EVALUATION OF SAFETY AND IMMUNOGENICITY OF RIFT VALLEY FEVER VACCINES MP-12 AND arMP-12ΔNSm21/384 IN SHEEP, GOATS AND CALVES ADMINISTERED INTRADERMALLY

ESTER KASISI ADAMSON

A DISSERTATION SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF PHILOSOPHY IN VIROLOGY OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.

2018

ABSTRACT

Rift valley fever (RVF) is a zoonotic disease caused by Rift Valley fever virus (RVFV) with impact on animal and human health. Vaccines developed against RVF have safety and efficacy concerns and administered by needles posing risks of RVFV transmission. This study reports the safety and immunogenicity of attenuated RVF MP-12 and its recombinant arMP-12ΔNSm21/384 vaccines. A total of 32 sheep, 15 goats and 23 zebu calves were vaccinated with $1x10^5$ plaque forming units (PFU)/ml of MP-12 or arMP-12ΔNSm21/384 RVF vaccine candidates intramuscularly or intradermally. Six animals from each species were vaccinated with Eagle's Minimum Essential Medium (EMEM) as negative controls. RVFV neutralizing antibody was tested in serum samples collected on days 0, 3, 5, 7, 14, 21, 28, 35, 70, 84 and 87. Rectal temperatures were taken on days of blood collection and signs of illness were observed daily post vaccination (PV). On day 87 PV, all intramuscularly vaccinated animals were re-vaccinated with 1×10^4 PFU/ml of the MP-12 vaccine and blood samples were obtained on days 7, 14, and 21 PV. The vaccines were found to be safe and all vaccinated animals produced neutralizing antibodies against RVFV. Higher antibody response was observed in animals that were vaccinated intradermally with arMP-12ΔNSm21/384 RVF vaccine with geometric mean antibody titers (GMT) of 1113 in goats, 210 in sheep and 22 in zebu calves as compared to intramuscularly vaccinated animals with GMT of 160 in goats, 51 in sheep and 63 in zebu calves. On re-vaccination, the antibody titers increased rapidly, reaching maximum titers of 470 in sheep, 640 cattle and 640 in goats. Overall, this study indicates that these RVFV vaccines are promising candidate for the prevention of RVF among domestic ruminants, however intradermal vaccination works better for sheep and goats while intramuscular vaccination works better for zebu calves.

DECLARATION

I, ESTER KASISI ADAMSON, do hereby declare to the Senate of Sokoine University of Agriculture that this thesis is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

_____ _____

Ester Kasisi Adamson Date

The above declaration is confirmed by;

_____ _____

Prof. P. Wambura Date

(Supervisor)

_____ _____

Dr. D. M. Watts Date

(Supervisor)

COPYRIGHT

ACKNOWLEDGEMENTS

I would like to express my sincerely gratitude to my supervisors Prof Philemon Wambura and Dr. Douglas Watts for their enormous support, guidance and provision of valuable comments and suggestions that contributed immensely to the successful completion of my study. Furthermore I would like to thank Dr. George Bettinger, Dr. Mirende Matiko, Miss Jessica Rowland and Mr. Pedro Palermo for providing me with the ideas, materials and technical support that were required to complete the study.

I would also like to thank the USAID government for funding this study and provide me with all the materials I needed for my research as well as support for my well-being during the period of my study.

In addition, I would also like to thank Mr. Anthony Mhando for providing technical support with the animal experiments, Shida Hussein and Peter Marwa for taking care of the experimental animals by providing them with food, water and making sure the animals stayed in clean condition, and my colleagues, Salama Burhan and Linda Salekwa for providing me with technical support I needed during my work.

Last but not least I would also like to thank all staff and students at college of Veterinary Medicine and Bio Medical Science, Sokoine University of Agriculture for providing me with important comments and suggestions during all of my presentations that has helped to strengthen my work.

This study was funded under a subcontract from the University of Texas at El Paso (UTEP) under a Cooperative Agreement (AID-OAA-A-13-00084) from the United States Agency for International Development. The author's views expressed in this publication do not necessarily reflect the views of the United States Agency for International Development or the United States Government.

DEDICATION

I dedicate this work to my mother Maria Joel Mbene for her never ending support and for always being my motivation and strength. Also, my brother Marko Kasisi and my sister Catherine for always were being my reason to work harder.

But also I would like to dedicate this work to all the farmers in the world it is my hope that these findings will one day help them in real life.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS AND ACRONOMY

ABSL	Animal Biosafety level
APC	Antigen presenting cells
CD	Cluster of differentiation
CDC	Centre for disease control and prevention
CO_2	Carbon dioxide
DC	Dendritic cell
DIVA	Differentiating infected from vaccinated Animals
DNA	Deoxy ribonucleic acid
EBME	Eagle’s Basal Medium with Earle’s salts
ELISA	Enzyme Linked Immunosorbent assay
EMEM	Eagle's Minimum Essential Medium
et al.	And others
FAO	Food and Agriculture organization
FBS	Fetal Bovine Serum
GMT	Geometric Mean Titer
HBSS	Hanks’ Balanced Salt Solution
IACUC	Institutional Animal Care and Use Committees
IL	Interleukin
M	Medium segment
MHC	Major Histocompatibility Complex
NSm	Nonstructural protein from medium segment
OIE	World Organization for Animal Health
PCR	Polymerase chain reaction
PFU	Plaque forming unit

PRNT	Plaque reduction Neutralization test
RNA	Ribonucleic acid
RT-PCR	Reverse transcription Polymerase Chain Reaction
RVF	Rift Valley Fever
RVFV	Rift valley Fever Virus
TNF	Tumor necrosis factor
WHO	World Health Organization
α	Alpha
β	Beta

CHAPTER ONE

1.0 INTRODUCTION

Rift Valley Fever (RVF) is an arthropod borne zoonotic disease caused by Rift valley fever Virus (RVFV). The Virus is a member of the Bunyaviridae family in the genus Phlebovirus consisting of a tripartite negative single stranded RNA genome with the diameter of approximately 80-120nm. The virus genome consist of three segments, a large segment (L) that encodes for an enzyme RNA dependent RNA polymerase which helps in virus transcription and replication, Medium segment (M) which encodes for non-structural protein (NSm) and structural protein Gc and Gn where by NSm helps virus replication in cell culture, and also act as an ant apoptotic protein and Gc and Gn that helps the virus in receptor recognition, entry and budding. The last segment is a small segment (S) which is an ambisense having positive and negative sense, whereby negative sense encodes for nucleo-capsid protein which binds the viral RNA, provide structural stability to the genome and helps in virus replication and assembly while positive sense encodes for nonstructural protein NSs which is serve as a virulent factor for the virus by inhibiting the host innate immune response (Balkhy and Memish, 2003; Caplen *et al*., 1985; Morrill and McClain, 1996; Pepin *et al.*, 2010; Peters *et al.*, 1989).

Since first isolation of RVFV in 1930 in Kenya following an epidemic in sheep (Daubney *et al.*, 1931), RVFV outbreaks have been reported in most sub-Saharan countries, especially the Rift Valley in Kenya and Tanzania. Subsequent outbreaks with human cases have been reported in South Africa and the Nile Valley from Sudan to the Egyptian delta. The disease spread from continental Africa to Madagascar in 1991 and in the Arabian Peninsula in 2000. In Madagascar, RVFV was isolated for the first time in 1979 from pools of mosquitoes captured during the rainy season in the primary rain forest of Perinet,

Moramanga district. The most recent RVF outbreaks were detected in Somalia (2006–2007), Kenya (2006–2007), Tanzania (2007), Sudan (2007–2008), Madagascar (2008–2009), South Africa (2008, 2009, and 2010), Mauritania (2010), Botswana (2010) and Namibia (2010) (Boushab *et al.*, 2016; Nguku *et al.*, 2010; Sayed-Ahmed *et al*., 2015; Sindato *et al.,* 2014; Woods *et al.,* 2002).

The virus causes hemorrhagic disease associated with abortion storms and mortality in affected animals and self-limiting febrile illness in human. RVFV is classified as a Category A agent because the virus can cause high morbidity and mortality rates and has the potential for major veterinary and public health impact (Mandell and flick, 2011). In Mayotte, sporadic cases in livestock have been recorded since 2004 with human cases detected in 2007–2008 and 2011 (Balenghien *et al.*, 2013). All these outbreaks have caused social economic loss. In 1977, an outbreak of RVFV in Egypt caused an estimated 200 000 human infection were approximately 598 death reported (Abdel-Wahab, 1978). In 2007 during an outbreak in Tanzania, the estimated economic losses due to death of livestock and control of the disease were approximately USD 6 million (Sindato *et al*., 2011).

RVFV is transmitted by *Aedes* spp. of mosquitoes both horizontally and vertically, thus, vertical transmission to the eggs enables the virus to stay in the eggs of the *Aedes* mosquito during dry season. Wet seasons facilitate hatching of mosquito eggs hence following a period of heavy rainfall these eggs hatch and produce large populations of adult mosquitoes, including RVFV infected mosquitoes. The infected female mosquitoes ingest blood by feeding on vertebrates, including ruminants at the same time, transmits the RVFV to these animals, which then develop high viremia to serve as a source of virus for infecting more mosquitoes, thus serving as virus amplifying host for the virus. Other

species of mosquito such as Culex helps in transmission of the virus between susceptible species (Clark *et al.*, 1988; Jupp *et al*., 2002; Turell *et al.*, 2008a; Turell *et al*., 2008b; Turell *et al.*, 1996).

Human gets RVFV infection following mosquito bites, contact with body fluids such as blood containing infectious virus especially during the handling of dead fetuses, meat in the abattoir, helping animals with delivery, and also by ingesting raw milk or eating uncooked meat which makes infected animals and their products a major source of RVFV infection among humans (Ashford *et al.*, 2004; Boushab *et al.*, 2016; Niklasson *et al.*, 1985; Pittman *et al*., 2016).

Infection with RVFV results in fever, abortion storm, fetal malformation, mortality in young animals, hemorrhagic fever, encephalitis, retinitis, nasal discharge, coagulative necrosis, fulminant hepatitis, anorexia, depression, weakness, epistaxis and swelling of liver, lymph nodes and spleen in animals. In humans the disease causes fever, weakness, back pain, chills, malaise, severe headache, elevated body temperature, insomnia and constipation (Daubney *et al*., 1931; Findlay *et al*., 1936; Morrill and McClain, 1996; Pepin *et al.*, 2010). Normally human infected with this disease recover after short period of time but in severe cases involving about 1% or less, the disease results in encephalitis, retinitis, thrombosis, vision loss and sometimes death. Risk factors associated with disease include heavy rainfall, handling of infected animals and their products e.g. in the abattoir, veterinarians, sleeping without mosquito nets and working with infectious agent (Ashford *et al.*, 2004; Chengula et al., 2013; Jupp *et al.*, 2002; Woods *et al.*, 2002).

1.1 Problem Statement and Justification

Outbreaks associated with RVFV are still a great challenge in Africa and continue to be a threat to other parts of the world. The presence of susceptible and competent mosquito

vectors, climate change, and an increased international trade and disease outbreaks in RVFV non-enzootic areas raise an alarm on the importance of having a well-established control method against the disease (Bird and Nichol, 2012; Sindato *et al.*, 2014; Turell *et al.*, 2008a; Turell *et al.*, 2008b). Vector and animal movement control, disease surveillance and vaccination are the promising controls strategies for this disease. Lack of enough equipment, trained personnel and proper record system for source of animals together with lack of differentiating naturally infected from vaccinated animals (DIVA) vaccine makes it difficult to have an effective disease surveillance system. Cost and health effects associated with the use of pesticides hinder control of vectors that transmit the disease and there are no effective therapeutics, thus as proposed by FAO, vaccination is the only promising prevention measure against the disease (Bird and Nichol, 2012).

Availability of safe, efficacious and easy to deliver RVFV vaccine is an important criteria for control of RVF (Bird and Nichol, 2012). Vaccination can protect both animals and human against the RVF by preventing an outbreak occurrence and preventing spread of disease which helps in reducing the magnitude of outbreaks. However, most of the available RVF vaccines have safety concerns such as Smith burn vaccine which causes abortion in gestating ewes and fetal malformation(Botros *et al.*, 2006; Smithburn, 1949; Von Teichman *et al.*, 2011), Formalin Inactivated RVF vaccine provides poor immune response on single dose vaccination hence needs multiple boosters (Flehmig *et al.*, 1997). A safe Clone 13 vaccine which is current conditionally licensed in South Africa provides long time protection but experimental studies showed that the virus can cause fetal infections, malformations and stillbirths, and this vaccine is not DIVA compatible. (Dungu *et al*., 2010; Lo *et al.*, 2015; Njenga *et al.*, 2015; Von Teichman *et al.*, 2011; Makoschey *et al.*, 2016).

RVF MP-12 is a live-attenuated vaccine candidate that was developed by 12 serial passages of wild type RVFV ZH548 strain in human diploid lung (MRC-5) cells in the presence of the chemical mutagen 5-fluorouracil (Caplen *et al.,* 1985; Lokugamage *et al*., 2012). MP-12 is safe and highly efficacious in ruminants (Morrill *et al.*, 1991, 1987; Morril *et al.,* 1997a, 1997b). Also this vaccine was tested in human and found to be safe and immunogenic (Pittman *et al.,* 2016 a,b) However, it lacks a marker to differentiate infected from vaccinated animals (DIVA). To develop a MP-12-based vaccine that allows for DIVA testing, arMP-12 viruses were created that lack the non-structural region of the NSm-coding regions, referred to as **arMP-12ΔNSm21/384** (Morrill *et al.,* 2013a). Studies done with **arMP-12ΔNSm21/384** in America showed it to be safe and efficacious but there have not been any studies done using indigenous animals in Tanzania as animal breed of animals might influence the immune response to the vaccine (Bird *et al*., 2011, Morrill *et al.,* 2013a).

In addition, another concern with the available vaccines is that most of them cannot differentiate between naturally infected and vaccinated animals, which is an important criteria for disease surveillance and trade restriction especially during outbreaks where RVFV affected areas are not allowed to transport animals. In order to address the concern, this study evaluated the safety and immunogenicity of the arMP-12ΔNSm21/384 vaccine candidate which is DIVA promising so that the vaccine could be used in both enzootic and non-enzootic areas (Morrill *et al.,* 2013a). Furthermore, to address vaccination coverage especially in rural areas associated with lack of enough skilled labor this study evaluated needle free intradermal delivery of vaccine using a commercially available Bioject device that can be used even by illiterate person without affecting the safety and immunogenicity of the vaccine (Giudice and Campbell, 2006; Mousel *et al*., 2008).

1.2 Objectives of the Study

1.2.1 Overall objective

To evaluate the safety and immunogenicity of Rift Valley Fever vaccine MP-12 and arMP-12ΔNSm21/384 in sheep, goats and zebu calves using needle and needle free devices administered intradermal.

1.2.2 Specific objectives

i. To evaluate the safety and immunogenicity of RVFV MP 12 and arMP-12ΔNSm21/384 vaccine candidates in sheep, goats and zebu calves vaccinated intramuscularly by using needle.

ii. To evaluate the safety and immunogenicity of RVFV arMP-**12ΔNSm21/384** vaccine candidates in sheep, goats and zebu calves vaccinated intradermally using a Bioject ZetaJet™ needle free vaccine delivery device.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Rift Valley Fever

Rift Valley Fever (RVF) is an enzootic disease that has an enormous impact on the health of humans and domestic ruminants. This disease was first identified on a farm in the Rift Valley of Kenya in 1930s following an outbreak among humans and sheep (Daubney *et al.*, 1931). The disease was characterized by heavy mortality in new born lambs reaching 100%, animals became listless and disinclined to feed before death, high mortality in adult ewes, high abortion rate, mucol purulent and nasal discharge, blood in the stool and fever. The causative agent of the disease was found to be Rift Valley Fever Virus (RVFV). Following this outbreak it was known that the disease infected only cattle, sheep and goats.

The disease in humans presented as a flu like illness with headache, back pain, fever due to increase in temperature, shivering and malaise, photophobia and nose bleeding (Smithburn, 1949). Another study done by showed the ability of the virus to infect rodents and non-human primates such as rhesus macaques (Findlay *et al.,* 1936; Mackenzie *et al.,* 1936). Since then a lot of studies to evaluate effect of the virus or vaccine against virus in human has been done by using rhesus macaques.

2.2 Rift Valley Fever Virus Cycle

RVFV replication consists of three major cycles which are transcription, translation and assembly. Replication of the virus in an animal body is initiated by the attachment of the viral structural proteins Gc and Gn to the cell of the immune system. Immature dendritic cells located in the dermis are believed to be the first type of cells to be infected by the

virus where by, heparin sulfate serves as an attachment factor for the virus to bind and accumulates on the cell surfaces which facilitate virus entry into the cell (De Boer *et al*., 2012).

The virus enters the cell via endosome mediated pathway into the cytoplasm of the cell where it releases its viral genome (Lozach *et al.*, 2011). Transcription of the virus genome replication occurs in the cytoplasm of the host cell. An enzyme RNA dependent RNA polymerase (RdRp) is used in transcription of the viral genome making more copies of its complementary RNA which serves as template for synthesis of new viral RNA. The negative sense RNA strands is used as genetic material. The positive sense RNA strand, act as mRNA for protein synthesis. The mRNAs produced are translated into their proteins where by large (L) segment mRNA is translated into RNA dependent RNA polymerase an enzyme responsible for replication of the virus genome.

The small (S) segment mRNA is translated into Nucleocapsid (N) protein which coats the viral genomes and nonstructural NSs protein which is the major virulence factor for the virus which inhibit production of IFN Y. The medium (M) segment mRNA is translated into two structural proteins Gn and Gc and one nonstructural protein NSm. The two structural proteins play vital role in virus attachment to the host cell and the nonstructural protein plays a role in the pathogenesis of the virus by inducing an anti-apoptotic function (Ikegami, 2012; Ikegami *et al.*, 2015; Ikegami *et al.*, 2009; Lozach *et al.*, 2011).

The positive strands RNA can also be used as a template to make negative strands RNA. Negative RNA strands combine with capsids and viral RdRp to form new negative RNA viruses. Virus maturation and assembly occurs in the Golgi complex where the localization of glycoproteins occurs. After budding in the lumen of Golgi complex the

newly made RVFV are transported to the cytoplasm where they are released from the cell and spread in the body(Billecocq *et al*., 2004; Ikegami *et al.*, 2009; Vialat *et al.*, 2000).

2.3 Immune Response to RVFV

2.3.1 Innate immunity to RVFV

The innate immune system consists of soluble factors such as complement and lysozyme and cellular effectors such as macrophages and dendritic cells. Innate cells originate in the bone marrow and roams to different parts of the body such as in the tissue, blood and lymphatic system. Effectors of the innate immune system includes TNF, IFN, chemo attractant and soluble chemical factors (Clem, 2011).

RVFV is recognized by the body innate immune system via pathogen associated molecular pattern (PAMPS) which can be viral RNA or its structural and nonstructural proteins. Upon binding of these PAMPS to the pathogen recognition receptors (PPRS) which are located on the surface of immune cells such as dendritic cells that become activated and these cells start to process antigen and releasing chemokines and cytokines which in turn activate other cells of the immune system in order to remove the virus. Studies done in goats, rodents and rhesus monkeys have shown the role of innate immunity in RVFV clearance. Interferons, interleukins and cells of the innate immune system are major factors in protecting against RVFV infection before the onset of antibody production (Dodd *et al.*, 2013; Lathan *et al.*, 2017; Terasaki and Makino, 2015).

In the study done in rhesus monkey, IFN α was shown to be a crucial innate response in protecting against RVFV infection because monkey that received this interferon 12 hours before challenge with wild type virus were protected from disease (Morrill *et al.*, 1990), however RVFV has developed the mechanism by using its NSs segment which hinders IFN α production allowing virus replication and viremia.

In addition, a role of other innate responses has been demonstrated in the study done in goats and mice. Goats were protected from developing viremia after infection before neutralizing antibodies were detected suggesting the role of innate immunity in protecting against the disease. In these goats IL-12 and IFN_{γ} was produced immediately post infection although IFNα which was demonstrated to be protective in monkey was not produced (Nfon *et al.*, 2012).

IL-12 is believed to activate bovine and ovine natural killer cells to secrete IFN_{γ} which in turn activates natural killer cells to cause cytotoxicity, which protected goats before production of neutralizing antibodies at day 5 (Charles *et al*., 2012). In addition, the role of IFN_{γ} was further supported by a mouse model study where by the expression of IFNAR receptor on leukocyte was found to be crucial for the functioning of innate cells rather than production of IFNα. IFNAR helps in preventing viral spread by activating innate cells and IFN mediated response (do Valle *et al.*, 2010; Lathan *et al.*, 2017; Terasaki and Makino, 2015).

Furthermore, IFN has been shown to activate other cells of the immune system to produce antiviral factor against the virus which results in reduction of spread of infection. Additionally, innate immunity has been associated with susceptibility and resistance of mice to RVFV infection. This was shown in comparison of pathogenicity between Balb mice and MBT mice (Rashida *et al.*, 2017, Tania *et al*., 2010).

2.3.2 Adaptive immunity against RVFV

Despite the role of innate immune response in protection against RVFV infection, adaptive immunity mediated by humoral and cell mediated immunity has been shown to be important in long term protection against infection by the virus. This is because adaptive immune system works by creating memory system in order to remember the pathogen it

has encountered in the past which helps in eliciting higher immune response upon subsequent encounter with the same or similar pathogens. During innate immune response, once RVFV binds to antigen presenting cells such as immature dendritic cells, these cells process this antigen and present an epitope on the surface of Antigen Presenting cells (APC) via major histocompatibility complex (MHC) I or II and migrate to the peripheral lymph nodes or spleen where naïve T cells and B cells are present (Clem, 2011; Le Borgne *et al.*, 2006; Lenz *et al.*,1993).

For cell mediated immunity, an epitope presented on the surface of an APC via MHC II activates CD4+ T cells which in turn secrete soluble cytokines which act against the virus and activate other immune cells to perform their task (Dodd *et al.*, 2013). Cytokines like IFN $_{\gamma}$ are secreted by Th1 cells which limit the spread of the virus and activate cells capable of destroying virus infected cell; this has been shown in mouse model where production of IFN protected mice from developing viremia. Th2 cells produce cytokines such as IL-4, IL-5, IL-13 which activates innate cells and Follicular T helper cell to secrete IL-21 which helps B cell differentiation and proliferation in the follicular structure.

On the other hand, an epitope presented on the surface of an APC via the MHC I activates cytotoxic T cells (CD8+ T cell). Once activated CD8+ T cells differentiate into effector cytotoxic T cells and memory T cells. Cytotoxic T cells recognize a virus infected cells and acts on them by secreting proteins that creates pores in the membrane of infected cell and induces signaling pathway which leads to cell lysis or apoptosis.

Humoral immunity is mediated by B cells, where by production of antibodies occur in the follicular region and extra follicular region. During the extra follicular action of antibody production, B cells acts as an antigen presenting cells, where by this cell captures an

antigen via B cell receptor (BCR), processes it and presents it on the surface via MHC II molecule to CD4+ T helper cells (Reif *et al*., 2002).

Upon presentation the activated CD 4+ Th1 and Th2 cells secrete cytokines that stimulate B cells proliferation and differentiation into antibody producing plasma cells and memory B cells (Reif *et al*., 2002; Linterman and Vinuesa, 2010). During this phase the antibodies produced are usually rapid and short lasting usually IgM antibodies. In the follicular region antibody production is aid by follicular helper T cells which secretes IL-21 which causes B cell proliferation and differentiation into antibodies producing plasma cells and memory B cells (Crotty, 2011). During this action, the antibodies produced are believed to be more specific and with long lasting effects. After antibodies have been produced they migrate to the survive niches in the bone marrow where they confer long lasting immunity.

For plasma cells that remain in the peripheral lymph nodes they keep producing antibodies but because they have short life span they eventually die leaving only memory cells, which become activated upon an encounter with similar pathogen and produce strong immune response. Produced antibodies can act on the virus by binding to the surface of pathogen and act as a signal attracting other immune cells such as macrophage to destroy the pathogen, or can neutralize the virus binding to the virus and prevent attachment to cells receptor hence inhibiting infection, or can recruit effector molecules such as complements and can activate effector cells via binding of an Fc region to the specific receptor on the surface of innate cells and facilitate pathogen internalization and destruction a mechanism known as opsonization (Flehming *et al*.,1997).

2.4 Control of Rift Valley Fever

The control of RVF outbreaks requires various actions, from limiting the movement of animals, to reducing human risk through health and hygiene awareness campaigns and

targeted interventions for populations at risk. FAO and WHO have a common strategy to implement contingency plans during RVF outbreaks, and vaccination is an important tool (Bird and Nichol, 2012; Faburay *et al.*, 2017; Indran and Ikegami, 2012). Currently, there are two classical RVFV vaccines that are available in South Africa, which have been used to control recent outbreaks. The first is based on an inactivated whole RVFV vaccine. For optimal efficacy, this vaccine requires a booster vaccination and annual re-vaccination (Ahmed, 2011; Flehmig *et al*., 1997). The second vaccine is the live-attenuated Smithburn vaccine. This vaccine can provide lifelong immunity and is, therefore, a less expensive and more effective alternative to the inactivated vaccine. However, due to residual virulence, the Smithburn virus can cause abortion and foetal malformations when administered to gestating animals (Botros *et al.*, 2006; Smithburn, 1949; Von Teichman *et al.*, 2011).

There is need for a vaccine of equal, or greater, efficacy than the live-attenuated Smithburn vaccine that is as safe as the inactivated vaccine. A study suggested that humoral immunity is sufficient for protection against RVFV. Furthermore, newborn lamb acquires protective immunity after raising neutralizing antibody by having colostrum of immunized ewes (Morrill *et al.*, 2013a). In contrast, the significance of cell-mediated immunity in protection remains unknown. Another important aspect is that RVFV has relatively narrow genetic diversity. These past studies provide evidence that the ideal RVFV vaccine for both humans and animals would be one that is safe, elicits rapid humoral immune responses that neutralize known RVFV strains, and induces long-term protective immunity (Caroline *et al.*, 2014).

Vaccination can prevent the amplification of the virus and protect animals and humans from getting the disease (Bird and Nichol, 2012). There is however no point of vaccinating

during an outbreak, since it might intensify the outbreak due to the risk of iatrogenic transmission with reused needles. Also, vaccinating viremic animals with live attenuated vaccine might also result in a reassortant virus consisting of field-strains and vaccine viruses (Grobbelaar *et al.,* 2011).

2.5 Rift Valley Fever Vaccines

Since human infections originate from animal infection with RVFV, hence protecting animals against infection is an important tool in controlling human infections. Vaccination is an important tool in preventing RVF disease as it can reduce or prevent occurrence of outbreaks because vaccines can induce both call mediated and humoral immunity that can prevent virus replication in an animal's body. Several RVFV vaccines have been made, including inactivated, live attenuated vaccines such as smith burn and clone 13, recombinant vaccines such as arMP-12ΔNSm21/384, R566, virus like particles, DNA vaccines, vector based vaccines and replicon (Faburay *et al.,* 2017; Indran and Ikegami, 2012) .

Formalin inactivated RVFV vaccine is made by inactivating the virus in formalin, the vaccine is known to be safe in pregnant and non-pregnant animals since there is no risk of residual virulence. But this vaccine was shown to induce poor immune response; as a result multiple booster was needed three to four weeks after initial vaccination and annual revaccination in order to induce the required immune response. The preparation of RVFV formalin inactivated vaccines involve handling of wild type virulence virus in the manufacturing process which poses a risk to the workers (Ahmed, 2011; Barnard and Botha, 1977; Flehmig *et al*., 1997).

The live attenuated Smith Burn vaccine was used in Tanzania during 2007 RVFV outbreak was made from a Ugandan RVFV isolate and was attenuated by making serial

passage in mice and amplified in baby hamster kidney cells. This vaccine offered long term protection after the initial vaccination, especially in sheep but a poor immune response in cattle. Although the vaccine affords protection to sheep, it causes teratogenicity due to residual virulence and caused abortion in pregnant animals. In addition, since this vaccine is made from a virulent strain there is a risk of reversion to virulence and vaccinated animals cannot be distinguished from vaccinated animal, thus making the vaccine unsuitable for use to prevent RVF in domestic ruminants (Botros *et al*., 2006; Smithburn, 1949; Von Teichman *et al*., 2011).

Another live attenuated vaccine that is conditionally licensed in South Africa, Namibia, Botswana, Zambia and Mozambique is Clone 13 which is made from an isolate from infected patient. The vaccine contains mutation in the NSs segment of the virus which is a major virulent factor. When tested in ruminant, this vaccine induced long term protection after a single vaccination, but an overdose of this vaccine was found to cross placenta and cause fetal malformations in ewes vaccinated at 50 days of gestation, thus, the vaccine is not safe for vaccinating pregnant animals. Also, the vaccine needs appropriate storage to maintain its immunogenicity, however, scientists have since created a more thermostable Clone 13 vaccine which was found to be safe and immunogenic (Dungu *et al*., 2010; Lo *et al*., 2015; Makoschey *et al.*, 2016; Muller *et al*., 1995; Njenga *et al*., 2015; Von Teichman *et al*., 2011).However, this vaccine could not differentiate between naturally infected from vaccinated animals . Due to shortcomings of the available vaccines, scientist developed several promising, vaccine candidates, including live attenuated RVF MP-12 and recombinant arMP-12ΔNSm21/384.

2.6 MP 12 and Recombinant arMP-12ΔNSm21/384

MP-12 is a live attenuated RVFV vaccine which was prepared by 12 serial passage of a virulent ZH 548 RVFV isolate in a diploid human cell culture line composed of lung

fibroblast hence called medical research council cell strain 5 (MRC-5 cells) in the presence of chemical mutagen 5-fluorouracil. The ZH 548 virus was isolated during the 1977 Egyptian RVFV outbreak from a non-fatal human case. After doing 12 serial passages, the virus was found to contain 23 mutations of which 11 mutation led to the attenuation of the virus making it less virulent and suitable for use as a vaccine candidate (Caplen *et al.*, 1985; Ikegami *et al*., 2015; Indran and Ikegami, 2012; Lokugamage *et al.*, 2012).

Several studies have been done in ruminants, non-human primates, animal models and human to evaluate the safety and efficacy of the RVF MP-12 vaccine candidate. Studies done in ruminants, including sheep and calves showed this vaccine candidate to induce long lasting neutralizing antibodies against RVFV which protected the vaccinated animals from challenge with wild type RVFV ZH 501. The wild type virus used in challenge studies with this vaccine candidate was isolated from fatal human case during 1977 Egyptian outbreak (Morrill *et al*., 1987,1991, 1997a; Morrill *et al.*, 2013).

Since RVFV is known to cause abortion storms and teratogenic effects in new born animals, studies have been done to evaluate the safety of the MP-12 vaccine in pregnant animals. Vaccination of pregnant ewes during the late stage of pregnancy showed the vaccine to be safe, and that lactating lambs were able to acquire maternal antibodies against RVFV from colostrum. A study also showed the vaccine to be safe for vaccinating 2 days old lambs (Baskerville *et al.*, 1992; Morrill *et al.,* 1991, 1987; Morrill *et al.*, 2013).

Although RVFV also infect humans, there are no commercially available human vaccines. However, the RVF MP-12 has been shown to be safe and efficacious for use in human volunteers (Pittman *et al.,* 2016a,b). All vaccinated humans developed neutralizing

antibodies against the virus with $PRNT_{80}$ titer of up to 1:40 which was shown to be protective and maintained up to 5years post vaccination without a booster dose.

In addition, the vaccine has been evaluated in Rhesus macaques monkeys and upon challenge following vaccination with MP-12, all animals were protected. Also, since RVFV can be transmitted via the aerosol route, Rhesus macaques vaccinated with MP-12 were also challenged with RVFV ZH 501 wild type strain via aerosol route post vaccination and they were protected from infection (Morrill and Peters, 2011a; Morrill and Peters, 2003; Morrill and Peters, 2011b).

The concern of the potential global spread of RVFV is because of the presence of competent mosquito vectors and movement of animals especially during trade; therefore, there is also a need for an effective vaccine that could be used even in non-enzootic areas. A vaccine that can differentiate naturally infected animals and vaccinated animals (DIVA) would be of great importance. MP-12 vaccine candidate is not DIVA compatible because it lacks a marker to differentiate naturally infected from vaccinated animals. As a result, scientist deleted two genes from this candidate vaccine to create two recombinant vaccine candidates, the recombinants arMP-12ΔNSm21/384 and arMP-12ΔNSs16/198.

Studies were done to evaluate safety and immunogenicity of the newly made recombinant vaccine candidates as compared to the parent MP-12 vaccine candidate. It was shown that the recombinant arMP-12ΔNSs16/198 elicited a poor immune response in sheep as compared to MP-12 and arMP-12ΔNSm21/384 candidates which led to the discontinuation of further testing of this candidate vaccine (Morrill *et al.,* 2013a). Since arMP-12ΔNSm21/384 produced an antibody response similar to the parent MP-12 vaccine candidate and has a marker which makes it potentially DIVA compatible, several studies

have been done and are being done to evaluate safety and efficacy of this candidate vaccine in ruminants to provide more data that will enable its licensing for use in selected countries of Africa and other RVFV enzootic countries.

Studies have shown the arMP-12ΔNSm21/384 vaccine to be safe and immunogenic in sheep and calves. When pregnant sheep were vaccinated, none of the animals had abortion or teratogenic effects which show that this vaccine was safe in this domestic ruminant (Morrill *et al.*, 2013a,b; Weingartl *et al.*, 2014a,b).

2.7 Needle Free Vaccine Delivery

Needles and syringes are the most commonly used a vaccination tool that leads to avoidance of vaccination and phobic behaviors in a large proportion of people (Andrews, 2011). Needle phobia makes vaccination stressful, moreover needle-stick injury, improper and unsafe use, such as re-use of needle or syringe cause transmission of blood-borne pathogens. (Mitragotri,2005). Needle free vaccine delivery is the method by which the vaccine is administered into the body without using conventional needle. It includes method such as jet injectors, nasal sprays and oral administration. Needle free vaccine administration offers several advantages such as disease prevention caused by reuse of needles and needle stick injury during vaccination, aids in antigen dispersion as a result presenting vaccine antigen to more cells of the immune system called antigen presenting cells which increases immune response following vaccination. But it also ensures consistent vaccine delivery and maintains the quality of animal skin since there is no needle penetration into the skin. Due to fact that RVFV is a blood borne pathogen with the potential route of being transmitted due to reuse of needles there is a need to develop a vaccine that can be administered without using needle or non-invasive method (Chase *et al.*, 2008; Giudice and Campbell, 2006; Kumar, 2012; Mousel *et al.*, 2008).

Studies have evaluated safety and immunogenicity of MP-12 vaccine candidate in rhesus macaques when administered intranasal, the vaccine induced an immune response which protected these animals after challenge with wild type RVFV virus. However, due to the high clearance of vaccine antigen in the nasal cavity, large amount of vaccine is required to induce the required immune response. As a results there is a need to evaluate other vaccination routes that can be used vaccinate a large number of animals safely and rapidly, especially during outbreaks where a lot of animals need to be vaccinated (Morrill and Peters, 2011a; Morrill and Peters, 2011b).

2.8 Intradermal Route of Vaccination

The upper second layer of the skin below epidermis is called intradermal. Studies have demonstrated that the area is rich in antigen presenting cells (APC) such as immature dendritic cells and macrophages, which are the main enhancer of the innate and humoral immune response. When the vaccine is administered intradermally, tissue resident immature dendritic cell in the dermis immediately capture and process this antigen and express it on its surface via MHC molecule and subsequently matures and migrates to regional lymph nodes a process governed by IL-1-β and TNFα (Hickling and Jones, 2009; Hickling *et al.*, 2011; Kim *et al.*, 2011).

In the lymph nodes, DC acts as APCs. The type of MHC molecule determines the outcome of the type of adaptive response. That is, MHC II molecules leads to humoral mediated and MHC I molecule leads to cell mediated immune mediated responses by CD 8+ T cells. Intradermal vaccination has been shown to improve recruitment of DCs from the blood stream into dermis and their migration to lymph nodes (Clem, 2011; Koutsonanos *et al.*, 2015). As a result, administering the vaccine into this area leads to the induction of a high

immune response as compared to other target areas such as subcutaneous and intramuscular routes of vaccination.

Studies with other vaccines, such as rabies in cattle, porcine circo virus in pigs, hepatitis B and influenza vaccine in humans have shown the potential for intradermal vaccine delivery to induce immune response equivalent or higher as compared to other standard vaccination route (Alarcon *et al*., 2007; Belshe *et al*., 2004; Kulkarni *et al.*, 2013; Roukens *et al.*, 2008). Also these studies have evaluated the immune response following vaccination with small doses of the vaccine administered intradermally as compared to when standard doses were administered by other routes. The results showed that despite vaccinating animals with lower dose intradermally these animals mounted equivalent or higher antibody response as compared to the use of full doses. These findings indicated that the intradermal route of vaccination to be a promising route for vaccinating humans and animals.

Infection by the bite of RVFV is known to use DC-SIGN as receptor for internalization into host cell which are rich in intradermal cells hence administering vaccine into this area will mimic natural infection to elicit even better and more specific immune response (Lozach *et al.*, 2011). Hence, there is a need to test the safety and immunogenicity of RVFV vaccine via this route as a potential standard route for administering candidate RVFV vaccine.

2.9 Test for Rift Valley Fever Virus and Antibodies

Several methods have been established in order to detect RVFV antigen such as Revere transcription polymerase chain reaction (PCR) assay and Virus isolation cell culture assays (Garcia *et al.*, 2001; Ibrahim *et al.*, 1997; Wilson *et al.*, 2013; Wommack *et al*., 2009).

Virus isolation involves testing clinical or surveillance human and/or animal sera and/or other samples using cell culture or animals suspected of containing virus. This procedure is performed in the appropriate biosafety level laboratory to provide a safe working environment, Virus isolates can be identified using virus specific fluorescence antibody, or by neutralization test, ELISA and other techniques. PCR can be used but in many laboratory this technology is not available. confirmation is done by RT-PCR and sequencing, although due to problems associated with degradation of RNA samples virus isolation is preferred as it can easily amplify virus nucleic acid in cell culture and can be used even in vaccine development. RT-PCR is being used in several laboratories because it is not time consuming and is a sensitive Other methods using cell culture and animals can also be used to detect virus soon after infection, in fact for arthropod-borne viruses, like RVFV produces a viremia for about 3 to 5 days on day 2 to about day 5, and this is the only time any technique can be used to detect virus.

Techniques such as Haemaglutination inhibition, complement fixation assays and ELISA are used in detection of RVFV antibodies (Mansfield *et al.*, 2015; Wilson *et al.*, 2013), but there is problem of cross reactivity associated with those techniques. The only OIE approved gold standard for detecting and measuring RVFV antibodies is the plaque reduction neutralization test (PRNT) which is highly specific and sensitive and detects neutralizing antibodies (Swanepoel,1986). Hence, in order to evaluate efficacy and immunogenicity of vaccine this technique is recommended.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area and Design

The study was conducted at the Sokoine University of Agriculture (SUA) in Morogoro, Tanzania (6°49′S 37°40′E/ 6.817°S 37.667°E) in a biosafety level 2 (BSL-2) virology laboratory and animal biosafety level 2 (ABSL-2) facility located on the university farm. The BSL-2 virology laboratory is equipped with general laboratory equipment, class II biosafety cabinets for protecting personnel, and an autoclave for proper decontamination of waste. The ABSL-2 facility is screened to prevent the entrance and exit of flying insects, such as mosquitoes, fenced with a locked gate and a 24- hour guard, equipped with an incinerator to dispose of animal waste, and is located in close proximity to the ABSL-2 facility.

3.2 Ethical Approval

Animal experiment was performed according to an experimental protocol that was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) at University of Texas at El Paso, Texas (ref No. 559105-08) and Sokoine University of Agriculture Ethics Committee (ref. No. SUA/CMVBS/R.1/2017/02). All laboratory experimental procedures were performed in accordance with the guidelines of working in the Biosafety Level 2 laboratory. All personnel involved in the study received biosafety and animal care and use training from a qualified member of the SUA virology laboratory.

3.3 Vero E6 Cells and Vaccine Viruses

The Vero E6 cells used in this study were provided by the University of Texas at El Paso (UTEP), Texas. Aliquots of 1.0 ml in freeze dried form of the arMP-12ΔNSm21/384

vaccine (Lot No 15/3/2017) were provided by the Multi-chemical industry (MCI) Santé Animale Biopharmaceutical Company in Mohammedia, Morocco. The identity of arMP-12ΔNSm21/384 virus was confirmed at MCI by qualitative real time polymerase chain reaction assay (QPCR) (Garcia *et al.*, 2001) that targeted the L and M viral RNA segments of the virus (Garcia *et al.*, 2001; Nguku *et al.*, 2010) and then sequenced in Genewiz laboratories (GENEWIZ Global Headquarters; USA), using Next Generation Sequencing technology (NGS) Illumina method 1x50bp SR, HiSeq2500, High Output, per lane (V4 chemistry). The infectivity titer of the arMP-12ΔNSm21/384 vaccine virus was $10^{5.5}$ tissue culture infectious dose ($TCID_{50}$)/ml in Vero E6 cells.

The MP-12 virus was originally obtained 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 UTEP, the identity of the MP-12 vaccine virus was confirmed by plaque reduction neutralization test using RVF MP-12 specific monoclonal antibody (Mab). The Mab neutralized the infectivity titer of the MP-12 virus from 10^6 plaque forming units (PFU)/ml to 10^2 PFU/ml, but did not neutralize the infectivity titer of Sindbis and/or West Nile viruses. A stock virus of RVF MP-12 with an infectivity titer of 1.4 x 10^7 PFU/ml was prepared at UTEP in Vero E6 cells and stored in 0.5 ml aliquots at -80°C. Of this stock, 10 aliquots of 0.5 ml each were provided to the SUA virology laboratory for used to prepare working virus stocks to support this study. At SUA, a working stock of the MP-12 virus was prepared in Vero E6 cells that had an infectivity titer of 1.0 ×10^7 PFU/ml.

3.4 Bioject ZetaJet™ Injection device

This device was given as a courtesy to support the study by Dr. George Bettinger. The Bioject ZetaJet™ is a needle free injection device that delivers injection intramuscularly,

subcutaneously or intradermally by using spring pressure generated by the injector. This device uses a sterile, single syringe for individual injection in order to prevent cross contamination. The device consisted of the ZetaJet injector, sterile single use auto disabling syringe and vial adapter. The ZetaJet injector device holds the plunger and syringe during vaccination but it also contains the trigger button and spring. The plunger in the device is pushed forward when the trigger button is pressed which then forces the inoculum out through the opening of the syringe and then disable itself after injection preventing reuse of the syringe. Vial adapter holds the vaccine vial facilitating reconstitution and withdraw the inoculum without the use of a needle (Giudice and Campbell, 2006; Kumar, 2012).

3.6 RVF PCR

3.6.1 RNA extraction

The RNA of each of the animal serum samples was extracted following the manufacturer's instructions using the QIAamp® Viral RNA Mini kit. 140 ul of serum was added to 560 ul of buffer AVL containing carrier RNA followed by incubation for 10 min at room temperature. After brief centrifugation, 560 of ethanol (99.9%) was added followed by vortexing for 15s. later 630 ul of lysed sample solution was added into the QIAMP Mini column (in a 2ml collection tube) then centrifugation was done at 600 Xg for 1min. After centrifugation, the collection tubes was replaced with a new collection tube, then 500 ul of AW1 was added into the QIAMP Mini column followed by centrifugation at 6000 Xg for 1min. The tube containing filtrate was replaced with new 2ml collection tube followed by addition of 500 ul of AW2 and then centrifuged at full speed for 3min. The mini column was placed in a new collection tube and then centrifuged at full speed for 1min. lastly, the QIAMP Mini column was placed in the new 2ml collection tube and 60 ul of buffer AVE

was added into the column and then centrifuged at 6000 Xg for 1min after 1 min incubation. The RNA samples were stored at -80◦C in an ultra-low freezer.

3.6.2 Reverse transcription PCR (RT PCR)

Qiagen one step RT PCR kit was used to conduct RT-PCR assays by following the manufacturer's protocol. Performance of the PCR assay used the following set of primers; RVF f: 5' TGTGAACAATAGGCATTGG 3' and RVF r: 5' GACTACCAGTCAGCTCAGCTCATTACC 3' (Ibrahim *et al.*, 1997) which amplify the RVFV M segment with an expected band size of 550bp. Reaction mix was prepared by the addition of 3ul of Qiagen One step RT-PCR buffer 5x, 0.6ul of dNTP's (10Mm each), 0.15ul of RVF f, 0.15ul of RVF r, and 7.5 RNAse free water and 3ul RNA sample into each PCR tube. Cycling conditions were done as per manufacturer recommendations where by Reverse transcription was done at 50 ^{o}C for 30min, Initial PCR activation at 95 ^{o}C for 15 min, Denaturation at 94 ^{o}C for 1min, Annealing at 58 ^{o}C for 1min, Extension at 72 ^{o}C for 1min, Number of cycles 40 and final extension at 72 ^{o}C for 10 min. After getting PCR products agarose gel electrophoresis was done in 1.5% agar, 120Volts and 45minutes. Bands were then visualized in the UV Trans illuminator.

3.7 Virus Isolation

Testing for RVFV in blood samples obtained from the animals before and after vaccination was done in Vero E6 cells. Cells were seeded in 24 well plates and incubated in a CO_2 incubator at 37°C, 5% CO_2 for 4 days until 80% confluent. Sera samples were diluted 1:2 in 4% FBS Eagle's Minimum Essential Medium (EMEM) media. Each sample in a volume of 50 ul each was inoculated onto the monolayers of medium free cells MP-12 vaccine virus in 4%FBS EMEM media was used as positive control and 4% FBS EMEM was used as negative control. After inoculation, cells and inoculum were incubated at

37°C, 5% CO_2 for 1 hour while agitating plates after every 15 minutes to allow virus adsorption onto the cells. After 1 hour, 0.5mls of 4%FBS, EMEM media was added into each well followed by incubation of cells in a CO_2 incubator at 37°C, 5% CO_2 for 10days. Cells were observed daily for any visible cytopathic effect (CPE) and the results were recorded in the notebook.

After 10 days, the cell cultures and inoculum were frozen at -80 °C followed by doing a blind passage. Cultures were thawed at a room temperature and 50uL of the diluted mixture of cells and cell debris (1:2) was inoculated into Vero E6 cells. After inoculation cell cultures were incubated at 37 °C, 5% CO_2 for 1 hour while agitating plates every 15 minutes to allow virus adsorption onto the cells. After 1 hour, 0.5mls of 4% FBS, EMEM media was added onto each culture followed by incubation in a CO_2 incubator at 37°C, 5% CO_2 for 10days. Cells were observed daily for any visible cytopathic effect (CPE) and the results were recorded in the notebook. If found positive for the presence of RVFV the culture would immediately be autoclaved and incinerated

3.8 Plaque Reduction Neutralization Test ($PRNT_{80}$)

Sera samples obtained from all animals before and after vaccination were tested by the PRNT for RVFV neutralizing antibody. Each PRNT assay included the test sera, and a known RVFV antibody positive serum sample and a RVFV antibody negative serum sample from sheep. Each animal test serum samples was diluted in Hanks' Balanced Salt Solution (HBSS) supplemented with one % each of HEPES, penicillin and streptomycin and heat-inactivated FBS. The dilutions were made in 96 well plates beginning with a 1:5 dilution in the first wells followed by 4-fold serial dilutions of 1:20, 1:80, 1:320, 1:1280, and 1:5120 in each of subsequent wells.

Each diluted serum sample was then mixed with an equal volume of 60 to 80 PFU of MP-12 vaccine virus. The number of PFU 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 equal volume of 60-80 PFU and a 1:10 dilution of antibody positive test serum. The antibody negative control consisted of a mixture of equal volume of 60-80 PFU a 1:10 dilution of RVFV antibody negative test serum. The virus dose –serum dilution mixtures were incubated at 37 °C in the absence of CO_2 for one hour. Next, Vero E6 cells were seeded in 24-well tissue culture plates and incubated for 4-5 days at 37 °C and 5% CO_2 to provide about 90% confluence monolayers.

The growth media was then discarded from the Vero cell monolayers and 50ul of each virus dose – serum dilution mixture was inoculated onto each of 2 cell monolayers per sample. The mixture of the virus dose and the antibody positive control serum mixture were inoculated onto each of 20 cultures and the virus dose - antibody negative control serum mixture was inoculated onto 4 cultures. After the cultures and inoculum were incubated for one hour at 37°C with 5% CO_2, each culture was overlaid with 0.5 ml of a Seakem agarose (1%) with an equal volume of 2X Eagle's Basal Medium with Earle's salts (EBME) supplemented with 8% FBS and one % penicillin/streptomycin, and Glutamine+8g/l HEPES.

After 2 more days incubation at 37°C with 5% CO_2, each culture was overlaid with 0.5 ml of a mixture of an equal volume of agarose (1%) and 2X EBME supplemented with 5% neutral red, 8% FBS, and penicillin and streptomycin (1%) and Glutamine+8g/l HEPES, and incubated overnight at 37°C with 5% CO_2. On the next day, the plaque forming units (PFU) were counted and recorded for both the controls and sheep sera test samples. The

neutralizing antibody titer was the dilution the sera samples that reduced the number of PFU by 80% based on the number of PFU observed for the virus dose and antibody negative serum sample.

3.9 Specific Objective 1

Evaluation of Safety and immunogenicity of MP 12 and arMP-12ΔNSm21/384 vaccine in sheep and Zebu calves vaccinated intramuscularly with a needle.

3.9.1 Experimental animals

Healthy 20 local breeds of black head fat tailed sheep (*Ovis aries*) and 11 zebu calves (*Bos Taurus indicus*) 6-9 months old were purchased from local producers in the Mvomero district located in Morogoro, Tanzania. All animals were then transported to the animal facility located at SUA. Prior to housing, all the animals were ear tagged and treated with ®Steladone 300 EC Acaricide and 2.5% Albendazole orally in order to remove ectoparasites and endoparasite respectively. Later, the animals were acclimatized for two weeks in the ABSL-2 facility. Animals were fed ad libitum with fresh grasses, water, and mineral blocks and monitored daily for elevated body temperature as a possible indication of illness.

Blood was collected on days -14 before vaccination and the sera tested for RVFV by using RT-PCR and virus isolation. Sera samples were tested for RVFV antibodies by a plaque reduction neutralization test ($PRNT_{80}$).

3.9.2 Vaccine preparation

RVFV vaccines inoculum doses of 1×10^5 PFU/ml were prepared 2 hours before vaccination of the animals for the RVF arMP-12ΔNSm21/384 and MP-12 vaccines. Each

vial of lyophilized arMP-12ΔNSm21/384 vaccine was reconstituted in 2 ml of EMEM containing 4% fetal bovine serum (FBS) to yield a dose of 1×10^5 PFU/ml. The MP-12 vaccine stock (1.0×10^7 PFU/ml) was diluted 1:100 in EMEM to yield a final concentration of 1×105/ml of PFU. After preparation, one ml doses of each vaccine were loaded into 5 ml syringes in a class II A2 biological safety cabinet. EMEM medium supplemented with 4% FBS was prepared to administer to the control animals. The loaded syringes were kept at 4°C and transported to the ABSL2 facility in a refrigerated container.

3.9.3 Vaccination

Immediately prior to administering the vaccine, on day 0, a 3 ml venous blood sample was collected from the jugular vein of each animal with an 18 gauge vacutainer needle attached to 5 ml vacutainer tube. Each of 9 sheep and 6 zebu calves was then vaccinated intramuscularly (IM) with one ml in the neck area with 1×10^5 PFU/ml of the arMP-12ΔNSm21/384 vaccine candidate, and each of 6 sheep and 3 zebu calves were inoculated IM with one ml in the same area with 1×10^5 PFU/ml of the MP-12 vaccine, and 2 sheep and 2 zebu were vaccinated IM each with one ml of EMEM media supplemented with 4% FBS to serve as controls. Information was recorded for each animal, including the date of inoculation, vaccine dose and route, identification numbers, sex, and animal pen number. All animals were housed in the same room of the ABSL 2 animal facility.

Blood samples were obtained from each animal on days -14, 0, 3, 4, 5, 7, 14, 21, 28, 35, 70, 84, and 87 post vaccination (PV). One to 2 ml of sera were obtained from 3 mL of venous blood samples after leaving the samples overnight at 4°C followed by centrifugation at 1200×G for 10 minutes. An aliquot of each serum sample was stored at -80°C for antibody testing. Also, rectal temperatures were recorded for each animal at

weekly interval PV. On day 87 PV, all animals including the 4 EMEM control animals received a booster dose of one ml of 1×10^4 PFU/ml of the MP-12 vaccine virus. All animals were observed for sign of illness and rectal temperatures were recorded once a week. Blood samples were obtained on days 7, 14 and 21 PV from the re-vaccinated animals to determine the RVFV antibody response by the plaque reduction neutralization test (PRNT).

3.9.4 Safety

In order to assess the safety of vaccine, all animals were observed daily for any signs of disease throughout the study, the temperature was recorded on days 0, 3, 5, 7, 14, 21, 28 and 35 PV. The negative control group was kept in one pen together with vaccinated animals. Sera samples obtained on days 3, 4 and 5 PV were tested for virus by RT PCR and cell culture assays.

3.9.5 Immunogenicity

Sera samples obtained from all animals on day 14 before vaccination and on day 0 with the MP-12 and arMP-12ΔNSm21/384 vaccines, and on days 5, 7, 14, 21, 28, 35, 70, 84, and 87 PI were tested by the plaque reduction neutralization test (PRNT) for RVFV neutralizing antibody. Also, sera samples obtained from the same animals on days 7, 14 and 21 PI after revaccination on day 87 with MP-12 were tested by the same technique for neutralizing antibody.

3.10 Specific objective 2

Safety and immunogenicity of RVF arMP-12ΔNSm21/384 candidate vaccine in sheep, goats and zebu calves vaccinated intradermally using a Bioject ZetaJet™ needle free injection device.

3.10.1 Experimental animals

Healthy local breeds of black head fat tailed sheep (*Ovis aries*), domestic goats (*Capra aegagrus hircus*) and Zebu (*Bos Taurus Indicus*) 6-9 months old were purchased from local producers in the Mvomero district located in Morogoro, Tanzania. A total of 12 sheep, 15 goats and 12 zebu were transported to the animal facility. Prior to housing the animals in the ABSL-2 facility, the animals were ear tagged with individual identification numbers and treated with Steladone 300® EC Acaricide, and given 2.5% Albendazole orally in order to remove ectoparasites and endoparasite respectively. Animals were then left to acclimatize for two weeks in the facility. Throughout the experiment, the animals were fed "ad libitum" with fresh grasses, water, and mineral blocks and monitored daily for elevated body temperature to rule out any illnesses.

3.10.2 Vaccine preparation

The arMP-12ΔNSm21/384 vaccine dose for inoculating the animals was prepared in the Biosafety cabinet of the SUA Virology BSL 2 Laboratory two hours before inoculation. The dose was prepared by adding 2mls of EMEM supplemented with 4% FBS into each vial containing the lyophilized vaccine followed by vortexing to obtain a final concentration of $1x10^5$ PFU/ml of the vaccine virus. The inoculum used to vaccinate each of the control animals was one ml of EMEM plus 4% FBS. After preparation, the vaccine, control animal inoculum and syringes were transported in a cool box with ice packs to the animal facility. The Bioject ZetaJet™ device with the needle free syringe were also taken to the animal facility.

3.10.3 Inoculation

Immediately before vaccination of the animals on day 0, , blood samples were collected from the jugular vein of each animal using 6 ml plain vacutainer tubes. Each animal was

then vaccinated with 0.3 mls each of the respective vaccine intradermally on the neck using Bioject ZetaJet™ device with needle free syringe. A total of 10 sheep, 10 goats and 10 zebu were vaccinated with arMP-12ΔNSm21/384 vaccine using the Bioject ZetaJet™ device on the left side of the neck, and 2 sheep, 2 goats and 2 zebu were vaccinated with EMEM plus 4% FBS using the Bioject ZetaJet™ device, 3 goats were vaccinated with 1mls of arMP-12ΔNSm21/384 by using needles intramuscularly. Rectal temperature was also recorded at the time of inoculation. Animal data was recorded in a log book at the time of inoculation, including the animal number, sex, pen number, and device used to inject the vaccine. Animals were monitored for neutralizing antibody weekly up to day 35 PV.

3.10.4 Specimen collection and preparation

Animal sera were obtained from 3 mL venous blood samples collected in 6 ml plain vacutainer tubes on day 0 before inoculation, days 3, 4, 5, 7, 14, 21, 28, 35 post inoculations (PV), One to 2 ml of serum was harvested from each blood sample after leaving the samples overnight at 4°C followed by centrifugation at 1200 × G for 10 minutes to allow the serum to be separated. Aliquot of each serum sample was kept in one labeled Cryo-vial tube and two 1.5ml eppendorf tubes and stored at -80 °C freezer in labeled Cryo-vial boxes. Temperatures were recorded from each animal on days 0, 3, 5, 7, 14, 21, 28 and 35.

3.10.5 Vaccine Safety

In order to assess the safety of vaccine, all animals were observed daily for any signs of disease throughout the study; the temperature was recorded on day's 0, 3, 5, 7, 14, 21, 28 and 35 PV. The negative control animals were kept in the pen together with vaccinated

animals. Viremia testing by virus isolation was done on serum samples collected on days 3, 4 and 5 post inoculations.

3.10.6 Immunogenicity of the vaccine when administered intradermally

Sera samples collected on days 14 and 0 before inoculation, on days, 5, 7, 14, 21, 28, 35 PV were tested for RVFV antibody by the PRNT. .

3.11 Statistical Analysis

All serological data were analyzed by using R software 3.1.4. Welch two-sample t-test to compare antibody responses in animal vaccinated with MP-12 and arMP-12ΔNSm21/384 for statistical significance at $\alpha=0.05$. In addition, it was used to compare antibody responses between the animals vaccinated either intradermally or intramuscularly. One way Anova was used to compare antibody responses between species for each vaccine.

.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Specific Objective 1: Evaluation of Safety and immunogenicity of MP 12 and arMP-12ΔNSm21/384 vaccine in sheep and zebu calves vaccinated intramuscularly with a needle

4.1.1 Screening animals

Sera samples obtained from sheep and zebu calves before vaccination and on day 0 immediately prior to vaccination with the MP-12 and arMP-12ΔNSm21/384 were negative for RVFV determined by RT-PCR and by isolation in Vero cells. Also all animals were negative for RVFV neutralizing antibodies by PRNT.

4.1.2 Safety

All sheep and zebu calves remained healthy based on the absence of any clinical signs of fever, nasal and ocular discharge, weakness and death throughout the study. The body temperature of the animals did not exceed 41 ^{o}C throughout the study (Figures 1-2). All animals maintained their daily activities such as drinking, eating, and locomotion. None of the vaccinated animals had any visible swelling at injection site. The MP-12 and arMP-12ΔNSm21/384 vaccine viruses were not detected in sera collected on days 3, 4 and 5 PV based on the absence of any CPE in Vero cells, thus indicating that the animals did not develop a detectable viremia following vaccination. All control animals remained antibody negative throughout the study period suggesting that the vaccinated animals did not shed the viruses during the study.

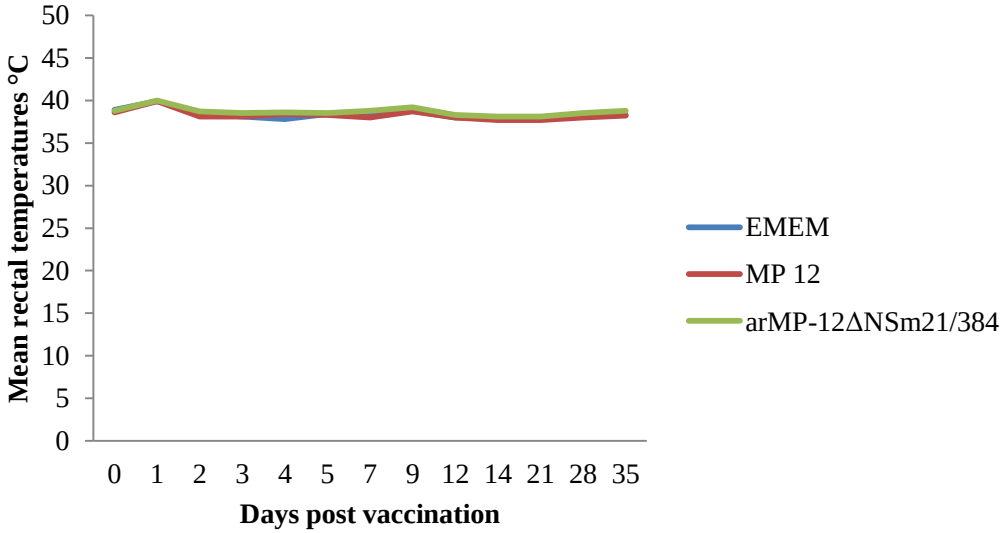

Figure 1: Mean rectal temperatures in sheep vaccinated intramuscularly with RVF MP-12 and arMP-12ΔNSm21/384 vaccine candidates, and EMEM using a needle.

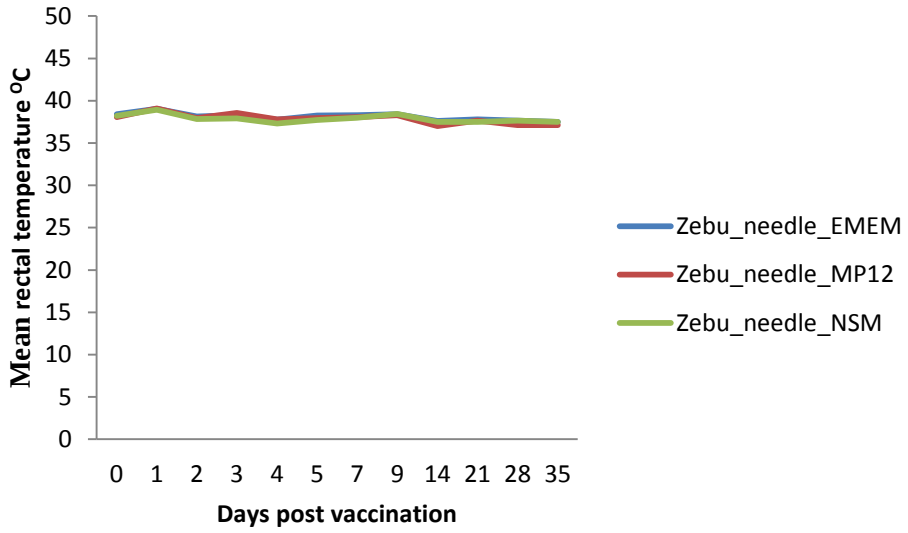

Figure 2: Mean rectal temperatures in zebu calves vaccinated intramuscularly with RVF MP-12 and arMP-12ΔNSm21/384 vaccine candidates, and EMEM using a needle.

4.1.3 Immunogenicity

All sheep and zebu calves that were vaccinated with MP 12 and arMP-12ΔNSm21/384 vaccine candidates developed neutralizing RVFV antibody. In the group of sheep that were vaccinated intramuscular with MP 12 and arMP-12ΔNSm21/384, antibody were detected on day 5 PV and then the titers increased through day 14 with geometric mean titer (GMT) of 51 and 44, respectively (Fig.4). For MP 12 and arMP-12ΔNSm21/384 vaccine groups the highest peak of antibody production was detected on day 35 post vaccination reaching GMT of 79 and 50 respectively. Until this time, none of the negative control sheep had neutralizing antibodies against RVFV. There was no statistical significant difference in antibody response between sheep vaccinated with MP 12 vaccine and sheep vaccinated with arMP-12ΔNSm21/384 vaccine candidate (P value = 0.3704).

Following revaccination with MP 12 vaccine virus on day 87, all sheep elicited an amnestic antibody response on day 7 with GMT of 253 and 159 for MP 12 and arMP-12ΔNSm21/384 vaccinated sheep, respectively (Fig. 4). Negative control sheep that were vaccinated with EMEM had a lower antibody response on day 7 with GMT of 79. Neutralizing antibody titers continued to increase PV reaching the highest GMT of 843, 469 and 159 for MP 12, arMP-12ΔNSm21/384 and EMEM vaccinated sheep, respectively by day 21PV. However, there was significant difference in antibody response in sheep vaccinated with MP 12 between first vaccination and revaccination (P value = 0.00877). There was also a significant difference in the antibody titer when the animals received the first vaccination with arMP-12ΔNSm21/384 and when were revaccinated with the MP-12 vaccine (P value = 0.0081). In addition, during revaccination study there was a significant difference in antibody response in sheep vaccinated with EMEM and sheep vaccinated with arMP-12ΔNSm21/384 (P value = 0.0164) and also there was difference between

sheep vaccinated with MP 12 and EMEM (P value = 0.01802). In both cases EMEM vaccinated animals had lower antibody response.

For the group of zebu calves that were vaccinated intramuscularly with either MP 12 or arMP-12ΔNSm21/384 vaccine candidates during first vaccination study, antibody was detected on day 5 and 7, respectively with GMT of 0.5 and 22 (Fig.3). Neutralizing antibody titers continued to increase reaching the peak titers on day 28 and 35 PV with GMT of 100 and 62 for MP 12 and arMP-12ΔNSm21/384, respectively. There was not a significant difference in antibody response between zebu calves vaccinated with MP 12 and arMP-12ΔNSm21/384 (P value = 0.2325).

Following revaccination with MP 12, all zebu calves elicited a rapid and higher antibody response on day 7 with GMT of 1014 and 451 for MP 12 and arMP-12ΔNSm21/384 vaccine group, respectively (Fig.3). EMEM vaccinated zebu calves had GMT of 9 by day 7 PV and titers increased to GMT of 160 by day 21 PV. Among the MP 12 vaccinated calves, the GMT was 252 and for thearMP-12ΔNSm21/384 vaccinated animals, a GMT of 451 was detected by day 21 PV. There was not a significant difference in the antibody response between the MP 12 and arMP-12ΔNSm21/384 vaccinated calves (P value = 0.61).

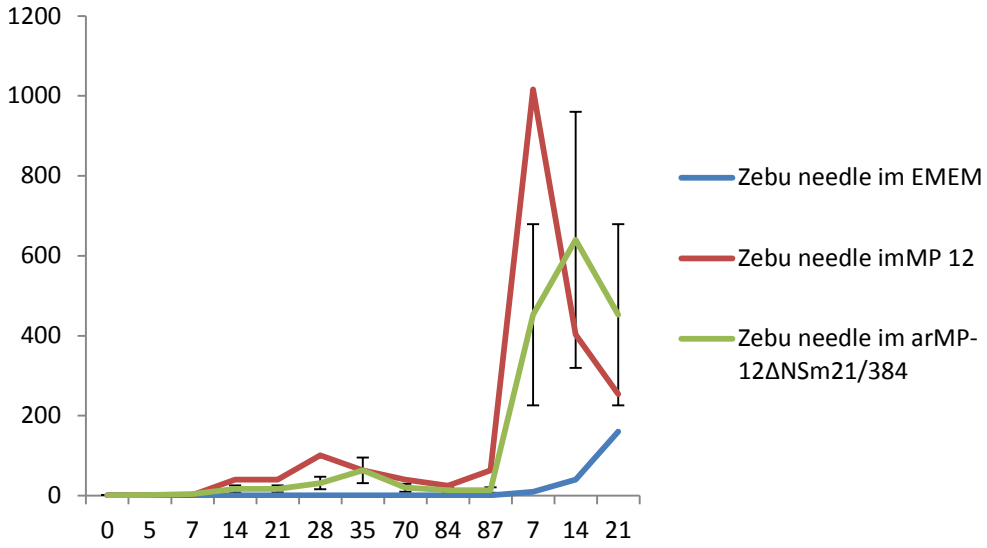

Figure 3: Geometric mean antibody titer in zebu calves vaccinated with MP 12, arMP-12ΔNSm21/384, EMEM using a needle intramuscularly

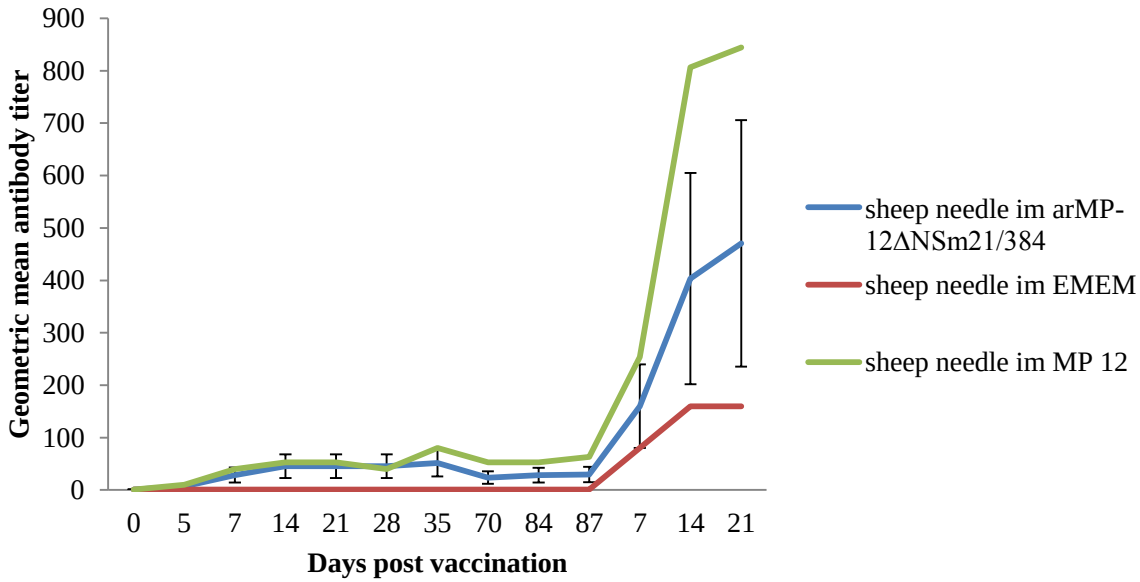

Figure 4: Geometric mean antibody titer in sheep vaccinated with MP-12, arMP-12ΔNSm21/384, EMEM by using a needle intramuscularly

4.2 Specific objective 2: Evaluation of safety and immunogenicity of RVF vaccine candidate arMP-12ΔNSm21/384 in sheep, goats and zebu calves vaccinated intradermally using Bioject ZetaJet™ device

4.2.1 Safety

All sheep, goats and zebu calves vaccinated intradermally with arMP-12ΔNSm21/384 vaccine maintained normal temperature throughout the study (Figures 5-7). No animal had detectable viremia. There was no virus shedding. Animals maintained their daily activities such as drinking, eating, and locomotion. None of the vaccinated animals had any visible swelling at injection site post vaccination.

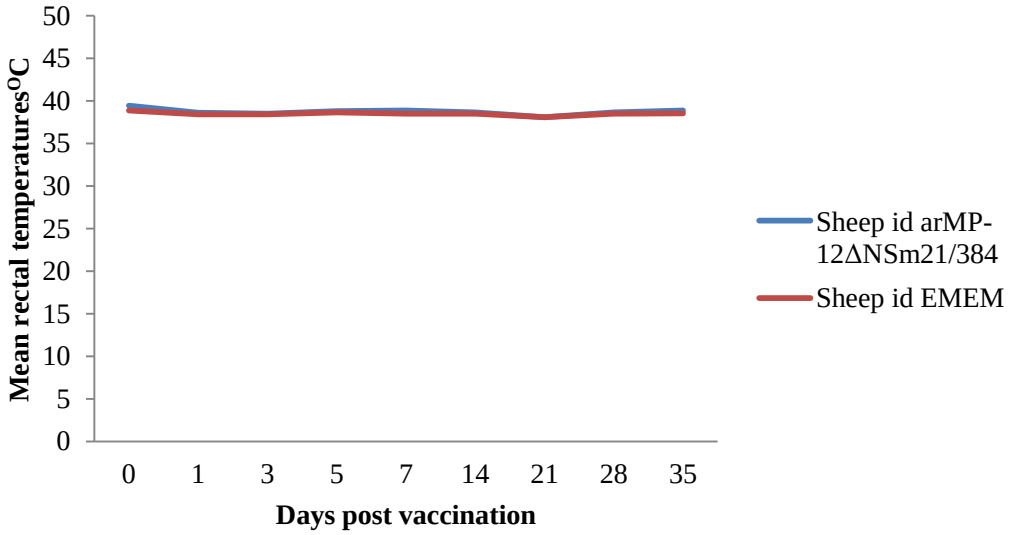

Figure 5: Mean rectal temperature in sheep vaccinated with RVF arMP-12ΔNSm21/384 vaccine candidate and EMEM intradermally.

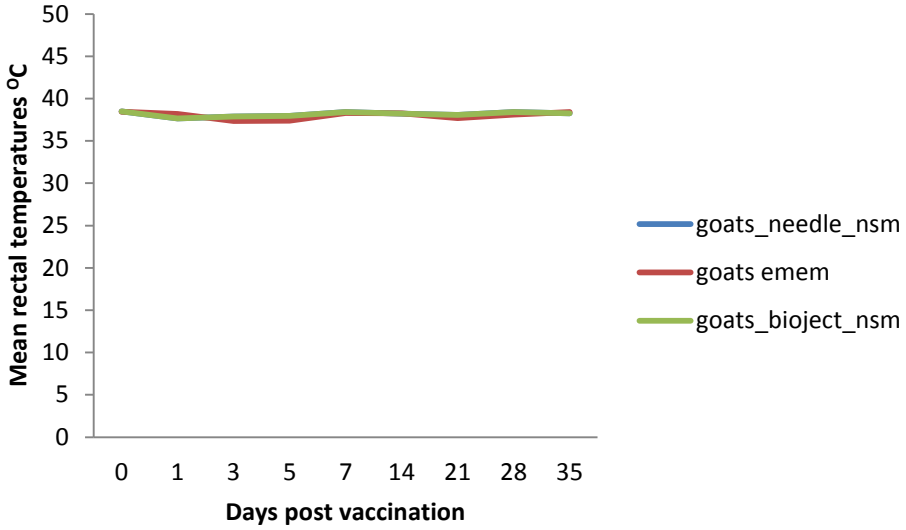

Figure 6: Mean rectal temperatures in goats vaccinated with RVF arMP-12ΔNSm21/384 vaccine candidate and EMEM intradermally

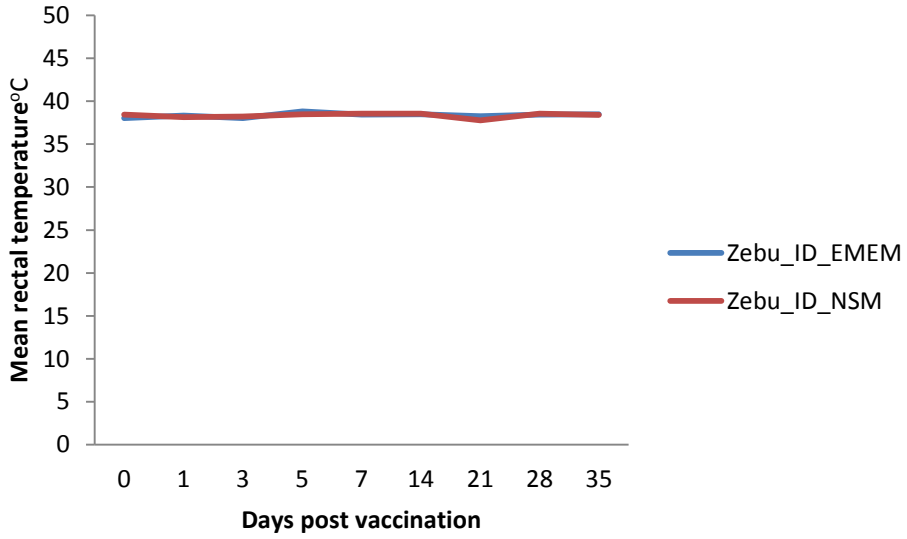

Figure 7: Mean rectal temperatures in zebu calves vaccinated with RVF arMP-12ΔNSm21/384 vaccine candidate and EMEM intradermally

4.2.2 Immunogenicity

Similar to what has been observed when sheep were vaccinated intramuscular with arMP-12ΔNSm21/384, intradermally vaccinated sheep had detectable neutralizing antibodies on day 5 PV with a GMT of 3. A steady increase in neutralizing antibodies was seen in these animals with the highest peak on day 21 with a GMT of 211 (Fig. 9). As compared to the

antibody response of sheep vaccinated with the same vaccine intramuscularly, there was a significant difference (P value = 0.03) with high antibody response in the intradermal vaccinated sheep.

Intradermally arMP-12ΔNSm21/384 vaccinated zebu calves had similar antibody response pattern with antibody being detected on day 5 with a GMT of 1.5 which increased steadily through day 35 PV reaching the highest titer on day 35 with a GMT of 62 (Fig. 8). However, there was no significant difference in antibody production between zebu calves vaccinated with arMP-12ΔNSm21/384 intradermally and intramuscularly (P value = 0.89). Intradermally vaccinated goats had an antibody response comparable to that of goats vaccinated intramuscularly with antibody being detected on day 5 with a GMT of 8 and titers increase reaching the highest titer on day 35 with a GMT of 1113 (Fig. 10).

However, overall, goats had the highest antibody response as compared to animals when vaccinated intramuscularly or intradermally with arMP-12ΔNSm21/384 vaccine candidate. There was significant difference in antibody response between goats and zebu calves vaccinated intradermally (P value = 0.002) and between goats and sheep vaccinated intradermally (P value = 0.006). In addition there was a significant difference in antibody response between goats and sheep and zebu calves vaccinated intramuscularly (P value = 0.0011).

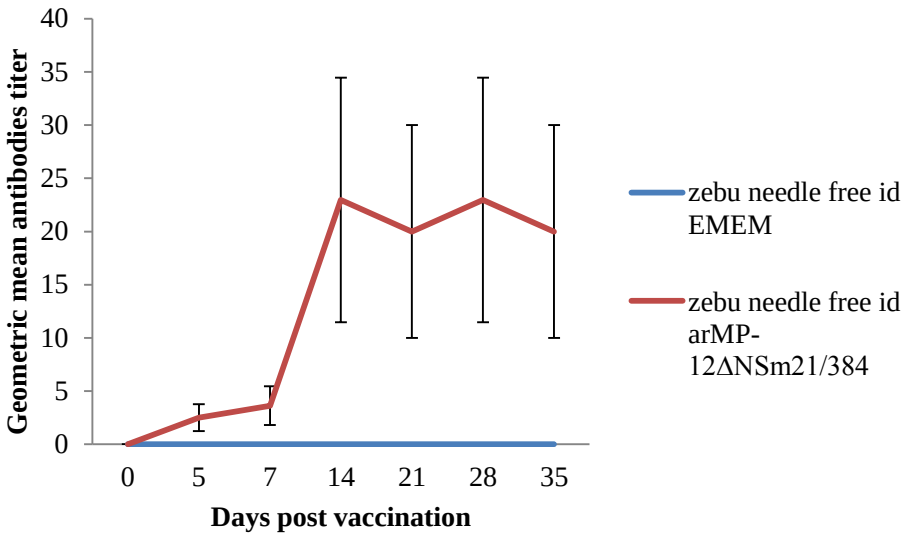

Figure 8: Geometric mean antibody titer in arMP-12ΔNSm21/384 and EMEM vaccinated zebu calves using a needle free vaccine delivery device

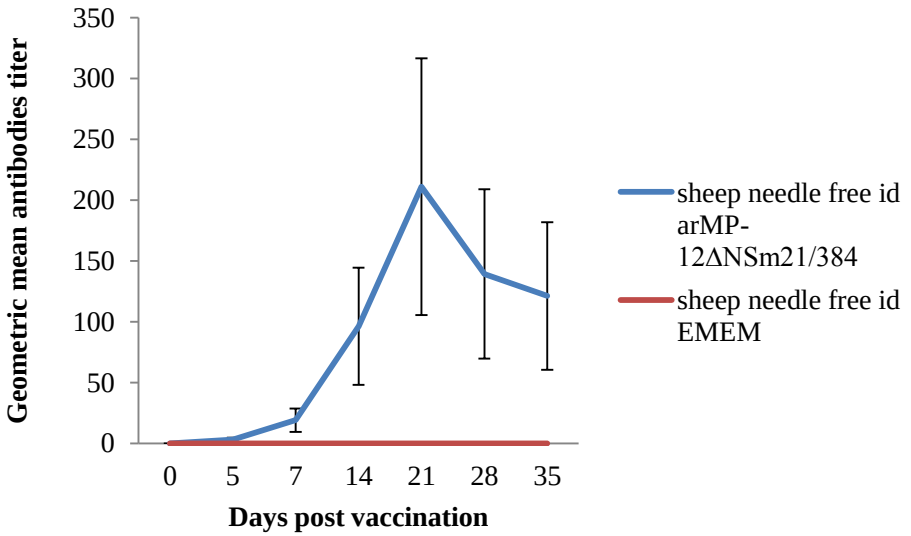

Figure 9: Geometric mean antibody titer in sheep vaccinated with arMP-12ΔNSm21/384 and EMEM by using a needle free vaccine delivery device

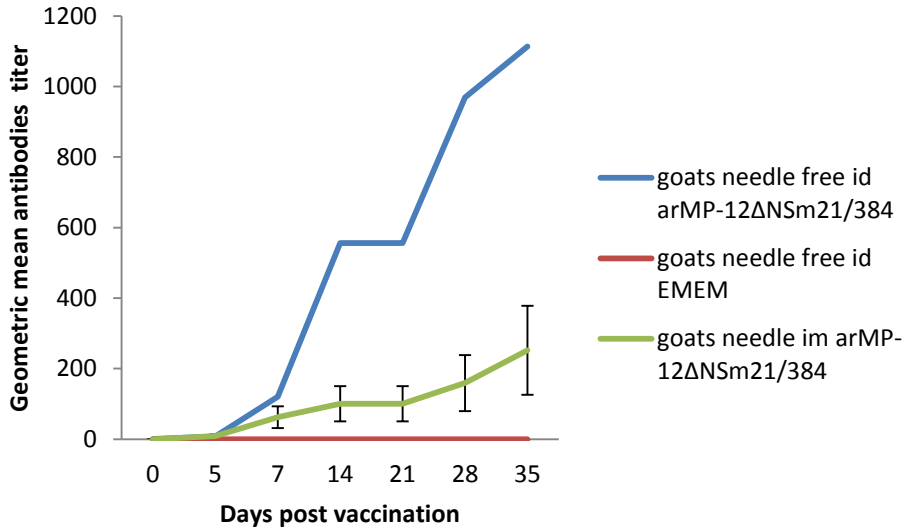

Figure 10: Geometric mean antibody titer in arMP-12ΔNSm21/384 and EMEM vaccinated goats using a needle and needle free vaccine delivery device

4.2 Discussion

The results of this study indicated that the RVF MP-12 and arMP-12ΔNSm21/384 vaccine candidates were safe in sheep, goats and zebu calves vaccinated via the intramuscular route or intradermal route. The safety of the candidate vaccines was confirmed as all animals maintained normal body parameters such as gait, appetite, and normal rectal temperature. Other clinical manifestations associated with infection by wild type RVFV, such as hemorrhage, diarrhea, nasal and ocular discharge were not observed during the study (Daubney *et al.*,1931; Mackenzie *et al.*, 1936; Pepin *et al.*, 2010).

There was no evidence that the vaccine viruses were shed by the vaccinated animals as the control animals remained negative for virus while being confined in the same pens with vaccinated animals. In addition, that both the RVF MP-12 and arMP-12ΔNSm21/384 vaccine candidates are promising for the prevention of RVF was further supported by the observations that none of the animals experienced any adverse effects and none developed

a detectable viremia. None of the animals developed swelling at injection site post vaccination.

The detection of neutralizing antibodies on day 7 in MP 12 vaccinated zebu calves showed the potential for this candidate vaccine to induce a rapid antibody response which may protect animals even during an outbreak, although only one zebu calf had detectable antibodies on that day. The high antibody response in all zebu calves vaccinated intramuscularly with MP 12 animals on day 14 makes them slower responds rather than non-responders (Dungu *et al*., 2010; Morrill *et al*., 2013b; Von Teichman *et al*., 2011). However, the late detection of neutralizing antibodies on day 14 in this group differ from what has been observed in another study using the same vaccine where calves had neutralizing RVFV antibody by day 4 post vaccination, the difference might be attributed by the difference in species and age of animals used in the two studies (Wilson *et al*., 2014).

Despite late detection of antibodies, these animals elicited high antibody response similar to what was observed in the group of sheep that was vaccinated with similar vaccine, indicating potential for this vaccine to provide protection in all susceptible species, unlike what was observed with other vaccine such as smithburn vaccine which provided good response in sheep but poor in cattle (Banard and Botha, 1977) throughout the study.

An amnestic antibody response in MP 12 vaccinated zebu calves following revaccination with MP 12 vaccine virus, increases the possibility of protection from wild type RVFV challenge in these vaccinated animals, because it shows after vaccination that these animals were able to mount sufficient memory cells which helped them to respond rapidly with strong antibody response following an encounter with the same virus. Also, because

MP 12 vaccine virus contains all the three segments presents on the wild type RVFV, there is a huge chance that the same pattern of response will be mounted upon encounter with the virulent virus, although there is still a need to conduct challenge study in order to confirm this possibility.

Furthermore, The poor onset of antibody response of zebu calves following vaccination with arMP-12ΔNSm21/384 similar to what has been observed in studies with (Morrill *et al.,* 2013a) where calves had detectable antibodies by day 10 and 14 upon vaccination with arMP-12ΔNSm21/384, but also it was shown that neutralizing antibodies in calves were dose dependent, hence, there is a possibility that the dose used during this study was not sufficient for this particular species of cattle to induce a high and rapid antibody response in all zebu calves, although individual variation in antibody response is something that cannot easily be prevented.

However, zebu cattle vaccinated with arMP-12ΔNSm21/384 elicited a similar pattern of antibody response which is in agreement with the results of a study done in calves (Bos Taurus) vaccinated with the arMP-12ΔNSm21/384 vaccine. The animals had antibody titers ≥40 by day 14 PV, suggesting that domestic livestock vaccinated with MP-12 and arMP-12ΔNSm21/384 vaccines would not experience any adverse effect and would be protected against infection by the wild type RVFV (Morrill *et al.,* 1997a).

Observations during a study involving calves vaccinated with the Smithburn and Clone 13 RVFV vaccine, the antibody response was poor, but these animals were protected upon challenge with wild type RVFV (Von Teichman *et al*., 2011). When the zebu calves were revaccinated with MP 12 vaccine virus, high titers of neutralizing antibodies was observed on day 7 similar to what has been observed in sheep, which means that if these animals

were to be infected after vaccination, they will mount a stronger antibody response which will protect them from infection.

The rapid immune response by sheep vaccinated intramuscularly with MP-12 vaccine by day 5 demonstrated that the vaccine could possibly protect these sheep even if administered after the onset of a RVF outbreak (Dungu *et al*., 2010). Also, an overall sustained, as well as an increase in the pattern of neutralizing antibody titers indicated that the vaccine activated antibody producing B cells with the highest antibody titers on days 14 - 35 in most animals The antibody response following revaccination with MP 12 vaccine virus was characterized by a rapid increase and high antibody titers on day 7 PV, followed by an increasing pattern to maximum titers on day 21 PV, thus, demonstrating that the animals were likely to be protected if exposed to virulent RVFV.

According to a previous study done using MP-12 and the arMP-12ΔNSm21/384 vaccine in sheep and other animals, an antibody titer of 1:100 or less afforded protection against challenge with wild type RVFV (Lokugamage et *al.,* 2012; Morrill *et al.,* 1991; Morrill *et al.,* 2013b; Weingartl *et al.,* 2014a,b). Hence, the results of this study indicated that an African breed of sheep vaccinated with MP-12 vaccine were likely to be protected if exposed to wild type RVFV.

Sheep vaccinated intramuscularly with the arMP-12ΔNSm21/384 developed an early neutralizing antibody PV, which was consistent with results reported previously (Morrill *et al*., 2013a; Weingartl *et al*., 2014a,b). Also, antibody titers increased in all sheep with peak titers on day 14 PV which were sustained through day 87 PV in most animals. These findings demonstrated the potential of the vaccine to induce high antibody titers within short period of time, and therefore increased the likely hood of vaccinated animals to be

protected almost immediately during epizootics. Also, the results of studies reported by others in sheep, including gestating animals that received the arMP-12ΔNSm21/384 revealed that the vaccine elicited an antibody response that afforded protection and did not cause abortions, thus providing promising evidence in support of this RVFV vaccine candidate for the prevention of RVF among sheep in Africa (Ikegami *et al*., 2015; Morrill *et al.*, 1987; Morrill *et al*., 2013a; Weingartl *et al.*, 2014a,b).

The validity of the immune response to the initial vaccination of sheep with the arMP-12ΔNSm21/384 was supported by the pattern of the secondary immune response to the booster vaccination with the MP-12 vaccine. The results showed that the antibody titers increased rapidly in all sheep and were 12 fold higher than titers observed among the animals that received the initial vaccination. These findings show the potential of these vaccines to elicit a strong and likely protective immune response in animals if exposed to wild type RVFV in the field. That the humoral immune system was primed by the initial vaccination is further supported by the observation that the antibody response in sheep #117 and 64 was poor during first vaccination but the response to revaccination with MP-12 was similar to that of the other animals that had a much stronger antibody response to the initial vaccination. When an animal is exposed to a virus, plasma cells start to differentiate and produce antibodies, and as they multiply the more the antibody are produced, but because these cells have a short life span they differentiate into antibody producing plasma B cells and into memory cells where by the antibody producing cells eventually dies while memory cells remain and serve to afford protection following secondary exposure to a similar pathogen (Flehmig *et al.*, 1997). Hence, if animals are vaccinated with these RVF vaccines they are likely to be protected when they are exposed to the wild type RVFV under field conditions.

Since there was no significance between the immune response based on antibody titers for sheep vaccinated with the MP-12 and the arMP-12ΔNSm21/384 vaccines, this indicated that either the MP-12-NSm-del or the MP-12 vaccine could be used to vaccinate sheep. Furthermore, the timing of seroconversion and antibody titers were similar to results reported for the immune response of sheep to these vaccines in the United states with antibody being first detectable on day 5 PV (Morrill *et al.*, 2013a) Other data that support the use of either vaccine was generated by studies that showed MP 12 vaccinated sheep with antibody titer of ≥1:40 were protected from clinical disease following challenge by wild-type virulent ZH501 RVFV (Faburay *et al.*, 2016; Ikegami *et al.*, 2015; Weingartl *et al.*, 2014a,b). However, the DIVA potential of the arMP-12ΔNSm21/384 vaccine candidate, if shown to be effective could provide an advantage over using the MP-12 vaccine candidate.

In contrast to other studies involving the vaccination of sheep and calves in the United States and Canada with MP-12 and arMP-12ΔNSm21/384 which reported antibody titer as high as ≥ 1:10240, which were substantially higher than titers observed for animals in this study (Morrill *et al.*, 1991, Morrill *et al.* 1987; Weingartl *et al.*, 2014a,b). According to the results of studies involving arMP-12ΔNSm21/384, vaccinated sheep challenge with a wild type RVFV, the clinical and pathological response to experimental RVFV infection in ruminants was dependent on the strain of RVFV used to inoculate the animals , the species, breed and age of host animals (Busquets *et al.*, 2010; Faburay *et al.*, 2016; Weingartl *et al.*, 2014a,b). Therefore, the lower antibody titers observed in our study may have in part been due to the fact that the species and age of sheep and calves used in our study differed from those used in the previous studies (Faburay *et al.*, 2016). Also, difference in the nutritional background of the animals and possible differences in health status might have affected the immune response status of the animals. The animals used in

our study were free ranging animals that were at greater risk to infestation of endo- and ecto- parasites as well as other pathogens making them prone to various infections that might have interfered with their immune response as compared to sheep held in feed lots in the United States and Canada. However, the use of locally bred and reared sheep and zebu cattle in Africa is likely to provide a more realistic understanding of the immune response to the MP-12 and arMP-12ΔNSm21/384 vaccine candidates.

Our observations that the antibody response of local Tanzanian species sheep (*Ovis aries)* and zebu calves to intramuscularly vaccination with MP-12 and arMP-12ΔNSm21/384 was more comparable to the response of sheep vaccinated with the RVFV Clone 13 vaccine. The antibody titers reported for sheep vaccinated with clone 13 in Kenya ranged from a low of ≥ 40 to ≥480 while in zebu calves ranged from <10 to <80 (Njenga *et al*., 2015). The vaccination of sheep with Clone 13 in Senegal showed that 70% of the animals started seroconverting on day 60 PV with titers ≥1:80, however, antibody data before day 60, and titers above 1:80 are not presented, and therefore, it is not possible to consider comparison of the observations to this study (Lo *et al.,* 2015) .

Although the exact date of seroconversion and the antibody titers were not readily discernible, sheep vaccinated with Clone 13 vaccine were protected against challenge with wild type RVFV. Also study with live attenuated thermostable clone 13 in cattle showed that seroconversion started on day 28 PV when similar vaccine dose was used, indicating the potential for the vaccines used in this study to provide early protection. Although the immune response of an African species of sheep and zebu calves based on antibody titers was not as robust as that reported for sheep and calves in the United States and Canada, but apparently the lower titers did not interfere with the efficacy of the Clone 13 vaccine, nor the safety based on the absence of clinical manifestations and abortions.

Since the findings of this study show the MP-12 and arMP-12ΔNSm21/384 vaccines to be promising for preventing RVF in African species of sheep and zebu calves, further studies were done to evaluate the safety and immunogenicity of a DIVA potential arMP-12ΔNSm21/384 vaccine candidate when vaccinated intradermally using Bioject ZetaJet™ needle free device in sheep, goats and zebu calves. This is because the intradermal route of vaccine administration is believed to offer an more advantage in terms of safety and immunogenicity (Giudice and Campbell, 2006; Hickling and Jones, 2009; Mousel *et al.*, 2008) as compared to the standard route used in this study which have less antigen presenting cells and therefore, a lowered immune response.

The rapid and higher antibody response in the group of sheep, goats and zebu calves intradermally vaccinated with arMP-12ΔNSm21/384 vaccine by day 5 demonstrated the potential for the use of intradermal vaccination as the standard route for administering RVFV vaccines as more antigen presenting cells were likely to be stimulated which led to a stronger immune response, different from what was seen when these animals were vaccinated intramuscularly with a needle using the same vaccine with the same dose. This ensures protection of this susceptible species; especially in Africa where mostly vaccines against RVF are being administered during outbreaks period hence there is a need of having a fast acting RVFV vaccine in all susceptible species, whereby intradermal vaccination could be used as standard alternative. Also, intradermal vaccination mimic natural infection to RVFV caused by mosquito transmission, according to study done in goats it was shown that insect cell derived RVFV (IN-RVFV) caused severe infection in infected goats as compared to mammalian cell derived RVFV (MAM-RVFV), which means mosquito infection causes the severe form of disease (Weingartl *et al.,* 2014a), Hence vaccination using the same route that produces the severe form of disease is more

likely to produce specific type of response which will protect animals from the severe form of disease and prevent transmission of the virus upon challenge.

High antibody response in intradermally vaccinated sheep, goats and zebu calves demonstrated in this study despite using low vaccine dose as compared to when the vaccine was administered intramuscularly in sheep and zebu cattle, shows the potential for intradermal route for inducing high antibody titers as compared to the intramuscular route. Since antibodies undergo decay over time, high antibody response in intradermally vaccinated animals ensures longer protection time. As the higher the antibody titer, the longer it will take to decay, and regard to herd immunity, the high antibody response in goats and sheep will help minimize the extent of outbreaks, by protecting human, cattle and other susceptible species since the rate of virus replication will be reduced in these susceptible hosts.

The higher antibody response in intradermally vaccinated animals was most likely achieved due to the richness of antigen presenting cells, the main enhancer of the adaptive immunity (Halperin *et al.,* 1979; Kulkarni *et al.*, 2013; Roukens et al., 2008). Also, using the Bioject ZetaJet™ device might have facilitated the response because the vaccine was propelled into an animal body by using force in the injector which may have led to the dispersion of antigen, (Kim *et al.*, 2011; Kumar, 2012; Roukens *et al.,* 2008). Hence reaching more cells of the immune system as compared to when needle was using via the intramuscular route, as compared to using a needle. Needles deliver the vaccine in form of a drop, hence only few cells are targeted at one time and because the intramuscular route target few antigen presenting cells, the immune response is dependent on diffusion of antigen to the blood vessel in order to reach cell of the immune system, as a result high vaccine dose is used to produce the required amount of response. Hence, suggesting

intradermal delivery of arMP-12ΔNSm21/384 vaccine to be the more effective potential route for inducing a robust immune response in the animals.

Similar results on intradermal delivery of vaccine was also observed in study done in pigs during evaluation of porcine circovirus type 2 vaccine (Chase *et al*., 2008), In this study, it was found that the vaccine was safe and immunogenic when immunized via intradermal route and offered protection for at least 4 months PV. In cattle vaccinated with rabies vaccine, the intradermal route of vaccination with a lower vaccine dose produced an equivalent antibody response as compared to when full dose was administered intramuscularly, In other species vaccinated via the intradermal route using a lower vaccine dose produced equivalent or higher antibody response as compared to when the vaccine was administered by the intramuscular and subcutaneous routes (Amori *et al.*,2010; Hickling and Jones, 2009; Roukens *et al*., 2008; Verma *et al.*, 2011). In a study done with influenza vaccine, it was shown that intradermal vaccination increased seroconversion rate as compared to using small dose of 9 ug of influenza B strain vaccine which produced a better antibody response than the standard 15ug which was administered intramuscularly. Also, another study showed the antibody response of the HINI train was higher in the intradermal vaccinated human. These findings, together with the results of this study warrant further research to evaluate animal and human vaccines using the intradermal route (Amorij *et al.,* 2010; Belshe *et al.,* 2004; Halperin *et al.,* 1979; Koutsonanos *et al.,* 2015).

Furthermore, since RVF affects poor marginalized livestock community, a cost effective vaccine is required for successful vaccination program (Chengula *et al.*, 2013; Sindato *et al*., 2011). The ability of intradermal vaccinated sheep and goats to induce high neutralizing antibody using a low vaccine dose as compared to needle vaccination showed

its potential as a potential cost effective approach in comparison to many vaccines used in developing countries which hinders successful vaccination programs (Verma *et al*., 2011). This is because lowering vaccine dose helps to lower production cost which later affects the price of the vaccine. High production cost leads to high price of the vaccine. Hence, vaccinating animals via the intradermal route will make vaccine affordable and allow successful vaccination programs even in poor communities. Not only will this lower the required dose, but it will make it easier for manufacturing companies to meet all the demand, especially during outbreaks when the vaccine needed to protect the health of the animals.

In addition, during inoculation, immediately when the vaccine was injected into an animal, the plunger broke which made it difficult to reuse the syringe hence ensuring that once the needle free delivery device is used in the field and there will be no disease transmission that will be caused by reuse of needles. Also since Bioject ZetaJet™ device does not use needle, this ensures prevention of needle stick injuries which previously caused people to become infected with zoonotic disease when taking care of animals (Kumar, 2012, Ashford *et al*., 2004). Also the device is user friendly, durable and easy to use as compared to the use of needles.

Additionally, this result showed that intradermal inoculation of arMP-12ΔNSm21/384 vaccine using Bioject device to be a potential route for administering this vaccine after successful registration. Also, this will cover problems associated with most of the available vaccine as it will be safe, immunogenic, use low doses, and hence will be cost effective, easily administered preventing needle stick injuries and can be used even by layman with minimum supervision which will help with mass inoculation.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This study indicated that both MP 12 and arMP-12ΔNSm21/384 RVF vaccines candidates are safe and immunogenic and hence are promising for use to prevent RVFV outbreaks. However, arMP-12ΔNSm21/384 RVF vaccine should be used instead of MP 12 since it has DIVA potential which enables the vaccine to avoid livestock trade embargoes and be used even outside endemic areas, thus offering improvement over the MP 12 vaccine. In addition, antibody response following vaccination is dependent on the route of vaccination; hence for sheep and goats higher antibody response would be achieved if these animals are vaccinated intradermally, however, the intramuscular route is likely to be a more promising route for zebu calves.

5.2 Recommendations

i. The efficacy of RVFV vaccine should be evaluated by conducting challenge studies with wild type RVFV in order to see if the neutralizing antibody elicited is really protective.

ii. Long term field study should be conducted in order to determine persistence of neutralizing antibody in vaccinated animals which will help to determine if a booster dose is needed to effectively protect the animals.

iii. A dose escalation study should be done, especially for zebu calves in order to determine the optimal dose that elicits the highest antibody response in this species.

iv. A study should be done using large number of animals to provide more supportive data to support the hypothesis that antibody response is dependent on route of vaccination.

v. Animals should be vaccinated intradermally using needle free devices to avoid disadvantages associated with the use of needles.

vi. Additional studies should be done to evaluate safety of the vaccine in pregnant animals and newly born animals.

REFERENCES

Abdel-Wahab,E. K. ., El Baz, L. M., El Tayeb, E. M., Omar, H., Moneim Ossman, M. A. and Yasin, W. (1978). Rift Valley fever virus infections in Egypt: pathological and virological findings in man. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *72* (4), 392-396.

Ahmed, K. S. (2011). Observations on rift valley fever virus and vaccines in Egypt. *Virology Journal*, 8 (1): 532.

Andrews, G. J. (2011). 'I had to go to the hospital and it was freaking me out': needle phobic encounter space. *Health Place,* 17 (4): 875-884.

Alarcon, J. B., Hartley, A. W., Harvey, N. G. and Mikszta, J. A. (2007). Preclinical Evaluation of Microneedle Technology for Intradermal Delivery of Influenza Vaccines. *Clinical and Vaccine Immunology*, 14(4): 375–381.

Amorij, J. P., Hinrichs, W. L., Frijlink, H. W., Wilschut, J. C. and Huckriede, A. (2010). Needle-free influenza vaccination. *The Lancet Infectious Diseases*, 10(10): 699–711.

Ashford, D. A., Di Pietra, J., Lingappa, J., Woods, C., Noll, H., Neville, B. and Tappero, J. (2004). Adverse events in humans associated with accidental exposure to the livestock brucellosis vaccine RB51. *Vaccine,* 22 (25): 3435–3439.

Balkhy, H. H. and Memish, Z. A. (2003). Rift Valley fever: an uninvited zoonosis in the Arabian peninsula. *International Journal of Antimicrobial Agents*, 21 (2): 153–157.

Barnard, B. J. H. and Botha, M. J. (1977). An inactivated rift valley fever vaccine. *Journal of the South African Veterinary Association*, 48 (1): 45-48.

Baskerville, A., Hubbard, K. A. and Stephenson, J. R. (1992). Comparison of the pathogenicity for pregnant sheep of Rift Valley fever virus and a live attenuated vaccine. *Research in Veterinary Science*, 52 (3): 307–311.

Balenghien, C. E., Chevalier, V., Elissa, N., Failloux, A., Ninna, T., Nipomichene, J.J., Nicolas, G., Rakotohannome, M. V., Roger, M and Zombo, B. (2013). Towards a better understanding of rift valley fever epidemiology in southwest of the Indian ocean. *Veterinary research*, 44 (1):78.

Belete, T. B., and Abebe Mengesha, D. N. (2013). Safety and Immunogenicity of Ethiorab Rabies Vaccine. *Journal of Vaccines and Vaccination*, 04 (195):2

Belshe, R. B., Duane, C. and Howe, B. J. (2004). Serum Antibody Responses after Intradermal Vaccination against Influenza. *The New England Journal of Medicine*, 351(22):2286-2294.

Billecocq, A., Spiegel, M., Vialat, P., Kohl, A., Weber, F., Bouloy, M. and Haller, O. (2004). NSs protein of Rift Valley fever virus blocks interferon production by inhibiting host gene transcription. *Journal of Virology*, 78 (18): 9798–9806.

Billecocq, A., Guillemot, L., Alberts, R., Gommet, C., Geffers, R. and Panthier, J. J. (2010). A new mouse model reveals a critical role for host innate immunity in resistance to Rift Valley fever. *The Journal of Immunology*, 1000949.

Bird, B. H., Maartens, L. H., Campbell, S., Erasmus, B. J., Erickson, B. R., Dodd, K. A. and Knust, B. (2011). Rift Valley fever virus vaccine lacking the NSs and NSm genes is safe, non-teratogenic, and confers protection from viremia, pyrexia, and abortion following lethal challenge in adult and pregnant sheep. *Journal of Virology*, JVI–06046.

Bird, B. H. and Nichol, S. T. (2012). Breaking the chain: Rift Valley fever virus control via livestock vaccination. *Current Opinion in Virology*, 2 (3): 315–323.

Botros, B., Omar, A., Elian, K., Mohamed, G., Soliman, A., Salib, A. and Earhart, K. (2006). Adverse response of non-indigenous cattle of European breeds to live attenuated Smithburn Rift Valley fever vaccine. *Journal of Medical Virology*, 78 (6): 787–791.

Boushab, B. M., Fall-Malick, F. Z., Baba, O., El Wafi, S., Ould Salem, M. L., Belizaire, M. R. D. and Basco, L. K. (2016). Severe human illness caused by Rift Valley Fever virus in Mauritania, 2015. In; *Open Forum Infectious Diseases* (Vol. 3). Oxford University Press.

Busquets, N., Xavier, F., Martín-Folgar, R., Lorenzo, G., Galindo-Cardiel, I., del Val, B. P. and Solanes, D. (2010). Experimental infection of young adult European breed sheep with Rift Valley fever virus field isolates. *Vector-Borne and Zoonotic Diseases*, 10 (7): 689–696.

Caplen, H., Peters, C. J. and Bishop, D. H. (1985). Mutagen-directed attenuation of Rift Valley fever virus as a method for vaccine development. *In Journal Genetics Virology*, 66 (10): 2271-2277.

Caroline, A.L., Hartman, A.L., Bethel, L.M., Powell, D.S., Reed, D.S, D.S. and Schmid, R.D (2014). Aerosolized Rift valley fever virus causes fatal encephalitis in African green monkeys and common mammoset. *Journal of virology*, 88 (4): 2235-2248

Charles, N. K., Marszal, P., Zhang, S., and Weingartl, H. M. (2012). Innate immune response to Rift Valley fever virus in goats. *PLoS neglected tropical diseases*, *6* (4), e1623.

Chase, C. C., Daniels, C. S., Garcia, R., Milward, F. and Nation, T. (2008). Needle-free injection technology in swine: Progress toward vaccine efficacy and pork quality. *Journal of Swine Health and Production*, 16 (5): 254–261.

Chengula, A. A., Mdegela, R. H. and Kasanga, C. J. (2013). Socio-economic impact of Rift Valley fever to pastoralists and agro pastoralists in Arusha, Manyara and Morogoro regions in Tanzania. *Journal of Health, Medicine and Nursing*, 2 (1): 13-17.

Clem, A. (2011). Fundamentals of vaccine immunology. *Journal of Global Infectious Diseases*, 3 (1): 73-78.

Daubney, R., Hudson, J. R. and Garnham, P. C. (1931). Enzootic hepatitis or Rift Valley fever. An undescribed virus disease of sheep cattle and man from East Africa. *The Journal of Pathology and Bacteriology*, 34 (4): 545–579.

De Boer, S. M., Kortekaas, J., de Haan, C. A. M., Rottier, P. J. M., Moormann, R. J. M. and Bosch, B. J. (2012). Heparan sulfate facilitates Rift Valley fever virus entry into the cell. *Journal of Virology*, 24: 13642-13652.

do Valle, T. Z., Billecocq, A., Guillemot, L., Alberts, R., Gommet, C., Geffers, R. and Panthier, J. J. (2010). A New Mouse Model Reveals a Critical Role for Host Innate Immunity in Resistance to Rift Valley Fever. *The Journal of Immunology*, 185 (10): 6146–6156.

Dodd, K. A., McElroy, A. K., Jones, M. E. B., Nichol, S. T. and Spiropoulou, C. F. (2013). Rift Valley Fever Virus Clearance and Protection from Neurologic Disease Are Dependent on CD4+ T Cell and Virus-Specific Antibody Responses. *Journal of Virology*, 87 (11): 6161–6171.

Dungu, B., Louw, I., Lubisi, A., Hunter, P., Von Teichman, B. F. and Bouloy, M. (2010). Evaluation of the efficacy and safety of the Rift Valley Fever Clone 13 vaccine in sheep. *Vaccine*, 28 (29): 4581–4587.

Faburay, B., Gaudreault, N. N., Liu, Q., Davis, A. S., Shivanna, V., Sunwoo, S. Y. and Drolet, B. (2016). Development of a sheep challenge model for Rift Valley fever. *Virology*, 489: 128–140.

Faburay, B., LaBeaud, A. D., McVey, D. S., Wilson, W. C. and Richt, J. A. (2017). Current status of Rift Valley fever vaccine development. *Vaccines*, 5 (3): 29-49.

Findlay, G. M., MacKenzie, R. D. and Stern, R. O. (1936). Studies on neurotropic Rift Valley fever virus: the susceptibility of sheep and monkeys. *British Journal of Experimental Pathology*, 17 (6): 431.

Flehmig, B., Staedele, H., Xueref, C., Vidor, E., Zuckerman, J. and Zuckerman, A. (1997). Early appearance of neutralizing antibodies after vaccination with an inactivated hepatitis A vaccine. *Journal of Infection*, 35 (1): 37–40.

Garcia, S., Crance, J. M., Billecocq, A., Peinnequin, A., Jouan, A., Bouloy, M. and Garin, D. (2001). Quantitative real-time PCR detection of Rift Valley fever virus and its application to evaluation of antiviral compounds. *Journal of Clinical Microbiology*, 39 (12): 4456–4461.

Gargan II, T. P., Clark, G. G., Dohm, D. J., Turell, M. J. and Bailey, C. L. (1988). Vector potential of selected North American mosquito species for Rift Valley fever virus. *The American Journal of Tropical Medicine and Hygiene*, 38 (2): 440–446.

Giudice, E. L. and Campbell, J. D. (2006). Needle-free vaccine delivery. *Advanced Drug Delivery Reviews*, 58 (1): 68–89.

Grobbelaar, A.A., Weyer, J., Leman, P.A., Kemp, A., Paweska, J.T. and Swanepoel, R. (2011). Molecular epidemiology of Rift Valley fever virus. *Emerging Infectious Diseases*, 17 (12): 2270–2276.

Halperin, W., Weiss, W. I., Altman, R., Diamond, M. A., Black, K. J., Iaci, A. W. and Goldfield, M. (1979). A comparison of the intradermal and subcutaneous routes of influenza vaccination with A/New Jersey/76 (swine flu) and A/Victoria/75: report of a study and review of the literature. *American Journal of Public Health*, 69 (12):1247–1251.

Hickling, J. and Jones, R. (2009). Intradermal delivery of vaccines: a review of the literature and the potential for development for use in low-and middle-income

countries. *Program for Appropriate Technology in Health (PATH), Ferney Voltaire*.

Hickling, J. K., Jones, K., Friede, M., Zehrung, D., Chen, D. and Kristensen, D. (2011). Intradermal delivery of vaccines: potential benefits and current challenges. *Bulletin of the World Health Organization*, 89 (3): 221–226.

Ibrahim, M. S., Turell, M. J., Knauert, F. K. and Lofts, R. S. (1997). Detection of Rift Valley fever virus in mosquitoes by RT-PCR. *Molecular and Cellular Probes*, 11 (1): 49–53.

Ikegami, T. (2012). Molecular biology and genetic diversity of Rift Valley fever virus. *Antiviral Research*, 95 (3): 293–310.

Ikegami, T., Hill, T. E., Smith, J. K., Zhang, L., Juelich, T. L., Gong, B. and Freiberg, A. N. (2015). Rift Valley fever virus MP-12 vaccine is fully attenuated by a combination of partial attenuations in the S, M, and L segments. *Journal of Virology*, 89 (14): 7262–7276.

Ikegami, T., Narayanan, K., Won, S., Kamitani, W., Peters, C. J. and Makino, S. (2009). Rift Valley fever virus NSs protein promotes post-transcriptional downregulation of protein kinase PKR and inhibits eIF2α phosphorylation. *PLoS Pathogens*, 5 (2): e1000287.

Indran, S. V. and Ikegami, T. (2012). Novel approaches to develop Rift Valley fever vaccines. *Frontiers in Cellular and Infection Microbiology*, 2: 131-138.

Jupp, P. G., Kemp, A., Grobbelaar, A., Leman, P., Burt, F. J., Alahmed, A. M. and Swanepoel, R. (2002). The 2000 epidemic of Rift Valley fever in Saudi Arabia: mosquito vector studies. *Medical and Veterinary Entomology*, 16 (3): 245–252.

Kim, Y. C., Jarrahian, C., Zehrung, D., Mitragotri, S. and Prausnitz, M. R. (2011). Delivery systems for intradermal vaccination. *Intradermal Immunization*, 2011; 77–112.

Koutsonanos, D. G., Esser, E. S., McMaster, S. R., Kalluri, P., Lee, J. W., Prausnitz, M. R. and Compans, R. W. (2015). Enhanced immune responses by skin vaccination with influenza subunit vaccine in young hosts. *Vaccine*, 33 (37): 4675–4682.

Kulkarni, V., Rosati, M., Bear, J., Pilkington, G. R., Jalah, R., Bergamaschi, C. and Pavlakis, G. N. (2013). Comparison of intradermal and intramuscular delivery followed by in vivo electroporation of SIV Env DNA in macaques. *Human Vaccines and Immunotherapeutics*, 9 (10): 2081–2094.

Kumar, R. B. (2012). Needle free injection systems. *The Pharma Innovation*, 1 (9): 57-72.

Lathan, R., Simon-Chazottes, D., Jouvion, G., Godon, O., Malissen, M., Flamand, M. and Panthier, J. J. (2017). Innate Immune Basis for Rift Valley Fever Susceptibility in Mouse Models. *Scientific Reports*, 7 (1): 7096.

Le Borgne, M., Etchart, N., Goubier, A., Lira, S. A., Sirard, J. C., van Rooijen, N. and Dubois, B. (2006). Dendritic Cells Rapidly Recruited into Epithelial Tissues via CCR6/CCL20 Are Responsible for CD8+ T Cell Crosspriming In; *Vivo*. *Immunity*, 24 (2): 191–201.

Lenz, A., Heine, M., Schuler, G. and Romani, N. (1993). Human and murine dermis contain dendritic cells. Isolation by means of a novel method and phenotypical and functional characterization. *Journal of Clinical Investigation*, 92 (6): 2587–2596.

Linterman, M. A. and Vinuesa, C. G. (2010). Signals that influence T follicular helper cell differentiation and function. *Seminars in immunopathology*,32 (2):183-196.

Lo, M. M., Mbao, V., Sierra, P., Thiongane, Y., Diop, M., Donadeu, M. and Dungu, B. (2015). Safety and immunogenicity of Onderstepoort Biological Products' Rift Valley fever Clone 13 vaccine in sheep and goats under field conditions in Senegal. *Onderstepoort Journal of Veterinary Research*, 82 (1): 01–06.

Lokugamage, N., Freiberg, A. N., Morrill, J. C. and Ikegami, T. (2012). Genetic subpopulations of Rift Valley fever ZH548, MP-12 and recombinant MP-12 strains. *Journal of Virology*, 2012; 02081-02112.

Lozach, P.-Y., Kühbacher, A., Meier, R., Mancini, R., Bitto, D., Bouloy, M. and Helenius, A. (2011). DC-SIGN as a receptor for phleboviruses. *Cell Host and Microbe*, 10 (1): 75–88.

Mackenzie, R. D., Findlay, G. M. and Stern, R. O. (1936). Studies on neurotropic Rift Valley fever virus: the susceptibility of rodents. *British Journal of Experimental Pathology*, 17 (5): 352-361.

Makoschey, B., van Kilsdonk, E., Hubers, W. R., Vrijenhoek, M. P., Smit, M., Schreur, P. J. W. and Moulin, V. (2016). Rift Valley fever vaccine virus clone 13 is able to

cross the ovine placental barrier associated with foetal infections, malformations, and stillbirths. *PLoS Neglected Tropical Diseases*, 10 (3): e0004550.

Mansfield, K. L., Banyard, A. C., McElhinney, L., Johnson, N., Horton, D. L., Hernández-Triana, L. M. and Fooks, A. R. (2015). Rift Valley fever virus: A review of diagnosis and vaccination, and implications for emergence in Europe. *Vaccine*, 33 (42): 5520–5531.

Mandell, R. B. and Flick, J. (2011). Rift Valley Fever Virus; A Real Bioterror threat. *Journal of Bioterrorism Biodefence*, 2 (2): 108-113.

Mitragotri, S. (2005). Immunization without needles. *Nature Reviews Immunology*, *5* (12), 905.

Morrill, J. C., Carpenter, L., Taylor, D., Ramsburg, H. H., Quance, J. and Peters, C. J. (1991). Further evaluation of a mutagen-attenuated Rift Valley fever vaccine in sheep. *Vaccine*, 9 (1): 35–41.

Morrill, J. C., Jennings, G. B., Caplen, H., Turell, M. J., Johnson, A. J. and Peters, C. J. (1987). Pathogenicity and immunogenicity of a mutagen-attenuated Rift Valley fever virus immunogen in pregnant ewes. *American Journal of Veterinary Research*, 48 (7): 1042–1047.

Morrill, J. C., Jennings, G. B., Johnson, A. J., Cosgriff, T. M., Gibbs, P. H. and Peters, C. J. (1990). Pathogenesis of Rift Valley fever in rhesus monkeys: role of interferon response. *Archives of Virology*, 110 (3–4): 195–212.

Morrill, J. C. and McClain, D. J. (1996). Epidemiology and Pathogenesis of Rift Valley Fever and Other Phleboviruses. In; *The Bunyaviridae*. 281–293.

Morrill, J. C., Mebus, C. A. and Peters, C. J. (1997a). Safety and efficacy of a mutagen-attenuated Rift Valley fever virus vaccine in cattle. *American Journal of Veterinary Research*, 58 (10): 1104–1109.

Morrill, J. C., Mebus, C. A. and Peters, C. J. (1997b). Safety of a mutagen-attenuated Rift Valley fever virus vaccine in fetal and neonatal bovids. *American Journal of Veterinary Research*, 58 (10): 1110–1114.

Morrill, J. C. and Peters, C. (2003). Pathogenicity and neurovirulence of a mutagen-attenuated Rift Valley fever vaccine in rhesus monkeys. *Vaccine*, 21 (21–22), 2994–3002.

Morrill, J. C. and Peters, C. J. (2011a). Protection of MP-12-Vaccinated Rhesus Macaques Against Parenteral and Aerosol Challenge With Virulent Rift Valley Fever Virus. *Journal of Infectious Diseases*, 204 (2): 229–236.

Morrill, J. C. and Peters, C. J. (2011b). Mucosal immunization of rhesus macaques with Rift Valley fever MP-12 vaccine. *Journal of Infectious Diseases*, 204 (4): 617–625.

Morrill, J. C., Laughlin, R. C., Lokugamage, N., Pugh, R., Sbrana, E., Weise, W. J. and Peters, C. J. (2013a). Safety and immunogenicity of recombinant Rift Valley fever MP-12 vaccine candidates in sheep. *Vaccine*, 31 (3): 559–565.

Morrill, J. C., Laughlin, R. C., Lokugamage, N., Wu, J., Pugh, R., Kanani, P. and Peters, C. J. (2013b). Immunogenicity of a recombinant Rift Valley fever MP-12-NSm deletion vaccine candidate in calves. *Vaccine*, 31 (43): 4988–4994.

Mousel, M. R., Leeds, T. D., White, S. N. and Herrmann-Hoesing, L. M. (2008). Comparison of traditional needle vaccination with pneumatic, needle-free vaccination for sheep. *Journal of Animal Science*, 86 (6): 1468–1471.

Muller, R., Saluzzo, J. F., Lopez, N., Dreier, T., Turell, M., Smith, J. and Bouloy, M. (1995). Characterization of clone 13, a naturally attenuated avirulent isolate of Rift Valley fever virus, which is altered in the small segment. *The American Journal of Tropical Medicine and Hygiene*, 53 (4): 405–411.

Nfon, C. K., Marszal, P., Zhang, S. and Weingartl, H. M. (2012). Innate Immune Response to Rift Valley Fever Virus in Goats. *PLoS Neglected Tropical Diseases*, 6 (4): e1623.

Nguku, P. M., Sharif, S. K., Mutonga, D., Amwayi, S., Omolo, J., Mohammed, O. and Rao, C. (2010). An investigation of a major outbreak of Rift Valley fever in Kenya: 2006–2007. *The American Journal of Tropical Medicine and Hygiene*, 83 (2): 05–13.

Niklasson, B., Peters, C. J., Bengtsson, E. and Norrby, E. (1985). Rift Valley fever virus vaccine trial: study of neutralizing antibody response in humans. *Vaccine*, 3 (2): 123–127.

Njenga, M. K., Njagi, L., Thumbi, S. M., Kahariri, S., Githinji, J., Omondi, E. and Ithondeka, P. M. (2015). Randomized controlled field trial to assess the

immunogenicity and safety of rift valley fever clone 13 vaccine in livestock. *PLoS Neglected Tropical Diseases*, 9 (3): e0003550.

Pepin, M., Bouloy, M., Bird, B. H., Kemp, A. and Paweska, J. (2010). Rift Valley fever virus (Bunyaviridae: Phlebovirus): an update on pathogenesis, molecular epidemiology, vectors, diagnostics and prevention. *Veterinary Research*, 41 (6): 61-101.

Peters, C. J., Lin, C. T., Anderson Jr, G. W., Morrill, J. C. and Jahrling, P. B. (1989). Pathogenesis of viral hemorrhagic fevers: Rift Valley fever and Lassa fever contrasted. *Reviews of Infectious Diseases*, 11: 743–749.

Pittman, P. R., McClain, D., Quinn, X., Coonan, K. M., Mangiafico, J., Makuch, R. S. and Peters, C. J. (2016). Safety and immunogenicity of a mutagenized, live attenuated Rift Valley fever vaccine, MP-12, in a Phase 1 dose escalation and route comparison study in humans. *Vaccine*, 34 (4): 424–429.

Roukens, A. H., Vossen, A. C., Bredenbeek, P. J., Van Dissel, J. T. and Visser, L. G. (2008). Intradermally administered yellow fever vaccine at reduced dose induces a protective immune response: a randomized controlled non-inferiority trial. *PLoS One*, 3 (4): e1993.

Sayed-Ahmed, M. Z., Nomier, Y. and Shoeib, S. (2015). Epidemic situation of Rift valley fever in Egypt and Saudi Arabia. *Journal of Dairy, Veterinary and Animal Research*, 2 (3): 23–25.

Sindato, C., Karimuribo, E. D., Pfeiffer, D. U., Mboera, L. E., Kivaria, F., Dautu, G. and Paweska, J. T. (2014). Spatial and temporal pattern of Rift Valley fever outbreaks in Tanzania; 1930 to 2007. *PLoS One*, 9 (2): e88897.

Sindato, C., Karimuribo, E. and Mboera, L. E. G. (2011). The epidemiology and socio-economic impact of rift valley fever in Tanzania: a review. *Tanzania Journal of Health Research*, 13 (5).

Smithburn, K. C. (1949). Rift Valley fever: the neurotropic adaptation of the virus and the experimental use of this modified virus as a vaccine. *British Journal of Experimental Pathology*, 30 (1): 1-16.

Swanepoel, J. K., Struthers, M. J., Erasmus, S. P. and Shephered, G. M. (1986). Comparative pathogenicity and antigenic cross reactivity of Rift Valley Fever and other African phleboviruses in sheep. *Journal of Hygiene*. 97: 331-346.

Terasaki, K. and Makino, S. (2015). Interplay between the Virus and Host in Rift Valley Fever Pathogenesis. *Journal of Innate Immunity*, 7(5): 450–458.

Turell, M. J., Dohm, D. J., Mores, C. N., Terracina, L., Wallette Jr, D. L., Hribar, L. J. and Blow, J. A. (2008a). Potential for North American mosquitoes to transmit Rift Valley fever virus. *Journal of the American Mosquito Control Association*, 24 (4): 502–507.

Turell, M. J., Linthicum, K. J., Patrican, L. A., Davies, F. G., Kairo, A. and Bailey, C. L. (2008b). Vector competence of selected African mosquito (Diptera: Culicidae) species for Rift Valley fever virus. *Journal of Medical Entomology*, 45 (1): 102–108.

Turell, M. J., Presley, S. M., Gad, A. M., Cope, S. E., Dohm, D. J., Morrill, J. C. and Arthur, R. R. (1996). Vector competence of Egyptian mosquitoes for Rift Valley fever virus. *The American Journal of Tropical Medicine and Hygiene*, 54 (2): 136–139.

Verma, R., Khanna, P., Prinja, S. and Rajput, M. (2011). Intra-dermal administration of rabies vaccines in developing countries: at an affordable cost. *Human Vaccines*, *7* (7): 792–794.

Vialat, P., Billecocq, A., Kohl, A. and Bouloy, M. (2000). The S segment of rift valley fever phlebovirus (Bunyaviridae) carries determinants for attenuation and virulence in mice. *Journal of Virology*, 74 (3): 1538–1543.

Von Teichman, B., Engelbrecht, A., Zulu, G., Dungu, B., Pardini, A. and Bouloy, M. (2011). Safety and efficacy of Rift Valley fever Smithburn and Clone 13 vaccines in calves. *Vaccine*, 29(34): 5771–5777.

Wangmo K. (2018). Comparison of antibody titres between intradermal and intramuscular rabies vaccination using inactivated vaccine in cattle in Bhutan: A Thesis for Award degree of Master of Veterinary Science at Massey University, Palmerston North, New Zealand. at Massey University. 92pp.

Weingartl, H. M., Miller, M., Nfon, C. and Wilson, W. C. (2014a). Development of a Rift Valley fever virus viremia challenge model in sheep and goats. *Vaccine*, 32 (20): 2337–2344.

Weingartl, H. M., Nfon, C. K., Zhang, S., Marszal, P., Wilson, W. C., Morrill, J. C. and Peters, C. J. (2014b). Efficacy of a recombinant Rift Valley fever virus MP-12

with NSm deletion as a vaccine candidate in sheep. *Vaccine,* 32 (20): 2345–2349.

Wilson, W. C., Weingartl, H. M., Drolet, B. S., Davé, K., Harpster, M. H., Johnson, P. A. and McVey, D. S. (2013). Diagnostic approaches for Rift Valley fever. In *Vaccines and Diagnostics for Transboundary Animal Diseases,* 135: 73–78.

Wilson, William C., Bawa, B., Drolet, B. S., Lehiy, C., Faburay, B., Jasperson, D. C. and Richt, J. A. (2014). Evaluation of lamb and calf responses to Rift Valley fever MP-12 vaccination. *Veterinary Microbiology*, 172 (1–2): 44–50.

Wilson, William C., Romito, M., Jasperson, D. C., Weingartl, H., Binepal, Y. S., Maluleke, M. R. and Paweska, J. T. (2013). Development of a Rift Valley fever real-time RT-PCR assay that can detect all three genome segments. *Journal of Virological Methods*, 193 (2): 426–431.

Wommack, K. E., Williamson, K. E., Helton, R. R., Bench, S. R. and Winget, D. M. (2009). Methods for the Isolation of Viruses from Environmental Samples. In M. R. J. Clokie & A. M. Kropinski (Eds.), *Bacteriophages,* 501: 3–14.

Woods, C. W., Karpati, A. M., Grein, T., McCarthy, N., Gaturuku, P., Muchiri, E. and Swanepoel, R. (2002). An outbreak of Rift Valley fever in northeastern Kenya, 1997-98. *Emerging Infectious Diseases*, 8 (2): 138.

APPENDICES

Appendix 1: Rift Valley fever virus neutralizing antibody titers for individual sheep and zebu cattle following vaccination with MP-12 and arMP-12ΔNSm21/384 and Eagle's Minimum Essential Medium and a booster vaccination on day 87 post vaccination with the MP-12 vaccine.

NT: Not tested, this is because serum samples were not collected from these animals on those days

				SCREENING		**FIRST VACCINATION**									**MP 12 REVACCINATION**			
Species	Vaccine	Animal number	sex	Virus Isolation	PRNT80	DPVO	DPV5	DPV7	DPV14	DPV21	DPV28	DPV35	DPV70	DPV84	DPI0	DPI7	DPI14	DPI21
Sheep local	MP-12-NSm-deletion	63	F	Negative	Negative	0	10	40	40	40	40	160	40	40	40	40	160	160
Sheep local	MP-12-NSm-deletion	64	F	Negative	Negative	0	0	0	10	10	40	10	10	10	10	160	640	640
Sheep local	MP-12-NSm-deletion	65	F	Negative	Negative	0	10	40	160	640	160	160	40	40	40	640	640	640
Sheep local	MP-12-NSm-deletion	113	M	Negative	Negative	0	10	40	40	40	160	160	160	40	40	160	40	640
Sheep local	MP-12-NSm-deletion	115	F	Negative	Negative	0	10	40	40	40	40	40	40	40	40	256 0	640	640
Sheep local	MP-12-NSm-deletion	116	M	Negative	Negative	0	10	40	160	160	160	40	40	40	40	160	256 0	640
Sheep local	MP-12-NSm-deletion	117	F	Negative	Negative	0	10	40	40	10	10	10	10	160	160	160	256 0	256 0
Sheep local	MP-12-NSm-deletion	119	M	Negative	Negative	0	10	40	160	160	40	40	10	10	10	40	160	160
Sheep local	MP-12-NSm-deletion	72	F	Negative	Negative	0	10	40	10	10	10	10	10	10	10	40	160	160
Sheep local	EMEM	52	F	Negative	Negative	0	0	0	0	0	0	0	0	0	0	160	160	160

Sheep local	EMEM	61	F	Negative	Negative	0	0	0	0	0	0	0	0	0	0	40	160	160
Sheep local	MP 12	51	F	Negative	Negative	0	10	160	160	160	160	160	40	40	40	40	640	640
Sheep local	MP 12	53	M	Negative	Negative	0	10	10	40	40	40	160	160	40	160	640	640	2560
Sheep local	MP 12	54	M	Negative	Negative	0	10	40	40	40	40	40	40	160	160	640	2560	640
Sheep local	MP 12	55	F	Negative	Negative	0	10	40	40	40	40	40	40	40	40	640	640	640
Sheep local	MP 12	62	F	Negative	Negative	0	10	40	10	10	40	160	160	40	40	160	640	640
Sheep local	MP 12	118	F	Negative	Negative	0	10	40	40	40	10		40	40	40	160	640	DEAD
Zebu	EMEM	39	F	Negative	Negative	0	0	0	0	0	0	0	0	0	0	10	40	160
Zebu	EMEM	107	M	Negative	Negative	0	0	0	0	0	0	0	0	0	0	10	40	160
Zebu	MP-12	103	F	Negative	Negative	0	0	10	40	40	40	40	10	10	10	160	160	160
Zebu	MP-12	104	F	Negative	Negative	0	0	0	40	40	160	160	160	160	160	640	160	160
Zebu	MP-12	105	F	Negative	Negative	0	0	0	40	40	160	40	40	10	160	1020	2560	640
Zebu	MP-12-Nsm-Deletion	47	M	Negative	Negative	0	0	0	40	40	40	40	40	40	40	640	640	640
Zebu	MP-12-Nsm-Deletion	48	F	Negative	Negative	0	0	10	0	0	10	10	10	10	10	640	640	160
Zebu	MP-12-Nsm-Deletion	49	M	Negative	Negative	0	0	0	10	40	40	40	40	10	10	640	640	640
Zebu	MP-12-Nsm-Deletion	101	M	Negative	Negative	0	0	40	40	10	40	40	10	10	10	160	640	640
Zebu	MP-12-Nsm-Deletion	925	M	Negative	Negative	0	10	10	40	40	40	160	NT	NT	NT	NT	NT	NT
Zebu	MP-12-Nsm-Deletion	905	M	Negative	Negative	0	0	0	40	40	40	640	NT	NT	NT	NT	NT	NT

Appendix 2: Rift Valley fever virus neutralizing antibody titers for individual sheep, goat and zebu cattle following vaccination with arMP-12ΔNSm21/384 and Eagle's Minimum Essential Medium by using Bioject ZetaJet™ device administered intradermally.

Species	sex	Animal number	Device	Route	Vaccine	DPVO	DPV5	DPV7	DPV14	DPV21	DPV 28	DPV 35
Zebu	F	909	Bioject	Intradermal	EMEM	0	0	0	0	0	0	0
Zebu	M	912	Bioject	Intradermal	EMEM	0	0	0	0	0	0	0
Zebu	F	901	Bioject	Intradermal	NSM	0	0	0	10	10	10	10
Zebu	M	903	Bioject	Intradermal	NSM	0	0	0	10	10	10	10
Zebu	F	904	Bioject	Intradermal	NSM	0	10	10	40	160	160	640
Zebu	F	906	Bioject	Intradermal	NSM	0	0	10	160	40	40	40
Zebu	F	910	Bioject	Intradermal	NSM	0	0	0	10	10	10	10
Zebu	M	914	Bioject	Intradermal	NSM	0	10	40	40	40	10	10
Zebu	F	917	Bioject	Intradermal	NSM	0	10	10	40	10	40	10
Zebu	F	920	Bioject	Intradermal	NSM	0	0	0	10	10	10	10
Zebu	M	929	Bioject	Intradermal	NSM	0	0	0	10	10	40	40
Zebu	M	985	Bioject	Intradermal	NSM	0	10	10	40	40	40	10
Sheep	F	940	Bioject	Intradermal	NSM	0	0	10	160	160	40	40
Sheep	F	953	Bioject	Intradermal	NSM	0	0	10	40	160	160	160
Sheep	F	932	Bioject	Intradermal	NSM	0	0	0	0	160	160	160
Sheep	F	945	Bioject	Intradermal	NSM	0	0	160	160	160	160	160
Sheep	F	944	Bioject	Intradermal	NSM	0	10	160	640	160	160	160
Sheep	M	931	Bioject	Intradermal	NSM	0	10	40	160	640	640	160
Sheep	M	956	Bioject	Intradermal	NSM	0	10	40	160	160	160	160
Sheep	M	952	Bioject	Intradermal	NSM	0	0	0	40	40	40	160
Sheep	F	946	Bioject	Intradermal	NSM	0	10	40	640	640	160	160
Sheep	F	948	Bioject	Intradermal	NSM	0	10	40	160	640	160	40
Sheep	M	941	Bioject	Intradermal	EMEM	0	0	0	0	0	0	0
Sheep	M	934	Bioject	Intradermal	EMEM	0	0	0	0	0	0	0
Sheep	F	936	Needle	Intramuscular	NSM	0	10	10	40	40	40	160

Sheep	M	937	Needle	Intramuscular	NSM	0	0	40	40	160	40	40
Sheep	F	947	Needle	Intramuscular	NSM	0	10	160	160	160	160	640
Goats	F	966	Bioject	Intradermal	NSM	0	10	160	640	2560	2560	2560
Goats	F	983	Bioject	Intradermal	NSM	10	10	40	2560	2560	2560	2560
Goats	F	962	Bioject	Intradermal	NSM	0	0	160	2560	2560	2560	2560
Goats	F	964	Bioject	Intradermal	NSM	0	10	40	160	160	640	640
Goats	M	971	Bioject	Intradermal	NSM	0	0	10	160	160	160	160
Goats	F	967	Bioject	Intradermal	NSM	0	0	640	640	640	640	2560
Goats	M	963	Bioject	Intradermal	NSM	0	10	40	160	160	160	160
Goats	M	960	Bioject	Intradermal	NSM	0	40	160	160	160	640	640
Goats	M	981	Bioject	Intradermal	NSM	0	160	160	640	640	2560	2560
Goats	F	970	Bioject	Intradermal	NSM	0	40	2560	2560	640	2560	2560
Goats	F	975	Bioject	Intradermal	EMEM	0	0	0	0	0	0	0
Goats	F	959	Bioject	Intradermal	EMEM	0	0	0	0	0	0	0
Goats	F	972	Needle	Intramuscular	NSM	0	10	160	160	160	160	160
Goats	M	978	Needle	Intramuscular	NSM	0	10	40	40	40	160	160
Goats	F	973	Needle	Intramuscular	NSM	0	10	40	160	160	160	640