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Research Article

Evaluation of a Needle-Free Vaccine Delivery Device for Vaccinating Rats with Rift Valley Fever Vaccine Candidates

Pedro M. Palermo*, George Bettinger and Douglas M. Watts

Department of Biological Science Border Biomedical Research Center, University of Texas at El Paso, El Paso, Texas, 79968
***Corresponding author:** ppalermo@utep.edu

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ABSTRACT

Rift Valley Fever (RVF) is a major public health and veterinary concern in Africa and the neighboring regions. One approach that is a priority for preventing epidemics of RVF is to develop and deliver an effective vaccine for livestock and humans. The aim of this study was to evaluate the Bioject ZetaJet needle-free vaccine delivery device for use to vaccinate animals with a very promising live recombinant RVF MP-12ΔNSm vaccine candidate. A preliminary assessment of the device was conducted in young adult Wistar Furth rats with and without a spacer, suggesting an subcutaneous or intramuscular route of delivery, respectively. Two doses of the RVF MP-12ΔNSm vaccine of 1 x 10^3 and 1 x 10^5 plaque forming units (PFU) were administered using the needle-free device (NFD) to each of 2 groups of 5 animals each. Also, a dose of 1 x 10^5 PFU of the RVF MP-12ΔNSm vaccine and of the RVF MP-12 parent vaccine was administered via the intraperitoneal (IP) route to groups of rats using a needle. Blood samples collected from rats before vaccination and at 7, 11, 15, and 25 days post-vaccination (DPV) were tested for antibody by the plaque reduction neutralization assay. Most animals (80-100%) vaccinated with the NFD developed detectable neutralizing antibody by 7 DPV that persisted through 25 DPV or the duration of the experiment, with antibody titers ranging from 1:20 to 1:1280, with no significant difference in the titers observed for the groups IP vaccinated with RVF MP-12 ΔNSm and RVF MP-12 vaccine versus the groups that received RVF MP-12 ΔNSm using the NFD at 25 DPV. These results suggest that needle-free vaccine delivery may be a more convenient and effective method of vaccinating animals with RVF vaccines.

Keywords: Rift Valley fever virus, MP-12 ΔNSm vaccine, Needle-free device, Wistar Furth rats

INTRODUCTION

Rift Valley Fever (RVF) is an acute mosquito-borne viral disease caused by a Rift Valley fever virus (RVFV) of genus Phlebovirus (*Family Bunyaviridae*) that affects the health of hundreds to millions of livestock and humans in Africa and the Middle East (Bird *et al*., 2009; Peyre *et al*., 2015). The livestock industry has lost millions of dollars due to RVF epidemics, affecting millions of those whose livelihood depends on livestock and increasing poverty in already deprived communities. Accumulated evidence indicates that the epidemics of RVF in East Africa and the Middle East are associated with severe climate oscillations related to the El Nino South Oscillation (Anyamba *et al*., 2009).

RVFV causes mortality approaching 100% in young sheep and abortions in 90– 100% of pregnant ewes and 30% to 40% mortality rate in adult livestock (McMillen *et al*., 2018; Peters *et al*., 1981; Pepin *et al*., 2010). Among humans, more than 90% of the infections are asymptomatic and most symptomatic infections cause a self-limited flu-like illness. About 1% to 3% experience disease that progresses to more severe forms, including hemorrhagic fever, blindness and/or neurological disorders, with a fatality rate that can approach 50%. The primary route of RVFV transmission is by mosquito bites, but transovarial transmission can occur, and also the virus can be easily transmitted by aerosols to animal caretakers and others who have contact with infected animals, such as during animal husbandry activities, and the handling of fluids or tissues of infected animals, especially during slaughter (Pepin *et al*., 2010; Linthicum *et al*., 2016). RVFV is classified as an enhanced biosafety level 3 agent and a select agent by the United States Department of Homeland Security's (DHS) and is also considered as a potential bioterrorism threat worldwide (Borio *et al*., 2002; CDC, 2005).

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RVFV is a negative strand ribonucleic nucleic acid (RNA) virus that contains a large, medium and small RNA segment (Pepin *et al*., 2010; Schmaljohn *et al*., 2007). The large segment encodes the RNA polymerase gene involved in RNA replication. The medium segment encodes 2 glycoproteins (Gn and Gc), the 78-kDa protein and the non-structural M (NSm) protein. The small segment encodes the nucleoprotein and non-structural S (NSs) protein.

Although several vaccines have been developed and used to prevent RVF, none have been considered effective because of safety and efficacy problems (Pepin *et al*., 2010). As a next-generation promising candidate, RVF MP-12 is a live attenuated safe and efficacious vaccine for use in humans and small laboratory animals as well as in sheep, cattle and non-human primates (Caplen *et al*., 1985; Morrill *et al*., 1987; Morrill *et al*., 1991; Morrill *et al*., 1997a; Morrill *et al*., 1997b; Morrill *et al*., 2003; Morrill *et al*., 2011). However, as a potential veterinary vaccine, RVF MP-12 is not considered appropriate for use in Africa because it does not contain biomarkers required to distinguish naturally infected animals from vaccinated animals (DIVA). A DIVA vaccine is preferred in Africa to avoid the trade embargo on the exportation of naturally infected RVFV animals in Africa. As a strategy to develop a DIVA vaccine, reverse genetic technology was used to develop a recombinant RVF MP-12 vaccine by deleting nucleotides 21-384 from the non-structural genes of the medium viral RNA genome segment (NSm) to serve as a potential DIVA marker (Won *et al*., 2007). This vaccine referred to as RVF MP-12ΔNSm candidate is safe, immunogenic and efficacious in key ruminant species and is under industry evaluation for licensure to prevent RVF in Africa (Morrill *et al*., 2013a; Morrill *et al*., 2013b; Weingartl *et al*., 2014).

As an approach based on the reported advantages of using needle-free vaccine delivery devices to vaccinate animals (Daniels, 2010), this study was conducted to evaluate such a device for inducing an immune response to RVF MP-12ΔNSm vaccine in Wistar Furth (WF) rats.

MATERIALS AND METHODS

Animals

A total of 32 male and female WF rats (10-12 weeks old) were obtained from Envigo Co. (Indianapolis, Indiana). These animals were housed in 6 groups of 5 each and one group with 2 animals in individually ventilated cages in an Animal Biosafety Level 3 Laboratory. Animal husbandry support was provided by the University of Texas at El Paso (UTEP) Department of Laboratory Animal Resources according to standard operating procedures described in an animal use protocol approved by the UTEP Institutional Animal Care and Use Committee (Protocol number A- 201401-1).

Injection of animals

A Bioject Zetajet™ needle-free injection device obtained from Bioject Medical Technologies Inc. (Portland, Oregon) was used to inject animals with the RVF MP-12ΔNSm vaccine candidate. The device is compact, spring-powered and designed to deliver vaccines either subcutaneously or intramuscularly. The syringe assembly has a unique "auto-disable" feature to prevent re-use of the syringe. Group 1 and 2 rats, 5 per group were vaccinated in the shaven posterior area just above the tail with 50 µL per animal containing 1 x 10^3 plaque forming units (PFU) and 1 x 10^5 PFU of the RVF MP-12ΔNSm vaccine candidate, respectively using the Bioject device with a 10 mm spacer attached to the nozzle part of the syringe that resulted in a subcutaneous (SC) route of delivery. Group 3 and 4 rats were each vaccinated with 50 µL in the same area as mentioned above, containing 1 x 10^3 PFU and 1 x 10^5 PFU of the RVF MP-12ΔNSm vaccine candidate respectively using the Bioject device without a spacer that resulted in an intramuscular (IM) route of delivery. Group 5 and 6 rats were each vaccinated via the intraperitoneal (IP) route with a 23-gauge needle with a dose of 1 x 10^5 PFU of the RVF MP-12ΔNSm and the RVF MP-12 parent vaccine candidates, respectively. Group 7 rats were injected with a single dose of phosphate buffer saline (PBS), using the Bioject device without the spacer.

Each animal was anesthetized by exposure to the vapor of isoflurane, and then sterile Pasteur pipettes were used to obtain blood samples from the retro-orbital sinus complex of each animal prior to vaccination and at 7, 11, 15 and 25 days post vaccinations (DPV). A volume of 0.2 mL of blood was obtained from each animal and diluted directly in 0.8 mL of Earle Modified Eagle Medium (EMEM) containing 2% fetal bovine serum and 1% of penicillin and streptomycin. Samples were stored at -20ºC until tested for RVF MP-12 virus neutralizing antibody assay.

Plaque reduction neutralization test $_{80}$ (PRNT$_{80}$)

Each blood samples were heat inactivated at 56°C for 30 minutes and tested for neutralizing antibody to RVF MP-12 virus. Briefly, equal volumes of 2-fold dilutions ranging from 1:10 through 1:1280 of each blood samples were prepared in EMEM and incubated overnight at 4°C with an equal volume of 50-100 PFU of the RVF MP-12 virus. An equal volume of RVF antibody negative sample was mixed with an equal volume of 50–100 PFU of RVF MP-12 virus to estimate the number of PFU used as the virus dose in the assay. On the following day, 50 µL of the virus/blood mixture was inoculated in duplicate onto a confluent monolayer of Vero E-6 cells grown in 24 well-plates, and after 1 hour incubation, the cells were overlaid with 0.5 mL of the agarose-EMEM mixture. After 3 days of incubation at 37°C with 5% CO_2 atmosphere, the cells were stained with a 0.33% neutral red solution to identify and enumerate plaques. The dilution of blood that reduced the RVF MP-12 virus dose by 80% was considered as the neutralizing antibody titer.

Statistical analysis

Antibody neutralizing titers over the period (Day 0 to 25) in each group were analyzed by using the longitudinal nonlinear Mixed (fixed and random) effects model in the R software package (R version 3.4.4) with a significance level of α=0.05. Finally, nonparametric multiple

comparisons were used to determine antibody titer differences between all the groups at the end time point (25 DPV) with a significance level of α =0.05.

RESULTS

The immune response of rats to the RVF MP-12 ΔNSm vaccine using the needle-free vaccine delivery device (with or without a spacer) was compared to the response to the RVF MP-12 and RVF MP-12ΔNSm vaccines following intraperitoneal injection with a needle. The individual antibody neutralization responses of the rats are presented in Table 1. The fitted trends of log neutralizing antibody $PRNT_{80}$ response of the groups are shown in Fig. 1.

Neutralizing antibodies appeared by 7 DPV and persisted at or above the titers on 7 through 25 DPV with titers ranging from 1:20 to 1:1280. The antibody titers over the period of the rats vaccinated with the RVF MP-12ΔNSm by using the NFD did not differ significantly from that of the rats vaccinated via the IP route with RVF vaccines, with exception of the group that received 1 x 10^5 PFU of the RVF MP-12ΔNSm vaccine without spacer, which had higher antibody titers ($p<0.05$) than the rats IP vaccinated with the RVF MP-12 vaccine only.

Neutralizing antibodies were detected in all the rats IP vaccinated with the RVF MP-12ΔNSm and RVF MP-12 vaccines at 25 DPV. In case of the rats vaccinated with RVF MP-12ΔNSm using the NFD with or without a spacer, neutralizing antibody was detected in 80% (8/10) and 100% (10/10) respectively. No significant differences ($P>0.05$) between all the seroconverted and vaccinated rats (Group1 to 6) were found in the neutralizing antibody titers at 25DPV.

DISCUSSION

This study showed that a needle-free vaccine delivery device was effective for inducing an immune response to the recombinant RVF MP-12ΔNSm vaccine in WF inbred rat model, which is highly sensitive to virulent RVFV (Anderson *et al*., 1987). These findings are consistent with previous reported data that showed needle-free vaccine delivery devices to be reliable for inducing an immune response. Also, these promising findings will support further plans to evaluate NFD as a method for vaccinating livestock with RVF MP-12ΔNSm. This method of vaccination will sustain the quality of animal hide, require a smaller volume of a vaccine, reduce the mechanical spread of infectious agents and reduce the risk of accidental needle sticks associated with the use of needles and syringes as reported in previous studies (Daniels, 2010).

Previous studies in cattle vaccinated with RVF MP-12ΔNSm by a subcutaneous or intramuscular route using a needle induced neutralizing antibody but did not show any difference in the protective neutralizing antibody titers (Morrill *et al*., 2013b). Neutralizing antibody titers in WF rats vaccinated with a NFD without a spacer, or a suggested intramuscular route were higher than in animals IP vaccinated with a needle, suggesting that the intramuscular route was a more efficient delivery route. Also, only two animals that did not have neutralizing antibody after delivery of the RVF MP-12ΔNSm vaccine using the device with the spacer were male rats which have twice thicker skin than females and could have affected the route of delivery of the vaccine with the spacer attached to the device.

Neutralizing antibody is considered the main response of the immune system to RVFV infection (Anderson *et al*., 1987; Peters *et al*., 1988), but is not the only mechanism as was stated previously. Antibody titers to RVF MP-12 vaccine at 25 DPV using the NFD were higher than 1:40, a dilution of antibody which has been reported to prevent encephalitis and clinical disease in WF rats after challenging with wild-type RVFV (Anderson *et al*., 1991). Neutralizing antibody titers ≥1:40 to RVF MP-12 found in WF rats were also reported to be protective after challenge with wild-type RVFV in ruminants species (Miller *et al*., 2015; Morrill *et al*., 1987), thus indicating that the use of a NFD to deliver RVF MP-12ΔNSm would induce high enough antibody titers required to protect susceptible animals against RVFV infection. Also, passive transfer of RVF neutralizing antibody (1:40) to rhesus

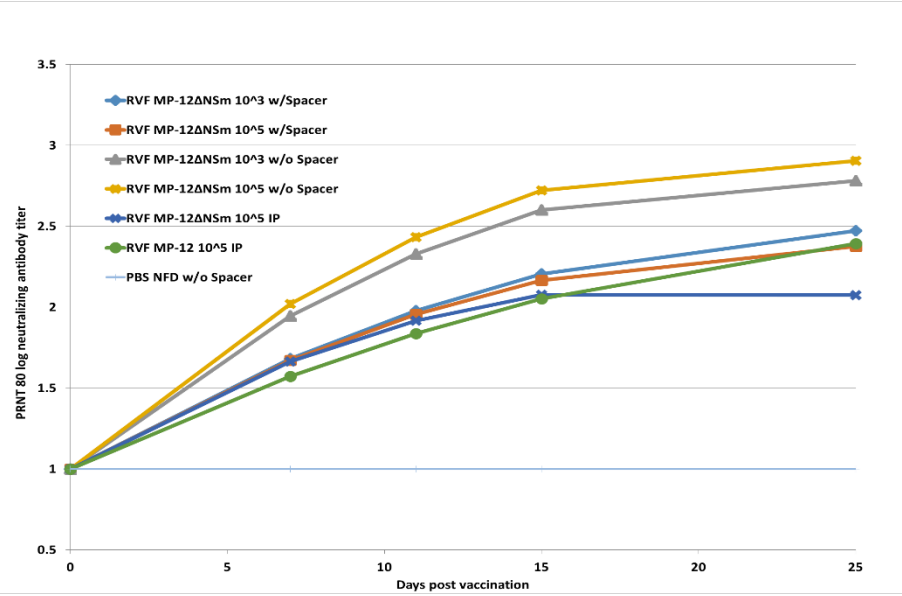

Fig. 1: Fitted trend of neutralizing antibody response in WF rats vaccinated with RVF MP-12 vaccine candidates by the needle-free device or the needle via IP injection.

Table 1: Serum neutralizing antibody responses of rats inoculated with different doses of RVF MP-12 and RVF MP-12ΔNSm vaccines*

		Days post-vaccination				
Vaccine	ID	0	7	11	14	25
1 x 10^3 PFU RVF-MP12ΔNSm	# 1	< 1/10	1/80	1/320	1/320	1/640
NFD with Spacer	# 2	< 1/10	1/40	1/320	1/160	1/1280
Group I	# 3	< 1/10	1/40	1/640	1/320	1/1280
	# 4	< 1/10	1/40	1/160	1/80	1/320
	# 5	< 1/10	< 1/10	< 1/10	< 1/10	< 1/10
1 x 10^5 PFU RVF-MP12ΔNSm	# 6	< 1/10	1/20	1/320	1/80	1/320
NFD with Spacer	# 7	< 1/10	1/20	1/320	1/320	1/320
Group II	# 8	< 1/10	1/40	1/320	1/320	1/1280
	# 9	< 1/10	1/80	1/160	1/320	1/320
	# 10	< 1/10	< 1/10	< 1/10	< 1/10	< 1/10
1 x 10^3 PFU RVF-MP12ΔNSm	# 11	< 1/10	1/10	1/320	1/320	1/640
NFD without Spacer	# 12	< 1/10	1/40	1/640	1/320	1/640
Group III	# 13	< 1/10	1/80	1/80	1/1280	1/320
	# 14	< 1/10	1/1280	1/80	1/320	1/320
	# 15	< 1/10	1/20	1/320	1/640	1/640
1 x 10^5 PFU RVF-MP12ΔNSm	# 16	< 1/10	1/160	1/320	1/1280	1/640
NFD without Spacer	# 17	< 1/10	1/40	1/640	1/320	1/640
Group IV	# 18	< 1/10	1/160	1/1280	1/1280	1/1280
	# 19	< 1/10	< 1/10	1/80	1/80	1/640
	# 20	< 1/10	1/80	1/640	1/320	1/640
1 x 10^5 PFU RVF-MP12ΔNSm	# 21	< 1/10	1/20	1/40	1/40	1/80
IP	# 22	< 1/10	1/40	1/640	1/80	1/80
Group V	# 23	< 1/10	1/40	1/40	1/40	1/80
	# 24	< 1/10	1/20	1/320	1/80	1/320
	# 25	< 1/10	1/10	1/640	1/80	1/320
1 x 10^5 PFU RVF-MP12	# 26	< 1/10	1/160	1/80	1/80	1/1280
IP	# 27	< 1/10	1/40	1/80	1/80	1/80
Group VI	# 28	< 1/10	1/10	1/40	<1/40	1/160
	# 29	< 1/10	1/40	1/320	1/80	1/320
	# 30	< 1/10	1/40	1/40	1/320	1/320
PBS	# 31	< 1/10	< 1/10	< 1/10	< 1/10	< 1/10
NFD without spacer/Group VII	# 32	< 1/10	< 1/10	< 1/10	< 1/10	< 1/10

*Data are expressed as the reciprocal of 80% plaque-reduction neutralization titer

macaques protected against the RVFV challenge of these animals (Peters *et al*., 1988), thus, affording a protective role for antibody against RVF disease and that our preliminary results indicated that a NFD route would be effective for inducing an immune response that would be protective against wild-type RVFV infection.

Conclusions

These preliminary data are very promising in regards to our goal of further evaluation of a needle-free delivery system for vaccinating livestock in Africa with RVF vaccines candidates. However, the skin thickness of the animals, such as sheep, goat, and cattle needs to be considered, with the potential use of NFD without the spacer for the proper delivery of the vaccine through the animal skin.

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Disclaimer

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