Mouse model for the Rift Valley Fever virus MP12 strain infection

Running title: Mouse model for MP12 infection

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Abstract

Rift Valley fever virus (RVFV), a Category A pathogen and select agent, is the causative agent of Rift Valley fever. To date, no fully licensed vaccine is available in the U.S. for human or animal use and effective antiviral drugs have not been identified. The RVFV MP12 strain is conditionally licensed for use for veterinary purposes in the U.S. which was excluded from the select agent rule of Health and Human Services and the U.S. Department of Agriculture. The MP12 vaccine strain is commonly used in BSL-2 laboratories that is generally not virulent in mice. To establish a small animal model that can be used in a BSL-2 facility for antiviral drug development, we investigated susceptibility of six mouse strains (129S6/SvEv, STAT-1 KO, 129S1/SvImJ, C57BL/6J, NZW/LacJ, BALB/c) to the MP12 virus infection via an intranasal inoculation route. Severe weight loss, obvious clinical and neurologic signs, and 50% mortality was observed in the STAT-1 KO mice, whereas the other 5 mouse strains did not display obvious and/or severe disease. Virus replication and histopathological lesions were detected in brain and liver of MP12-infected STAT-1 KO mice that developed the acute-onset hepatitis and delayed-onset encephalitis. In conclusion, the STAT-1 KO mouse strain is susceptible to MP12 virus infection, indicating that it can be used to investigate RVFV antivirals in a BSL-2 environment.

Key words: Rift Valley fever virus; Mouse model; MP12 vaccine strain

Introduction

Rift Valley fever virus (RVFV), the causative agent of Rift Valley fever (RVF), belongs to the genus *Phlebovirus*, in the *Bunyaviridae* family and is classified as a Category A pathogen designed by the NIH/NIAID (Bishop and Shope, 1979; Georgiev, 2009). RVF is an acute, fever-causing zoonotic disease that affects humans and animals such as cattle, buffalo, sheep, goats, and camels. RVFV was first reported in 1931 from a farm of Rift Valley in Kenya (Daubney et al., 1931). The virus has been circulating in the countries of the African continent and Madagascar, causing great economic loss and human deaths. The first RVF outbreak outside Africa was confirmed in 2000 in the Arabian Peninsula, resulting in loss of livestock and more than 200 human deaths out of approximately 2,000 hospitalized patients (Balkhy and Memish, 2003; Shoemaker et al., 2002). This fact raised concern that the disease could spread worldwide (Ahmed et al., 2009).

The RVFV MP12 attenuated strain provides a BSL-2 model virus for preliminary investigations of RVFV prior to using virulent RVFV strains that are required to be handled in an expensive high biosecurity BSL-3 facility (Gaudreault et al., 2015). This attenuated strain was obtained through 12 serial passages of a virulent isolate ZH548 in the presence of 5-fluorouracil and has been shown to be safe and efficacious against virulent RVFV challenge in sheep (Morrill et al., 1991; Morrill et al., 1987), cattle (Morrill et al., 1997a, b) and macaques (Morrill and Peters, 2003). However, a previous study showed that this vaccine still can cause abortion in pregnant ewes (Hunter et al., 2002). MP12 is classified as Risk group 2 pathogen and commonly used in BSL-2 laboratories (Ikegami et al., 2015). MP12 is highly attenuated in mice at immunogenic doses (Caplen et al., 1985). However, it may cause tissue pathology in some strains of mice such as BALB/c and C57BL/6 mice when administered at high doses (Indran et al.,

2013; Lihoradova et al., 2013). When RVFV natural hosts, such as cattle and lambs, were infected with a higher dose (10^6 PFU) of MP12 virus, clinical disease and viremia were not observed in inoculated animals (Wilson et al., 2014), indicating that they are not good models for evaluating antivirals using RVFV MP12 virus in a BSL-2 environment. It has been reported that all strains of RVFV remain closely related at the nucleotide and amino acid level (Ikegami, 2012), suggesting that antiviral drugs or vaccines effective against one strain may also be effective against all strains. To date, there is no fully licensed vaccine for human or animal use in the U.S. and effective antiviral drugs have not been identified (Eddy et al., 1981; Hunter et al., 2002; Ikegami, 2012; Liu et al., 2008). It is urgent to develop efficacious antivirals and vaccines to protect public and animal health. However, limited laboratories can perform efficacy testing of RVF antivirals and vaccines and have access to the virulent RVFV strains, since high biocontainment facilities are required and the cost for performing antiviral test is also very expensive. Based on above knowledge, our objective was to establish a mouse model susceptible to infection with the MP12 vaccine strain in order to identify and evaluate antiviral compounds against RVFV in a BSL-2 level facility. BALB/c mice are highly susceptible to the virulent RVFVs such as the ZH501 strain, displaying acute-onset liver disease and delayed-onset encephalitis (Smith et al., 2010). Mouse strains including NZW/LacJ, 129S1/SvImJ, and C57BL/6J have been reported to be susceptible to infection with Punta Toro virus, another phlebovirus in the *Bunyaviridae* family (Ashley et al., 2011; Mendenhall et al., 2009). Furthermore, MP12 is able to infect and cause mortality in Sv129 IFN α/β receptor $-/-$ (interferon α/β receptor knockout) mice that have been used to study vaccine efficacy (Boshra et al., 2011; Bouloy et al., 2001; Lorenzo et al., 2010). Therefore, we selected and screened six strains of mice [129S6/SvEv, STAT-1 KO (129S6/SvEv-Stat1^{tm1Rds}), 129S1/SvImJ,

C57BL/6J, NZW/LacJ, and BALB/c] to test their susceptibility to the MP12 strain in this study. The mouse strain most susceptible to the MP12 vaccine strain infection will likely be selected for further testing the efficacy of antiviral compounds in future studies.

Materials and Methods

Virus and Cells

African green monkey kidney epithelial (Vero) E6 cells (ATCC-CRL 1586) were grown in 1x DMEM medium containing 10% fetal bovine serum (FBS, Atlanta Biologicals). The RVFV MP-12 strain was kindly provided by the US Army Medical Research Institute for Infectious Diseases. The virus was propagated and passaged 3 times in Vero E6 cells. Cells were infected with 0.01 multiplicity of infection (MOI) of the virus in the medium of DMEM with 10% FBS. Cell cultures were collected when 80–95% of the infected cells showed cytopathogenic effect (CPE). Titer of viral stock was determined and calculated as tissue culture infective dose 50 (TCID₅₀) based on the presence of cytopathic effect

Animals

Six groups (14 mice/group) of seven-week-old female mice were obtained from different companies. 129S6/SvEv and STAT-1 KO mice were purchased from Taconic, Hudson, NY; 129S1/SvImJ, C57BL/6J and NZW/LacJ mice were purchased from Jackson Lab, Harbor, ME; BALB/c mice were purchased from Charles River Laboratories, Wilmington, MA. All animal procedures used in this study complied with guidelines set by the USDA and were approved by Kansas State University Animal Care and Use Committee.

Mouse Infection Experiment

Mice were maintained in a mouse vivarium at the USDA, ARS, ABADRU, Manhattan, KS. Twelve mice of each group were intranasally inoculated with 1.58×10^6 TCID₅₀ of the MP12 virus in a volume of 70 μ l (35 μ l per nostril) under anesthesia using isoflurane. The remaining two mice of each group served as negative controls. Mice were weighed every 2 days and observed daily for clinical symptoms. On 3 and 6 days post inoculation (dpi), three inoculated mice and one negative control from each group were euthanized and necropsied. The remaining six mice of each group were kept for 14 days. If a mouse has more than 25% weight loss or showed signs of neurologic disease, it was humanely euthanized and necropsied. During necropsy, livers, brains, spleens, and blood from each mouse were collected under sterile conditions. The half of liver or brain from each mouse collected and stored at -80°C for later virus detection, and the part of these tissues and spleen was fixed in 10% formalin for histopathologic analysis (Liu et al., 2011). Tissues were homogenized twice in PBS for 1 min in a Mini BeadBeater-8 (Biospec Products) to make 10% tissue homogenates. The homogenate was centrifuged at 640 g for 5 min, and the supernatant was transferred to 1.5 ml reaction tubes for virus isolation and titration in Vero E6 cells as described previously (Richt et al., 2003). The serum was isolated from collected blood samples for further viremia analysis using the real-time RT-PCR assay.

RNA Extraction and Real-time RT-PCR

Total RNA was extracted from serum samples collected from each mouse using the Applied Biosystems MagMAX-96 total RNA Isolation Magnetic-bead capture kit according to the manufacturer's protocols. Briefly, 130 μ l of lysis/binding buffer was

mixed with 50 µl of sera followed by shaking in a 96-well plate. Then 20 µl bead mix was added and the mixture was shaken for 5 min. After washing (150 µl each) for four times, the RNA was eluted in 50 µl of elution buffer at 65°C. RNA was quantitated using UV spectroscopy.

Real-time RT-PCR was performed to detect viral RNA of serum samples using the AgPath ID rRT-PCR Kit (Life Technologies, Inc., Grand Island, NY) as described previously (Wilson et al., 2013). Briefly, the RVFV triplex rRT-PCR procedure was designed to contain L, M and S primers and probes and one of the external RNA control combinations using the following cycling conditions: 45°C for 10 min, 95°C for 10 min, followed by 40 cycles of 95°C for 10 sec and 60°C for 1 min. The Ct value of at least two of the three RVFV segments from one sample below 35 cycles was considered positive.

Histopathological and Immunohistochemical Analysis

Tissues including liver, brain and spleen in 10% formalin buffer were routinely processed, sections were cut at 4 microns thick on a microtome and stained with hematoxylin and eosin. The amount of necrosis and inflammation were graded microscopically. In the spleen and liver samples, a 5 point scale was used: 0=No necrosis, 1=Occasional scattered single cell necrosis, 2=Scattered 0.5-2 mm foci of random necrosis, 3=Scattered larger random area of necrosis, often coalescing with each other, 4=Diffuse lobular necrosis. In the spleen, necrosis was seen as lymphocytolysis in periarteriolar sheaths. In the brain samples, a 4-point scale was used: 0=No necrosis, 1=one focal area of necrosis and inflammation, 2=2-5 areas of necrosis and inflammation, 3≥6 areas of necrosis and inflammation.

For immunohistochemical analysis, tissues were deparaffinized and rehydrated, antigen retrieval was performed in pH 9.0 EDTA buffer, Bond ER Solution 2 (Leica Microsystems, Buffalo Grove, IL) for 20 minutes at 100° C on the Leica Bond Max autostainer. The primary polyclonal rabbit anti-RVSV antibody (Drolet et al., 2012) was diluted 1:3000 in Bond Primary Antibody Diluent (Leica Microsystem) and incubated for 15 minutes at ambient temperature with the tissue on the slide. Polymerization was done with Anti-rabbit Poly-HRP-IgG (Leica Microsystems, Buffalo Grove, IL) for 25 min at ambient temperature. Slides were blocked with 3% hydrogen peroxide for 5 minutes, visualized with DAB, and counterstained with hematoxylin. Immunohistochemical positivity was graded as follows: 0=No staining, 1=Scattered individual cell staining, not more than 15 cells, 2=Moderate numbers of scattered cells, and 3=Diffuse multifocal staining, severe, greater than 75% of sectional area with staining.

Statistical analysis.

The student t-test was used to analyze differences between groups for data collected at a given time point. A $p \leq 0.05$ was considered statistically significant.

Results

Clinical symptoms, morbidity and mortality of different mouse strains infected with the RVSV MP12 virus

RVSV can be transmitted to human by aerosol exposure and intranasal infection with RVSV induces earlier and more severe neuropathology in the mouse model compared with other infection routes (Reed et al., 2013). Therefore, six different mouse strains of 12 mice/group were intranasally inoculated with 1.58×10^6 TCID₅₀/mouse of the

RVFV MP12 virus in this study. Two control mice euthanized on day 3 and 6 dpi in each group did not show any clinical symptoms and gained weight normally. All infected STAT-1 KO mice started to exhibit weight loss at 2 dpi (**Figure 1A**) and obvious clinical signs including decreased activity, huddling, hunched posture, and ruffled fur as early as 4 dpi. Three STAT-1 KO mice were humanly euthanized as they developed neurological symptoms such as continual cage circling and head pressing at 8 (1 mouse) and 10 dpi (2 mice), and their weight loss were also close to 25%. The remaining 3 mice began to gain weight at 10 dpi, but did not recover until the end of the experiment (14 dpi). In contrast, the BALB/c and C57BL/6J mouse strains started to gain weight post inoculation of RVFV MP12 virus and did not show clinical signs except for 2 BALB/c mice that developed vestibular neurological symptoms (continual cage circling and head pressing) at end of the study (14 dpi) and were euthanized. The infected NZW/LacJ mice showed a slight weight loss at 2 dpi and a severe weight loss at 12 dpi, then started to recover (**Figure 1A**). No obvious clinical signs were observed in these infected mice. Both infected 129S1/SvImJ and 129S6/SvEv mice showed approximately 10% weight loss at later time points (8 dpi or 10 dpi) when they displayed clinical signs such as decreased activity and ruffled fur. One of 6 infected 129S1/SvImJ mice showed neurological symptoms at 8 dpi and was humanly euthanized. In summary, RVFV MP12 infection caused weight loss and clinical signs in 3 mouse strains including STAT-1 KO, 129S1/SvImJ and 129S6/SvEv, but the STAT-1 KO mice developed more severe disease with 50% mortality than other tested mouse strains (**Figure 1A and 1B**).

Viral replication in mouse livers and brains

Three infected and one negative control mice from each group were sacrificed on 3 and 6 dpi and viral titers of their livers and brains were determined. Virus was not detected in both liver and brain samples from control mice of all strains on either day. Virus was detected in liver samples of each infected mouse strain on both days (**Figure 2A**). Virus titer of liver samples in infected STAT-1 KO mice on 3 dpi was higher than those of other 5 mouse strains. The virus titer in liver of the 129S6/SvEv mice was significantly lower than those of the NZW/LacJ and C57BL/6J mouse strains (**Figure 2A**), but the C57BL/6J mice did not show clinical symptoms. Although a higher virus titer was detected in the liver of the 129S1/SvImJ on 6 dpi, no significant difference was observed among groups (**Figure 2A**). Virus was recovered from the brains of each infected mouse strain on both days, but statistically significant lower titers were found in both NZW/LacJ and C57BL/6J mouse strains when compared to the other 4 mouse strains (**Figure 2B**). Furthermore, a significant lower virus titer was detected in brain samples of infected 129S1/SvImJ mice when compared to those in STAT-1 KO, 129S6/SvEv and BALB/c mice at 3 dpi (**Figure 2B**). The results indicated that the RVFV MP12 was able to infect each tested mouse strain via the intranasal infection, but it showed different replication in the mouse livers and brains.

Viremia of serum samples collected from infected different mouse strains

Real-time RT-PCR assay was performed to detect viral RNA in serum samples collected from inoculated mice that were necropsied on 3 and 6 dpi and those that displayed neurological symptoms were humanely euthanized. Viral RNA was detected in all samples from STAT-1 KO mice on 3 and 6 dpi, but not in all 3 samples collected from humanely euthanized on 8 and 10 dpi (**Table 1**), indicating that the virus was cleared from blood at later time points. All samples collected from infected NZW/LacJ

and C57BL/6J mice on both days were negative. One of 3 serum samples from infected 129S6/SvEv group on 3 dpi was positive; whereas the infected 129S1/SvImJ group had 2 positives out of 3 samples on 6 dpi (**Table 1**). Viral RNA was detected in serum sample of one mouse of infected BALB/c group on 6 dpi, and in one of 2 humanely euthanized animals on 14 dpi (**Table 1**).

Microscopic lesions of tissues collected from infected different mouse strains

Liver, spleen and brain collected from each infected and control mouse were examined for lesions and scored. Five out of 6 groups of mice, which include the STAT-1 KO, BALB/c, 129S6/SvEv, C57BL/6J and 129S1/SvImJ, had histopathological lesions in one or more organs when compared to each respective control animals. Lesions were similar between groups but varied in severity. Histopathological scores for mice are summarized in **Table 2**. In the liver, there were random foci of hepatocellular necrosis and apoptosis with small numbers of inflammatory cells, which included neutrophils, Kupffer cells and a few lymphocytes (**Figure 3B**). In the spleen, 3 STAT-1 KO and 1 129S1/SvImJ mice had mild lymphocytolysis in the white pulp (**Figure 3D**) and 1 STAT-1 KO mouse had a few individual scattered cells in the red pulp. The remainder of the mice in this study had normal spleens, which did not have necrosis or inflammation. Mice in this study had mild lymphoid hyperplasia and extramedullary hematopoiesis in the spleen in both control and infected animals of each strain; these findings were interpreted as incidental in the mouse splenic tissues and the role RVFV infection played in the development of hyperplasia cannot be determined. Lesions in the brain were consistent with varying degrees of encephalitis that included areas of pyknotic and karyorrhectic cellular debris with gutter cells and neutrophils. In addition, vessels were cuffed by neutrophils, lymphocytes and plasma cells (**Figure 3F**). On

histopathological exam, three STAT-1 KO mice, which were humanely euthanized at 8 or 10 dpi due to neurological symptoms, had the most severe lesions in the brain, followed by the liver and then the spleen. Two BALB/C mice, which were humanely euthanized at 14 dpi, also had lesions in the brain. In the mice scheduled to be euthanized on 3 dpi, lesions were observed in both the liver and spleen in STAT-1 KO and in the liver of the BALB/c group. The infected groups of BALB/c, 129S6/SvEv, C57BL/6J and 129S1/SvImJ on 6 dpi had liver lesions. Both 129S6/SvEv and 129S1/SvImJ groups had lymphocytolysis in the spleen on 6 dpi while 129S6/SvEv was the only one to have encephalitis in 6 dpi.

Immunohistochemistry results

To detect RVFV MP12 antigens, immunohistochemistry staining was performed for the tissues including liver, brain and spleen collected from mice euthanized on 3 and 6 dpi and from those that were euthanized later due to neurological symptoms (**Table 3**). No RVFV MP12 antigen was detected in liver, brain and spleen tissues collected from control mice euthanized on 3 and 6 dpi. In the liver, most RVFV MP12 antigen detected was seen in the STAT-1 KO group (**Table 3, Figure 4B**) with 3 mice euthanized on 3 dpi and 2 mice euthanized later. One 129S6/SVEV mouse and one 129S1/SVLMJ mouse had RVFV MP12 antigen detected in the liver. RVFV MP12 antigen detected was seen in the spleen of STAT-1 KO mice that were euthanized at 3 dpi (**Figure 4D**) or euthanized later due to neurological symptoms. One BALB/c mouse and two 129S6/SvEv mice had RVFV MP12 antigen detected in the spleen at 3 dpi (**Table 3**). RVFV antigen detected was also present in the brain of 3 STAT-1 KO mice (**Table 3, Figure 4F**), 2 BALB/C mice, and 1 129S1/SvImJ mouse that were euthanized later due to neurological symptoms. No RVFV MP12 antigen was detected in the brain of all

mice euthanized on 3 dpi (**Table 3**). Immunohistochemistry scores are summarized in **Table 3**.

Discussion

The aim of this study was to establish a mouse model susceptible to infection with RVFV MP12 virus that can be used in the development and evaluation of antiviral therapeutics in BSL-2 facilities in future studies. This mouse model using RVFV MP12 that can be used in a BSL-2 level facility will make RVF research more affordable and facilitate identification of effective antivirals against RVFV for human and animal use. Our results showed different susceptibilities of six mouse strains to RVFV MP12 vaccine strain infection based on clinical symptoms, mortality, viremia, virus replication, histopathological, and immunochemistry analysis. The STAT-1 KO mice were the most susceptible of the tested 6 mouse strains as supported by that infected STAT-1 KO mice displayed obvious clinical signs including weight loss and neurological disease, 50% mortality, viremia, virus replication and histopathological lesions detected in liver and brain. Especially, the infected STAT-1 KO mice developed both the acute-onset hepatitis and delayed-onset encephalitis that are very similar to the severe consequences of human RVFV infection (Smith et al., 2010). In contrast, both 129S1/SvImJ and 129S6/SvEv mouse strains showed slight weight loss and clinical signs at later time points. Other 3 mouse strains including C57BL/6J, NZW/LacJ and BALB/c did not show any clinical signs; however, the virus was detected in livers and brains of both NZW/LacJ and BALB/c strains, suggesting that they are not resistant to infection. Previous studies have reported that NZW/LacJ, 129S1/SvImJ and C57BL/6J mice are susceptible to infection with Punta Toro virus with approximately 100% mortality, another phlebovirus in the *Bunyaviridae* family (Ashley et al., 2011;

Mendenhall et al., 2009); however, they are not susceptible to infection of RVFV MP12 strain. This fact suggests that different phleboviruses show differential pathogenicity in the same mouse strain.

Although the 129S6/SvEv mice are the genetic background of STAT-1 KO mice, they are not as susceptible to RVFV MP12 infection when compared to the STAT-1 KO mice. The STAT-1 KO mice have a deficient immune cell response to alpha (α) and gamma (γ) interferons (Meraz et al., 1996), resulting in failure to induce type I IFN response and enhanced susceptibility to MP12 infection. Previous studies also showed that a low dose of the MP12 strain (approximately 10^4 PFU) can cause 100% mortality in Sv129 IFN α/β receptor $-/-$ mice that are defective for the IFN- α/β receptor (Boshra et al., 2011; Bouloy et al., 2001; Lorenzo et al., 2010); whereas the Sv129 IFNGR $^{-/-}$ mice, which are defective for the IFN γ receptor, did not show any disease symptoms when infected with a same dose of the MP12 virus (Bouloy et al., 2001). It has been shown that MBT/Pas mouse cells induced an impaired induction of type I IFN response, leading to its extreme susceptibility to infection with the virulent RVFV ZH548 and Kenya 98 strains compared with BALB/cByJ mice (do Valle et al., 2010). All these data indicate that the IFN response plays an important role in controlling RVFV infection, but IFN- α/β is absolutely required when compared to the IFN- γ . BALB/c mice are susceptible to infection with the virulent RVFV (Mendenhall *et al.*, 2009; Smith *et al.*, 2010) and die due to severe hepatitis and encephalitis after infection (Smith et al., 2010). However, our results showed that they are not susceptible to RVFV MP12 strain infection although several BALB/c mice developed similar neurologic symptoms at end of the experiment as those observed in the infected STAT-1 KO mice. The neurologic symptoms observed in BALB/c mice most likely attribute to the intranasal inoculation as a previous study has reported that

aerosol exposure to RVFV induces earlier and more severe neuropathology in the mouse model when compared to the subcutaneous exposure (Reed et al., 2013). Aerosol exposure of wild-type RVFV results in uniformly fatal disease in mice, which is similar to subcutaneous infection with the same virus (Brown et al., 1981; Reed et al., 2013). Furthermore, RVFV can be transmitted to humans by aerosol exposure, which is evident by several laboratory workers who have become infected (Smithburn et al., 1949). This indicates the potential high risk of infection of veterinarians and abattoir workers who handle infected animals. In our study, the STAT-1 KO mice developed acute-onset hepatitis and delayed-onset encephalitis after intranasal infection with attenuated MP12 vaccine strain, which is consistent with the neurological fatal disease of C57BL/6 mice intranasally infected with an attenuated RVFV lacking NSs (Dodd et al., 2014). The neurologic disease is most likely associated with the intranasal infection, not due to immune-mediation (Dodd et al., 2014), which is consistent with findings in infected BALB/c and 129S1/SvImJ mice that also developed neurologic symptoms in our study.

Histopathological lesions and RVFV antigen detected were seen in the STAT-1 KO, 129S6/SvEv, BALB/c, C57BL/6J and 129S1/SvImJ, which means these mice strains have varying degrees of susceptibility to RVFV MP12. Lesions in all groups of mice in this study were consistent with those that have been previously published and included hepatic necrosis with neutrophils, lymphocytolysis in the spleen, and multifocal encephalitis in a BALB/c mouse model challenge with RVFV strain ZH501 (Smith et al., 2010). All control and infected mice had lymphoid hyperplasia in the spleen which can be found in normal mice and can depend on location of the lymph node, age, health status and plane of section (Elmore, 2006). Since the control mice were negative by IHC test, the lymphoid hyperplasia of the spleen was not

interpreted as a lesion of rift valley fever infection. The most severe lesions, histopathological scores, and RVFV antigen detected were present in the STAT1-KO mice which coincides with increased mortality and weight loss supporting that STAT1-KO mice are a good mouse model for MP12 infection.

Despite of the STAT-1 KO mouse strain exhibiting severe diseases post infection with the RVFV MP12 strain, it is still not an ideal model to evaluate vaccine efficacy as it is immunodeficient, leading to failure to induce host type I IFN responses against virus infection. However, it could be a very useful small animal model at BSL-2 level facility to initially screen and elevate antiviral compounds against RVFV prior to using virulent RVFV strains in a high biocontainment facility.

In conclusion, the STAT-1 KO mouse strain is susceptible to MP12 virus infection and develops representative disease including acute-onset hepatitis and delayed-onset encephalitis, indicating that STAT-1 KO mouse model for the MP12 has potential to be used to develop and evaluate RVF antivirals in a BSL-2 environment.

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Figure legends

Figure 1: Mouse weight loss and survival rate post infection with the MP12 virus.

Each mouse strain was intranasally infected with 1.58×10^6 TCID₅₀/mouse of the MP12 vaccine strain and was monitored for 14 days post infection. A) Mouse weight loss: the percentage weight from each group and each time point are presented as means $\pm$ SD, and B) Mouse survival rate.

Figure 2. Virus titers of liver and brain samples collected from each mouse infected with the MP12 virus. A) virus titer in liver samples, and B) virus titer in brain samples. Three mice from each mouse strain were necropsied at 3 and 6 days post infection. All animals were positive for virus isolation at indicated time points. Error bars represent $\pm$ SEM (* P < 0.05).

Figure 3. Microscopic liver, brain and spleen sections from control or infected STAT-1 KO mice with the MP12 virus.

A) This liver photomicrograph does not have inflammation or necrosis, which is consistent with normal liver in a control STAT-1 KO mouse that was euthanized at 3 day post infection. B) Liver of an infected STAT-1 KO mouse that was euthanized at 3 day post infection. Two areas of inflammatory cells consisting of viable and degenerate neutrophils, Kupffer cells, and lymphocytes admixed with necrotic and degenerate cells. C) No necrosis or inflammation in normal spleen of a control STAT-1 KO mouse that was euthanized at 3 day post infection. D) Spleen from an infected STAT-1 KO mouse that was euthanized at 3 day post infection. There is extramedullary hematopoiesis, a lymphoid follicle and the red pulp. There are no obvious lesions present. Macrophages containing hemosiderin (brown cytoplasmic pigment from the break down of red blood cells) are present. E) No necrosis or

inflammation consistent with normal brain of a control STAT-1 KO mouse that was euthanized at 6 day post infection. F) Brain of an infected STAT-1 KO mouse showed neurological symptoms and euthanized at 8 day post infection. Encephalitis was characterized by necrosis and cellular debris. An inflammatory infiltrate composed of degenerate and intact neutrophils, macrophages and lymphocytes, which also cuff vessels. Neuronal necrosis and astrocytosis is present. The magnification of each image is 400X.

Figure 4. Immunohistochemistry staining results of liver and brain sections from control or infected STAT-1 KO mice with the MP12 virus.

A) No RVFV MP12 antigen was present in the liver of a control STAT-1 KO mouse that was euthanized at 3 day post infection; B) Liver of an infected STAT-1 KO mouse that was euthanized at 3 day post infection. Foci of degenerate and viable hepatocytes have positive cytoplasmic RVFV labeling. C) No RVFV MP12 antigen was present in the spleen of a control STAT-1 KO mouse that was euthanized at 3 day post infection. D) Spleen from an infected STAT-1 KO mouse that was euthanized at 3 day post infection. Scattered cells in the lymphoid follicle positive and there are is a group of several cells in the red pulp with positive cytoplasmic labeling. E) No RVFV MP12 antigen was present in the brain of a control STAT-1 KO mouse that was euthanized at 6 day post infection. F) Brain of an infected STAT-1 KO mouse showed neurological symptoms and euthanized at 8 day post infection. Scattered RVFV MP12 antigen among necrotic cells that have ruptured releasing antigen into the neuropil and in the cytoplasm of neurons. The magnification of each image is 400X.

Table 1. Viraemia test results based on real-time RT-PCR.

Mouse strain	3 dpi	6 dpi	Early humane euthanasia
STAT-1 KO	3/3 ^a	3/3	0/3
129S6/SvEv	1/3	0/3	N/A ^b
NZW/LacJ	0/3	0/3	N/A
BALB/c	0/3	1/3	1/2
C57BL/6J	0/3	0/3	N/A
129S1/SvImJ	0/3	2/3	0/1

a. Positive animals/total animals; b. Not applicable.

Table 2. Histopathological scores for scheduled euthanized mice and early humane euthanized mice.

	Scheduled Euthanasia						Early Humane Euthanasia		
	3dpi			6dpi					
	Liver	Brain	Spleen	Liver	Brain	Spleen	Liver	Brain	Spleen
STAT-1 KO	3/3 ^a (1.5±0.5)	0/3	2/3 (1.5±0.5)	0/3	0/3	0/3	2/3 (2.0±0.0)	3/3 (3.0±0.0)	1/3 (1.0)
129S6/SvEv	0/3	0/3	0/3	1/3 (1.0)	1/3 (1.0)	0/3	N/A ^b	N/A	N/A
NZW/LacJ	0/3	0/3	0/3	0/3	0/3	0/3	N/A	N/A	N/A
BALB/c	1/3 (1.0)	0/3	0/3	2/3 (2.0±0.0)	0/3	0/3	0/2	2/2 (1.5±0.5)	0/2
C57BL/6J	2/3 (1.5±0.5)	0/3	0/3	2/3 (1.5±0.5)	0/3	0/3	N/A	N/A	N/A
129S1/SvImJ	0/3	0/3	0/3	3/3 (2.0±0.0)	0/3	1/3 (1.0)	0/1	1/1 (2)	0/1

a. Positive animals/total animals (mean histological score ± absolute deviation); b. Not applicable.

1 **Table 3. Immunohistochemistry scores for scheduled euthanized mice and early humane euthanized mice.**

	Scheduled Euthanasia						Early Humane Euthanasia		
	3dpi			6dpi					
	Liver	Brain	Spleen	Liver	Brain	Spleen	Liver	Brain	Spleen
STAT-1 KO	3/3 (2.5±0.5) ^a	0/3	3/3 (2.0±0.0)	2/3 (1.0±0.0)	0/3	0/3	2/3 (1.0±0.0)	3/3 (2.0±0.0)	3/3 (1.5±0.5)
129S6/SvEv	1/3 (1.0)	0/3	2/3 (1.0±0.0)	3/3 (1.0±0.0)	0/3	1/3 (1.0)	N/A ^b	N/A	N/A
NZW/LacJ	0/3	0/3	0/3	1/3 (2.0)	0/3	1/3 (1.0)	N/A	N/A	N/A
BALB/c	0/3	0/3	1/3 (1.0)	1/3 (1.0)	0/3	0/3	0/2	2/2 (3.0±0.0)	0/2
C57BL/6J	1/3 (2.0)	0/3	0/3	3/3 (1.5±0.5)	0/3	1/3 (1.0)	N/A	N/A	N/A
129S1/SvImJ	1/3 (1.0)	0/3	0/3	3/3 (2.5±0.5)	0/3	0/3	0/1	1/1 (3)	0/1

2 a. Positive animals/total animals (mean histological score ± absolute deviation); b. Not applicable.

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Figure 1

Figure 2

Figure 3

Figure 4