial to delineate
73 improved control programs, prioritizing competent populations.

74 Although wild populations of the main vector, *Ae. aegypti*, represent the natural
75 dynamics of the ZIKV infection process in the invertebrate host, the determination of the
76 vector competence of different laboratory strains, which are well-adapted in captivity
77 and readily available for experiments, is also essential to support basic and applied
78 studies in different research fields related to vector-virus interactions. Moreover, some
79 strains are also known to be standard in many laboratories in the world and because the
80 experimental reproducibility is more robust than with field populations, they are used as

81 reference mosquito strains [8]. Important advances on mosquito immune responses to
82 dengue virus (DENV) and other human pathogens have been performed on *Ae. aegypti*
83 laboratory strains and natural mosquito populations for the purposes of comparison [8–
84 10] as well as for the evaluation of insecticide resistance [11,12]. Furthermore, the
85 characterization of the vector competence is relevant for more applied purposes. The
86 development of transgenic mosquitoes mainly uses laboratory reference strains for
87 transformation and the genetic background related to pathogen susceptibility is
88 incorporated to the established lines. Thus, the vector competence of laboratory strains
89 to ZIKV must be also considered in the context of the production and release of
90 transgenic mosquitoes [13].

91 To generate basic information about the interaction between ZIKV^{BR} and three
92 important laboratory-maintained *Ae. aegypti* strains, Rockefeller, Higgs white eyes and
93 Rexville mosquitoes were analyzed. The ROCK strain, an insecticide-susceptible strain
94 of Caribbean origin that was established in 1930 [14], is commonly used as a reference
95 for insecticides resistance trials and in DENV infection experiments [15,16]. The HWE
96 strain is an eye-pigment-deficient *Ae. aegypti*, a variant of the Rex-D strain, and it is
97 used as the recipient for mosquitoes germ-line transformations, because the lack of
98 pigment is a desirable phenotype for visual screening of transgenic individuals, which
99 are often marked with a fluorescent protein expressed in the eyes [17]. The RED strain
100 of *Ae. aegypti*, also a variant from Rex-D strain, is widely used to investigate pathogen-
101 host susceptibility [18].

102 This report details the ZIKV^{BR} infection, dissemination and saliva detection rates
103 in these three mosquito strains and reveals the viral kinetics in the ROCK reference
104 strain.

105

106 **Material and methods**

107 **Ethics Statement**

108 The use of human blood or its derivatives products were conducted
109 according to the principles expressed in the Declaration of Helsinki for mosquito
110 feeding experiments. The approval of the protocol was provided by the
111 Institutional Review Board in Human Research (IRB) (Comissão de Ética em
112 Pesquisa com Seres Humanos do Instituto de Ciências Biomédicas/USP -
113 CEPESH) and National Committee for Ethics in Research (Comissão Nacional de
114 Ética em Pesquisa – CONEP), protocol #503. Number of approval: 914.876
115 (CAAE 38518114.0.0000.5467).

116 The Brazilian Zika virus strain, named as ZIKV^{BR}, was previously isolated
117 from a Brazilian clinical case [2] and a lyophilized virus sample was gently
118 provided by the Evandro Chagas Institute in Belém, Pará. The use of the
119 anonymized viral samples were approved by our Institutional Review Board
120 (IRB). The research was approved by the Ethics Committee on the Research
121 with Humans (CEPESH - Off.011616) of the Institute of Biosciences of the
122 University of São Paulo.

123

124 **Mosquito rearing**

125 The experiments were performed using three *Ae. aegypti* laboratory strains:
126 HIGGS white-eye (HWE), Rexville D (RED) and Rockefeller (ROCK). Those mosquitoes
127 were maintained in a BSL-2 insectary facility in Institute of Biomedical Sciences from
128 University of São Paulo. The rearing conditions were $27 \pm 1^\circ\text{C}$, 75-80% relative humidity
129 with 12/12 hour (light/dark) photoperiod. Adult mosquitoes were maintained *ad libitum*
130 on 10% sucrose solution (w/v).

131

132 **Viral amplification and titration**

133 The Brazilian Zika virus strain, named previously as ZIKV^{BR}, was isolated from a
134 Brazilian clinical case [2] and a lyophilized virus sample was gently provided by the
135 Evandro Chagas Institute in Belém, Pará. ZIKV^{BR} was amplified and titrated as recently
136 described [2]. Viral titrated aliquots (5.0×10^6 plaque forming unit [pfu]/mL) of fourth
137 subculture (T4) was provided by the ZIKV São Paulo task force.

138

139 **Mosquito Infection**

140 Pre-mated 7-9 day old *Ae. aegypti* females were sucrose 10%-deprived for 24
141 hours prior blood meal. Starved females received ZIKV^{BR} infectious blood meal by using
142 the Glytube artificial feeder [19]. Human concentrated erythrocyte was mixed with
143 ZIKV^{BR} supernatant and inactivated human serum in a 10:10:1 proportion, respectively
144 and the ZIKV^{BR} final titer in this mixture was 2.2×10^6 pfu/mL. The infected-blood was
145 offered to female mosquitoes for 45 minutes. Non-engorged females were removed and

146 engorged ones were kept in plastic cups maintained with 10% sucrose solution until the
147 collection times.

148

149 **Mosquito saliva and tissue samples.**

150 Saliva, heads and bodies from individual mosquitoes (10 each), were collected at
151 7 and 14 dpi for virus detection. For saliva, the forced salivation technique as used as
152 previously described [20,21], with modifications. Mosquitoes were CO₂ anaesthetized,
153 transferred to a Petri dish on ice and legs and wings were removed with forceps. A
154 glass slide with a strip of modeling clay was used to support micropipette tips filled with
155 10 µL of Leibovitz's L-15 medium (Gibcotm) and the proboscis of each live mosquito
156 were inserted into the tip. Insects were allowed to salivate for 45 minutes and total
157 volume was ejected into the 1.5 mL microtube. After salivation, heads were separated
158 from the rest of the bodies using a McPherson-Vannas Scissors #501234 (World
159 Precision Instruments, Sarasota, FL) and each tissue was transferred to a 1.5 mL
160 microtube. Bodies, heads and saliva samples collected at 7 and 14 dpi were
161 immediately frozen in dry ice and stored at -80°C.

162

163 **RNA extraction of mosquito samples**

164 Total RNA from individual heads, bodies and saliva were extracted using QIAmp
165 Viral RNA Mini Kit (Qiagen, Valencia, CA, USA) following manufacturer's
166 recommendations. Total RNA was eluted in 60 µL of elution buffer and kept in -80°C
167 until qRT-PCR analyses.

168

169 **One-step qRT-PCR analysis**

170 To detect ZIKV and to quantify viral copy numbers, the Power SYBR[®] Green
171 RNA-to-C_T[™] 1-Step kit (Applied Biosystems, Foster City, CA, USA) was used in qRT-
172 PCR reactions as described by the manufacturer. Each sample was analyzed in
173 technical duplicates in a 20 µL final volume reactions containing 5 µL of total RNA and
174 0.5 µM for each ZIKV 835 and ZIKV 911c primers [22]. Negative (RNase free water)
175 and positive controls (500 ng of total RNA extracted from ZIKV cell culture supernatant
176 aliquot) were used in each assay. Samples were considered positive for ZIKV only
177 when detected in both analyses. The assays were performed in Mastercycler Realplex 2
178 thermocycler (Eppendorf) with the following conditions: 48°C for 30 min and 95°C for 10
179 min followed by 40 cycles of 95°C for 30 sec, 60°C for 30 sec and a melting curve step
180 of 95°C for 1 min, 60°C for 30 sec and 95°C for 1 min, with a temperature ramping from
181 60°C to 95°C at 0.02°C/sec. Amplicon specificity was evaluated by the peak of the
182 melting curve (79 ± 1°C) and primer pair efficiency ranged from 1.01 to 1.02.

183 For ZIKV copy number estimation, a standard curve was generated as described [23],
184 with some modification. Briefly, a target plasmid containing a 76 bp ZIKV fragment
185 amplified with ZIKV 835 and ZIKV 911c primer pairs was linearized and nine serial
186 dilutions ranging from 10⁻⁹ to 10⁻¹⁷ g were used to produce a standard curve. The limit of
187 detection was experimentally established in 23 copies (10⁻¹⁶ g dilution). The ZIKV
188 absolute copy numbers were extrapolated from the standard curves (R² ranged from
189 0.990 to 0.996), adjusted by back-calculation to the total RNA volume (60 µL) and were
190 expressed as copies per tissue.

191

192 **Infection, dissemination and saliva detection rate analysis**

193 Following the definitions proposed in [5]. The adopted infection rate (or
194 prevalence) is determined as the proportion of mosquitoes with virus detected in body
195 (abdomen and thorax) among tested ones. Dissemination rate corresponds to the
196 number of mosquitoes with infected heads among infected bodies (abdomen and thorax
197 positive). Since a method to detect viral RNA in saliva was used, the term saliva
198 detection rate was applied in place of transmission rate (which refers to infectious
199 particles present in saliva), but with equivalent meaning. The saliva detection rate was
200 estimated as the proportion of mosquitoes with positive saliva among mosquitoes with
201 disseminated infection (positive heads) and was expressed as percentages.

202

203 **Data analysis**

204 Statistical analyses were performed in GraphPad Prism5 software package
205 (Version 5.00) for Windows (San Diego, California, USA) and confidence intervals of
206 95% were defined for all analyses. Fisher's exact test were applied in conformity with
207 [24] to detected significant differences in ZIKV positive proportions of the same tissues
208 (bodies or heads or saliva) at 7 or 14 dpi from different mosquito strains and to compare
209 infection proportions during ZIKV kinetics in ROCK strain. Viral levels between different
210 *Ae. aegypti* lines were compared by using Two-way ANOVA and Bonferroni posttests.

211

212 **Results**

213 **ZIKV^{BR} infection prevalence and dissemination rates in orally challenged** 214 **mosquito strains**

215 To analyze and compare the infection susceptibility of *Ae. aegypti* laboratory
216 strains to a Brazilian ZIKV, we orally exposed ROCK, HWE and RED mosquitoes with a
217 low-passage ZIKV^{BR} strain. Viral RNA levels were quantified by qRT-PCR assay in
218 individual mosquito bodies and heads at 7 and 14 days post-infection (dpi). These
219 intervals are well-characterized indicators for infection establishment in the abdomen
220 and viral dissemination to the head, respectively, during flavivirus replication
221 progression in *Ae. aegypti* mosquitoes [25].

222 The three strains exhibited high viral levels in the bodies at 7 and 14 dpi, with the
223 mean viral copy numbers fluctuating from 10^7 at 7 dpi to 10^8 at 14 dpi (Fig 1A). At 7 dpi,
224 the ROCK and RED strains showed the highest body infection rates (number of positive
225 bodies/total mosquitoes tested) (90%) compared to HWE females (70%). At 14 dpi, the
226 body infection proportion increased in HWE samples and all the strains showed the
227 same infection rate (90%) at this time. However, the infection rate of the heads
228 exhibited variation in the number of positive samples among the strains at 7 dpi, with
229 ROCK at 90%, HWE at 50% and RED at 60%. At 14 dpi, the ROCK strain remained
230 with 90% of the tested heads infected and the HWE and RED strains had increases in
231 the percentage of infected heads to 80% and 90%, respectively. With regard to the
232 mean viral levels observed in the head, we observed that the RED strain had 10^5 ZIKV
233 copies at 7 dpi and this number increased to 10^7 at 14 dpi, indicating an increase of 2
234 logs after 7 days, while the ROCK and HWE strains had infection level increases only 1

235 log (Fig 1B). When analyzing the dissemination rate (the number of infected
236 heads/number of infected bodies), the ROCK strain had the highest index (100%)
237 analyzed at any time (Fig 2).

238

239

240 **Fig 1. ZIKV^{BR} infection rates and viral levels in bodies and heads from *Ae. aegypti***

241 **laboratory strains.** The infection rate and viral levels per tissue were individually

242 recorded in ROCK, HWE and RED females at 7 and 14 days following a ZIKV-infected

243 blood meal (dpi). **(A)** Viral prevalence and infection levels in the bodies. Each body

244 sample is represented by a solid circle. **(B)** Viral prevalence and infection levels in the

245 heads. Individual heads are indicated by open triangles. Black bars indicate the mean

246 viral copy numbers and the dashed grey line demonstrates the detection limit.

247 Significant differences were not observed between the bodies or heads from the three

248 strains at 7 and 14 dpi in the infection prevalences by Fisher's exact test ($p>0.05$), or in

249 the viral infection levels by two-way ANOVA with Bonferroni post-tests ($p>0.05$)

250

251 **Fig 2. ZIKV^{BR} infection, dissemination and saliva detection rates in *Ae. aegypti***

252 **laboratory strains.** The infection rate (number of infected bodies/total bodies

253 analyzed), dissemination rate (number of infected heads/number of infected bodies) and

254 saliva detection rate (number of infected saliva sample/number of infected heads) were

255 estimated in females from ROCK, HWE and RED strains at 7 and 14 days following a

256 ZIKV-infected blood meal (dpi). The results are represented by percentages.

257

258 Although strain variations in the infection rates and mean levels of ZIKV^{BR} were
259 characterized in the bodies and heads at 7 and 14 dpi, no significant differences were
260 observed in tissue infection prevalence (Fisher's exact test, $p>0.05$) and viral intensity
261 between the ROCK, HWE and RED samples (two-way ANOVA followed by Bonferroni
262 post-tests, $p>0.05$).

263

264

265 **Prevalence and detection rate of ZIKV^{BR} in mosquito saliva**

266 The presence of ZIKV^{BR} and viral level quantitation were assessed directly for
267 each collected saliva sample using the same qRT-PCR assay. ROCK strain females
268 showed one positive saliva sample (10%) at 7 dpi. However, no ZIKV^{BR} was detected in
269 saliva from HWE and RED mosquitoes at this time point. In contrast, saliva samples
270 from the three strains presented detectable viral levels at 14 dpi. HWE samples were
271 40% positive for ZIKV^{BR}, while ROCK and RED samples were 20% and 10%,
272 respectively (Fig 3). Accordingly, the highest ZIKV^{BR} saliva detection rate (number of
273 positive saliva samples/total number of infected heads) was observed in the HWE strain
274 (50%), followed by ROCK (22.22%) and RED (11.11%).

275

276 **Fig 3. ZIKV^{BR} prevalence and viral levels in collected saliva from *Ae. aegypti***
277 **laboratory strains.** The prevalence and viral levels per saliva sample were individually
278 recorded in ROCK, HWE and RED strains at 7 and 14 days following a ZIKV-infected
279 blood meal (dpi). Each saliva sample is represented by a solid star. Black bars indicate
280 the mean viral copy numbers and the dashed grey line demonstrates the detection limit.

281 Significant differences were not observed between samples from the three strains at 7
282 and 14 dpi in saliva infection prevalences by Fisher's exact test ($p>0.05$), or in viral
283 detection levels by two-way ANOVA and Bonferroni post-tests ($p>0.05$).

284

285

286 The ZIKV^{BR} prevalence was low in the ROCK and RED saliva samples. The
287 mean viral levels in ROCK saliva increased 1 log (10^1 to 10^2) between 7 and 14 dpi. In
288 comparison with ROCK, It was observed higher mean ZIKV levels in the RED and HWE
289 samples (10^4) (Fig 3) at 14 dpi.

290 As observed in the bodies and heads, saliva samples showed strain variations
291 related to the detection rate and the mean ZIKV^{BR} levels (Figs 2 and 3, respectively).
292 However, no statistically significant differences were detected in virus prevalence
293 (Fisher's exact test, $p>0.05$) or levels in the saliva (two-way ANOVA followed by
294 Bonferroni post-tests, $p>0.05$) between the ROCK, HWE and RED strains at 7 and 14
295 dpi.

296

297

298 **ZIKV^{BR} invasion and establishment kinetics in the ROCK strain**

299 ZIKV^{BR} infection, dissemination and saliva detection rates were higher in the
300 ROCK strain at 7 dpi (Fig 2). This result led us to perform an independent experiment in
301 order to determine the kinetics of ZIKV^{BR} during the establishment and dissemination of
302 infection in females from this strain. Five time-points post-infected blood meal (1, 4, 7,

303 11 and 14 dpi) were used to perform a quantitative analysis of the viral levels in the
304 individual bodies and heads (Fig 4).

305

306 **Fig 4. ZIKV^{BR} kinetics in bodies and heads of *Ae. aegypti* from the ROCK strain.**

307 The infection rate and viral levels per tissue were individually recorded at 1, 4, 7, 11 and
308 14 days following a ZIKV-infected blood meal (dpi). Solid circles and open triangles
309 represent each body and head sample, respectively, from the ROCK females. Solid
310 (body) and dashed (head) blue lines indicates the viral infection progression (mean
311 levels) during the time-course experiment. Dashed blue bars indicate early (1 to 4 dpi)
312 and late (7 to 14 dpi) infection stages in heads in which a significant difference in
313 infection prevalence was observed between the two stages using Fisher's exact test (*,
314 1 dpi x 7 dpi - p=0.0031; 1 dpi x 11 dpi - p=0.0007; 1dpi x 14 dpi - p<0.0001; 4 dpi x 7
315 dpi - p=0.0198; 4 dpi x 11 dpi - p=0.0055; 4 dpi x 14 dpi - p=0.0001). The dashed grey
316 line demonstrates the detection limit.

317

318

319

320 As expected, all the body samples analyzed were positive for ZIKV^{BR} at 1 dpi and
321 the mean viral levels was 7.68×10^4 copies. These results probably reflect the viral load
322 ingested during the oral infection of the ROCK females and ZIKV^{BR} particles still present
323 in the infectious blood remaining in the blood bolus inside the midgut. Not surprisingly,
324 none of the heads showed detectable levels of ZIKV in this period.

325 The detected viral copies increased gradually in the bodies and heads over the
326 experimental time course. At 4 dpi, when the blood had been completely digested, the
327 prevalence was 70% in the bodies and 10% in the heads, indicating that in some ROCK
328 females, ZIKV^{BR} infection can evolve more rapidly, reaching the head and producing
329 high infection intensity in the nervous tissues (10^5) over a short period after infection
330 (Fig 4).

331 The amount of infected bodies remained at the same level (70%) at 7 dpi,
332 however, the number of virus-infected heads increased to 70% and the mean viral
333 levels were enhanced by approximately 2 logs. ZIKV^{BR} was detected in the bodies of all
334 analyzed mosquito samples at 11 and 14 dpi. Furthermore, a viral level peak was
335 reached at 11 dpi, and an infection plateau was maintained in the body tissues between
336 the last two sampling times (Fig 4).

337 The ZIKV^{BR} prevalence was 80% in the heads at 11 dpi and 100% at 14 dpi. The
338 mean viral levels increased during the 14 days of infection from 0.0 to 3.13×10^7 copies
339 in the heads of mosquitoes fed with a viral titer of 2.27×10^6 pfu/mL (final
340 concentration). The infection rates of the ROCK head samples varied significantly
341 between the early (1 dpi and 4 dpi) and late time points of infection (7, 11 and 14 dpi)
342 (Fisher's exact test, $p < 0.05$).

343

344 Discussion

345 Basic knowledge on the interactions ZIKV with its vectors is one of the priorities
346 in order to create a solid scientific foundation supporting traditional and innovative
347 methods to face the Zika challenge [26]. The literature in this field is expanding with
348 recent studies uncovering the main species naturally infected with ZIKV [27–29] and
349 characterizing the viral susceptibility of the natural populations in regions with the
350 potential for urban transmission [5,30]. As important as these studies including wild or
351 recently colonized mosquitoes are, well-adapted laboratory vector strains will provide a
352 consistent basis for reliable cellular and molecular studies of the virus-mosquito
353 interaction, in which execution feasibility and reproducibility are essential.

354 Recently, the laboratory-adapted mosquito strains, HWE and Orlando (ORL),
355 were used to describe the infection pattern of chikungunya virus (CHIKV). Both
356 mosquito strains were susceptible to the CHIKV, and viral particles were detected in the
357 saliva only two days after an infectious blood meal. The CHIKV infection pattern in
358 midguts and dissemination rate were significantly lower for the ORL in comparison to
359 the HWE strain until 3 dpi, although the HWE and ORL mosquitoes showed similar
360 rates of virus in the saliva (60 and 65%, respectively) at 7 dpi [31].

361 Our study also found variations between laboratory-adapted strains during the
362 ZIKV infection. Although *Ae. aegypti* infection dynamics is more rapid for the CHIKV (an
363 alphavirus member from the *Togaviridae* family) than the ZIKV (a flavivirus from the
364 *Flaviviridae* family), the HWE strain demonstrates lower saliva prevalence in
365 comparison to the ROCK strain in early sampling time during ZIKV^{BR} infection. The

366 same pattern was observed in the HWE mosquitoes in relation to the ORL strain when
367 exposed to the CHIKV [31].

368 CHIKV prevalence into saliva of the HWE increases from 20% at 2 dpi to 60% at
369 7 dpi, differing from the ORL strain (55% at 2 dpi to 65% at 7 dpi)[31]. Our study also
370 demonstrates that the increase of the ZIKV^{BR} saliva detection rate was more
371 pronounced in the HWE infected mosquitoes (0% at 7 dpi to 50% at 14 dpi) in relation to
372 the RED (0% at 7 dpi to 11.1% at 14 dpi) and ROCK (11.1% at 7 dpi to 22.2% at 14
373 dpi). This result is surprising since the HWE has the lowest infection rate among the
374 strains at 7 dpi while the ROCK mosquitoes showed ZIKV^{BR} susceptibility that results in
375 faster infection establishment and dissemination. More interestingly, the HWE strain
376 showed the highest ZIKV^{BR} load in the saliva at late infection stage and a similar result
377 was demonstrated for the HWE mosquitoes infected with CHIKV [31].

378 American populations of *Ae. aegypti* were orally exposed to an Asian genotype of
379 ZIKV and viral infection and dissemination were observed in the early days post-
380 infection. Although the infection rates were high, dissemination and transmission rates
381 were comparatively low [5]. The infection and saliva detection rates were similar to our
382 results, but we found high dissemination rates in all *Ae. aegypti* strains tested.

383 Consistent with our findings with ZIKV^{BR} infection in *Ae. aegypti* laboratory
384 strains, other studies found the same high susceptibility in wild mosquito populations
385 infected with different ZIKV strains, highlighting that the reference strains can mimic the
386 infection pattern of wild population [5,32].

387 The present work adopted qRT-PCR as a rapid and efficient method to
388 characterize vector competence [7] and to precisely measure the viral levels during the

389 infection process [24,33]. Studies have shown a consistent correlation between viral
390 RNA levels and infectious viral particles of different flaviviridae [34,35]. Based on
391 ZIKV^{BR} genome amplification, we measured the saliva detection rates to verify the
392 ZIKV^{BR} competence of three *Ae. aegypti* laboratory strains. The detection of virus RNA
393 in the mosquito saliva indicates that salivary gland infection and escape barriers were
394 overcome and implies that *Ae. aegypti* mosquitoes from the ROCK, HWE and RED
395 strains are competent to ZIKV^{BR}.

396

397 **Conclusions**

398 The results from our study confirm that ROCK, HWE and RED laboratory strains not
399 only sustain the development of the Brazilian Zika virus but are also competent for virus
400 transmission. These findings provide useful comparisons for future researches and will
401 dictate the strains that suits best for desired experiments. In this sense, this knowledge
402 is fundamental for Zika-invertebrate host studies, especially because we determined the
403 main infection aspects of the ZIKV^{BR} strain in reference *Aedes aegypti* laboratory
404 mosquitoes. This knowledge is the first step to support the researches aiming to
405 understand ZIKV-vector biology focusing innovative solutions on vector control.

406

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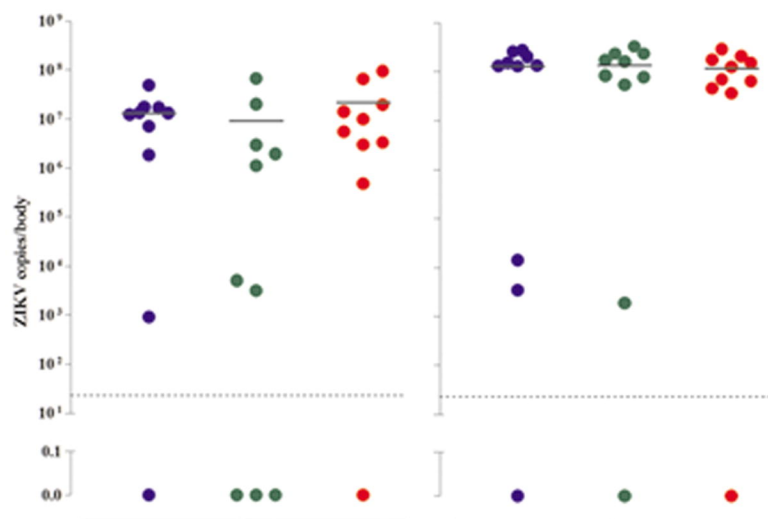
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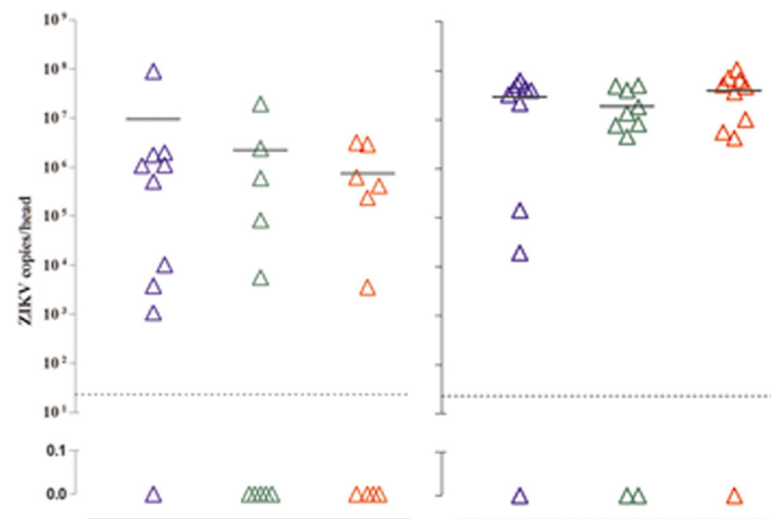
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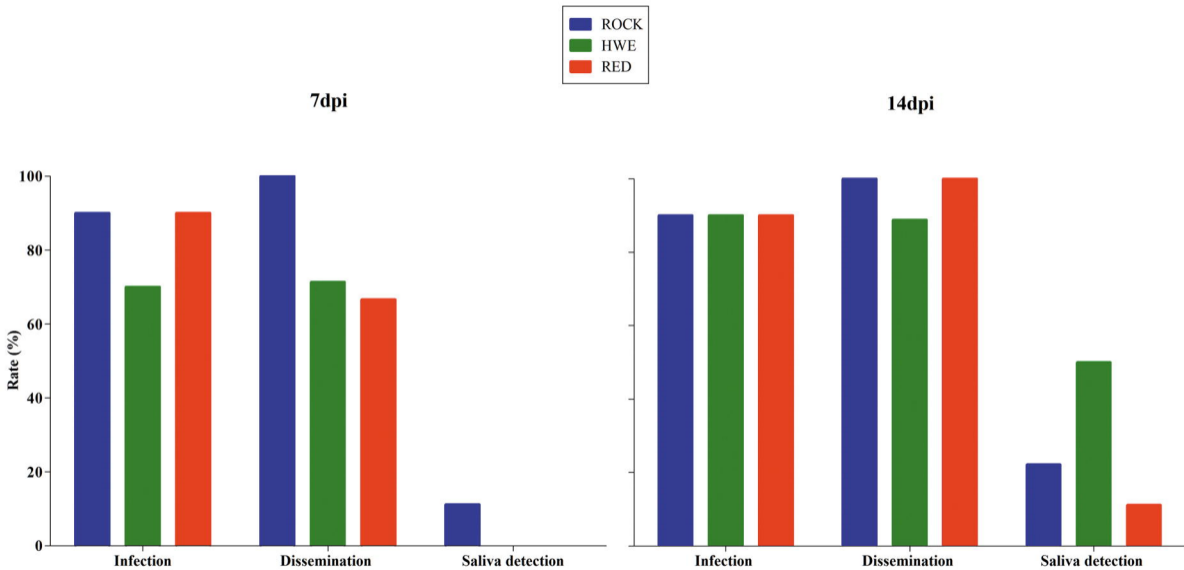
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A**Bodies**

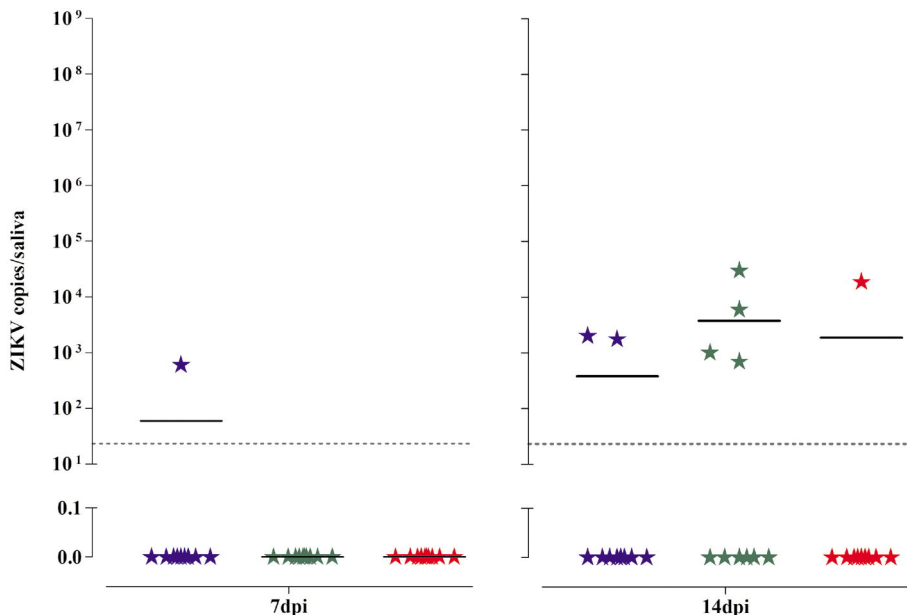
Lab. strains	7dpi			14dpi		
	ROCK	HWE	RED	ROCK	HWE	RED
Total samples	10	10	10	10	10	10
Number Infected	9	7	9	9	9	9
Infection rate (%)	90	70	90	90	90	90
Mean ZIKV ^{BR} copies	1.31×10^7	9.26×10^6	2.16×10^7	1.28×10^8	1.35×10^8	1.16×10^8
Standard deviation	1.43×10^7	2.11×10^7	3.22×10^7	1.02×10^8	1.10×10^8	9.02×10^7
Standard error	4.51×10^6	6.67×10^6	1.02×10^7	3.21×10^7	3.48×10^7	2.85×10^7

B**Heads**

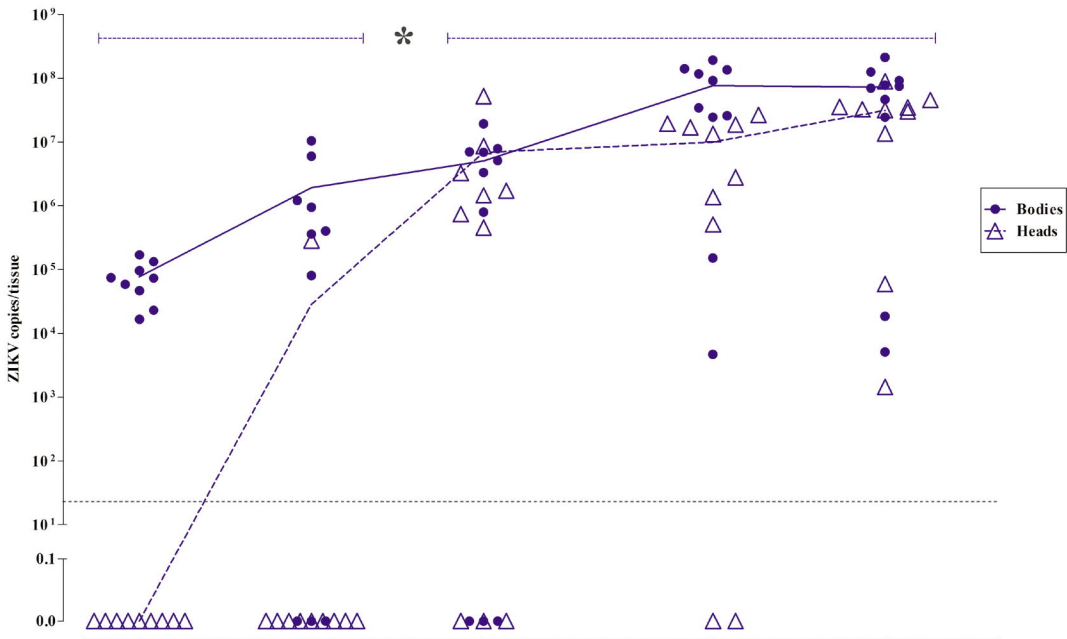
Lab. strains	7dpi			14dpi		
	ROCK	HWE	RED	ROCK	HWE	RED
Total samples	10	10	10	10	10	10
Number Infected	9	5	6	9	8	9
Infection rate (%)	90	50	60	90	80	90
Mean ZIKV ^{BR} copies	9.58×10^6	2.23×10^6	7.30×10^5	2.97×10^7	1.93×10^7	3.99×10^7
Standard deviation	2.80×10^7	6.01×10^6	1.22×10^6	2.31×10^7	2.00×10^7	3.53×10^7
Standard error	8.86×10^6	1.90×10^6	3.87×10^5	7.32×10^6	6.32×10^6	1.12×10^7



Saliva



Lab. strains	ROCK	HWE	RED	ROCK	HWE	RED
Total samples	10	10	10	10	10	10
Number Infected	1	0	0	2	4	1
Infection rate (%)	10	0	0	20	40	10
Mean ZIKV^{BR} copies	5.98×10^1	0.00	0.00	3.79×10^2	3.76×10^3	1.88×10^3
Standard deviation	1.89×10^2	0.00	0.00	8.01×10^2	9.37×10^3	5.94×10^3
Standard error	5.98×10^1	0.00	0.00	2.53×10^2	2.96×10^3	1.88×10^3



ROCK	1 dpi		4 dpi		7 dpi		11 dpi		14 dpi	
	Bodies	Heads	Bodies	Heads	Bodies	Heads	Bodies	Heads	Bodies	Heads
Total samples	9	9	10	10	10	10	10	10	10	10
Number Infected	9	0	7	1	7	7	10	8	10	10
Infection rate (%)	100	0	70	10	70	70	100	80	100	100
Mean ZIKV^{BR} copies	7.68×10^4	0.00	1.94×10^6	2.85×10^4	5.04×10^6	6.86×10^6	7.61×10^7	9.94×10^6	7.21×10^7	3.13×10^7
Standard deviation	5.00×10^4	0.00	3.48×10^6	9.01×10^4	5.95×10^6	1.62×10^7	6.80×10^7	1.01×10^7	6.32×10^7	2.56×10^7
Standard error	1.67×10^4	0.00	1.10×10^6	2.85×10^4	1.88×10^6	5.12×10^6	2.15×10^7	3.18×10^6	2.00×10^7	8.10×10^6