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Research

Evaluation of the Immunogenicity of a Mutagenized Rift Valley Fever MP-12 and a Recombinant Rift Valley Fever arMP12ΔNsm21/384 Vaccine Candidates in Indigenous Species of Cattle, Sheep, and Goats in Tanzania

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ABSTRACT

Objective: Rift valley fever virus (RVFV) is the cause of devastating outbreaks among domestic ruminants and humans in most African countries and in the Arabian Peninsula. Current efforts to develop and evaluate an improved veterinary vaccine to prevent RVF disease among domestic ruminants includes a live attenuated RVFV MP-12 candidate and a recombinant vaccine referred to as arMP12ΔNsm21/384 that was derived from the RVF MP-12 vaccine candidate. The aim of this study was to evaluate these RVFV vaccine candidates in domestic ruminants in Tanzania.

Methods: Six to nine months old goats (*Capra aegagrus hircus*), calves (*Bos taurus indicus*) and sheep (*Ovis aries*) were vaccinated subcutaneously with one ml each of 1×10^5 plaque forming units (PFU)/ml of the RVF MP-12 and/or the arMP-12ΔNSm21/384 candidate vaccines. The controls included six animals, two of each species which received 1 ml of Eagle's Minimum Essential Medium as a placebo. Blood samples were collected on day 2 before vaccination, and on the day (0) immediately before vaccination were tested for RVFV antibody to insure that only antibody negative animals were used in the vaccine trials. Samples collected on days, 3, 4, 5, 7 post-vaccination (PV) to determine the possibility of a vaccine induced viremia, and on days 4, 5, 7, 14, 21, 28, 35, 42 and 67 to determine the immune response of the vaccinated animals. Sera samples were tested for RVFV RNA by RT-PCR and for infectious virus in Vero E6 cells. The immune response was determined by testing sera samples for antibody using a commercial ID-VERT ELISA kit (Montpellier-France) as well as a plaque reduction neutralization test (PRNT). Animals were observed daily for adverse effects and rectal temperature was recorded at the time of blood collection.

Results: All vaccinated animals developed RVFV neutralizing antibodies with titers ranging from 1:10 in some animals as early as day 4 and 5 PV to as high as 1:160-1:2560 over the 67 day study period with no adverse effect observed in any of the animals. The antibody titers of goats to both MP-12 and arMP-12ΔNSm21/384 vaccines was significantly higher than the response observed for sheep and cattle. A viremia was not detected in any of the vaccinated and control animals.

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Conclusions: The findings of this study demonstrated that the RVFV MP-12 and arMP12ΔNsm21/384 vaccine candidates administered by the SC route elicited RVFV neutralizing antibodies in indigenous species of cattle, sheep, and goats in Tanzania, and therefore warrants further studies to assess the safety and protective efficacy of these vaccine candidates in domestic ruminants.

Keywords: Rift valley fever (RVF); Virus; Sheep; Goats; Calves; RVF MP12 vaccine; arMP-12ΔNSm21/384; Tanzania

INTRODUCTION

Rift valley fever virus (RVFV) is classified as Category A Select Agent by the CDC and the USDA and is a major zoonotic threat to humans and livestock in most African countries and in the Arabian Peninsula [1]. RVFV is an enveloped RNA virus with a tripartite genome of approximately 11.9kb which utilize an ambisense mode of transcription [2]. The virus is enzootic in most of African countries and the Arabian Peninsula as a high-consequence pathogen of serious socioeconomic and veterinary and public health concern with the potential for international spread (List A) (OIE 2009) (WHO, 2015). Also, the economic impact is enormous due mainly to international restrictions on trading livestock that have been infected with RVFV [3]. Mosquitoes of *Aedes* and *Culex* species are the known biological vectors of the virus, but the virus has been isolated from other arthropods, including phlebotomine, sandflies and ticks [4-12]. Humans are infected through the bite of RVFV infected mosquitoes and direct contact and/or aerosol associated with infected animals or body fluids [5,13-18]. RVFV outbreaks have been reported following heavy rains at intervals of 5-15 years in most of the east African countries [19,20]. Outbreaks in West Africa have been associated with imported cattle and closing of the Diama dam on the Senegal River and in Egypt with irrigation of crops following the suspected introduction of the RVFV via infected animals from Sudan [21,22]. Since its original detection in the Rift Valley of Kenya, RVFV has crossed extensive geographic barriers, including the Sahara desert, the Red Sea and the Indian Ocean, and has spread in Africa from the Cape of Good Hope in South Africa to the Nile Delta in Egypt and to the Madagascar, Mayotte, Western Africa, and Arabian Peninsula [23-42]. Due to the presence of competent mosquito vectors in non-enzootic countries, there is a potential for the virus to spread to Europe, Asia, and the Americas if no control measures are established [43-49]. The prevention of RVF relies heavily on immunization of domestic ruminants with safe and cost-effective vaccines that confer long-term protective immunity against RVF to reduce the spread of the virus [50]. However, the risk of inducing abortion in pregnant animals using LAV as well as the possibility of virulence reversion are the challenges of using LAV [51]. Although the inactivated vaccines are safe and stable with less risk of virulence reversion, the duration of protection is less and they require multiple vaccinations to confer protection against RVF as compared to LAV [3,11,44,51,52]. Also, local reactogenicity and risk of incomplete inactivation of are other shortcomings of inactivated vaccines [53].

The RVFV MP-12 is a live attenuated vaccine that was developed by mutagenesis of a virulent virus isolates RVFV ZH548 by 12 passages in human fetal lung fibroblast cells (MRC) in the presence of the chemical mutagen 5-fluorouracil [54]. MP-12 is the only dual-use vaccine that has attenuation in all three segments, including a small (S) Large (L) and medium (M) segments of RVFV genome. Evaluation of MP-12 in human volunteers showed the vaccine to be safe and to induce a protective serological immune response [55-57]. The vaccine is safe in pregnant sheep and cattle in that neither abortions nor teratogenicity was observed when challenged with the virulent RVFV [58-62]. The vaccine was also safe in golden Syrian hamsters which are known to be exquisitely sensitive to RVFV animals. The possibility of reversion to virulence by vaccination with MP-12 was ruled out in a number of studies [56,61,63]. Though MP-12 has some advantages, the vaccine does not have antigenic markers to differentiate naturally infected animals from vaccinated animals (DIVA). As a potential DIVA vaccine, the MP-12 vaccine was modified by reverse genetics technology to produce a recombinant vaccine candidate (arMP-12NSm21/384) by deleting the nucleotides in the nonstructural region of the RVFV RNA M segment (Nsm) in the pre-Gn region of the viral genome [64-66]. The arMP-12NSm21/384 vaccine was shown to be efficacious and immunogenic and did not cause abortion or fetal malformation when tested in pregnant sheep in the United States [67]. The vaccine afforded protection to sheep challenged with, virulent RVFV ZH501 during experimental studies in Canada [68]. The absence of the *Nsm* gene did not effect RVFV replication and immunogenicity and therefore may serve as a DIVA vaccine [65-67,69-71]. Also, this candidate vaccine retained the original MP-12 attenuations in the L, M and S viral RNA segments and was stable by both phenotypic and genetic sequence analysis [63,68,72]. Therefore, the possibility of acquiring the *NSm* deleted gene by reassortment from other Phleboviruses would not alter the attenuations, thus, these findings indicated that the vaccine would not revert to virulence and the MP-12 and arMP-12NSm21/384 vaccines could be used in RVFV non-enzootic areas with very low risk of introducing a virulent virus. This study will, therefore, test the immunogenicity of the RVFV MP12 and arMP-12NSm21/384 vaccine candidates in the indigenous species of cattle, sheep, and goats in Tanzania following subcutaneous vaccination.

MATERIALS AND METHODS

Animals

The study was conducted at Sokoine University of Agriculture (SUA) in an insect proof Animal Biosafety Level 2 (ABSL 2) large animal facility. Goats (*Capra aegagrus*), calves (*Bos taurus indicus*) and sheep (*Ovis aries*), 6 to 9 months old were purchased from local livestock keepers in Morogoro region of Tanzania, and screened for RVFV and RVFV antibody and kept in the facility for 2 weeks prior to implementing the vaccine trials. Animals were initially treated with ®Steladone 300 EC and 4 ml 2.5% Albendazole and were fed with ad libitum fresh grasses, water, and mineral blocks throughout the study. All animals were managed and treated in accordance with a protocol approved by the Institutional Animal Care and Use Committee, University of Texas at El Paso (UTEP), Texas. A total of 36 goats, sheep, and calves, including 12 animals of each species were used in this study.

Vaccine strains

The vaccine candidate arMP-12NSm21/384 was obtained from Multi-chemical industry (MCI) Santé Animale Biopharmaceutical Company in Mohammedia, Morocco in a lyophilized form. Each dose of the vaccine was reconstituted in 2 ml of Eagle's Minimum Essential Medium (EMEM) containing 4% fetal bovine serum (FBS). The infectivity titer of the arMP-12ΔNSm21/384 vaccine virus was 105.5TCID50/ml in Vero E6 cells.

The MP-12 vaccine virus was originally obtained at a concentration of 1.4×10^7 PFU/ml by UTEP from the World Reference Centre for Emerging Viruses and arboviruses, Department of Microbiology and Immunology, University of Texas Medical Branch, Galveston, Texas. At SUA, a working stock of the MP-12 virus was prepared in Vero E6 cells with infectivity titer of 1.0×10^7 plaque forming units (PFU/ml). The Vero E6 cells used in this study were provided by UTEP.

Vaccination

Goats, calves and sheep were manually restrained and vaccinated subcutaneously (SC) with RVFV MP-12 and arMP-12ΔNSm21/384 vaccines. The vaccines were diluted in Eagle's minimum essential media (EMEM) to achieve the vaccine dose. One ml of 1×10^5 PFU/ml of the MP-12 vaccine was used to vaccinate each of 5 goats, 5 calves and 6 sheep. Similarly, 5 goats, 5 calves and 4 sheep each received 1 ml of 1×10^5 PFU of the arMP12ΔNSm21/384 as previously described [63,69]. The controls consisted of six animals, two of each species which received 1 ml of EMEM as a placebo. All animal handlers as well as veterinarians involved in the vaccination process adhered to international biosafety practices appropriate for ABSL 2 laboratory including wearing personal protective gears during vaccination and sample collection.

Sample collection and testing plans for virus and antibodies

Blood samples were collected from control and vaccinated goats, sheep and calves on day 2 before vaccination, and on day 0 immediately before vaccination for any previous serological evidence of RVFV infection and on days 3, 4, 5, and 7 post vaccination (PV) to determine the possibility of a vaccine induced viremia and on days 4, 5, 7, 14, 21, 28, 35, 42, and 67 PV to determine the immune response of the vaccinated animals. Serum was obtained from blood collected after centrifugation at 1000 g and duplicates of each sample were stored at -80°C until tested for the virus and/or antibody. As described below, sera samples obtained from blood on days 3, 4, 5, and 7 PV were tested for virus by RT-PCR and for virus in Vero E6 cells. Sera samples obtained on days 4-67 PV were tested for RVFV antibody by a commercial ID-VERT ELISA kit (Montpellier-France) ELISA and for neutralizing antibody by a plaque reduction neutralization test (PRNT). The animals were observed daily for adverse effects and rectal temperature was recorded during blood collection.

Euthanasia

After 67 days of the study, all animals used in the vaccination study were humanely euthanized using a captive bolt and pentobarbital sodium administered as 120 mg/kg intravenously (IV).

Virus isolation

Serum samples were tested for the virus in confluent Vero cell monolayers propagated in 25-cm^2 flasks. The samples were diluted 1:10 in Hanks balanced solution with Earl's salt (HBSS) supplemented with 2% FBS and 1% of penicillin/streptomycin antibiotics. The media was discarded from the cell monolayer and 1 ml of the each diluted serum sample was added per culture for each sample. The cells and inoculum were then incubated for 1 hour at 37°C and rocked gently every 15 minutes. Thereafter, 3 ml of EMEM supplemented with 8% FBS and 1% penicillin/streptomycin was added to each culture and incubated in 5% CO_2 at 37°C. Cell cultures were observed for cytopathic effect (CPE) for 10 consecutively days before blind passage into a new flask of cells that were observed for another 10 days for CPE.

The antibody response of the animals vaccinated with the two vaccines, MP-12 and arMP-12NSm21/384 were initially determined by testing sera samples obtained on days 4-67 using a commercial competition Inhibition Enzyme-Linked Immunosorbent Assay (cELISA) kit(Montpelier-France). Samples were tested according to the manufacturer instructions. For each sample, the competition percentage (S/N%) was calculated and any value equal to or less than 40% was considered positive. A value greater than 50% was a negative result and the values between 40% and 50% indicated doubtful results.

All sera samples were tested for neutralizing antibody by a PRNT. The PRNT has performed in 4 days old Vero E 6 confluent monolayers propagated in 24 well plates. Antibody

titers of the sera samples were determined by diluting each sample 1:10 to the maximum dilution of 1:10,240. Each PRNT included the test sera, and a known RVFV antibody positive serum sample and an RVFV antibody-negative serum sample from sheep, goats, and calves. The number of plaque forming units (PFU) used as the virus dose in each PRNT was confirmed by plaque assay based on testing a mixture of equal volumes of the 60-80 PFU and HBSS to confirm that the final virus dose ranged from 30-40 PFUs. The antibody positive control consisted of a mixture of an equal volume of 60-80 PFU and a 1:10 dilution of antibody positive serum. Serum samples were diluted in HBSS and mixed with equal volume of the (1/4000) diluted MP-12 vaccine virus (75 µl each) in a 96 well plate. The mixture of sera samples and virus was then incubated at 37°C for 1 hour. A total of 50 µl of each dilution of the serum-virus mixture was then inoculated onto each of 2 Vero E6 cells monolayers. The cells and inoculum were incubated at 37°C. After 1 hour of incubation at 37°C, 0.5 ml of an agarose overlay consisting of equal parts of 1% agarose and 2x basal Eagle medium with Earle salts (2XEBME), 17 mM HEPES [N-2-hydroxyethylpiperazine-N'-2-ethane sulfonic acid], 8% fetal bovine serum, and 1% of penicillin and streptomycin was added to each culture. After incubation at 37°C in a 5% CO_2 atmosphere for 3 days, a second agarose overlay containing 5% of neutral red stain was added to each culture, 0.5 ml per culture. PFU were enumerated the next day using a lightbox. The dilution of each serum sample that reduced the virus dose by 80% reduction was recorded as the virus neutralization antibody titer.

Reverse transcription polymerase chain reaction (RT-PCR)

RT-PCR was used to test serum samples for RVFV before vaccination of the animals, as well as after vaccination to test for viremia. A reaction mix of 15 µl was used with 0.1 µM of primers designed to amplify the M segment of RVFV giving PCR amplicons of approximately 550bp. A pair of primers used for amplification of RVFV was RVF forward 5'TGT GAA CAA TAG GCA TTG G'3 and RVF reverse 3'GAC TAC CAG TCA GCT CAT TAC 5' [70] run with cycling conditions set at 50°C for 30 minutes, initial PCR activation at 95°C for 30 minutes, followed by 40 cycles of 95°C for 30 seconds, 58°C for 1 minute, and 72°C for 2 minutes, and lastly a final extension at 72°C for 10 minutes. The MP-12 RNA was used as a positive control which was included in each RT-PCR run. The PCR amplicons, together with Hi-Lo™ DNA Marker (Bio nexus, Inc.), were loaded and separated on a 1.5% agarose gel (stained with 10 µl of gel red) using electrophoresis at 120 volts for 45 minutes and visualized using a UV-transluminator. A ladder of 50pb was used to estimate the band size of the PCR amplicons.

Statistical analysis

Data collected in this study were analyzed by using R software 3.4.1. The immunologic response between vaccines and vaccinated species were tested using Welch two-sample t-test and two-way ANOVA test (95% Confidence level) to determine significance difference. Variables with p values <0.05 were considered to have a significant difference.

RESULTS

Vaccine safety

A viremia was not detected in any of the goats, sheep and/or calves based on assaying sera samples collected on days 3, 4, 5, and 7 PV in Vero E6 cells and for viral RNA by a RT-PCR assay. Evidence of clinical disease was not observed as the rectal temperatures were not above 39°C for all animals throughout the study. Moreover, all species of animals that received only EMEM were negative for RVFV antibodies on all PV days.

Immunogenicity

Antibody response in goats: Neutralizing antibody was first detected in 2 of 5 goats on day 5 and in all 5 goats vaccinated with MP-12 on day 7 PV. Subsequently, antibody was detected in all goats through day 67 PV or the last observation day with titers that ranged between 1:10 to 1:2,560 (Table 1a). The peak antibody response in goats vaccinated with MP-12 ranged from 1:640 to 1:2,560 on day 21 PV. Unlike neutralization assay, antibody was first detected by the ELISA in 2 of 5 goats on day 7 PV in the MP-12 vaccinated animals. All MP-12 vaccinated goats were positive by day 14, including the one that was doubtful on day 7 (Table 1b).

Table 1a: Neutralizing antibody response for 10 goats vaccinated with MP12 and arMP12Nsm del vaccines. Antibody titers are expressed as the reciprocal of PRNT 80% neutralization titers.

Goats vaccinated with MP12 vaccine								
Days post-vaccination	DPV 5	DPV 7	DPV 14	DPV 21	DPV 27	DPV 35	DPV 42	DPV 67
#27	68.2%	52.9%	25.6%	9.9%	6.7%	7.2%	8.7%	9.2%
#31	93.6%	38.9%	16.3%	11.7%	10%	12%	14.9%	10%
#35	70.1%	62.1%	10%	5.2%	4.7%	3.8%	3.5%	23.2%
#28	54.2%	43.3%	16.1%	5.8%	5.8%	4.2%	3.7%	3.5%

#29	72.9%	38.3%	10.6%	4.5%	5.8%	5.1%	4.1%	4.4%
Goats vaccinated with arMP12Nsm del vaccine								
#37	72%	49.3%	12.5%	8.1%	17.7%	13%	8%	8%
#36	80%	39.1%	19.3%	5.3%	5.6%	4.2%	3.3%	3.2%
#34	62%	47.3%	16.3%	17.1%	8.8%	8.4%	8.1%	8.0%
#33	74%	60.2%	28.5%	12.3%	8.9%	9.1%	9.3%	9.5%
#32	47.9%	38.9%	8%	4.1%	3.6%	4.1%	4.6%	4.8%
CUTOFF	cELISA (SN%), ≤ 40%=Positive, 40%-50%=Doubtful, ≥ 40%=Negative							

Among the goats vaccinated with arMP-12ΔNSm21/384, neutralizing antibody was detected in 3/5 animals on day 4 PV, and on day 5 PV, all 5 goats had neutralizing antibody with titers that ranged from 1:10 to 1:40 (Table 1a). Antibody titers peaked between days 14-28 PV with titers ranging from 1:40 to 1:2, 560. Similarly, 2/5 arMP-12ΔNSm21/384 vaccinated goats were positive on day 7 PV for antibody by the ELISA and one animal was doubtful on day 7 and by day 14, all five animals were antibody positive (Table 1b).

Table 1b: Antibody response for 10 goats vaccinated with MP12 and arMP12Nsm del vaccines obtained by a commercial cELISA kit. cELISA results are expressed in inhibition percent (S/N%).

Goats vaccinated with MP12 vaccine								
Days post-vaccination	DPV 5	DPV 7	DPV 14	DPV 21	DPV 27	DPV 35	DPV 42	DPV 67
#27	68.2%	52.9%	25.6%	9.9%	6.7%	7.2%	8.7%	9.2%
#31	93.6%	38.9%	16.3%	11.7%	10	12%	14.9%	10%
#35	70.1%	62.1%	10%	5.2%	4.7%	3.8%	3.5%	23.2%
#28	54.2%	43.3%	16.1%	5.8%	5.8%	4.2%	3.7%	3.5%
#29	72.9%	38.3%	10.6%	4.5%	5.8%	5.1%	4.1%	4.4%
Goats vaccinated with arMP12Nsm del vaccine								
#37	72%	49.3%	12.5%	8.1%	17.7%	13%	8%	8%
#36	80%	39.1%	19.3%	5.3%	5.6%	4.2%	3.3%	3.2%
#34	62%	47.3%	16.3%	17.1%	8.8%	8.4%	8.1%	8.0%
#33	74%	60.2%	28.5%	12.3%	8.9%	9.1%	9.3%	9.5%
#32	47.9%	38.9%	8%	4.1%	3.6%	4.1%	4.6%	4.8%
CUTOFF	cELISA (SN%), ≤ 40%=Positive, 40%-50%=Doubtful, ≥ 40%=Negative							

Antibody response in calves: Neutralizing antibody was detected in one of five calves vaccinated with MP-12 on day 4 PV (Table 2a). Three of the other calves were positive on day 5 PV and 7 PV. Antibody fluctuated between positive and negative results for one animal throughout the experiment. Despite the variation in antibody onset, all five calves vaccinated with MP-12 had developed RVFV neutralizing antibody by day 14 PV through day 67 PV. Antibody peak titers in MP-12 vaccinated calves ranged from 1:10 to 1:160 on day 14. The antibody was first detected in 2/5 calves by I-ELISA on day 14 PV in animals vaccinated with MP-12 (Table 2b). The other two calves had antibody on day 21 while antibody was not detected by cELISA

in one animal throughout the study. Although antibody was first detected on days 14 & 21, detectable antibody in two calves fluctuated between positive and negative results during the 67 days observation period.

Table 2a: Neutralizing antibody response for 9 calves vaccinated with MP12 and arMP-12ΔNSm21/384 vaccines Antibody titers are expressed as the reciprocal of PRNT 80% neutralization titers.

Calves vaccinated with MP12 vaccine									
Days post-vaccination	DPV 4	DPV 5	DPV 7	DPV 14	DPV 21	DPV 28	DPV 35	DPV 42	DPV 67
#6	<10	10	<10	160	160	160	160	160	160
#3	10	<10	<10	40	40	40	10	10	160
#13	<10	<10	10	160	40	10	40	160	40
#7	<10	10	10	40	10	10	10	10	10
#2	<10	<10	10	10	40	<10	<10	<10	<10
Calves vaccinated with arMP12Nsm del vaccine									
#8	<10	<10	10	160	160	40	40	40	40
#11	<10	10	10	40	40	40	40	40	10
#9	<10	10	10	40	40	10	10	10	40
#10	<10	<10	<10	40	40	160	10	10	10
#12	<10	<10	<10	10	10	10	10	40	40
CUTOFF	PRNT titer >10=Positive, <10=Negative								
# Animal Identity, DPV=Day post vaccination (DPV)									

Two of the five calves vaccinated with arMP-12ΔNSm21/384 had detectable neutralizing antibody on day 5 PV. Subsequently, antibody was detected in 1 of the remaining 3 animals on day 7 PV, and by day 14 PV, all five animals were positive for neutralizing antibody and remained positive until the last day of the experiment. Antibody titers for calves vaccinated with arMP-12ΔNSm21/384 ranged between 1:10 to 1:160 (Table 2a).

Antibody peak titers in vaccinated calves ranged from 1:10 to 1:160 on days 14 & 21 PV. Calves vaccinated with arMP-12ΔNSm21/384 had detectable antibody in 1/5 by I-ELISA on day 14 and 2/5 animals on day 21 (Table 2b). One calf was doubtful on 14 DPV before turning positive on day 21 and one of the five calves was negative throughout the 67 days of observations.

Table 2b: Antibody response for 9 calves vaccinated with MP12 and arMP-12ΔNSm21/384 obtained by a commercial cELISA kit. cELISA results are expressed in inhibition percent (S/N%).

Calves vaccinated with MP12 vaccine								
Days post-vaccination	DPV 5	DPV 7	DPV 14	DPV 21	DPV 28	DPV 35	DPV 42	DPV 67
#6	82%	72.5%	61.6%	39.2%	27.6%	28.2%	29.4%	24.2%
#3	71.8%	63.5%	39.6%	22.6%	25.6%	30.8%	33%	35%
#13	87.2%	80%	22.4%	35.9%	41.9%	40.9%	39.6%	32.9%
#7	68.1%	62.3%	54.3%	32.2%	44.4%	40.1%	35.8%	32.8%

#2	80%	85%	71.9%	54%	70.7%	68.2%	54.8%	43.7%
Calves vaccinated with arMP12Nsm del vaccine								
#8	64%	58.5%	47.4%	29.6%	27.3%	24.1%	23.4%	19.8%
#11	86%	85%	73.6%	51.3%	46.7%	60%	69.9%	71.9%
#9	69.4%	67.7%	44.1%	22.2%	27.5%	25.1%	20.1%	28%
#10	80.1%	72%	63.6%	49%	43.7%	43%	42.4%	40.9%
#12	64.3%	54.2%	39.6%	28.5%	35.5%	48.5%	52.8%	58.2%
CUTOFF	cELISA (SN%), ≤ 40%=Positive, 40%-50%=Doubtful, ≥ 40%=Negative							

Antibody response in sheep: Neutralizing antibody in sheep vaccinated with MP-12 was first detected on day 5 PV in 2 of 6 animals. By day 7 PV, all MP-12 vaccinated sheep had neutralizing antibody with titers ranging from 1:10 to 1:160 (Table 3a). The peak antibody response in sheep ranged from 1:40 to 1:160 PV on days 14. Antibody was first detected by cELISA on day 7 in 3/6 sheep vaccinated with MP-12. Later on day 14 PV, two other animals became positive including one animal which was doubtful on day 7. By day 21 PV all the animals had detectable antibody by cELISA. ELISA antibody was not detected in one sheep until day 42 PV (Table 3b).

Table 3a: Neutralizing antibody response for sheep vaccinated with MP12 and arMP-12ΔNSm21/384 vaccines. Antibody titers are expressed in reciprocal of PRNT 80% neutralization titers.

Sheep vaccinated with MP12 vaccine									
Days post-vaccination	DPV 4	DPV 5	DPV 7	DPV 14	DPV 21	DPV 28	DPV 35	DPV 42	DPV 67
#17	<10	10	40	160	160	160	40	40	40
#16	<10	<10	40	40	40	10	40	40	40
#15	<10	<10	10	40	40	160	40	40	10
#19	<10	10	40	160	160	40	160	10	160
#24	<10	<10	40	160	160	160	40	40	10
#14	<10	<10	40	40	40	40	40	10	40
Sheep vaccinated with arMP12Nsm del									
#18	<10	<10	40	160	160	160	160	40	40
#20	<10	40	10	640	10	10	10	10	10
#23	<10	<10	40	40	40	40	10	10	10
#25	<10	<10	10	40	640	640	160	160	10
CUTOFF	PRNT titer >10=Positive, <10=Negative								
# Animal Identity, DPV=Day post vaccination (DPV)									

Neutralizing antibody was detected in 1 of 4 arMP-12ΔNSm21/384 vaccinated sheep on day 5 PV (Table 3a). By day 7 PV, the other 3 animals had developed neutralizing antibody. The antibody titers for sheep vaccinated with arMP-12ΔNSm21/384 ranged from 1:10 to 1:640 (Table 3a). The peak antibody response in sheep vaccinated with the

arMP-12ΔNSm21/384 vaccine ranged from 1:40 to 1:640 on days 14-28 PV. Sheep vaccinated with an arMP-12ΔNSm21/384 developed antibody that were first detected by cELISA in 2 of 4 animals on day 7 PV, in one of 4 on day 14 PV and the last sheep on day 21 PV. Detectable antibody for one animal fluctuated between positive and negative throughout the experiment (Table 3b).

Table 3b: Antibody for sheep vaccinated with MP12 and arMP-12ΔNSm21/384 obtained by a commercial cELISA kit. cELISA results are expressed in inhibition percent (S/N%).

Sheep vaccinated with MP12 vaccine								
Days post-vaccination	DPV 5	DPV 7	DPV 14	DPV 21	DPV 28	DPV 35	DPV 42	DPV 67
#17	77.6%	17.1%	14.4%	6.9%	8.9%	12.8%	19.8%	15.8%
#16	82%	76.3%	31.7%	29.4%	38.2%	48.1%	53.9%	50.2%
#15	73.2%	64%	56.5%	32.2%	46.8%	40.2%	36.6%	35.9%
#19	104%	104%	102%	71%	97.7%	92.5%	86.5%	74.9%
#24	45.8%	19.2%	11%	5.3%	22.5%	15.8%	10.9%	9.7%
Sheep vaccinated with arMP12 Nsm del vaccine								
#14	87%	31.2%	25.9%	12.6%	27.2%	34.6%	48.3%	42.9%
#18	67.3%	39.1%	18.9%	4.6%	23.8%	18.9%	4.9%	3.8%
#20	74%	61.5%	45.8%	21.7%	20.8%	25.9%	26.5%	30.2%
#23	65.8%	52.5%	23.5%	15.1%	34.7%	48.9%	53.2%	50.3%
#25	55.7%	44.4%	23.9%	12.4%	14.6%	19.7%	15.1%	11.3%
CUTOFF	cELISA (SN%), ≤ 40%=Positive, 40%-50%=Doubtful, ≥ 40%=Negative							
# Animal Identity, DPV=Day post vaccination (DPV)								

Comparison between groups: Welch t-test did not reveal any significant difference (p=0.09) in immunologic response within species vaccinated with MP-12 and arMP-12ΔNSm21/384 (Table 4). Also, there was no significant difference in the immune response elicited in all animals vaccinated with MP-12 compared to those vaccinated with the arMP-12ΔNSm21/384 vaccine (p=0.08). ANOVA test showed that goats vaccinated with MP12 and arMP-12ΔNSm21/384 had a significantly higher immune response compared to cattle and sheep (Table 4).

Table 4: Statistical analysis by student t-test and ANOVA for the three species (goat, sheep, and calves) vaccinated with MP12 and arMP-12ΔNSm21/384 vaccines.

Student t-test at 95% Confidence level results			
Variables	Levels	Measures	p values
Species	Goat	MP12	0.04
		arMP-12ΔNSm21/384	
	Calves	MP12	1.22
		arMP-12ΔNSm21/384	
	Sheep	MP12	0.47

		arMP-12ΔNSm21/384	
Vaccinated animals	Sheep, Goats, and Cattle	MP12	
	Sheep, Goats, and Cattle	arMP-12ΔNSm21/384	0.09
ANOVA at 95% Confidence Level results			
Vaccines	arMP-12ΔNSm21/384	Sheep	
		Goats	
		Calves	0.0003*
	MP12	Sheep	
		Goats	
		Calves	0.0008*

* significant difference

DISCUSSION

The findings of this study showed that all sheep, goats and calves vaccinated SC with the RVFV MP-12 and arMP-12ΔNSm21/384 candidate vaccines developed varying titers of neutralizing antibodies. Variation of the immune response to these RVFV vaccines among sheep and calves species and individuals of these species has been reported previously [56,57,63,69]. Individual variation, as well as the genetic makeup of animals, was previously mentioned as factors which may lead to variability in immunologic response between species vaccinated with live attenuated vaccines [51]

Among the animal species vaccinated with the two RVF vaccines, MP-12 and the arMP-12ΔNSm21/384, all five goats had a stronger immunologic response than calves and sheep. The higher antibody titer for goats was more comparable to titers reported for sheep following vaccination with these vaccines in other studies [30,62,67,73-75]. The results of previous studies in sheep demonstrated an early and protective immunologic response in sheep vaccinated with MP 12 as well as arMP-12ΔNSm21/384. Similar to observations reported by others, all five sheep vaccinated with arMP-12ΔNSm21/384 and MP 12 in this study had detectable neutralizing antibodies within the first week PV, although the antibody titers were lower [61,63,75,]. An early and strong immune response in sheep increased the likely-hood of vaccinated animals being protected almost immediately during epizootics [61,67,75,76]. This observation was supported by challenge studies that showed antibody induced by the arMP-12ΔNSm21/384 vaccine protected sheep against virulent RVFV in previously reported studies. Therefore, suggesting that the higher antibody titers in goats as observed in this study would be protective against virulent RVFV infection [61,62]. Although the antibody titers in our study were lower compared to what was observed in calves in the USA, there was an agreement with the onset of antibody in calves vaccinated with arMP-12ΔNSm21/384 and MP12 which were first detected within 2 weeks of vaccination [61,62,69,70,73,74]. Even though calves had lower antibody titers, as previously observed with other RVF vaccine, the antibody titers were within the protective limit against RVFV. However, all species of animals developed varying antibody titers which are considered protective against RVFV by PRNT80 [51,66,77].

Our observations derived from this study demonstrated that the immune response elicited by the MP-12 and arMP-12ΔNSm21/384 vaccine candidates was likely to be protective for indigenous species of sheep, goats and calves against RVFV during the RVF outbreaks. While studies in the USA demonstrated that the vaccines elicited protective antibody in all 3 species for RVFV MP-12 and in sheep for arMP-12ΔNSm21/384, studies conducted in African species of domestic ruminants developed lower antibody titers when vaccinated with various RVFV vaccines including MP12, and arMP-12ΔNSm21/384 in Tanzania and Clone 13 in Senegal and Kenya [75,78]. Therefore, a more conclusive interpretation of the possible protective efficacy of antibody titers in African species will require the results of challenge of the vaccinated animals with virulent RVFV. If the MP-12 and arMP-12ΔNSm21/384 vaccines induce protective antibody, the duration of the response is unknown but another study using an inactivated RVFV vaccine suggested that one dose will elicit protective antibody for several years. [57]. While the animals in this study was vaccinated via the SC route, the use of the intra-muscular route for vaccinating Tanzanian goats with RVFV MP-12 and arMP-12ΔNSm21/384 was just as effective as using the SC route in this study [76].

A viremia was not detected in sheep, goats and calves vaccinated during this study with RVFV MP12 and arMP-12NSm21/384 vaccines. However, a viremia was detected in sheep and calves during studies following vaccination with the same two vaccines [61,67,73]. All through the viremia was very low and in only a few animals, a viremia in any of the vaccinated species is

preferred to eliminate the possibilities of transmission and dissemination of vaccine virus into the environment by RVFV vectors [67,79]. In addition to the absence of a viremia in animals during this study, preliminary findings indicated that the vaccine virus did not spread among the vaccinated and control animals housed in the same animal holding pens. The vaccine did not result in the development of any clinical signs of RVF disease as fever, diarrhea, nasal and ocular discharge. Also, the safety of arMP-12ΔNSm21/384 regarding the possibility of reversion to virulence which was previously reported in other vaccines is unlikely to occur since the vaccine is developed from a genotypic stable MP12 vaccine [80]. The acquisition of a deleted *NSm* gene by genome reassortment will result in MP12 which is an attenuated virus [81–84]. Furthermore, due to the absence of viremia in the vaccinated animals in this study, the chances of reassortment of arMP-12ΔNSm21/384 and other phlebovirus to produce a virulent variant strain of virus is not likely to occur.

In vaccination studies, neutralization assays and ELISA are the more commonly used methods to monitor the antibody kinetics in vaccinated animals [51,61–63,67,73,85]. Contrary to what was reported in other studies, this study, demonstrated a higher sensitivity of PRNT in detecting antibodies earlier than an ELISA [67,86]. Also, the current study demonstrated that the PRNT was capable of detecting neutralizing antibodies earlier than commercial ELISA [51,86]. Overall, false negative results were observed using the ELISA for 30 of 166 samples tested, thus resulting in a sensitivity of 75% for the commercial (ID VERT) ELISA kit [87]. These observations suggested the possibility of underestimation and delays in detecting antibody when samples are tested merely by the ELISA technique.

CONCLUSION

The finding of this study demonstrated for the first time that the candidate vaccines, arMP-12ΔNSm21/384 and MP12 was immunogenic following SC vaccination of Tanzanian sheep, goats and calves. The immunogenicity of the two vaccines was comparable in these animals vaccinated with MP12 and arMP-12ΔNSm21/384 candidates. However, goats were found to be the best responders to both of the vaccines and that a higher dose of vaccine will be required to elicit a more robust immune response in calves. The similarities in the immune response to the two vaccines indicated that the deletion of the *NSm* gene in the M segment of MP12 to enable potential DIVA capacity did not have any effect on the immunogenicity of the parent MP12 vaccine.

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CONFLICT OF INTEREST

The authors declare that they had no conflict of interest while conducting this study

AUTHORS' CONTRIBUTION

LP conceptualized the idea, prepared the study design and writes the manuscript. LP, SB, and EK performed the laboratory analysis of the samples under the supervision of Miss JR and Palermo P. MK led blood collection team. All the works throughout the study were supervised by DMW, GEB, and PN. All authors participated in preparation of this manuscript

DISCLAIMER

The contents are the responsibility of Sokoine University of Agriculture and UTEP and do not necessarily reflect the views of USAID or the United States Government

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