

# Infection of mice with a Brazilian clinical isolate of Mayaro virus results in mild disease

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## Abstract

### Background

Mayaro virus (MAYV) is the etiologic agent of Mayaro fever and is currently considered an emergent and neglected disease. Several factors indicate the possibility of urbanization of this virus that may configure the disease as a major public health problem. Cases of Mayaro fever have been confirmed in different Brazilian states, which supports the possible adaptation of the vector to the urban environment. The characterization of the Brazilian isolate of Mayaro virus (MT/SINOP/210/2011) in a murine model may help in the elucidation of disease mechanisms, pathogenesis, and contribute to advancing the development of treatments and prophylactic measures.

### Methods

IFN- $\alpha/\beta$  receptor-deficient (B6.129S2-Ifnar1) or congenital controls (129Sv/Ev) female and male mice at 21 days of age were inoculated via footpad with 105 TCID<sub>50</sub> of MAYV or PBS in a volume of 20  $\mu$ L. Survival, body weight, and paw edema were assessed daily. A clinical score was determined using a numerical value and employed in phenotypic disease monitoring. Three and six days after infection, blood, joints, and muscles were collected for hematology and histopathology evaluation, respectively.

### Findings

Knockout mice were susceptible to infection, developing clinical signs of the disease, which was not observed in animals with an intact immune system. There were no changes in the animals' body weights and the score evaluation demonstrated that

infected male mice showed more pronounced clinical signs compared to females, such as diarrhea, purulent conjunctivitis, and plantar cushion edema. MAYV infection in knockout mice led to a low lethality rate. Histopathological analysis revealed that there was a greater inflammatory response in the acute phase of the disease in infected knockout animals compared to wild-type animals. Mild edema and vasculitis were seen in both knockout and wild-type animals. Finally, there was an intense cellular infiltrate of lymphocytes in the acute phase of the disease in knockout and wild-type.

### Interpretation

The infected knockout animals showed mild clinical signs of the disease. The infection had a benign course and most were able to control the infection. The signs observed lasted only a few days, coinciding with the period of the acute phase of Mayaro fever in humans, therefore, the disease manifests itself mildly in humans and mice, demonstrating that the circulating strain MT/SINOP/210/2011 has low virulence and the experimental model is compatible with the course of infection in human patients.

## Introduction

Mayaro virus (MAYV) is the etiologic agent of Mayaro fever, an acute febrile infectious disease. MAYV is part of the antigenic complex called Semliki Forest, along with Chikungunya, Sindbis, and Ross River viruses [1]. MAYV belongs to the *Togaviridae* family and the *Alphavirus* genus. It is an arbovirus that single-stranded RNA of positive polarity with icosahedral capsid, enveloped and spherical. [2] MAYV can infect, replicate, and spread in both vertebrate and invertebrate hosts. In endemic regions of Latin America, the transmission cycle occurs mostly in sylvatic and rural areas. In this type of cycle, the hematophagous mosquito *Haemagogus janthinomys* is classified as the main vector and the non-human primates are considered primary hosts. However, to a lesser extent, other types of mosquitoes may participate as occasional vectors, such as those of the *Mansonia* and *Culex* genus; as well as other vertebrates may be secondary hosts (viral reservoir), such as rodents, reptiles, and birds. Infection in humans is classified as accidental, and occurs when humans are exposed to sylvatic reservoir habitats [3,4]

In 1954, MAYV was identified for the first time in blood samples from rural workers in Trinidad and Tobago, therefore it suggests that the virus is native to the Americas. In Brazil, MAYV was isolated the following year, in 1955, nearby the riverside of Guamá River, in the city of Belém. Mayaro virus was the cause of an outbreak of an acute febrile illness that affected 100 rural workers in the region. Since then, some sporadic cases, outbreaks, and small epidemics of Mayaro Fever have been reported in several countries in tropical regions of South and Central America. Clinical cases of the febrile illness and the isolation of the virus have already been reported in countries such as Brazil, Peru, Suriname, French Guiana, Guyana, Venezuela, Colombia, Ecuador, Panama, and Bolivia. Serological investigations also indicate the presence of the virus in Costa Rica, Guatemala, and Mexico [6,7]. In the Brazilian epidemiological context, cases of Mayaro Fever have been confirmed in states in the North, Midwest, and Northeast regions. Studies indicate the circulation of the virus in the Southeast, both in urban regions of Rio de Janeiro and in the state of São Paulo, suggesting the vector's adaptation to the urban environment. [6,8,9]

Phylogenetic studies of MAYV based on the E2/E1 gene sequence validate the existence of three genotypes. The D genotype is found in different countries in South America. The N genotype was isolated only in Peru. The L genotype, on the other side, is mostly restricted to the northern region of Brazil, where it was first isolated, specifically in the state of Pará in 1955. However, recent studies show the circulation

of this genotype in the central-western region, as in the state of Mato Grosso and, interestingly, in Haiti [10]. Mavian (2017) suggested that the 2015 Haiti isolate could be a recombination of strains from São Paulo and Acre, which came from Mato Grosso, this demonstrates the ability to generate new viral strains [11]. Among the strains coming from the Brazilian state of Mato Grosso, in 2011, the MT/SINOP/210/2011 (GenBank no. KF305672.1) was isolated in a case of human infection in the city of Sinop (MT) [12,13] and there are still no studies in the literature that characterize it *in vivo*. Besides the problem of the possibility that new strains may arise, MAYV is considered emerging and several factors indicate the viability of urbanization of this virus that can configure the disease as a major public health issue for being a possible cause of epidemics [14], among these the Mayaro virus is part of the same Semliki Forest Complex as Chikungunya, which has an established history of adaptation to the urban environment. In addition, studies have shown the possibility of MAYV transmission by *Aedes aegypti* [15] and the occurrence of cases of MAYV infection in regions near large cities infested by this same vector [10]. The feasibility assumption of urban adaptation of the vector, in conjunction with anthropogenic factors such as rapid urbanization, climate change, and increased population mobility, raise the risk of virus distribution to other continents, generating the possibility of disease outbreaks in countries with no history of infection [5,16].

Therefore, the study aims to characterize the Brazilian isolate of Mayaro virus (MT/SINOP/210/2011) in a murine model.

## Materials and methods

### Cells and virus

Vero CCL-81 cells from the cell bank of Aggeu Magalhães Institute-Fiocruz Pernambuco were used in the cultivation and titration of the Mayaro virus strain (MT/SINOP/210/2011, GenBank: KF305672.1) isolated from a patient in Sinop, Mato Grosso, Brazil [13]. Vero cells were cultured with DMEM culture medium (Dulbecco's Modified Eagle Medium) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin (10.000 U/mL) at 37°C and 5% CO<sub>2</sub> atmosphere. For making the viral stock, cells were infected with about 80-90% confluence of the cell mat and maintained with DMEM supplemented with 2% fetal bovine serum and 1% penicillin/streptomycin (10.000 U/mL) at 37°C and 5% CO<sub>2</sub> atmosphere for 48h or with 20% of the cellular mat adhered. The culture supernatant was stored at -80°C and the viral titer was defined by the TCID<sub>50</sub>/mL method.

### Mice

IFN- $\alpha/\beta$  receptor-deficient mice (B6.129S2-Ifnar1) and congenic control mice (129Sv/Ev) were obtained from the Rodent colony Animal Facilities Complex, Federal University of Uberlândia, Brazil.

21-day-old male and female mice raised in a specific pathogen-free facility were used for all *in vivo* experiments.

The animals were placed in mini isolators, appropriate for the species, and received autoclaved *Pinus ellioti* wood shaving as bedding, positioned in ventilated racks. Water and feed were autoclaved in the animal care unit (REBIR/UFU) and provided without restriction (*Ad libitum*). The animals were kept in a 12/12 hour light/dark cycle, respectively, in a room with temperature and humidity control (22°C  $\pm$  2°C/ 55%  $\pm$  5%).

All procedures with animals were approved by the Ethics Committee for the Use of Animals (CEUA) of the Federal University of Uberlândia, Brazil, and followed all current Brazilian legislation (Law No. 11.794/2008) of the National Council for the

Control of Animal Experimentation (CONCEA). Protocol CEUA 124/2021.

## Pathogenicity assessment

Ten male or female mice, knockout or wild-type, were inoculated with MAYV subcutaneously (footpad) with  $10^5$  TCID<sub>50</sub> of MAYV or phosphate-buffered saline (PBS) in a volume of 20  $\mu$ L. Survival, weight loss, and signs of illness were monitored. A score was adapted from Orozco (2012) [17] and assigned with a numerical value designated to a group of clinical signs as described: 0. healthy, 1. mild signs of lethargy, bristly hair, 2. increased lethargy, bristly hair, eye irritation, diarrhea, stooped posture, 3. increased lethargy, bristly hair, purulent conjunctivitis, diarrhea, stooped posture, 4. lethargic animal, bristly hair, purulent conjunctivitis, diarrhea, hunched posture, limited mobility, 5. dying with minimal mobility and inability to eat or drink water. In addition, each animal's clinical score was assigned to the durability and intensity of the illness signs presented.

The animals were weighed daily and the footpad measurements (height) were taken with the aid of a digital caliper. The degree of swelling was expressed as the relative increase in footpad size compared with pre-infection (day 0). Three and six days post-infection, three animals per group had 200  $\mu$ L of blood collected and were euthanized and necropsied. The tibiotarsal joint and its associated muscles were also collected. The muscles and joints were intended for histopathology studies.

## Histopathology evaluation

A histopathological examination was performed in the tibiotarsal joint. Tissues were fixated with 10% neutral buffered formalin. Tibiotarsal joints were decalcified with EDTA 10%, pH 7. After histological processing steps (dehydration, clearing, embedding), 3 $\mu$ m sections were obtained by microtomy, stained with hematoxylin and eosin, and scanned using Aperio AT2 (Leica Biosystems, Nussloch, Germany) with 400x magnification.

## Histological analysis of ankle joint

The evaluation of tissue response was based on qualitative and semiquantitative observations of aspects of inflammatory reaction [18]. Two independent observers were calibrated for the presence or absence of: inflammatory response, neutrophils, macrophages, lymphocytes, plasma cells, giant foreign body cells, necrotic tissue, edema, and fibroblasts/fibrosis. These characteristics were scored for intensity: (–) absent, (+) mild, (++) moderate, (+++) intense.

## Hematological characterization

To perform the hematological characterization, blood samples were collected three and six days post-infection from three animals per group, the collected volume was 200 $\mu$ L. Preceding the blood collection, the animals were submitted to Isoflurane inhalation anesthesia. The collection was conducted via retro-orbital plexus using a heparinized microhematocrit capillary tube (Precision®). The blood was poured into tubes with disodium salt of ethylenediaminetetraacetic acid (EDTA K2) 0,5ml (Vacuplast®). After the collection, the animals were euthanized by cervical dislocation.

The blood samples were submitted to the automatic hematology analyzer Hematoclin 2.8 VET-R666 (Bioclin/Midray®), the parameters analyzed were white blood cell count (WBC), red blood cell count (RBC), hemoglobin and hematocrit measures and platelet count (PLT). Manual differential leukocyte counts were performed on blood

smears stained with panoptic fast stain (LaborClin, Santa Bárbara, Brazil).

## Statistical analysis

All analyzes were performed using GraphPad PRISM software 6.0 (GraphPad Software, USA). ANOVA test, followed by Duckey's test and Dunnet's test were used for all graphs analyses. For hematological characterization, one-way ANOVA was used with Tukey's post hoc and Kruskal-Wallis with Dunn's post hoc test.

## Results

### The clinical manifestations developed from MAYV infection were self-limited enough not to lead to weight loss of the animals

Sudden weight loss can be a consequence of clinical manifestations of diseases or infections that enable animals to maintain their healthy habits. Arthritogenic diseases and diseases that affect the muscular system, such as some arboviruses, can evolve to a condition serious enough to be considered disabling. Thus, the body weight was measured daily as a way to monitor the development of the MAYV infection in mice. It was found that MAYV does not induce weight loss in infected adult animals, both wild-type and knockout, nor during the acute phase of the disease (3-5 days) (Fig 1A-B). Therefore, the clinical manifestations of the disease, although present, are self-limited and do not generate abrupt weight loss in the animals. The wild-type and knockout control groups (PBS) also showed no weight loss.

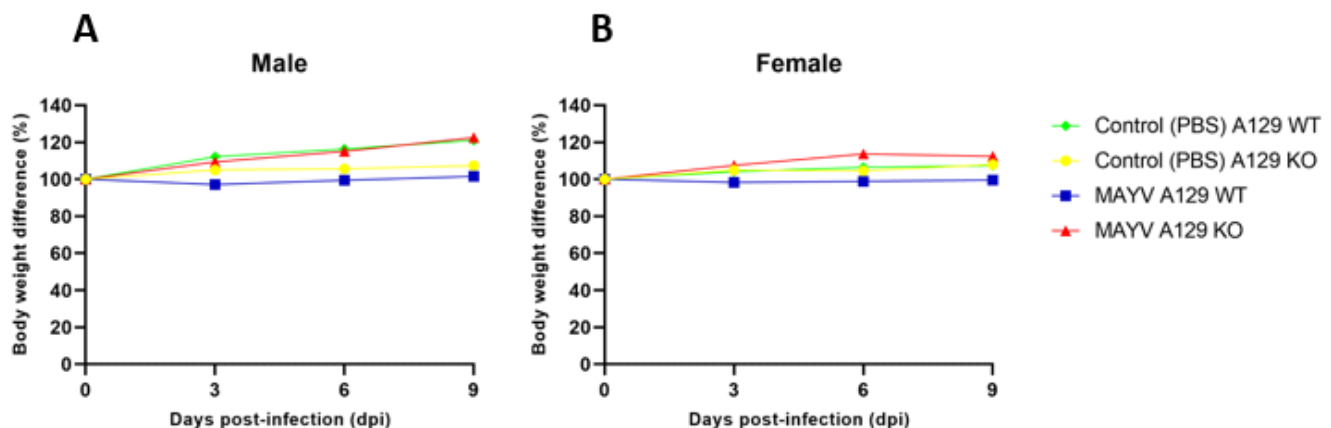


**Fig 1. MAYV did not induce weight loss in knockout and wild-type mice.** A: Male body weight difference. B: Female body weight difference. The daily weighing was performed up to 9 days post-infection. Infected knockout and wild-type mice showed no statistical difference in weight loss compared to the control groups.

### MAYV infection caused low lethality rate in knockout and wild-type animals

MAYV infection in knockout mice led to a low lethality rate. There was only one case of death in infected knockout male mice in the acute phase of the disease, when the clinical signs are more severe (3 days post-infection). All the infected knockout female mice survived the infection. In the group of infected wild-type mice, there were no cases of deaths, as seen in the control groups (Fig 2A-B).

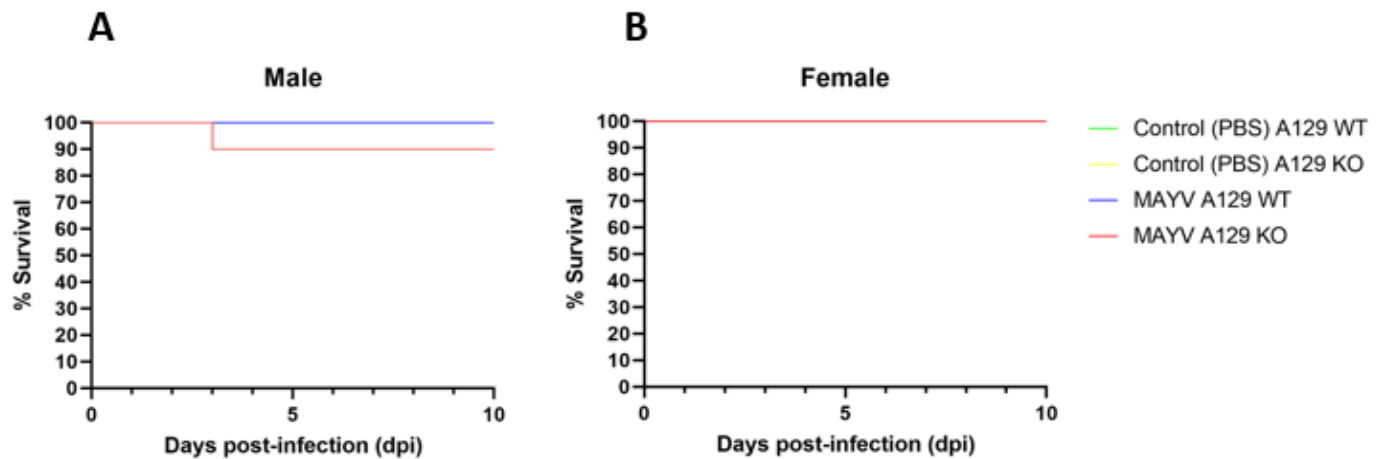


**Fig 2. The lethality rate associated with MAYV infection is low.** A: Survival (%) in males. B: Survival (%) in females. Survival was monitored daily, up to 10 days post-infection in each infectious and mock group. Only one case of death was reported in the group of infected knockout male mice.

### Infected knockout mice show evident clinical signs, with male animals having more accentuated phenotypes of MAYV infection

It was observed that only infected knockout females and males showed a clinical score of the disease, furthermore, males expressed more accentuated signs compared to females (Fig 5). Infected knockout female mice ( $n=8$ ) presented clinical scores of 0 (healthy), 1, and 2 (Fig 3B). Half of this group maintained healthy, 3 animals exhibited clinical score 1, with signs of lethargy, and bristly hair and only 1 animal presented clinical score 2, with increased lethargy and bristly hair and stooped posture (Fig 4A). There was a significant statistical difference in the clinical scores presented by the infected knockout females, when compared to the PBS controls and infected wild-type groups (Fig 3B). The intensity of the piloerection, stooped posture, and lethargy phenotypes displayed by the infected females remained between mild and medium (Fig 5). Infected knockout males ( $n=7$ , one case of death) had clinical scores of 0 (healthy), 2 and 3 (Fig 3B). Score 3 was only achieved by this group, 5 animals presented increased lethargy, bristly hair (Fig 4A), purulent conjunctivitis (Fig 4D), diarrhea (Fig 4C), and stooped posture (Fig 4A). Only one animal did not show disease phenotypes. The statistical significance of the latter group was higher about the female, when compared with the PBS controls and infected wild-type groups. The phenotypic intensity of infected males reached an accentuated level (Fig 5). The fact that only knockout animals show clinical scores with males showing more accentuated phenotypes and a case of lethality (Fig 2A), demonstrates that the IFN pathway plays a key role in controlling infection and raises the hypothesis that gender can influence the disease expression. However, although some infected knockout animals showed signs of the MAYV infection, most expressed mild signals and the lethality rate remained low. Thus, although they are animals with a compromised immune system in the interferon pathway, most were able to control the infection.

Wild-type mice infected with MAYV showed no clinical signs of the infection, all of them obtained a clinical score of 0, considered healthy, as well as wild-type and knockout control animals (Fig 3A-B).

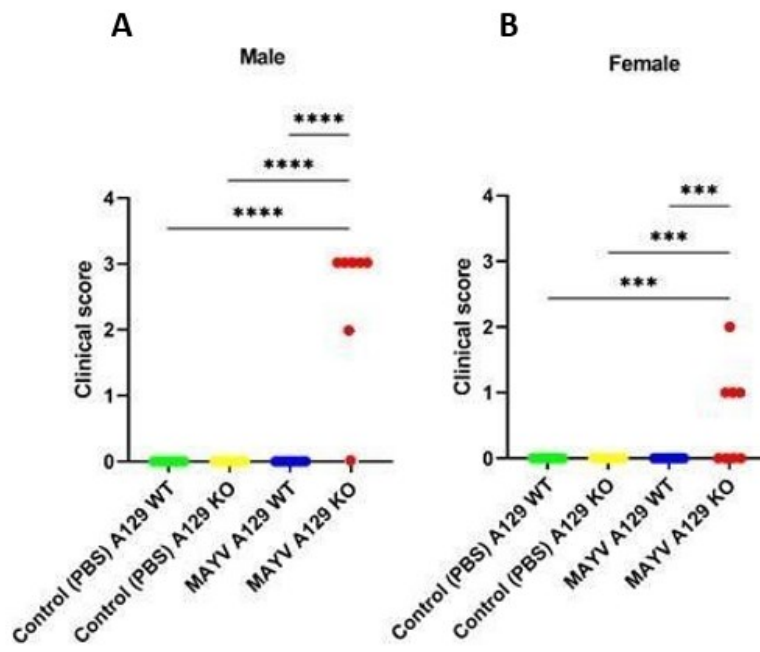


**Fig 3. Clinical scores rated from 1 to 5 are assigned daily for the infected knockout and wild-type animals or controls.** A: Clinical score presented by males. B: Clinical score presented by females. Infected male and female knockout mice showed clinical signs of the disease, but some of the males showed scores up to 3, with more accentuated signs compared to the females.

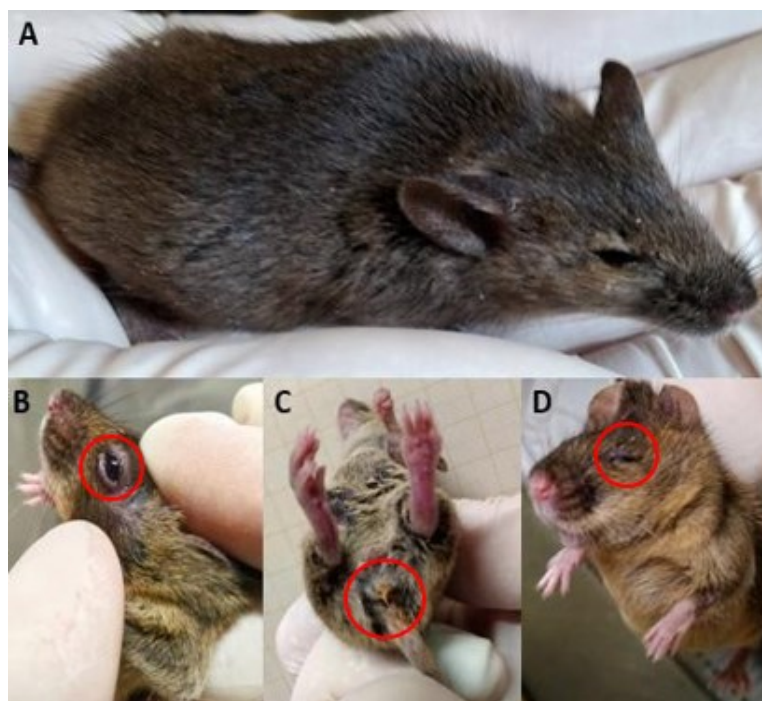


**Fig 4. Clinical signs presented by MAYV infected knockout male mice.** A: animal with bristly hair and stooped posture. B: eye irritation. C: diarrhea. D: purulent conjunctivitis.

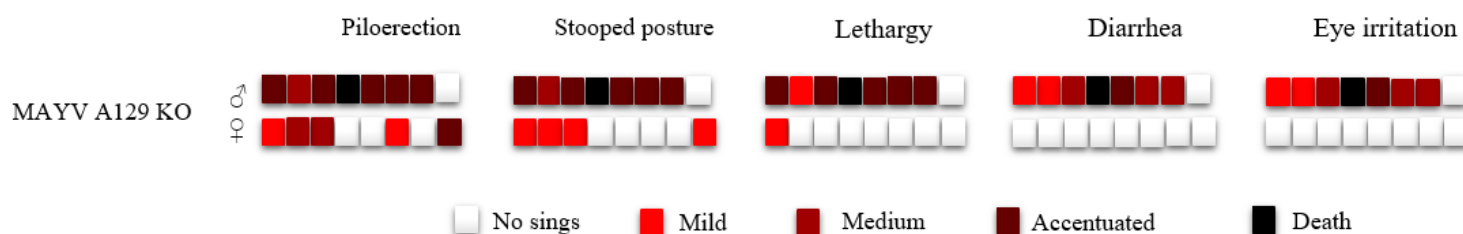


**Fig 5. Infographic of phenotypic intensity caused by MAYV infection in knockout mice.** The phenotypic intensity was classified as mild, medium, accentuated, and culminated in death. The classification was determined for each individual in the infected knockout groups. Male knockout animals showed medium and accentuated phenotypes, with one case of death. The phenotype in females, in contrast, was mild.

### The plantar swelling phenotype is apparent in MAYV infection

Although the results were not statistically significant (Fig 6A-B), the observation of the plantar swelling phenotype in infected wild-type and knockout animals was apparent (Fig 7C-D), the latter with a more intense phenotype. In some of these animals the swelling was visible even 6 days post-infection, not only in the acute phase of the disease (3 days post-infection). Swelling in the plantar region may be a strong indication of joint and muscle damage caused by MAYV, leading to an intense inflammatory process. During the process of measurement with the digital caliper, it was possible to notice the increased sensitivity that the infected animals presented in the region, through the immediate withdrawal reflex, indicating pain.

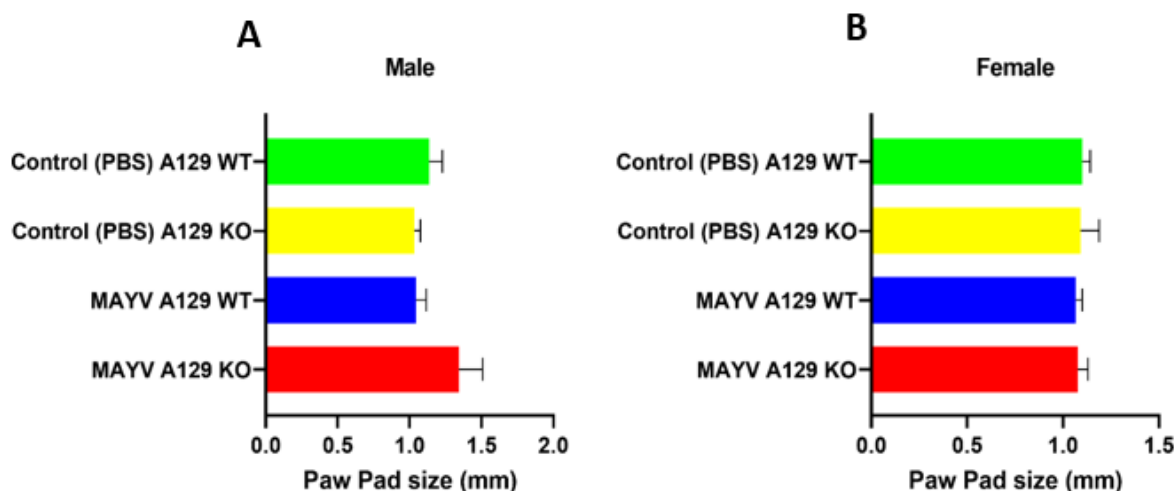


**Fig 6. Size evaluation of the paw pad to verify swelling.** A: Paw pad size of males. B: Paw pad size of females. The size of the paw pad was measured daily with the aid of a digital caliper to ascertain the swelling in the plantar region. Joint and muscular swelling is a classic symptom of Mayaro Fever. The results in both male and female animals graphs did not show statistically significant.

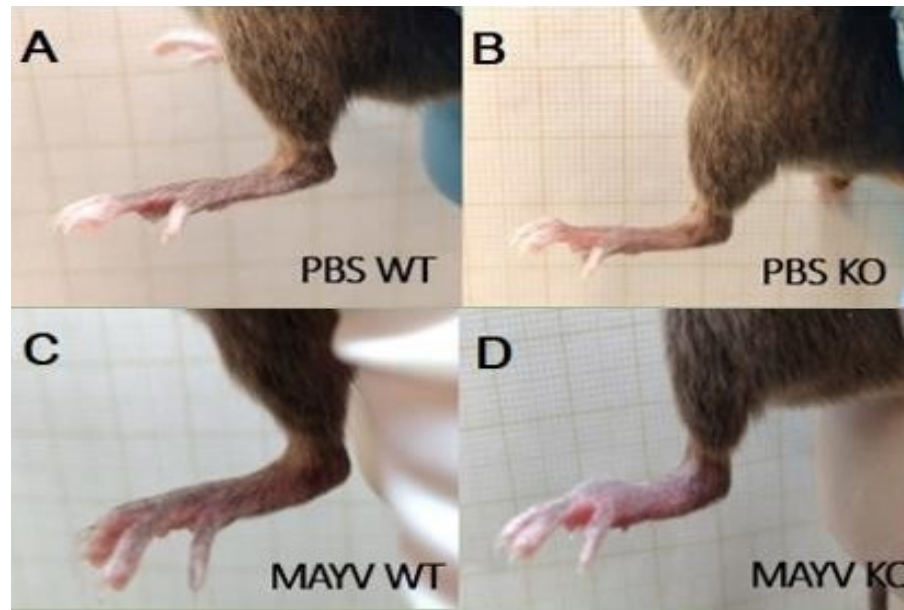


**Fig 7. The plantar swelling phenotype in infected knockout and wild-type mice.** A: No plantar swelling in the control (PBS) wild-type group. B: No plantar swelling in the control (PBS) knockout group. C: Plantar swelling in infected wild-type group. D: Plantar swelling in infected knockout group. Plantar swelling phenotype was observed in both infected groups, the knockout animals with higher expressivity in edema. The measurement was taken with the aid of a digital caliper.

### A129 knockout mice showed a more intense inflammatory response in the tibiotarsal joint

We also performed the qualitative histopathological analysis of the ankle joint (Table 1, Fig 8), and was observed a more intense inflammatory response in infected knockout mice than in wild-type mice. Furthermore, the inflammatory response was more intense in the acute phase of the disease (3 days post-infection) and it is marked by the presence of lymphocyte-predominant cells. Additionally, there was also the presence of edema and fiber degeneration in some samples.

**Table 1-Qualitative analyses of the cell infiltrate in the tibiotarsal joint**

|                          | MAYV A129 WT |       | MAYV A129 KO |       |
|--------------------------|--------------|-------|--------------|-------|
|                          | 3 DPI        | 6 DPI | 3 DPI        | 6 DPI |
| Inflammatory response    | ++           | +     | +++          | ++    |
| Vasculitis               | +            | +     | +            | +     |
| Neutrophilic leucocytes  | -            | -     | -            | -     |
| Macrophages              | -            | -     | -            | -     |
| Lymphocytes              | ++           | +     | +++          | ++    |
| Plasma cells             | -            | -     | -            | -     |
| Giant foreign body cells | -            | -     | -            | -     |
| Fiber degeneration       | +            | -     | -            | -     |
| Necrotic tissue          | -            | -     | -            | -     |
| Edema                    | +            | +     | +            | +     |
| Fibrosis                 | -            | -     | -            | -     |

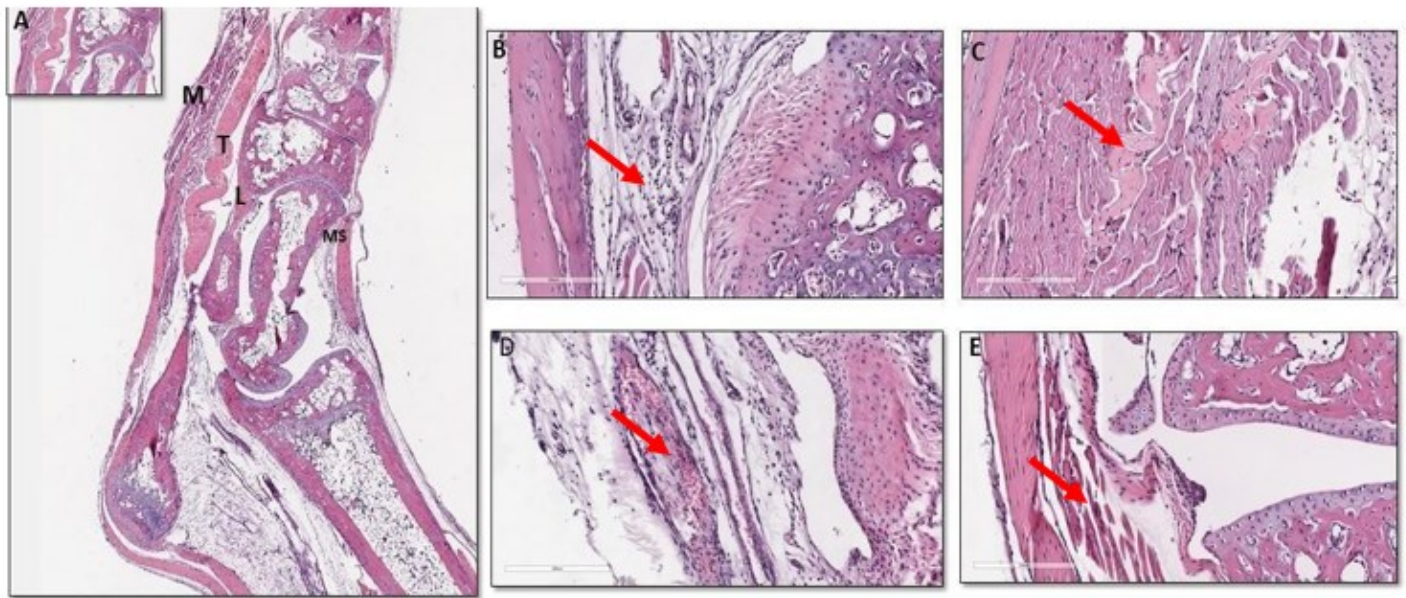


**Fig 8. Histological analysis.** After 3 or 6 days post-infection (dpi), mice were euthanized and histological sections of tibiotarsal joint (ankle joint) and associated tissues were stained in HE for analysis of the inflammatory response. Representative images of the evaluated alterations: A: Representative image of ankle joint. B: Infiltrated the synovial membrane (6 dpi, MAYV A129 KO). C: Edema and fiber degeneration (6 dpi, MAYV A129 WT). D: Vasculitis (3 dpi, MAYV A129 KO). E: Infiltrated in muscle (3 dpi, MAYV A129 KO). M: Muscle, T: Tendon; L: Ligament; MS: Synovial Membrane. Images A (200X magnification, scale bar = 3mm). Images B, C, D, E (200X magnification, scale bar = 200  $\mu$ m).

## Hematological profile

Studies report changes in hematological parameters in arbovirus infectious disease [19]. In our study, there were no significant differences in red cells, white blood cells, and platelet counts (Tables 2 and 3).

**Table 2- Hematological profile: red series**

| Parameters                                 | Experimental groups, median (range) |                       |              |                       |                       |              | p-value         |
|--------------------------------------------|-------------------------------------|-----------------------|--------------|-----------------------|-----------------------|--------------|-----------------|
|                                            | MAYV A129<br>WT (3DPI)              | MAYV A129<br>WT(6DPI) | Control (WT) | MAYV A129<br>KO(3DPI) | MAYV A129<br>KO(6DPI) | Control (KO) |                 |
| RBCC <sup>1</sup><br>(10 <sup>6</sup> /uL) | 10.67(1.0)                          | 10.04 (0.2)           | 11.47(2.7)   | 9.950(2.2)            | 9.780(2.2)            | 9.360(1.5)   | ns <sup>4</sup> |
| Hb(g/dL) <sup>2</sup>                      | 17.40(1.6)                          | 16.25(1.8)            | 19.00 (4.4)  | 16.85(3.9)            | 16.50(3.2)            | 15.75(2.9)   | ns <sup>5</sup> |
| Hct (%) <sup>3</sup>                       | 53.90(4.2)                          | 50.70(4.7)            | 59.19(13.8)  | 50.40(9.5)            | 48.50(10.4)           | 47.85(7.7)   | ns <sup>5</sup> |

N=4 (2 males and 2 females)

1RBCC: red blood cell count;2: Hemoglobin;3: Hematocrit; 4Kruskal-Wallis with Dunn's post hoc test,5 one-way ANOVA with Tukey's post hoc, p<0.05; ns: not significant

**Table 3- Hematological profile: white blood and platelet**

| Parameters                                | Experimental groups, median (range) |                       |              |                       |                       |              | <i>p</i> -value |
|-------------------------------------------|-------------------------------------|-----------------------|--------------|-----------------------|-----------------------|--------------|-----------------|
|                                           | MAYV A129<br>WT (3DPI)              | MAYV A129<br>WT(6DPI) | Control (WT) | MAYV A129<br>KO(3DPI) | MAYV A129<br>KO(6DPI) | Control (KO) |                 |
| WBCC <sup>1</sup> x (10 <sup>3</sup> /uL) | 4.850(1.0)                          | 5.800(2.6)            | 5.800(0.6)   | 4.300(4.4)            | 4.650(2.3)            | 10.65(3.1)   | ns <sup>4</sup> |
| Lymphocytes (%)                           | 82(8.0)                             | 80.5(14.0)            | 80.0(10.0)   | 79.0(16.0)            | 83.0(9.0)             | 85.0(12.0)   | ns <sup>4</sup> |
| Monocytes (%)                             | 0.0(6.0)                            | 0.0(1.0)              | 1.5(3.0)     | 0.5(2.0)              | 0.5(1.0)              | 2.7(3.0)     | ns <sup>3</sup> |
| Neutrophils (%)                           | 12.0(12.0)                          | 17.0(13.0)            | 18.5(7.0)    | 18.5(10.0)            | 15.5(11.0)            | 13.5(8.0)    | ns <sup>4</sup> |
| Eosinophils (%)                           | 0.0(6.0)                            | 0.5(4.0)              | 0.0(0.0)     | 0.0(0.0)              | 0.7(1.0)              | 0.0(0.0)     | ns <sup>3</sup> |
| Basophils (%)                             | 0.0(0.0)                            | 0.0(0.0)              | 0.0(0.0)     | 0.0(0.0)              | 0.0(0.0)              | 0.0(0.0)     | ns <sup>4</sup> |
| PCC <sup>2</sup> X (103/ uL)              | 801.0(140.0)                        | 787.5(137.0)          | 814.8(213.0) | 585.5(306.0)          | 844.0(67.0)           | 903.0(392.0) | ns <sup>4</sup> |

N=4 (2 males and 2 females)

1WBCC: white blood cell count; 2: platelets count; 3Kruskal-Wallis with Dunn's post hoc test, 4 one-way ANOVA with Tukey's post hoc, *p*<0.05; ns: not significant

## Discussion

Arboviruses such as Dengue, Zika, Chikungunya, and Mayaro are potential threats in generating outbreaks. Most patients who are infected by these viruses experience debilitating symptoms in the musculoskeletal system, with the possibility of symptoms lasting for months. Therefore, beyond being a serious public health problem, especially in emerging countries, arboviruses, such as Mayaro result in loss of productivity [20]. There are still no vaccines or effective treatments to combat MAYV, only palliative therapy. Furthermore, there are still mechanisms in the pathogenesis of MAYV infection that are not yet fully elucidated, making it difficult to find efficient treatments. Thus, we characterize the disease caused by the Mayaro virus in a murine experimental model, to assist research and scientific advancement in the pursuit of new therapeutic approaches, as well as to clarify the mechanisms of the disease.

The response mediated by type 1 Interferon has a major role in restricting the infection and dissemination of Alphaviruses [21,22]. This immune pathway exerts its effects on cells not yet infected, with four main activities: inhibit viral replication, suppress cell proliferation, increase the lytic potential of NK cells, and modulate the expression of MHC molecules [23]. The importance of IFN- $\alpha/\beta$  in antiviral defense was demonstrated through our *in vivo* findings. Mice deficient in the IFN-1 pathway were susceptible to the infection leading the animals to develop clinical signs of the disease, which was not observed in animals with an intact immune system. These findings help validate the important role of IFN in controlling infections caused by Alphaviruses. Furthermore, the influence of sex on MAYV infection was for the first time evaluated. Infected knockout male mice showed more pronounced clinical signs compared to the females, with increased lethargy, bristly hair, purulent conjunctivitis, diarrhea, and stooped posture. The disease was lethal for only one male individual and none of the females.

Although the infected knockout animals showed clinical signs of the disease, these were considered to be mild and MAYV strain MT/SINOP/210/2011 infection had a benign course, inducing neither weight loss nor a high lethality rate. In contrast to the

findings of [23], who demonstrated that inoculation of MAYV (TR 4675 strain) into adult type-I interferon receptor-deficient (IFNAR  $-/-$ ) mice resulted in early lethality and severe weight loss. Mota (2020) inoculated A129 $-/-$  animals with a high dose of live- attenuated Mayaro virus vaccine candidate (strain isolated from a human case in Peru, 2001), and even using an attenuated strain there was a 100% lethality rate [25]. Our findings demonstrate that even in the absence of an interferon 1 mediated response, the infected knockout animals were able to control viral replication to the extent that viremia was maintained at levels that did not generate a high lethality rate.

The phenotypes observed in immunodeficient A129 mice were also observed in immunocompetent BALB/ mice when infected with MAYV [26]. The presence of bristly hairs, bowed posture, and eye irritation were common signs in both studies. The fact that mice without deficiency in immune system pathways develop the infectious disease, raises the hypothesis that the strain used to infect these animals TR 4675 (patient serum isolate, 1954) is more virulent and possesses genetic characteristics that may facilitate permissiveness of infection to the murine host, than the MT/SINOP/210/2011 strain, since the wild-type A129SvEv animals in our study at clinical score 0, classified as healthy.

The histopathological findings of our study corroborate the picture of myositis and arthritis in MAYV infections in humans and a murine model from other studies [24-26]. The myositis in the MAYV-generated animals was characterized by the presence of cellular infiltrates, with a predominance of lymphocytes and degeneration of muscle fibers. Vasculitis was originated from the intense inflammatory process in the joint and muscle regions, in which occurs the invasion of immune system cells into the blood vessels. Castro-Jorge (2019) demonstrated that MAYV induces the expression of the NLRP3 protein that plays a role in the inflammatory response through inflammasome formation, along with ASC and caspase 1 molecules. The virus promotes the NLRP3 inflammasome response by inducing the production of reactive oxygen species. Therefore, the NLRP3 molecule is involved in the swelling, inflammation, and pain in the paws of infected animals [27]. The same mechanism of the NLRP3 molecule in the pathogenesis of MAYV infection, was observed in Chikungunya infections in mice and humans [20]. Thus, the commonness in the pathogenesis of MAYV and CHIKV infection justifies the similarity of debilitating clinical signs that both diseases express.

In humans, Mayaro Fever is characterized as an acute febrile disease, whose clinical picture is usually benign and similar to Dengue and Chikungunya. It begins as a non-specific acute febrile syndrome, accompanied by headache, myalgia, retro-orbital pain, rash, and gastrointestinal manifestations. The acute phase is between 3 and 5 days. The severe form of Mayaro Fever presents with arthralgia and swelling in the joint. MAYV-infected knockout animals showed human-like symptoms between 3 and 6 days post-infection [17, 28, 29]. Eye irritation was ascertained in infected knockout mice and is a frequent symptom in Mayaro patients, which they describe as retro-orbital pain and photophobia [17]. Gastrointestinal manifestations were also observed in infected knockout mice, which showed diarrhea. The swelling in the paws is related to the intense inflammatory process that occurs in regions of tropism of the virus, such as muscles and joints that also leads to signs of pain, also observed in patients with myalgia and arthralgia [21]. Knockout animals infected with the strain MT/SINOP/210/2011 showed mild and acute symptoms lasting only a few days, coinciding with the period of the acute phase of Mayaro fever in humans. Therefore, the disease manifests mildly in humans and mice, demonstrating that the experimental model is consistent with the course of the infection in human patients.

Although Mayaro Fever is a benign disease, with symptoms lasting only days, it should not be neglected. Mavian (2017) demonstrated the history of recombinant Mayaro virus strains, these recombination processes allow viruses to acquire key adaptive mutations that might enhance fitness, host tropism, or virulence. The

possibility of a more virulent strain of MAYV, generates the risk of developing an increase in the lethality rate and originating more severe and debilitating symptomatology, thus generating greater prejudice and overloading the public health system [12].

## Conclusions

In vivo assays have demonstrated that infection caused by the Brazilian Mayaro isolate MT/SINOP/210/2011 results in mild, non-lethal disease in a murine model. In addition, human patients infected with MAYV in regions where the strain MT/SINOP/210/2011 is circulating were also found to have mild, non-lethal disease. Therefore, IFN- $\alpha/\beta$  receptor-deficient mice (B6.129S2-Ifnar1) acted as a suitable experimental model generating a picture similar to the symptoms of Mayaro Fever in humans.

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