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# The influence of different sources of blood meals on the physiology of *Aedes aegypti* harboring *Wolbachia* wMel: mouse blood as an alternative for mosquito rearing

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## Abstract

**Background:** *Aedes aegypti* control programs have failed to restrain mosquito population expansion and, consequently, the spread of diseases such as dengue, Zika, and Chikungunya. *Wolbachia* infection of mosquitoes is a new and promising complementary tool for the control of arbovirus transmission. The use of *Wolbachia*-infected mosquitoes, mass reared using human blood, is currently being tested in several countries. However, the use of human blood for mass rearing mosquitoes, and thus expansion of this strategy, is problematic. With the aim of overcoming this problem, we tested the effect of different types of blood source on the fitness parameters of female *Ae. aegypti* and the *Wolbachia* titer over generations to be able to guarantee the suitability of an alternative source to human blood for mass rearing *Wolbachia*-infected mosquitoes.

**Methods:** We investigated and compared essential parameters of the vector capacity of laboratory strains of *Ae. aegypti* with and without *Wolbachia* that fed on blood of different types of host (human, guinea pig, and mouse). The parameters analyzed were fecundity, fertility, pupation dynamics, and adult survival. Also, we tested whether it is possible to maintain mosquitoes with *Wolbachia* on mouse blood over generations without losing the bacterium titer.

**Results:** The average number of eggs per female, egg viability and pupation dynamics in the *Wolbachia*-infected mosquito (wMelBr) strain were similar, regardless of the blood source. The F1 progenies of females that fed on mouse blood or human blood were analyzed. The longevity of males was lower than that of females. F1 female survival differed depending on the presence of *Wolbachia* in the mother. In subsequent generations analyzed up until F35, the relative *Wolbachia* density was even higher when mosquitoes fed on mouse blood in comparison to human blood.

**Conclusions:** Taken together, our results provide no evidence that the different types of blood influenced the fitness of the *Wolbachia*-infected mosquitoes. The presence of the bacterium in the colonies of *Wolbachia*-infected *Ae. aegypti* after 35 generations under the conditions evaluated indicates that they can be maintained on mouse blood. Based on these results, we show that it is possible to use mouse blood to feed female mosquitoes when using human blood for this purpose is problematic.

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**Keywords:** *Aedes aegypti*, *Wolbachia*, wMel strain, Blood feeding, Vector capacity

## Background

*Aedes aegypti*, a primary vector of arboviruses such as dengue, Zika, and chikungunya, has a wide geographical distribution and is found in tropical and subtropical regions around the world [1, 2]. Dengue, the main mosquito-borne arbovirus, is present in more than 100 countries, and there are an estimated 390 million cases of dengue infections in humans per year, according to the Pan American Health Organization [1]. A recent Zika virus pandemic had as its hallmark its rapid spread in the Western Hemisphere, where the vector *Ae. aegypti* is widely distributed [3]. Although first identified in the 1950s, there were only a few isolated cases of chikungunya virus before it reached the Americas in 2013; since then, it has spread throughout the world and become a major global concern [4].

Vector control programs have failed to restrain mosquito population expansion and, consequently, the spread of mosquito-borne diseases. One of the reasons for this is the increase of insecticide-resistant *Ae. aegypti* populations [5]. Furthermore, no specific treatment nor effective vaccines are yet available for these diseases [6].

A new complementary strategy to control arboviruses based on the endosymbiont *Wolbachia* has emerged, for which expectations are high. The bacterium *Wolbachia pipientis* is naturally present in individuals of most insect orders; however, it is not found in natural populations of *Ae. aegypti* [7, 8]. After a great deal of effort to artificially introduce *Wolbachia* into *Ae. aegypti*, it was found that the presence of certain strains of this bacterium strongly inhibit the replication of arboviruses, including dengue, Zika, and chikungunya [9, 10]. Due to a phenomenon called cytoplasmic incompatibility, reproduction leads to the rapid spread of *Wolbachia* through insect populations. Some countries have obtained approval for the release of mosquitoes infected with *Wolbachia* as a strategy to limit the transmission of arboviruses. Currently, this strategy is in the test phase in several countries, including Brazil [11].

Vector control programs, like the *Wolbachia* strategy, rely on mass rearing to enable the release of a substantial number of mosquitoes for the invasion of natural populations. *Ae. aegypti* females are usually obligatorily anautogenous; they need a vertebrate blood meal for egg production, and prefer human blood for this [12, 13]. McMeniman et al. [14] showed that *Ae. aegypti* females infected with a virulent *Wolbachia* strain (wMelPop)

were almost unable to produce viable eggs after a blood meal from non-human vertebrates such as chickens, guinea pigs, or mice (which are often used in laboratories for mosquito rearing), and only produced viable eggs when fed human blood. There is a considerable problem involved with the use of human blood for mosquito feeding: many countries do not have regulation to obtain volunteers that would accept to be bitten. Using blood from human donors, on the other hand, is also an obstacle to the mass rearing of mosquitoes. As a result, some studies have focused on the production of artificial diets for *Wolbachia*-infected mosquitoes to overcome the restrictions associated with them feeding solely on human blood. [15].

The aim of the present study was to test different kinds of laboratory animals on which *Ae. aegypti* harboring *Wolbachia* wMel can feed to find a suitable source of blood that does not alter their fitness. Here, we analyzed the fitness parameters of *Ae. aegypti* infected with the wMel strain when they fed on mouse, guinea pig, and human blood, compared with a natural population maintained under the same conditions, to establish an alternative blood source to human blood.

## Methods

### Mosquito lineages

Two lineages of *Ae. aegypti* without *Wolbachia* (Mosquitoes\_Br and Rockefeller), which were considered as experimental controls for fitness in all of the assays, and one *Wolbachia*-harboring lineage (wMelBr), were used in the experiments. The Mosquitoes\_Br strain was collected in the city of Rio de Janeiro, Brazil. To create the wMelBr lineage, males from the Mosquitoes\_Br population were crossed, for several generations, with Australian female mosquitoes from the *Wolbachia*-harboring wMel strain [5, 16]. Mosquitoes from the Rockefeller strain, an insecticide-susceptible reference lineage [17], were kindly provided by the Laboratório de Fisiologia e Controle de Artrópodes Vetores [Instituto Oswaldo Cruz (IOC), Fiocruz, Rio de Janeiro, RJ, Brazil].

### Mosquito maintenance

All assays were run under controlled laboratory conditions, as previously described [16]. Briefly, 300 mosquito larvae were raised at  $25 \pm 1$  °C and fed with fish food (Marine granules; Tetra, Germany). Adult mosquitoes were kept in cages at  $25 \pm 2$  °C and fed *ad libitum* with a 10% sucrose solution. Metamorphosis from the larval to

adult stage was synchronized under a 12:12-h light:dark photoperiod, at constant 25 °C and 40–80% relative humidity, as described in Rezende et al. [18]. Blood meals required for egg production were performed on anesthetized guinea pigs, mice (CEUA-FIOCRUZ LW-20/14), or donated human blood using a Hemotek membrane feeder (Hemotek, Blackburn, UK). The blood used in all experiments of infective feeding was obtained from a blood bank (Hemominas) through an agreement signed between Fiocruz and Hemominas (OF.GPO/CCO-Nr224/16). Eggs on filter paper were hatched according to Farnesi et al. [19]. In all experiments, a random pool of mosquitoes was tested for the presence of *Wolbachia* by reverse transcription quantitative–polymerase chain reaction (RT-qPCR), as described below.

#### **Analysis of the efficiency of oviposition after *Ae. aegypti* fed on different blood sources**

Five days after adult emergence, inseminated females were collected for blood feeding on various blood sources: guinea pigs, mice, or human blood bags, for 30–60 min. Following blood feeding, 20 females per experiment and blood condition were separated and distributed one-by-one among Petri dishes (150-mm diameter) lined with filter paper (Whatman no. 1). To each Petri dish, 4 mL filtered water was added to induce oviposition. After 1 h, the females were discarded, and the number of eggs per female was counted under a stereomicroscope (SteREO DiscoveryV.12; Zeiss). For each blood type, three independent experiments were performed. The methodology used to synchronize oviposition was adapted from Rezende et al. [18] and Vargas et al. [20].

#### **Analysis of egg viability**

Egg viability was calculated as the hatching of L1 larva, adapted from Farnesi et al. [19]. Briefly, a total of 150 eggs per blood condition was placed in Petri dishes lined with a moist Whatman no. 1 filter paper. A 0.15% yeast extract solution was used as a hatching stimulus. Petri dishes with eggs were maintained in an incubator at a constant temperature (25 ± 1 °C). The relative humidity inside the incubator ranged from 40 to 80%. For each experiment, 150 eggs, randomly split into three independent triplicates (50 eggs each), were used. Three experiments were performed for each blood condition tested, i.e., 450 eggs were used in total.

#### **Evaluation of pupation dynamics and adult survival**

The parameters were evaluated by simultaneously comparing Rockefeller, *wMelBr*, and *Mosquitoes\_Br* strains reared under identical conditions of initial larval density and feeding, temperature, and photoperiod regimens. Eggs were induced to hatch for approximately 1 h.

Three replicates of 300 newly hatched first-instar larvae were then randomly transferred to plastic trays (30 × 21 × 5 cm) with 1 L dechlorinated water and 0.15 g of fish food (Marine granules; Tetra). Fresh food was supplied every 3 days. The amount of food was sufficient for larval development.

#### **Pupae formation**

Pupation dynamics under the above conditions were examined daily as an indicator of cessation of larval development. This assay was performed three times.

#### **Male and female longevity**

The adults were randomly pooled in cylindrical cardboard cages (18 × 30 cm) 3 days after emergence and separated into groups of 50 couples, which received 10% sucrose solution ad libitum as the only food source. Mortality was scored every 2 or 3 days for approximately 2 months until all the infected mosquitoes had died. This assay was conducted three times.

#### **Selection of *Wolbachia*-infected *Ae. aegypti* fed on mouse blood after five and ten generations**

Two fitness parameters were analyzed for F5 and F10 generations of *Ae. aegypti* (*wMelBr*) feeding on mouse blood: fecundity and egg fertility (following the same methodology used in the previous experiment). These data were compared with those of mosquitoes of the same strain that fed on human blood. *Wolbachia*-free mosquitoes of the Rockefeller lineage (Rock) were fed mouse blood and defined as an external experimental control.

#### **Evaluation of *Wolbachia* density over generations in *Ae. aegypti* fed on mouse blood**

To ensure the presence of *Wolbachia* in the *Ae. aegypti* fed mouse blood throughout the generations, RT-qPCR was used for *Wolbachia* detection after 15, 20, 25, and 35 generations.

DNA extraction was performed by the squash buffer plus proteinase K method. After maceration of the mosquitoes in a Mini-BeadBeater (BioSpec, Bartlesville, USA) for 1.5 min, they were placed in a thermal cycler (Applied Biosystems, CA) at 56 °C for 5 min and then at 98 °C for 15 min. After this process, the samples were stored at – 20 °C.

RT-qPCR was performed using specific fluorescent probes in which the *Wolbachia wMel* strain and the ribosomal protein of *Ae. aegypti* (RPS17S) were detected in the same reaction (duplex) (Table S1). RT-qPCR was performed using a LightCycler96 (Roche). The conditions were as follows: an initial step of 95 °C for 6 min, followed by 95 °C for 20 s, and 45 cycles at 95 °C for 3 s and

60 °C for 30 s and 72 °C for 1 s for fluorescence acquisition. The final volume of the reaction was 10 µL TaqMan Mix 4× (Applied Biosystems), 1 µM of each probe and primer, and 1 µL of each sample. Relative quantification was performed by comparing the cycle threshold values obtained for the *Wolbachia* surface protein *TM513* compared to the values of the 40S ribosomal protein *S17* gene (*RPS17S*). Samples of *Wolbachia*-infected mosquitoes treated with an antibiotic (tetracycline) were used as a negative control.

### Statistical analysis

GraphPad Prism version 5.01 for Windows (GraphPad software, 1992–2007; GraphPad, San Diego, CA; [www.graphpad.com](http://www.graphpad.com)) was used for all statistical analyses. For all experiments, mean and SD were calculated. One-way ANOVA followed by Tukey's multiple comparison test, where  $P < 0.05$  was considered statistically significant, was used in the fecundity and egg viability experiment. Linear regression analysis was performed for the analysis of pupation dynamics and survival.

## Results

### Efficiency of oviposition with different blood sources

Figure 1 presents the overall female fecundity arising from various blood sources (guinea pig, mouse, and human blood bags) for all three lineages (Rockefeller, *wMelBr*, and Mosquitoes Br). *wMelBr* females laid fewer eggs than females without the bacterium ( $P < 0.0001$ ). However, the average number of eggs laid per female *wMelBr* was the same regardless of the blood source ( $P < 0.0001$ ). For mosquitoes without *Wolbachia*, in general, human blood lead to less egg production in comparison to the other blood sources.

### Egg viability

The egg viability was analyzed using a yeast solution as a hatching stimulus. In all lineages, with and without *Wolbachia*, there was no significant difference in the rate of viability after the mosquitoes fed on different blood sources (human, guinea pig, and mouse). On the other hand, the viability of *Wolbachia*-infected mosquitoes was, on average, lower (between 72.6 and 60.6%) than that of mosquitoes without the bacterium (between 97.6 and 89.6%) (Fig. 2).

### Pupation dynamics

When *wMelBr*, Rockefeller, and Mosquitoes\_Br strains were reared under the same controlled conditions, the pupation dynamics were not significantly different. As expected, the majority of *Ae. aegypti* larvae pupated between 6 and 7 days after egg hatching (Fig. 3). The

nonlinear regression analysis performed for all strains fitted a Gaussian curve ( $R^2 = 0.98$ ,  $R^2 = 0.97$ ,  $R^2 = 0.97$ ,  $R^2 = 0.98$ ,  $R^2 = 0.99$  for *wMelBr*\_human blood, *wMelBr*\_guinea pig blood, *wMelBr*\_mouse blood, Rock\_mouse blood, Mosquitoes\_Br\_mouse blood, respectively). There was no significant difference between the means (ANOVA followed by Tukey's multiple comparison test;  $F = 4.766$ ,  $P = 0.99$ ).

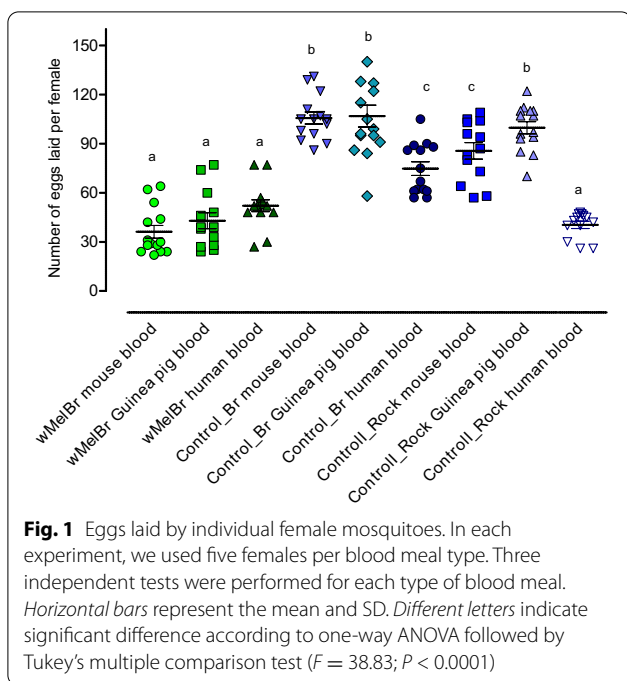
### Comparative adult longevity of F1 progeny of *Wolbachia*-infected and non-infected females fed on different blood sources

To simplify the methodology, we decided to use mouse blood as the blood source for F0 control female without *Wolbachia*, i.e., Rockefeller and Mosquitoes\_Br. We compared adult longevity (male and female) of the F1 progeny of females with and without *Wolbachia* that fed on mouse blood. For this assay, adults (both F1 males and females) were offered a 10% sucrose solution *ad libitum*. The results are shown in Fig. 4. As expected, male longevity was shorter than female longevity (cf. Fig. 4a, b). In F1 males, survival was not significantly different among all the tested conditions (ANOVA followed by Tukey's multiple comparison test,  $P = 0.06$ ).

The survival curves of F1 females of the control Rockefeller and *wMelBr* lineages were not significantly different (Fig. 4b) (ANOVA followed by Tukey's multiple comparison test;  $F = 10.57$ ,  $P > 0.05$ ). The survival of F1 *Wolbachia* females after the mothers fed on any kind of blood was significantly lower than that of females of the bacterium-free strain (ANOVA followed Tukey's multiple comparison test;  $F = 10.57$ ,  $P < 0.05$ ). The mortality of females without *Wolbachia* was only 30%, while it was 70% for females with *Wolbachia* after 30 days of observation (Fig. 4b). Taken together, our results did not provide any evidence that the type of blood affects the longevity of *Wolbachia*-infected mosquitoes.

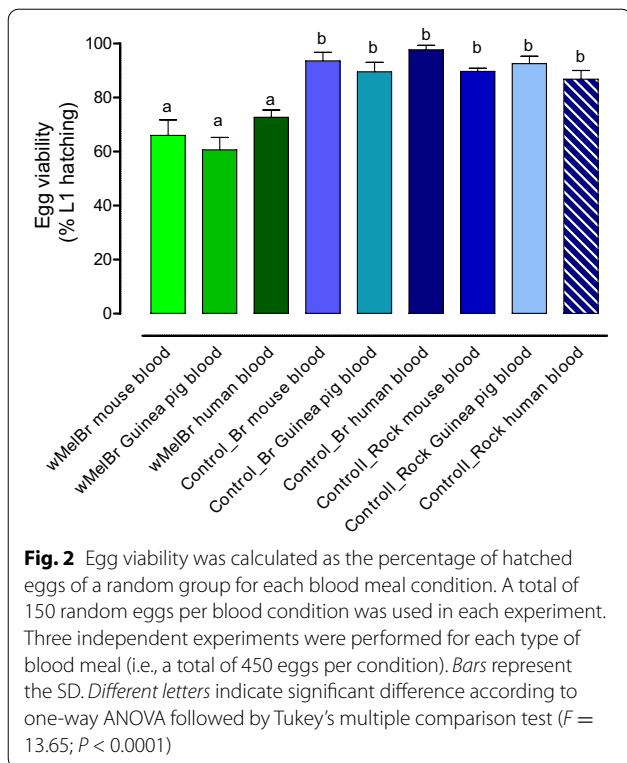
### Selection of *Wolbachia*-infected *Ae. aegypti* fed on mouse blood after five and ten generations

We analyzed some essential parameters necessary to maintain a prolific *wMelBr* lineage in the laboratory, such as fecundity (number of eggs per female) and fertility (percentage of hatched eggs) after five and ten generations. The fecundity and fertility of *Wolbachia* (*wMelBr*) females when fed mouse blood were satisfactory. In general, after five and ten generations, fecundity and fertility were similar for all treatments (Table 1). These results show that it is possible to artificially select and maintain *Ae. aegypti* with the *Wolbachia* *wMel* strain on mouse blood.



**Evaluation of *Wolbachia* density over generations in *Ae. aegypti* fed on mouse blood**

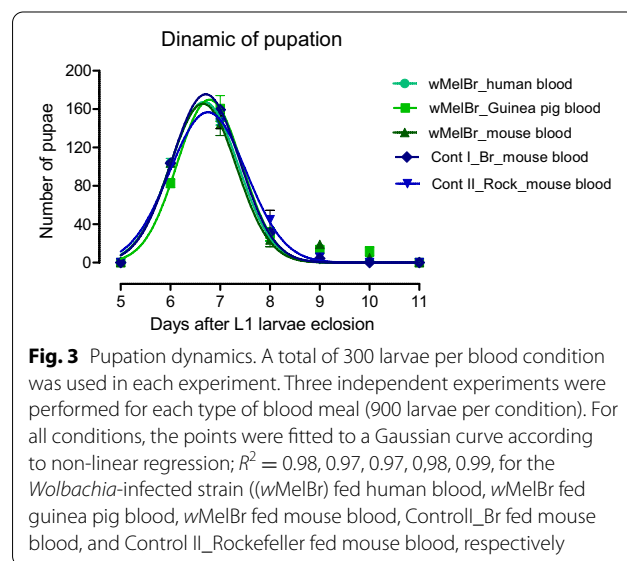
As it was possible to select *Ae. aegypti* with *Wolbachia* that fed on mouse blood and generated viable eggs, we

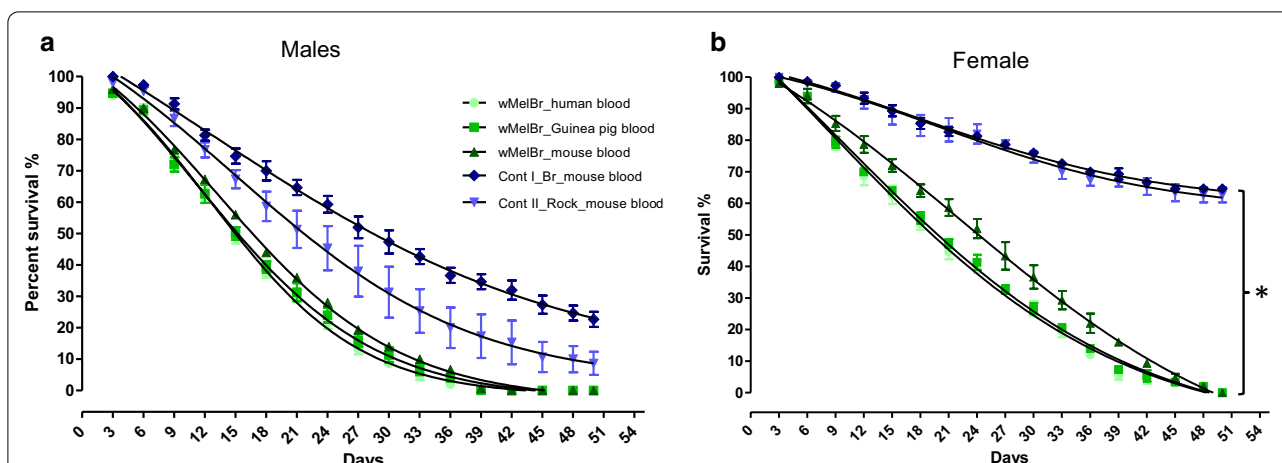


decided to investigate whether this lineage would maintain its level of *Wolbachia* infection over many generations using only this blood source. In general, feeding on mouse blood did not have a detrimental effect on *Wolbachia* density in *Ae. aegypti* over the generations (Fig. 5). The presence of *Wolbachia* in the colonies of the wMelBr strain after 35 generations indicated that it is safe to feed the female mosquitoes on mouse blood. There was a significant difference between the F15, F25, and F35 ( $P < 0.05$ ) generations according to the *t*-test following the Mann–Whitney test for pairwise comparisons (mouse blood  $\times$  human blood). In all cases, the relative *Wolbachia* density of mosquitoes that fed on mouse blood was higher than that of mosquitoes kept under the same conditions but fed human blood.

**Discussion**

*Aedes aegypti* is responsible for the transmission of many arboviruses worldwide, to many of which there are no specific antivirals or vaccines available [21, 22]. To overcome issues related to the massive use of insecticides, alternative and innovative approaches to the control of mosquito populations are currently being tested and evaluated, such as the use of bacteria of the genus *Wolbachia* [23–25]. Methodology based on the field release of *Ae. aegypti* infected with *Wolbachia* for dissemination amongst wild mosquito populations has been successfully tested by the World Mosquito Program [11] in many countries, e.g., Brazil, Colombia, Mexico, and Vietnam [27]. This strategy requires the large-scale dissemination of *Wolbachia*-infected mosquitoes, and the mass production of infected mosquitoes is mainly dependent on blood feeding. Laboratory strains of *Ae. aegypti* are





**Fig. 4a, b** Longevity of progeny of females fed different sources of blood. Survival of *Ae. aegypti* infected with *wMelBr* (green curves) was compared with that of naturally uninfected and uninfected laboratory strains (blue curves). For each strain, three replicate groups of 25 male (a) or female (b) mosquitoes were maintained at 25 °C, 70–80% relative humidity, under a 12:12-h light:dark photoperiod. Three independent experiments were carried out in each case. There was no significant difference in male longevity according to one-way ANOVA followed by Tukey’s multiple comparison test ( $F = 2.4, P = 0.06$ ). Regarding the females, there was no significant difference between the controls [*Wolbachia*-free mosquitoes of the Rockefeller lineage (*Rock*) × *wMelBr*] or among the *Wolbachia* strains. However, there was a significant difference between the progenies of infected mosquitoes and the controls according to one-way ANOVA followed by Tukey’s multiple comparison test ( $F = 10.57, P < 0.05$ )

generally able to withstand lab conditions when feeding on the blood of a wide variety of non-human vertebrates, including guinea pigs, chickens, mice, and sheep [17, 28]. However, feeding by a virulent strain (*wMelPop*-infected mosquitoes) on non-human animal blood sources (mouse, guinea pig, or chicken) resulted in the loss of reproductive capacity, reduced fecundity and hatching rate [14]. McMeniman et al. [14] showed that there is an interaction between the host blood and egg development in *wMelPop*-infected *Ae. aegypti* [14]. Based on this finding, the use of standard laboratory animal blood (chicken, mouse, guinea pig and sheep) was

not considered suitable for feeding mosquitoes carrying the *wMelPop* strain. Thus, because of the proven higher levels of egg viability and other physiological parameters analyzed in mosquitoes carrying the *wMelPop* strain, the World Mosquito Program currently uses human blood to this end [11, 13].

*Ae. aegypti* infected with the *wMel* strain is highly refractory against dengue, Zika, chikungunya, and Mayaro [5, 9, 10, 26, 27]. The use of this mosquito for population replacement strategies requires its stable mass production. In this study, we aimed to overcome the necessity for human blood for mass rearing *wMel*

**Table 1** Fecundity and viability of F5 and F10 *Wolbachia*-infected *Aedes aegypti* (*wMelBr*) females fed mouse blood

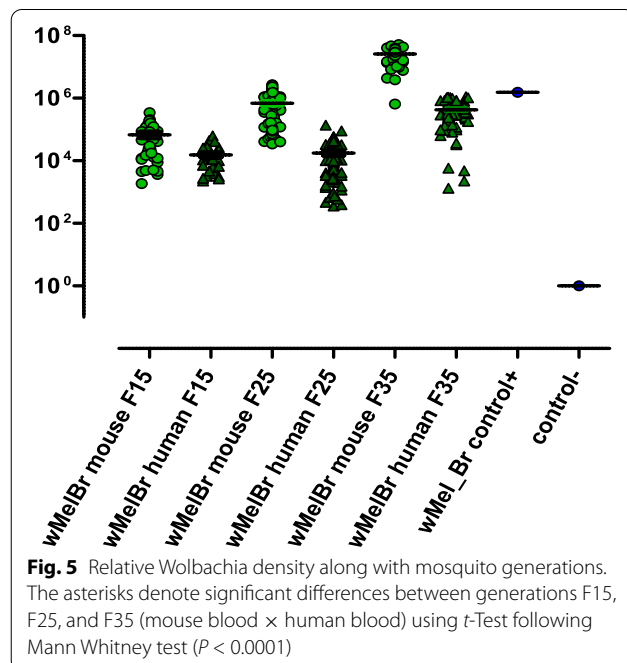
Generation	Strain	Nulls (%)	Fecundity (%)	Fertility (%)
F5 (n = 30)	<i>wMelBr</i> (mouse)	16.6	84.6 (± 15.7) a	72.0% (± 28.5%) a
	<i>wMelBr</i> (human)	10	47.2(± 16.7) b	87.4% (± 18.5%) a
	<i>wMelBr</i> (human to mouse)	10	66.4 (± 19.7) a	78.6% (± 20.0%) a
	Control_rock (mouse)	3	66.7 (± 9.9) a	84.6% (± 16.1%) a
F10 (n = 30)	<i>wMelBr</i> (mouse)	16.6	61.2 (± 37.6) a	77.5 (± 13.2) a
	<i>wMelBr</i> (human)	10	61.0(± 24.8) a	71.5% (± 17.5%) a
	<i>wMelBr</i> (human to mouse)	10	69.7(± 26.5) a	63.7% (± 11.5%) a
	Control_rock (mouse)	3	127.3(± 27.6) b	75.5% (± 14.9) a

Nulls Percentage of females that did not lay eggs, Fecundity mean number of eggs per female, Fertility mean number of eggs hatched, *wMelBr* *Wolbachia*-infected strain, *Rock* *Wolbachia*-free mosquitoes of the Rockefeller lineage

Different lowercase letters represent statistically significant differences among strains in each generation ( $P < 0.05$ )

mosquitoes. In addition to human blood, we tested the blood of guinea pigs and mice for mosquito feeding and assayed and compared some physiological parameters.

Our first experiment compared the fecundity of females using guinea pig and mouse as blood sources in comparison with human blood. Overall, the fecundity decreased significantly in *Wolbachia*-infected mosquitoes compared to mosquitoes without the bacterium. However, in females with *Wolbachia* (*wMel*), the number of eggs laid did not statistically differ, regardless of the blood source (Fig. 1). Using the same strain of *Wolbachia*-infected mosquitoes (*wMel*), Dutra et al. [15] observed a similar average fecundity when they fed the females with human blood. On the other hand, Paris et al. [29] found that the fecundity of *wMel* mosquitoes fed on pig or sheep blood decreased, although not significantly, compared to that of mosquitoes that fed on human blood. Taken together, these data suggest that it is not only the bacterial strain that affects fecundity when the blood meal comprises non-human blood, but also the type of host vertebrate used for the mosquito blood meal. Our data showed that egg viability was impaired when *Wolbachia* was present; however, there was no significant difference among the three sources of blood (Fig. 2). Paris et al. [29] showed that the viability of eggs of females infected with *wMel* that fed on sheep or pig blood was impaired. Egg viability is more critical than fecundity to guarantee the perpetuation of *Wolbachia*-infected mosquitoes in the field.



Some parameters can influence larval development time and pupae formation in *Aedes*, such as temperature, nutrient supply, and insect growth regulators [30, 31]. Interestingly, in our assays, neither *Wolbachia* presence nor the blood source offered to parent mosquitoes affected the development time of immatures, since the pupation dynamics were not significantly different (Fig. 3). Under the conditions used here, the majority of pupae emerged between days 6 and 7 after egg hatching, as expected for this species when larval breeding temperatures are between 24 and 28 °C [30, 31].

In all analyses of longevity, male survival was lower than that of females, as expected for *Ae. aegypti* according to studies carried out under similar laboratory conditions [32, 33]. In general, the *wMelBr* mosquitoes had lower survival rates than the naturally uninfected and uninfected laboratory strains. However, compared with the *wMelPop* strain [33], the life span of *wMel* was much improved. On the other hand, it is important to note there was no evidence that the type of blood influenced adult longevity (Fig. 4).

Paris et al. [29] found that *wMel*-infected *Ae. aegypti* raised on non-human blood sources exhibited reduced *Wolbachia* density compared to when they were raised on human blood. *Wolbachia* density of a mosquito could influence transmission blocking of arboviruses such as Zika, dengue and chikungunya. If low *Wolbachia* densities are inherited over generations, the use of *Wolbachia* infection as a strategy to reduce the transmission of arboviruses could become ineffective [34]. Due to their diverse and overlapping host-feeding patterns, important mosquito vectors such as *Culex pipiens* and *Ae. aegypti* can be kept under laboratory conditions with different sources of blood. Further studies should be carried out to examine the fitness of mosquitoes fed different kinds of host blood as a means of increasing the size of laboratory colonies of these vectors [35].

We were able to overcome problems associated with the use of human blood for mosquito rearing by using mouse blood to feed females, and proved, for the first time, that it is possible to maintain strains of *Ae. aegypti* infected with *wMel* on this type of blood without affecting the density of the bacterium over generations and without significant fitness costs to the mosquitoes.

#### Acknowledgments

We would like to thank Luiz Paulo Brito and Catia Cabral for their expert technical support, and Dr. José Bento Pereira Lima and his lab team (IOC-Fiocruz) for providing the Rockefeller strain. We would also like to thank Prof. Scott O'Neill (Monash University) for providing the original *wMel* line.

#### Authors' contributions

LCF, RVB, FDC, and LAM conceived and designed the experiments. LCF, FDC, and APSC performed the experiments. LCF, RVB and FDC analyzed the data. LCF, RVB, FDC, APSC, and LAM wrote and revised the manuscript. All the authors read and approved the final manuscript.

### Funding

This work was co-funded by the Brazilian Ministry of Health, the Brazilian National Council for Scientific and Technological Development, the Brazilian National Science and Technology Institute, IOC-Fiocruz and the Minas Gerais Research Funding Foundation.

### Availability of data and materials

Data supporting the conclusions of this article are included in the article. All the datasets used in this manuscript are available for consultation from the corresponding author upon request, and can be accessed at the Laboratório de Biologia Molecular de Insetos (IOC-FIOCRUZ).

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

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Received: 26 June 2020 Accepted: 5 November 2020

Published online: 06 January 2021

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