Lower survival rate, longevity and fecundity of *Aedes aegypti* (Diptera: Culicidae) females orally challenged with dengue virus serotype 2

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ABSTRACT

As the pathogenic effects of a parasite on its hosts can strongly influence its epidemiology, we compared the life-histories of dengue virus serotype 2 (DENV-2)-infected and uninfected *Aedes aegypti* females. Unexposed mosquitoes lived longer than exposed ones, but those infected lived longer than exposed but negative (as assayed by Real-Time quantitative Reverse Transcription PCR [qRT-PCR]) mosquitoes. Infected mosquitoes from a long-established laboratory colony presented more viral RNA copies at death than those from the F1-generation of a field population from Rio de Janeiro. The mortality of infected colony-mosquitoes was independent of the number of viral RNA copies at death, whereas in the field population, longevity decreased with the number of viral RNA copies, suggesting that F1 of field mosquitoes are less tolerant to infection than the laboratory-colony. Infected females had a lower fecundity than controls. F1 of field mosquitoes were more likely to lay eggs than the colony; egg-laying success was strongly affected by mosquito age for both mosquito populations: from 49.28 in the first clutch to 20.7 in the fifth. Overall, DENV-2 reduced *Aedes aegypti* survival and fecundity, clearly affecting vectorial capacity and consequently transmission intensity.

Keywords:
Aedes aegypti
Fitness
Dengue
Vectorial capacity
Cost of infection

1. Introduction

The World Health Organization (WHO) estimates that 50 million cases of dengue occur per year, with more than 2.5 billion people living at risk.[1] The geographic extension of dengue is essentially determined by the distribution of its primary vector, the mosquito *Aedes aegypti*. This species is commonly found in high numbers in urban areas, living close to human dwellings, where females usually breed in man-made containers.[2,3] It is believed that the intensity of dengue transmission is largely influenced by the parameters governing *Ae. aegypti* vectorial capacity such as the mosquito's population density, human biting rate, daily survival rate and the virus' extrinsic incubation period.[4]

Understanding the epidemiology of dengue is therefore difficult without good estimates of these factors and, in particular, how mosquito–dengue interaction itself influences epidemiologically relevant parameters. Like other arboviruses, dengue virus is able to invade the mosquito's brain, which may modify its physiology and metabolism, thereby changing vectorial capacity and the pattern of disease transmission.[5] Under this scenario, estimates of mortality, longevity and biting rate in dengue infected *Ae. aegypti* still need evaluation.[4,6] Most mathematical models assume there is no effect of dengue virus on these parameters,[7] and their effect, if any, is not well known. For instance, two studies considered the biting rate of dengue infected *Ae. aegypti*, but gave conflicting results. Using mosquitoes from long-established laboratory

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colonies, Putnam and Scott[8] found no evidence that dengue virus serotype 2 (DENV-2) influences the mosquito's feeding behavior (i.e. biting rate). On the other hand, Platt et al.[5] observed that the time required for blood-feeding and the time spent probing is longer in dengue-infected mosquitoes than in uninfected individuals.

The impact of the dengue virus on *Ae. aegypti* survival is almost unknown. It was noted in a previous study that intrathoracically dengue-infected mosquitoes survived less than uninfected siblings.[9] By exploring available data on virus-induced mortality on mosquitoes, it was observed that mosquitoes from the genus *Aedes* were not associated with significant virus-induced mortality if viruses are vertically transmitted.[10] However, strong vector mortality is often reported when viruses are transmitted by intrathoracic inoculation or oral infection induced.[10]

While traits other than survival and biting rate are less important for the epidemiology of a vector-borne disease such as dengue,[4,6] they can be relevant for the vector's population dynamics and for the evolutionary pressure on mosquitoes. One crucial aspect for mosquito population dynamics is age-specific fecundity. To our knowledge, no studies have estimated the impact of horizontally transmitted dengue on mosquito fecundity. Joshi et al.[9] observed that vertically dengue infected *Ae. aegypti* has significantly lower fecundity and egg hatching than controls. This is consistent with studies on various interactions of mosquito and virus genera, in which the vector's fecundity was reduced.[9,11–13]

In this study, we compared the effect of dengue infection on the mortality, longevity and age-specific fecundity of a laboratory-colony and a natural population of *Ae. aegypti*.

2. Materials and methods

2.1. Mosquitoes

Two *Ae. aegypti* populations were used. The field population was the F1-generation of mosquitoes collected in Caju, a district of Rio de Janeiro city (RJC), Brazil. In the 2007–2008 dengue epidemic this district had the highest incidence rate of dengue in RJC (12 681/100 000 inhabitants); therefore we assumed that its mosquito population transmits the virus efficiently. *Ae. aegypti* eggs from Caju were collected using 80 ovitraps filled with hay infusion in an area of 6.1 hectares. As a laboratory colony, we used the Paea strain, which is highly susceptible to oral dengue infections. The colony was initiated with mosquitoes caught in French Polynesia in 1994. Supposing two generations per month, the Paea strain has been maintained in the laboratory for approximately 300–360 generations.[14]

Larval populations were reared and maintained in identical laboratory conditions in all assays. Larvae were reared on fish food and raised in plastic basins at 25 ± 3 °C. After emergence, adults were maintained at 27 ± 2°C, 75 ± 5% relative humidity and nearly 12–12 h light-dark photoperiod in cages of 45 cm^3 and allowed to mate. They were fed ad libitum with cotton soaked with a 10% sucrose solution up to about 36 h before we offered a DENV-2 infectious blood-meal to *Ae. aegypti* females.

2.2. Virus

A C6/36 cell clone of *Aedes albopictus* (ATCC) was grown and maintained as monolayers at 28 °C on Dulbecco's modified medium (DMEM) buffered with sodium bicarbonate and supplemented with glutamine, penicillin-streptomycin and 5% inactivated fetal calf serum (iFCS). Green monkey kidney (Vero) cell clone (ATCC CCL81) was grown as monolayers and maintained at 37 °C on 199 medium with Earle's salts buffered with sodium bicarbonate and supplemented with 5% iFCS and gentamicin.

We used DENV-2 strain 16681 that had been provided by Dr. S. Halstead (Naval Medical Research Center, USA). The virus stock was obtained from four passages in Vero cell cultures. Culture monolayers were infected with a 10-fold diluted inoculum without iFCS and incubated at 37 °C for 90 minutes. After 7 days of infection, supernatant was centrifuged at 400 g for 10 minutes for cellular debris removal. The virus stock was stored at −70 °C with 30% iFCS. Virus was titrated by serial dilution cultures in microtiter plates and detected by immunofluorescence.[15,16]

2.3. Oral infection of mosquitoes with dengue virus serotype 2

Four to five days after emergence, 60 females were put into small cylindrical plastic cages, with no access to sugar. About 36 h later, they were offered a DENV-2 infectious blood meal. One ml of supernatant of infected cell culture was added to 2 ml of washed rabbit erythrocytes to prepare the infectious blood-meal, which was heated to 37 °C and provided to the mosquitoes in an artificial membrane feeding apparatus.[17] Mosquitoes were allowed to feed for 25 min on infectious blood that contained a viral titre of 3.6×10^5 PFU equivalent/ml. The same procedure and apparatus were used to feed control mosquitoes, but these received a non-infectious blood meal, with another 1 ml of rabbit blood replacing the viral supernatant. We conducted a total of four infection assays.

2.4. Experimental design

Those females that were visually fully engorged were isolated in labelled cylindrical plastic tubes (6.5 cm height, 2.5 cm diameter) containing moistened cotton overlaid with filter paper as substrate for oviposition on the bottom and closed on the top with mosquito netting. Three days after the infectious blood meal, the first egg clutch was laid by those engorged females. Later on, once a week, we offered non-infectious defibrinated rabbit blood with the membrane feeders described above to mosquitoes from the control and experimental groups. Three days after the blood meal, the filter papers were checked for eggs, which were counted, and a new filter paper was added as oviposition substrate. Survival was checked daily at 09:00 h and 16:00 h. When a dead mosquito was observed, it was removed from the plastic tube, and both wing lengths were measured as the distance from the axillary incision to the apical margin excluding the fringe.[18] Finally, samples were labelled and stored at −70 °C for Real-Time quantitative Reverse Transcription PCR (qRT-PCR) assays.

2.5. Dengue virus serotype 2 quantitative Real-Time RT-PCR

The concentration of viral RNA in each individual was estimated with a one-step RT-PCR using the ABI Prism® 7000 Sequence Detection System (SDS; Applied Biosystems, Foster City, CA, USA). Each mosquito was ground in 100 μl of Leibovitz medium (L-15) and 33 μl of foetal calf serum, and viral RNA was extracted using QIAmp Viral RNA Kit (Qiagen Sciences, Maryland, MA, USA). The reaction mixture was prepared using the Taqman® One-Step RT-PCR Master Mix Kit (Applied Biosystems, Foster City, CA, USA). Samples were assayed in a 30 μl reaction mixture containing 8.5 μl of extracted RNA, 0.63 μl of 40 × Multiscribe enzyme plus RNAse inhibitor, 12.5 μl TaqMan 2 × Universal PCR Master Mix and 300 nM of each specific primer and fluorogenic probe. Primer sequences (**DV3.U**: 5′-AGC ACT GAG GGA AGC TGT ACC TCC-3′; **DV.L1**: 5′-CAT TCC ATT TTC TGG CGT TCT-3′) and probe (**DV.P1**: 5′-CTG TCT CCT CAG CAT CAT TCC AGG CA-3′) were obtained from Houng et al.[19] and designed for the 3' noncoding sequences (3′NC). The TaqMan probe was labelled at the 5′ end with 5-carboxyfluorescein (FAM) reporter dye and at the 3′ end with 6-carboxy-*N*,*N*,*N*′,*N*′- tetramethylrhodamine (TAMRA) quencher fluorophore. The 5′ nuclease TaqMan assay relies on the 5′ exonuclease activity of the Taq polymerase to free the reporter dye in the quenched probe. DENV-2 viral stocks and water were set as positive and negative controls, respectively, and were included in every assay. The threshold cycle (Ct) represents the PCR cycle at which the SDS software first detects a noticeable increase in reporter fluorescence above a baseline signal.

The quantitative Real-Time PCR assays were standardized with a 10-fold-dilution series containing 10^7 RNA copies/ml. The number of viral RNA copies detected per sample was calculated with a standard curve from 10-fold dilutions of DENV-3 RNA, isolated from a known amount of local virus propagated in *Ae. albopictus* C6/36 cells, the titre of which was determined by plaque assay.[20] The same model of DENV-3 standard curve was applied to build a DENV-2 curve. Quantitative interpretation of the results obtained was performed by interpolation from the standard curve included in each independent run for each serotypes.

2.6. Statistical analysis

Mosquitoes were analyzed as belonging to three treatments: unexposed controls, which did not feed on DENV-2 infectious blood, and exposed (infected or negative). Exposed mosquitoes were blood-fed with a DENV-2 infective blood-meal and were classified as infected after qRT-PCR assays. Some of those mosquitoes exposed to DENV-2 were negative in qRT-PCR assays, even though they had.blood-fed on an infective blood-meal. Those females were classified from now on as 'exposed, but negative' mosquitoes.

Aedes aegypti longevity (which was assumed to be the day each mosquito died) was non-normally distributed, but the square root of longevity satisfied the assumption of normality (Shapiro-Wilk W = 0.9957, $P = 0.191$). We analyzed the effect of treatment (control, infected or exposed but negative), mosquito population (Paea or Caju), wing length and number of RNA copies (in infected specimens) on mosquito longevity with an ANOVA. We performed a log-rank test to compare on a two-sample basis the survival distribution of *Ae. aegypti* females from control, infected and exposed but negative treatments. Herein, we define survival rate as the number of individuals still alive as a function of time. In the infected treatment, we also considered the effect of the number of RNA copies on *Ae. aegypti* longevity, testing the hypothesis that higher dengue virus copies is harmful to mosquitoes. We initiated all analyses with all possible interactions, and backwards-eliminated them (starting with the highest interactions) if they had no effect at $P < 0.05$.

Fecundity was analyzed by considering the first five clutches of eggs laid, as only a small number of females laid eggs when they were more than five weeks old, precluding adequate numbers for analysis. We analyzed two aspects of fecundity. First, we analyzed the likelihood that a mosquito laid at least one egg (at a given clutch) with a logistic analysis that included treatment, population, wing length and clutch-number (i.e., age), again backwards-eliminating the insignificant interactions. Second, we analyzed the number of eggs of the successful mosquitoes with a repeated analysis. We square-root transformed the number of eggs to satisfy the assumptions of normality. We included clutch-number as the repeat and estimated the effects of treatment, wing length and population. All analyses were carried out with the statistical software JMP 7.0.2 (http://www.jmp.com/).

3. Results

3.1. Oral infection with dengue virus serotype 2

Of the 501 *Ae. aegypti* females used in this experiment, 297 mosquitoes were exposed to DENV-2 16681; 198 of these were infected and 99 were negative. Of the 501 mosquitoes, 276 belonged to Paea strain and 225 were from the field population.

3.2. Aedes aegypti longevity

The highest longevities were observed in the control treatment, in which six females lived more than 90 days; on average controls lived longer (42.7 days ± 23.2 SD) than the other treatments (infected: 26.3 days ± 12.1; exposed but negative: 22.1 days ± 12.7) (Figure 1). Among virus exposed mosquitoes, infected individuals survived longer than exposed but negative ones ($t = 2.58$, $df = 494$, $P = 0.0102$), but less than controls ($t = 9.414$, $df = 494$, $P < 0.001$). Figure 1 shows that infected mosquitoes had a similar survival rate to controls up to the 12th day after infection, whereupon their mortality rate increased. Negative mosquitoes had a higher mortality rate than controls and infected mosquitoes during the first few days after exposure.

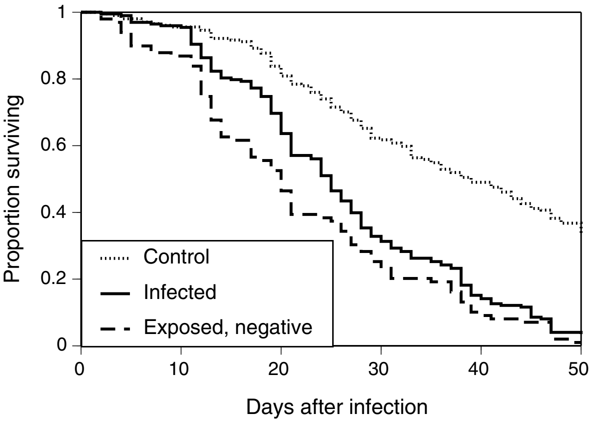

Figure 1. Survival curves of control, dengue virus serotype 2 infected, and exposed but negative *Aedes aegypti* females up to 50 days post-infection. Mosquitoes from the control treatment were alive up to the 109th day.

Mosquitoes with longer wings survived longer than small mosquitoes (Table 1). The Caju population survived longer than mosquitoes from the Paea strain (Table 1).

The survival analysis confirmed the results obtained in ANOVA. Individuals from the control group presented a lower mortality rate than infected ($\chi^2 = 86.27$, $P < 0.001$) and exposed but negative mosquitoes ($\chi^2 = 85.02$, $P < 0.001$). Finally, infected mosquitoes survived longer than exposed but negative ($\chi^2 = 4.22$, $P < 0.041$). Considering two single points in the mortality curves we observed 80.3 and 62.6% of mosquitoes from infected and exposed but negative group were alive on the 14th day after infection, when we expect dengue virus has completed it incubation period on mosquito body. On the 30th day post-infection, when the majority of mosquitoes have already died under field conditions, 31.3 and 23.2% of mosquitoes from the infected and from the exposed but negative treatments were alive.

In the infected treatment, we also considered the effect of the number of RNA copies on *Ae. aegypti* longevity. Mosquitoes from Paea population presented more RNA copies at death than Caju (95% interval limits for Caju: 4021.7–5658.7; Paea: 5418.3–7645.7), suggesting the Caju population was more resistant to higher virus titres. In addition to population (Table 2) and wing length, longevity was affected by the interaction between RNA copies and population (Table 2; Figure 2). In the Paea population, survival was independent of the number of RNA copies, whereas in the Caju population, longevity decreased with the number of RNA copies (Figure 2), suggesting that mosquitoes from Caju were less tolerant to infection.

Table 1
Analysis of variance of the square root of survival of control, infected and exposed but negative *Aedes aegypti* females

Source	d.f.	Sum of squares	*F*	*P-value*
Treatment[a]	2	198.83	44.47	<0.001
Wing size[b]	1	18.96	8.48	0.0037
Population[c]	1	44.09	19.72	<0.001
Error	494	1104.32		

[a] Unexposed controls, which did not feed on dengue virus serotype 2 (DENV-2) blood, infected, which were positive in quantitative Reverse Transcription PCR (qRT-PCR) and exposed but negative, which fed on DENV-s infectious blood but were negative in qRT-PCR.

[b] Distance from the axillary incision to the apical margin excluding the fringe.

[c] If insects came from a field or a lab colony, which has approximately 360 generations in confinement.

Table 2
Analysis of variance of the square root of survival of DENV-2 infected mosquitoes

Source	d.f.	Sum of squares	*F*	*P-value*
Population[a]	1	20.78	16.44	<0.001
Wing size[b]	1	6.55	5.18	0.0239
RNA copies[c]	1	1.46	1.15	0.2831
RNA copies AND[d] Population	1	4.97	3.93	0.0486
Error	191	241.33		

[a] If insects came from a field or a lab colony, which has approximately 360 generations in confinement.

[b] Wing size: the distance from the axillary incision to the apical margin excluding the fringe.

[c] Number of RNA copies at death determined by quantitative Reverse Transcription PCR assays.

[d] Interaction between sources.

3.3. *Aedes aegypti fecundity*

The first part of our analysis of fecundity considered whether females laid eggs (Table 3). Field mosquitoes (from Caju) were more likely than the Paea colony to lay eggs

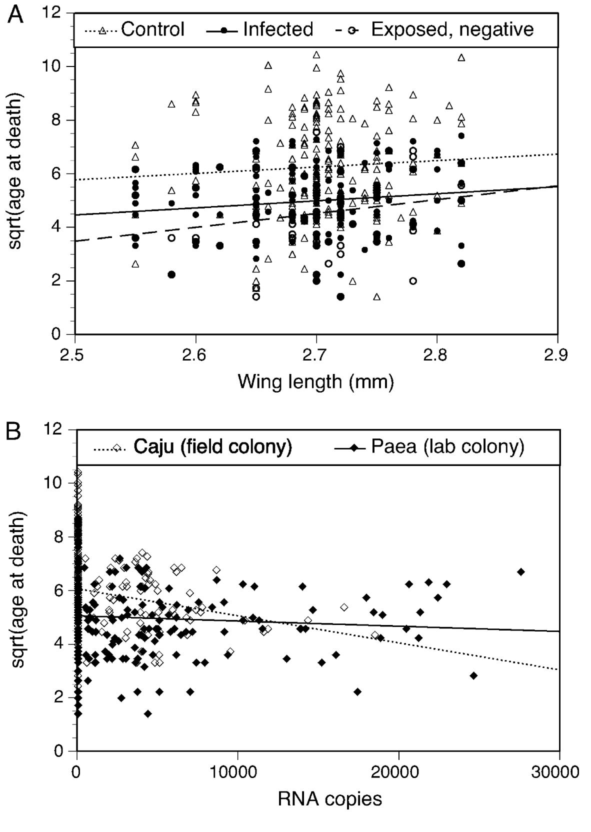

Figure 2. Influence of wing length (A) and RNA copies (B) on the survival of *Aedes aegypti* females.

Table 3
Logistic regression analysis of the influence of mosquito treatment, population, age when they lay eggs and wing size on the success of oviposition

Source	d.f.	χ^2	*P-value*
Treatment[a]	2	0.940	0.625
Population[b]	1	30.512	0.0001
Age[c]	1	50.626	<0.0001
Wing size[d]	1	0.887	0.346
Treatment AND[e] Wing size	2	0.448	0.799
Treatment AND[e] Age	2	2.239	0.326
Wing size AND[e] Age	1	0.014	0.906
Treatment AND[e] Wing size AND[e] Age	2	4.785	0.091

[a] Unexposed controls, which did not feed on dengue virus serotype 2 blood; infected, which were positive in quantitative Reverse Transcription (qRT)-PCR; and exposed but negative, which fed on DENV-s infectious blood but were negative in qRT-PCR;

[b] If insects came from a field or a lab colony, which has approximately 360 generations in confinement.

[c] Number of days mosquito survived.

[d] Distance from the axillary incision to the apical margin excluding the fringe

[e] Interaction between sources.

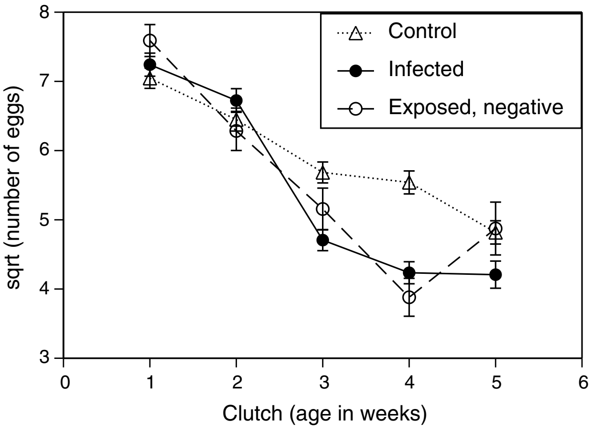

Figure 3. *Aedes aegypti* clutch size during the first five weeks of mosquito lifespan.

(90% of Caju, 82% of Paea laid eggs). Egg-laying success was strongly affected by mosquito age, decreasing with female age from 95% success at the first clutch to 75% at the fifth clutch. There was an interaction between treatment, wing size and age, with oviposition success dropping more rapidly with age in exposed (infected and negative) mosquitoes than in controls, but only if they were small (Table 3, Figure 3).

The second part of the analysis considered the number of eggs of females that laid at least one egg (Table 4). The number of eggs of ovipositing *Ae. aegypti* females from the three treatments decreased more than two-fold with time, from a mean of 49.28 in the first clutch to 20.7 in the fifth (Figure 3). The decrease of fecundity was more evident for infected and for exposed but negative mosquitoes than for unexposed controls (Figure 3). They laid similar numbers of eggs as the control in the first and second clutch, but laid fewer eggs in the later clutches (although in the fifth clutch the fecundity of the exposed but negative mosquitoes increased to a value similar to the controls).

4. Discussion

We showed that infection of *Ae. aegypti* females with DENV-2 considerably decreases mosquito longevity and fecundity, parameters that directly or indirectly (via modifications of vector population density) influence the intensity of dengue transmission. According to dengue transmission models,[6] the mosquito's survival and biting rates and the virus's extrinsic incubation period (EIP) greatly affect its vectorial capacity and thus the strength of disease transmission.[4,6,21] While the survivorship of *Ae. aegypti* females has been estimated under several field scenarios (socioeconomic status, rural or urban, crowded or low-density neighborhoods, wet vs dry season),[22–25] little is known about the *Ae. Aegypti*–dengue virus interactions. The observed negative effect on the fitness of dengue-infected *Ae. aegypti* is consistent with Lambrechts and Scott,[10] who observed that horizontally transmitted arboviruses generally induce significant fitness costs in mosquitoes.

Survival curves showed an intriguing result. During the first few days, the exposed but negative mosquitoes had the highest mortality rate, while infected mosquitoes died at a similar rate to the controls. After the first few days, the survival rates of the three treatments were similar. However, at 12–15 days after infection, exposed individuals (whether infected or exposed but negative) had increased mortality rates, while the controls continued to present a low mortality rate up to the end of the experiment. The reason for the initial mortality of exposed but negative mosquitoes is unknown, but we might speculate that the immune response is activated shortly after infection. If the immune response is strong enough, it could clear the virus, but it would also carry costs and might therefore increase

Table 4
Repeated analysis (with clutch taken as the repeat) of the square root of the number of eggs laid by *Aedes aegypti*. Interactions (apart from with Clutch) were insignificant, so are not shown

Source	Numerator d.f.	Denominator d.f.	*F*	*P*
Clutch[a] AND[b] Treatment[c]	8	170	2.475	0.0146
Clutch[a] AND[b] Wing size[d]	4	85	2.933	0.0253
Clutch[a] AND[b] Population[e]	4	85	0.850	0.4975

[a] Number of eggs laid per gonotrophic cycle per female.

[b] Interaction between sources.

[c] Unexposed controls, which did not feed on dengue virus serotype 2 blood (DENV-2); infected, which were positive in quantitative Reverse Transcription (qRT)-PCR; and exposed but negative, which fed on DENV-s infectious blood but were negative in qRT-PCR.

[d] Distance from the axillary incision to the apical margin excluding the fringe.

[e] If insects came from a field or a lab colony, which has approximately 360 generations in confinement.

vector mortality,[26] thus leading to the surprising result that exposed but negative mosquitoes are more likely to die than infected ones. The cellular and humoral immunity responses of *Ae. aegypti* mosquitoes to arboviruses such as DENV have only just recently begun to be examined.[27] The presence of midgut infection barriers seems to be the most efficient way mosquitoes can avoid virus dissemination.[28] Recent evidence has shown that RNA intereference, a major component of the mosquito innate immune response, may modulate infection by producing molecules to inhibit virus replication.[28–30] Up to now, there is no evidence that *Ae. aegypti* mosquitoes have a fitness cost due to mounting an immune response to dengue virus. Thus, we must recognize that with our data we are not able to confirm if negative individuals are free of virus due to an immune response that eliminated the pathogen or if virus titre was too low or even absent to be quantified in qRT-PCR assays.

The impact of dengue virus infection on *Ae. aegypti* fecundity has been little explored. A single paper observed that fertility and fecundity in vertically infected batches were lower than in control individuals.[9] Despite the differences regarding the mode of dengue infection, data in Joshi et al[9] were not gathered and analyzed for individual females as in our experiment, making any direct comparisons difficult. Our results considered horizontally infected mosquitoes and showed that the effect of the virus on fecundity varies over the mosquito's life. In the first two clutches, exposed and control mosquitoes laid similar numbers of egg. From the third clutch onwards, infected females had lower fecundity than the control treatment. Females laid the eggs of their second and third clutch around 11 and 18 days after infection. This is consistent with the observation that around 15 days after infection, several mosquito tissues such as midgut, nervous system and salivary glands are severely infected by dengue virus.[31] In contrast to the data on survival, egg laying in infected and in exposed but negative mosquitoes was affected similarly by the virus. Parasite-induced fecundity reduction in other vector/parasite interactions has already been observed, but not for *Ae. Aegypti*–dengue virus system.[32] For instance, parasite-induced fecundity reduction was observed in *Leishmania*-infected sandflies,[33] *Dirofilaria*-infected *Ae. trivittatus* mosquitoes[34] and *Onchocerca*-infected blackflies.[35]

Some of our conclusions must be carefully interpreted due to the experimental design we have chosen. For example, *Ae. aegypti* feeds almost exclusively on human blood, taking a blood meal about every day, and rarely feeds on sugar.[36,37] *Aedes aegypti* diet, i.e., frequency and availability of sugar and blood, has a major impact on mosquito survival and reproduction. For instance, females that feed more frequently on blood have higher fecundity, whereas those that feed more frequently on sugar have higher longevity.[38] The use of an artificial membrane feeding apparatus with rabbit blood meals once a week supplemented with sugar ad libitum might have influenced partitioning of energy and thus mosquito survival and reproduction. The use of human blood was discouraged due to ethical aspects and the blood feeding was done just once a week due to technical limitations. However, since all treatments were maintained under the same conditions we suggest that the differences observed in survival and fecundity between treatments were induced by infection rather than by diet after infection. Another potential source of bias may be the use of a dengue virus that does not coexist in the same geographical area in nature with the infected mosquito population. Recently, Lambrechts et al.[39] showed that vector competence for *Ae. aegypti* is largely governed by the interactiojns between the vector's and the virus's genotypes in natural populations. Thus, although our work focused on mosquito survival and fecundity rather than competence, the absence of previous local adaptation between *Ae. aegypti* from Paea and Caju strains and DENV-2 16681 may be a potential source of bias.

In the *Ae. aegypti*-DENV-2 association, infection with virus produced a significant deleterious impact on mosquito fitness, negatively affecting mosquito survival and fecundity, important traits of vector biology and components of disease transmission. Our study is the first quantitative evaluation of *Ae. aegypti* survivorship, longevity and fecundity in DENV-2 infected mosquitoes. Data gathered here may be seen as a preliminary attempt to develop more refined and precise dengue transmission models, since current models are based on biology of non-infected individuals.[6] Increasing the predictive accuracy of these mathematical models can help in the provision of public health resources where they will be needed most.

Taken together, we have: (1) observed that infected mosquitoes survived for a significantly shorter time and have lower longevity than unexposed individuals, (2) hypothesized that some infected mosquitoes might be able to elicit an effective immune response to dengue virus, with a cost that could also increase mosquito mortality, (3) observed that the genetic background of mosquito populations affects vector tolerance and resistance to dengue virus, (4) verified that mosquitoes were more likely to lay eggs and that egg-laying success was strongly affected by mosquito age for both populations, (5) observed that the number of eggs laid by *Ae. aegypti* females decreased more than two-fold with age and (6) observed that infected females had a lower fecundity than controls, but only from the third clutch onwards.

Authors' contribution: RMF and RLO designed the experiment; RMF conducted experiments; JCK and RMF analyzed data, RMF, JCK and RLO interpreted data and wrote the manuscript. RMF and RLO are guarantors of the paper.

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Ethical approval: Not required.

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