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Epidemiology of Rift Valley Fever Virus in South Africa: Assessing Herd Immunity, Viral Persistence and Drivers of Epizootics in Livestock (Volume 1)

Melinda K. Rostal, D.V.M., M.P.H.

Submitted in fulfilment of the requirements of the Degree of Ph.D.

Institute of Biodiversity, Animal Health & Comparative Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow
February 19, 2020

ABSTRACT

Rift Valley fever virus (RVFV) is a zoonotic arbovirus that can cause devastating outbreaks in ruminant livestock. These Rift Valley fever (RVF) outbreaks may be multinational or regional and occur every 5-36 years. While a significant amount of research has been conducted, there remain many questions about the epidemiology RVFV. This study aims to examine drivers of interepizootic and epizootic RVF in livestock in central South Africa. We hypothesized that interepizootic transmission of RVFV was occurring in central South Africa; that antibodies induced by vaccination will wane over time; that RVFV can persist in a single pan setting with two vector species and a single host species; and that factors associated with endemic RVFV circulation on the farm and importation of RVFV through trade would drive the occurrence of RVF on a farm during the 2010 RVF outbreak, while factors associated with vector amplification and host susceptibility would drive the number of abortions and mortalities during the outbreak.

We collected serological and questionnaire data from longitudinal and cross-sectional field studies. We analysed these data using a variety of statistical methods, including Generalized Linear Models, mathematical modelling and Bayesian statistical analysis. We determined that low-level interepizootic RVFV transmission is occurring in central South Africa. Despite this, herd immunity declined in unvaccinated cattle, while remaining relatively constant in sheep and goats. Our simulation of the underlying biological mechanisms, suggested that RVFV persistence in a single pan is possible. However, persistence was very sensitive to the vector bite rate, host-vector ratio and transovarial transmission. We also determined that vaccination with Smithburn is effective in inducing long-lasting antibodies. Despite this, only a small portion of reportedly vaccinated sheep were seropositive. This was likely due to suboptimal vaccine handling. If the vaccine were mishandled along the supply chain, prior to administration it could significantly reduce the impact of vaccination on herd immunity.

Our results also suggested that drivers of interepizootic RVF may be different than those previously identified for widespread epizootics. Likewise, drivers of widespread epizootics were insufficient to predict the probability of RVF occurring though we were able to identify drivers of the severity of RVF in livestock measured through the number of RVF-associated abortions and mortalities on a farm during the 2010 outbreak. Further research is needed to identify different drivers of interepizootic transmission and the number of RVF-associated abortion and mortalities during and epizootic.

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ACKNOWLEDGEMENT

I would like to thank EcoHealth Alliance for the opportunity, support and funding to complete my Ph.D. I would like to acknowledge the generous financial support that I received from the University of Glasgow.

I would like to thank all of my supervisors for their support. Prof. Sarah Cleaveland, thank you for all of your support. First, for letting me spend a summer vaccinating dogs in the Serengeti on one of your projects 15 years ago. That summer was a catalyst to my desire to work in the field of One Health. Thank you for responding to my email nearly ten years later and agreeing to be my Ph.D. advisor. I have learned a lot from working with you in the past four years. You conduct research and train people to conduct research in a manner that not only generates good science but also benefits the lives of the people in the communities in which you are working. I am very much looking forward to continuing our collaboration on our new project on CCHF in Tanzania. Prof. Louise Matthews, thank you for all of the time and help you provided while I was working on the SEIR model – which was most of my time while in the Ph.D. program. Given the number of ups and downs and ups and downs… that model took me on, I really appreciate your patience and support. Thank you also for teaching me how to tackle mathematical modelling and for your genuine enthusiasm for epidemiology and teaching. Thank you, Dr. Noam Ross, for all of your help. You have taught me so much about how to think with a different type of analytical mindset compared to what I have been used to in veterinary medicine. Despite your workload you have always made time for helping and teaching me, which is very much appreciated. Unfortunately for you and fortunately for me, you received the brunt of the questions because you were the only advisor in the EcoHealth offices with me in NY – but you patiently helped me through all of the coding and server issues as well as the higher-level statistical questions. Thank you also for stepping in when things felt like they were falling apart and helping to code the Bayesian model. Prof. Dan Haydon, thank you for generously giving your time to give me a personal class in advanced statistics, since I couldn't attend the actual course. Thank you also for helping me work through some tough spots when I just wasn't sure what to do. Thank you to Dr. Jamie Prentice, who with Louise, tirelessly helped me with my SEIR model – answering so many questions and patiently explaining the maths. I've enjoyed working with you and learned so much from you. Thank you also to Dr. Paul Johnson for tirelessly answering the never-ending statistics questions and for helping with other random things like developing simulations for power analysis. To Dr. Mafalda Viana, thank you for your help with the Bayesian model and for

the Skype calls answering various questions. Finally, I would like to thank my assessors – Dr. Tiziana Lembo and Prof. Neil Metcalfe. I appreciate that you were both genuinely interested in ensuring that I was getting enough support and was learning what I needed to accomplish my work. Thanks also for all of the tips on writing and managing this final year of work.

I would like to thank my wonderful husband, Joel, for all of his love and support. From the first time I mentioned that I would like to pursue a Ph.D. through this last year where I spent hour after hour, night after night, weekend after weekend solely working on this dissertation. You always encouraged me and told me that I could do this – and you were right! Thank you for your patience both with the amount of time I needed to work and with the endless stream of my talking about everything and anything related to my Ph.D. I want to thank Zoey Dog, you have been my constant companion from my first doctorate through the completion of my second! I want to thank my parents, you have always supported me through so much and I wouldn't be the person I am today without both of you. Thank you to Nicole and Katie for your support and helping me plan an amazing wedding while working to complete this degree – you are my best friends. I want to thank the rest of my friends and family – we have been through so much during the four years of this Ph.D. program. I thank you for your support and patience as I went radio silent for this past year while finishing my dissertation.

I would also like to thank my colleagues and co-workers at EcoHealth Alliance. I have been blessed to be in a working environment where I know everyone is genuinely working toward making this a better planet for people and animals alike. Thank you to Dr. Billy Karesh. I cannot tell you how much I have learned from working with you on the Rift Project. You have inspired me to always "make the world a better place and find joy along the way." Thank you for your patience and support as I completed this dissertation, it meant a lot to me. Dr. Peter Daszak, thank you for your support - ten years ago you asked me when I was going to get my Ph.D., thank you for helping to make that a reality when I came to you six years later and said now is the time. Catherine Machalaba, thank you for all of your encouragement as we went through this Ph.D. process together – I'm really glad we had each other's support when we were so far away from our fellow students. You, Catherine, are an inspiration and you have done and will continue to do amazing work! There are so many others at EcoHealth Alliance that have helped or supported me in this pursuit, including Aleksei Chmura, Evelyn Luciano, Evan Eskew, Emily Hagan and everyone else, I want to thank you all!

Finally, I want to thank everyone on the Understanding Rift Valley Fever in the Republic of South Africa Project! Prof. Janusz Paweska, it has been an honour to work with you as a co-PI on this project. I have learned so much from you over the years and appreciate all of the conversations, snacks, opportunities and support. Thank you to Dr. Claudia Cordel, your friendship and leadership on the field work are so greatly appreciated and critical to the research conducted by the project and in this dissertation! Thank you, Lara van Staden, for collecting thousands of cohort sheep samples and calling back all of those farmers to get the updated data for our Bayesian model. Thank you, Dr. Petrus Jansen van Vuren, Ms. Danai Kwende and Dr. Yusuf Ngoshe for analysing thousands of livestock samples. Thank you to Dr. Assaf Anyamba, Mr. Alan Kemp, Prof. Peter Thompson, Dr. Robert Brand, Prof. Cornie van Huyssteen and Ms. Veerle Msimang for all of the conversations about my models and RVF in general. You have all given me so much to think about from so many different perspectives on Rift Valley fever – I love it! Thank you to our entire One Health field team: Herman Zwiegers, Dr. Lida Avanant, J.C. Bester, Mariet Verster, Godfrey Kealeboga, Euthelia Masiza, Jerry Masiza, Estie Straus, Ettienne Theron, Gwendy Ambrose, Janine van Zyl, Zikhona Gqalaqha, Eunice Ntsheno, Caitlin McAllister, Alisa Saule Berg, Madre Brewis, Albie Loots and Mirriam Moleta.

I would like to acknowledge the weather station data provided by the South African Weather Service. I would also like to thank Dr. Assaf Anyamba and his team at the Universities Space Research Association/ National Aeronautics and Space Administration for providing the precipitation data from the African Rainfall Climatology dataset. I would like to acknowledge the “Understanding Rift Valley Fever in the Republic of South Africa” project for the opportunity it gave me to design my study, collect and analyse data as part of the field epidemiology work. The project was sponsored by the U.S. Department of Defense, Defense Threat Reduction Agency. The content of the information does not necessarily reflect the position or the policy of the federal government, and no official endorsement should be inferred. Finally, I would like to thank the USAID PREDICT-2 Project for financial support that was made possible by the generous support of the American people through the U.S. Agency for International Development Emerging Pandemic Threats PREDICT-2 project. The contents are the responsibility of the authors and do not necessarily reflect the views of the US Agency for International Development or the U.S. Government.

DEDICATION



[7]The fear of the LORD [is] the beginning of knowledge: [but] fools despise wisdom and instruction.

Proverbs 1:7

[7]But ask the beasts, and they will teach you; the birds of the heavens, and they will tell you; [8]or the bushes of the earth, and they will teach you; and the fish of the sea will declare to you. [9]Who among all these does not know that the hand of the Lord has done this? [10]In his hand is the life of every living thing.

Job 12:7-10

[19]Surely the fate of human beings is like that of the animals; the same fate awaits them both: As one dies, so dies the other. All have the same breath; humans have no advantage over animals.

Ecclesiastes 3:19

[6]Behold, I will bring to it health and healing, and I will heal them and reveal to them abundance of prosperity and security.

Jeremiah 33:6

AUTHOR'S DECLARATION

"I declare that, except where explicit reference is made to the contribution of others, that this dissertation is the result of my own work and has not been submitted for any other degree at the University of Glasgow or any other institution."

Printed Name: Melinda K. Rostal

Signature: _____

DEFINITIONS AND ABBREVIATIONS

Term	Definition
Adjusted seroprevalence:	The estimated seroprevalence adjusted for clustering and herd size.
Aedes:	A genus of mosquitoes. The term is used in the thesis to specifically refer to floodwater *Aedes* mosquitoes.
Aestivation:	Prolonged torpor or dormancy of an insect, fish, or amphibian during a hot or dry period.
AUC:	Area Under the Curve.
cELISA:	competitive Enzyme-Linked Immunosorbent Assay.
CI:	Confidence Interval or in relation to a Bayesian analysis (Chapter 5) Credible Interval.
Crude seroprevalence:	The seroprevalence as determined without adjusting for herd size, clustering or diagnostic test.
Culex:	A genus of mosquitoes. The term is used in the thesis to specifically refer to various species of *Culex* that can horizontally transmit Rift Valley fever virus.
ELISA:	Enzyme-Linked Immunosorbent Assay.
Enzootic:	Endemic in animals. An enzootic disease is constantly present in an animal population, but usually only affects a small number of animals at any one time.
Epidemic:	Affecting a disproportionately large number of people within a population, community, or region at the same time.
Epizootic:	Affecting a disproportionately large number of animals within a population, community, or region at the same time; in the thesis it will be used interchangeably with epizootic.
FAO:	Food and Agriculture Organization of the United Nations.
Figure SX:	Supplementary Figure that will be found after the conclusion of that particular chapter and before the references.
Figure X:	A regular figure that will be found in the results section of that chapter.
Gaussian process:	A stochastic process such that every finite collection of random variables has a multivariate normal distribution, i.e. every finite linear combination of them is normally distributed.
Gonotrophic cycle:	The reproductive cycle of the arthropod, includes the sequence of questing or searching for a host, blood feeding, blood meal digestion, egg maturation, and oviposition.
Herd immunity:	The protection of populations from infection which is brought about by the presence of immune individuals.
HIT:	Herd Immunity Threshold, is the minimum proportion of a population that must be immunized to eliminate an infection.
$IMLD_{50}$:	Infant Mouse Lethal Dose $_{50}$, the amount of a substance, when given all at once that causes death in 50% of infant mice.

Inclusion criteria: The criteria by which it was determined if a farm could be invited to enrol in the longitudinal study. It was: 1) Seropositive sheep were identified on the farm; 2) These sheep were born on the farm; and 3) The farmer never vaccinated his sheep before.

Interepidemic Period: The period between two Rift Valley fever epidemics that involve people, and during which there is low-level Rift Valley fever virus transmission.

Interepizootic Period: The period between two Rift Valley fever epidemics that involve animals, and during which there is low-level Rift Valley fever virus transmission.

Maternal immunity: A form of passive immunity from mother to neonate, which is the transfer of active humoral immunity of ready-made antibodies.

n: The sample size.

NOAA-CPC: National Oceanic and Atmospheric Administration-Climate Prediction Center.

OBP: Onderstepoort Biological Products.

ODE: Ordinary Differential Equation.

OIE: The World Organisation for Animal Health.

OR: Odds Ratio.

Outbreak: Affecting a disproportionately large number of individuals within a population, community, or region at the same time; in the thesis it will be used interchangeably with epizootic.

Pan: Shallow depressions in grasslands that fill with precipitation and there is no outlet so the water remains until it either evaporates or is absorbed into the ground. In other regions these are called playas and in East Africa they are called dambos.

Panveld: A South African term for plains that have multiple shallow depressions (pans) that fill with water during the rainy season creating a temporary wetland before they dry out via evaporation.

PCR: Polymerase Chain Reaction.

PRNT: Plaque Reduction Neutralization Test.

R_0: The basic reproduction number is the expected number of new cases of an infection caused by a typical infected individual in a population consisting of susceptible individuals only.

ROC curve: Receiver Operating Characteristic curve.

Routine vaccination: Refers to the population of ruminants from the cross-sectional study that the farmer reported vaccinating themselves.

RVF: Rift Valley Fever.

RVFV: Rift Valley Fever Virus.

SEIR: Susceptible-Exposed-Infected-Recovered compartmental model.

Seroconversion: Seroconversion was reported if a previously seronegative animal tested positive on two consecutive inhibition ELISAs. It was considered to have seroconverted prior to the first consecutive positive result.

Seronegative: An animal that tested negative on an inhibition ELISA.

Seropositive: An animal that tested positive on an inhibition ELISA.

Seroreversion: Seroconversion was reported if a previously seronegative animal tested positive on two consecutive inhibition ELISAs. It was considered to have seroconverted prior to the first consecutive positive result.

Serostatus: The serological status of an animal for RVFV antibodies, either seropositive or seronegative.

SNT: Serum Neutralization Test.

$TCID_{50}$: Median Tissue Culture Infectious Dose $_{50}$, the concentration at which 50% of the cells are infected when a test tube or well plate upon which cells have been cultured is inoculated with a diluted solution of viral fluid.

True seroprevalence: The estimated seroprevalence adjusted to incorporate the sensitivity and specificity of the diagnostic assay used.

Univoltine: Reproducing at a rate of one generation per year.

VNT: Virus Neutralization Test.

WAIC: Widely Applicable Information Criterion.

Chapter 1: Introduction

Rift Valley fever virus (RVFV) is a zoonotic arbovirus that was first detected in 1930 following an outbreak in sheep and cattle in Kenya (Daubney et al., 1931). RVFV causes large epizootics in ruminant livestock, particularly in sheep, cattle and goats (Swanepoel and Coetzer, 2004). These outbreaks can be widespread across a country or several countries or be isolated to a single farm (Nanyingi et al., 2015). Large epizootics tend to occur every 5-35 years across Africa (Nanyingi et al., 2015). In livestock, Rift Valley fever (RVF) is characterized by high mortality in young animals and abortion storms (Swanepoel and Coetzer, 2004). This can be economically devastating to the livestock owner as they may lose an entire year's cohort of young animals due to RVF. A recent outbreak in three provinces in South Africa was estimated to cost the sheep production sector approximately USD$29 million (ZAR203.4 million) (Mdlulwa and Ngwane, 2017). The 2007 outbreak in Kenya was estimated to cost USD$32 million (KSh 2.1 billion) and also caused food insecurity due to the number of animals lost (Rich and Wanyoike, 2010). In people RVFV usually causes a mild disease with influenza-like symptoms, though in severe cases people can suffer from retinitis, hepatitis, encephalitis or haemorrhagic fever (Van Velden et al., 1977). RVFV is a public and veterinary health threat and it remains difficult to predict when and where outbreaks will occur. Many aspects of RVFV ecology are still undetermined.

Here we will describe the current state of RVFV ecology and epidemiology, focused primarily on domestic ruminants. We will briefly provide a background briefly covering molecular epidemiology and outbreaks, clinical signs and risk factors. We will then describe the current state of the literature with a focus on the gaps that remain in our knowledge, briefly discussing the animal risk factors associated with RVFV seroprevalence; the ecology of Rift Valley fever virus vectors and interepizootic transmission; serological diagnostic methods for Rift Valley fever virus and Rift Valley fever virus vaccines. We will briefly summarise various epidemiological and ecological models applied to RVFV, including, seroprevalence and risk factor studies, modelling RVF outbreaks, mechanistic models of RVFV and a comparison to compartmental models used to understand the ecology and epidemiology of other arboviruses. We will also describe Rift Valley fever in South Africa; including the history of RVF epizootics in South Africa; previous research conducted in South Africa; the Understanding Rift Valley Fever in The Republic of South Africa Project, which supported the work conducted in this system; and

the study area where the field work for this thesis was undertaken. Finally, we will discuss the aims of the thesis, which include describing the herd immunity and interepizootic transmission of RVFV in South Africa, investigating the biological mechanisms that support interepizootic persistence and identify farm-level factors that contribute to the occurrence and severity of an epizootic on an individual farm.

BACKGROUND

Molecular Epidemiology

Rift Valley fever virus is a negative sense, RNA virus with three segments (Grobbelaar et al., 2011) that is in the Phenuiviridae family and Phlebovirus genus (Adams et al., 2017). The M segment encodes the envelop glycoproteins that produce the viral epitopes that are important for inducing neutralizing antibody responses (Battles and Dalrymple, 1988). One study compared the variation in the M segment RNA in 22 isolates of RVFV from six countries and collected over 34 years and they detected very little genetic variation in the M segment and determined that the same monoclonal antibodies are able to neutralize all but three strains of RVFV (Battles and Dalrymple, 1988). This indicates that neutralizing antibodies against one strain of RVFV should be highly protective against all of the strains that have been detected in animals thus far. Further analysis of 198 RVFV isolates from 17 countries over 67 years identified 15 lineages of RVFV confirmed that genetic diversity of the M segment was still quite low (Grobbelaar et al., 2011). Five of the 15 lineages were phylogenetically clustered within regional Rift Valley fever (RVF) outbreaks, though several outbreaks did include at least one isolate from a distant geographic origin. Six of the lineages were only isolated once (one was isolated twice). Lineage C was isolated from Madagascar in 1991, East Africa in 1996-97, Saudi Arabia in 2000-01, Kenya in 2007 and South Africa in 2008-2009. The final three lineages contained evidence of reassortment with a neurotropic Smithburn strain (a vaccine strain) (Grobbelaar et al., 2011).

Rift Valley Fever Outbreaks, Clinical Signs and Risk Factors

Historical Outbreaks

Although RVFV was first identified in Kenya, outbreaks have since occurred across Africa. In 1950-51, the first outbreak in South Africa was reported (Joubert et al., 1951). A second outbreak of RVF followed in 1974-75, which was the first outbreak in which

human deaths were attributed to infection with Rift Valley fever virus (Van Velden et al., 1977). In 1977, a large outbreak in Egypt was estimated to have affected 20,000-100,000 people (most have mild disease) (Laughlin et al., 1979) with at least 598 fatalities (WHO, 1978). This is thought to have been the first RVF outbreak outside of sub-Saharan Africa (Nanyingi et al., 2015) and was supported by serological evidence indicating that RVFV had not circulated in Egypt prior to 1977 (Hoogstraal et al., 1979). In 1987, following flooding caused by the installation of two dams on the Senegal River, a large outbreak of Rift Valley fever occurred in Mauritania (Jouan et al., 1988). In 1990, the virus moved off of the African continent when an outbreak was detected in Madagascar (Morvan et al., 1991). The virus was detected further afield during an outbreak in Saudi Arabia and Yemen in 2000 (Al-Hazmi et al., 2003). Though no RVF outbreaks have been reported in Mayotte or the Comoros, serological surveillance has detected infections in people and ruminants there through laboratory surveillance since 2007 (Daouda et al., 2009), which indicates the virus has been maintained in the absence of clinical outbreaks. While RVFV continued to spread geographically since 1930, recurrent large outbreaks were reported in East (1997-98, 2006-2007, 2018), West (1987, 1993-94,) and South Africa (1950-51, 1974-75, 2009-2011) (Nanyingi et al., 2015).

Large RVF outbreaks are reported to affect tens of thousands of animals and hundreds of people, likely more. We recently estimated that 2,635-5,374 farm workers living and working in the 200 x 200 km study area in South Africa, were previously infected with RVFV (Msimang et al., 2019), though only 302 people were diagnosed with RVFV across the entire country during the 2008-2011 outbreak (Archer et al., 2013). The 2006-07 outbreak in East Africa began in Kenya and affected at least three other countries, Tanzania, Somalia and Sudan, during which there were over 1,000 cases of RVF in people diagnosed in Kenya alone (Breiman et al., 2008) with 158 human deaths (Nanyingi et al., 2015). In animals, there are many fewer estimates of the number of animals impacted by RVF outbreaks. In South Africa in 2009-11 there were over 17,000 cases of RVF reported to the OIE in sheep, goats and cattle as well as camelids, buffalo (*Syncerus caffer*), kudu (*Tragelaphus strepsiceros*) and other wildlife (OIE, 2019). These findings indicate that RVFV may have a more significant toll on human and animal health than is currently understood.

Clinical Disease

Outbreaks of RVF are characterized by abortion storms in ruminant livestock and high mortality in very young livestock (Swanepoel and Coetzer, 2004). Sheep are more susceptible to RVFV, with adult mortality rates of up to 20% (Easterday, 1965), whereas the adult mortality rate in cattle and goats is closer to 10% (Swanepoel and Coetzer, 2004). There are four forms of clinical RVF in livestock (Alexander et al., 1951, Daubney et al., 1931, Easterday, 1965): the hyperacute/peracute form usually affects very young lambs (1-7 days of age), causes severe histopathological changes in the liver and is usually fatal within 36 hours (95-100%); the acute form also primarily affects younger animals and causes fever, hepatitis, nephritis, and abortion in pregnant animals, death usually occurs in 24-48 hours; the subacute form is more common in cattle and adult sheep and clinical signs include fever and abortion, with a low mortality rate (~10%); and the mild or inapparent form occurs in adult sheep and cattle and the only sign is a slight fever with viremia and seroconversion (Alexander et al., 1951, Daubney et al., 1931, Easterday, 1965). Rift Valley fever virus can be transmitted to humans through direct contact with an infected animal's fluids or tissues, as well as through the bite of an infected mosquito (Van Velden et al., 1977). Most people infected with RVFV have mild symptoms including fever and myalgia (Heymann, 2008). In approximately 10% of cases more severe symptoms can occur, including retinitis, hepatitis, encephalitis and haemorrhagic fever, which can result in death (Al-Hazmi et al., 2003).

Serological Evidence of RVFV Infection

There is serological evidence of RVFV transmission in animals and people in most African countries where RVFV has been studied, even in parts of Africa that have not reported RVF outbreaks (Zeller et al., 1995, van den Bergh et al., 2019, Daouda et al., 2009). Estimated seroprevalences in livestock range between 2-44.2% (Soumare et al., 2007, Blomström et al., 2016); however serosurveys conducted immediately following outbreaks have detected seroprevalences up to 80% (Thiongane et al., 1991). Amongst animal studies, the true seroprevalence has rarely been calculated (accounting for the diagnostic sensitivity and specificity of the assay used; see below) or adjusted for the clustering of animals sampled on the same farm. Exceptions to this include Lewerin et al.'s (2018) study that found a true herd-level seroprevalence in Uganda of 3.6%, adjusting for test performance, and Ngoshe et al.'s (submitted) study that adjusted their estimates of seroprevalence for herd size. Most studies also do not mention whether vaccination may

account for some of the seropositive samples from livestock. However, except for central South Africa (Ngoshe et al., submitted, Chapter 3), vaccination against RVFV has been rare.

Human Risk Factors Associated with RVFV Seroprevalence

Many risk factor analyses have assessed the risk of infection with RVFV in people. These have identified factors, such as older age, assisting with a livestock abortion, injecting livestock, slaughtering livestock (Msimang et al., 2019, Nicholas et al., 2014) etc., as having a higher odds of seropositivity. Fewer studies have identified human risk factors associated with mosquito transmission, but these include a higher odds of seropositivity in people that work or live on a farm that has a dam and a lower odds of seropositivity associated with taking actions to prevent mosquito bites (Msimang et al., 2019).

IDENTIFYING GAPS IN THE LITERATURE

Animal Risk Factors Associated with RVFV Seroprevalence

Amongst animal risk factors, there is a strong consensus that older animals have a higher odds of being seropositive for RVFV (Abakar et al., 2014, van den Bergh et al., 2019, Sumaye et al., 2013). The differences between species and sex are less clear as some studies have identified that the odds of seropositivity were significantly associated with these two factors (Abakar et al., 2014, Bett et al., 2018, Lwande et al., 2015, Nyakarahuka et al., 2018, Sumaye et al., 2013, Nakouné et al., 2016, Moiane et al., 2017), while others did not (Nanyingi et al., 2017, Matiko et al., 2018, Magona et al., 2013, Maganga et al., 2017, Fafetine et al., 2013). Farm-level risk factors have rarely been evaluated as risk factors for RVFV seropositive animals. Those risk factors that have been evaluated as having a higher odds of seropositivity in domestic ruminants include: animals located at ≤1000m above sea level (Sindato et al., 2013), animals that are kept at night in pens that are closer to the nearest permanent water bodies (Kanouté et al., 2017) and animals living on farms that purchase additional animals (Chevalier et al., 2011). Distance from water has a variable association with risk. One study found that animals that are 15 km or further from the edge of previous flooding (Sumaye et al., 2013) had a higher odds of seropositivity whereas another study found that as distance from a water point increased the odds decreased (Chevalier et al., 2011). One other study found that herd size was not found to be significantly associated with odds of seropositivity (Magona et al., 2013).

Most of the RVFV risk factors that have been described in livestock are either individual factors (age, sex, breed etc.) or environmental factors. Very few studies have investigated how farm-level management factors affect the likelihood of the farm having RVFV seropositive animals. A better understanding of how management factors, including trading and biosecurity practices, can increase or reduce the risk of RVFV infection could provide farmers with additional tools to prevent RVFV in their animals. One recent study has begun to fill this gap. Ngoshe et al. (submitted) examined 22 farm-level factors, following a thorough questionnaire regarding farming practices and identified several farm-level risk factors that were associated with a higher odds of seropositivity in livestock (Ngoshe et al. submitted). Surprisingly, none of the management practices examined were associated with seropositivity, except for whether the animals were grazed on private or communal land and the most recent year of vaccination. They also associated seropositivity with individual and environmental risk factors, including species (cattle, specifically), older age, and whether the animals could access a perennial source of water or a pan (a shallow depression in the land). Even with this study, a significant gap remains in our understanding of what drivers are associated with an RVF infection occurring on a specific farm.

The Ecology of Rift Valley Fever Virus Vectors and Interepizootic Transmission

Rift Valley fever virus is primarily transmitted between animals via mosquitoes. Many mosquito species have been implicated in horizontally transmitting RVFV to hosts through laboratory experiments (Turell and Bailey, 1987, Turell et al., 2008, Jupp and Cornel, 1988, McIntosh et al., 1973, McIntosh et al., 1980) and via field isolations/detection (Ba et al., 2012, Gear et al., 1955, Jupp et al., 2002, Linthicum et al., 1985b, Logan and Linthicum, 1992). Even though most regions have seasonal mosquito populations, there can be long interepizootic periods between outbreaks that last from 2-35 years (Nanyingi et al., 2015) during which no cases of clinical RVF have been reported in animals or people. The long interepizootic periods (Davies et al., 1985) and the inability to identify a reservoir host (Gear et al., 1955, Davies and Addy, 1979, Davis, 1957, Gora et al., 2000) despite significant effort and the isolation of RVFV from larvae and mosquitoes that had not taken their first blood meal (Linthicum et al., 1985b, Romoser et al., 2011, McIntosh, 1971) led researchers to propose that RVFV was transmitted and maintained transovarially through the eggs of floodwater *Aedes* spp. mosquitoes (Linthicum et al., 1985b). Under this proposed mechanism, the virus would remain in the drought resistant eggs until the next outbreak. When the eggs eventually hatched, the transovarially infected mosquitoes would

initiate an RVF outbreak, then other mosquito species, e.g. some *Culex* spp., that could transmit the virus horizontally would amplify the virus within ruminant (domestic and wild) and mosquito hosts, and eventually spill over into people (Linthicum et al., 2016).

The floodwater *Aedes* spp. mosquitoes (generally referred to as simply *Aedes* mosquitoes in the rest of the chapter) lay their eggs in shallow depressions that flood seasonally (called pans). The flooding *triggers* the eggs to hatch (Linthicum et al., 2016). Linthicum et al. (1985b) isolated RVFV from *Aedes lineapennis* larvae, which implicated them as hosts capable of transovarial transmission. This species was later reclassified as *Ae. mcintoshi* (Huang, 1985). Linthicum et al. (1984) reported that *Ae. mcintoshi* hatched within five days of a pan flooding. *Ae. mcintoshi* hatched for 5-10 days and then were absent for the remainder of the season (Linthicum et al., 1984, Linthicum et al., 1985a). Up to 90% of viable eggs hatched during the first flooding of a season and very few hatched during subsequent flooding (Logan et al., 1991). This suggests a univoltine life cycle, where the eggs only hatch one time per season (Crans, 2004).

Very little is known about the hatching triggers of vectors thought to be capable of RVFV transovarial transmission. Whereas, the eggs of other floodwater mosquito species have been thoroughly studied. In addition to water, most eggs require a specific pH, dissolved oxygen level and temperature to hatch (Borg and Horsfall, 1953). The RVFV vectors suspected to be capable of transovarial transmission continue to be extremely difficult to breed in a laboratory. However, it is necessary to establish a laboratory line to determine the rate of transovarial transmission (Logan and Linthicum, 1992, Gargan et al., 1988). Evidence for transovarial transmission includes isolating RVFV from larvae collected from the field and hatched in a laboratory as well as from unfed mosquitoes that could not have been infected horizontally (Linthicum et al., 1985b). Romoser et al. (2011). recently identified RVFV antigen in floodwater *Aedes* mosquitoes eggs collected from the field. The longevity of *Ae. mcintoshi* eggs is unknown. Logan and Linthicum (Logan and Linthicum, 1992) determined that they can survive at least nine months in a laboratory. Other flood water mosquito eggs have been shown to survive longer (Campos, 2008).

The hypothesis that floodwater *Aedes* eggs that only hatch every 5-15 years are the sole reservoir of RVFV (Linthicum et al., 1985b) (without interepizootic transmission) has been disproven. As described above, floodwater *Aedes* hatch annually (Logan et al., 1991). If transovarial transmission is an important part of the reservoir system maintaining RVFV, there should be some amount of RVFV infection occurring in hosts annually. Sufficient

evidence has been generated to support the hypothesis that low-level transmission of RVFV occurs in livestock (Rostal et al., 2010, Roger et al., 2011), wildlife (LaBeaud et al., 2011, Dondona et al., 2016) and even people (LaBeaud et al., 2008). Low-level, interepizootic transmission has been demonstrated through the identification of seropositive animals that were born after the most recent, known RVFV outbreak (Rostal et al., 2010); the identification of RVFV antibodies in animal and human populations in regions where an RVF outbreak has never been reported (Daouda et al., 2009, van den Bergh et al., 2019); and through one of several short-term longitudinal studies that identified seroconversion in serologically naïve animals (Zeller et al., 1997, Chapter 2) or people. Much of this evidence was collected in the absence of clinical signs of RVF (van den Bergh et al., 2019) Recall bias and a lack of farm records may have affected the detection of clinical cases outside of an outbreak period, as we known RVFV was circulating in Mayotte in people and animals before being identified on routine laboratory surveillance (Daouda et al., 2009).

In Madagascar on-going RVFV transmission has been identified in the absence of vectors known to be capable of transovarial transmission (Nicolas et al., 2014). This suggests that transovarial transmission may not contribute to RVFV maintenance in some ecosystems or that other species of mosquitoes are capable of transovarial transmission of RVFV. While it is widely accepted that low-level interepizootic RVFV transmission occurs, there are still many gaps in our understanding of RVFV maintenance. To fill these gaps the role of transovarial transmission in RVFV maintenance must be determined. This will involve breeding floodwater mosquitoes in a laboratory and demonstrating transovarial transmission under controlled conditions. The rate of transovarial transmission in infected vectors must also be determined. It will also be important to identify ecological differences between regions where transovarial transmission may be more or less important to viral maintenance (e.g. Kenya vs Madagascar). If it is determined that in certain regions RVFV persistence does not depend on transovarial transmission, whether the virus can persist through prolonged droughts must be investigated. It must also be determined whether transovarially transmitted RVFV is less pathogenic than horizontally transmitted RVFV, which might indicate why interepizootic transmission of RVFV usually undiagnosed. Finally, other vectors capable of transovarial transmission must be identified. Answering these questions will contribute toward a better understanding of why, when and how does low-level interepizootic transmission of RVFV manifest in massive outbreaks that impact wide geographic areas.

Serological Diagnostic Methods for Rift Valley Fever Virus

There are many serological and molecular diagnostic tests available to diagnose previous and current RVFV infections. Molecular tests include reverse transcriptase-PCR and quantitative real time-PCR (Njenga et al., 2009, Jupp et al., 2000). We will focus only on serological assays and their interpretation as these are more relevant to work presented here. One must understand the strengths and limitations of the diagnostic assay used to interpret the many RVFV seroprevalence studies. Here, we will briefly discuss the gold standard for RVFV serology, virus neutralization assays, and review commonly used ELISA assays. We will only discuss the sensitivity, specificity and validation of the ELISA assays currently recommended by the World Animal Health Organisation (OIE).

Specificity is the ability of an assay to identify samples that are truly negative as a negative test result. Whereas, sensitivity is the ability of an assay to correctly identify samples that are truly positive (Smith, 1995). If the specificity of an assay is 85%, then we would expect a false positive rate of 15%, which must be considered when evaluating a seroprevalence estimate. Often as either specificity or sensitivity increases, the other will decrease. It is rare to have a test with high sensitivity and specificity. Yet many RVFV ELISAs are reported to have a high sensitivity and specificity. Thus, it is worthwhile to further examine the subset of recommended assays in light of the previous discussion of interepizootic transmission and seroprevalence. We will first discuss assay validation based upon the OIE's standards to provide perspective on the sample sizes given for each assay, and aid in the interpretation of the study results.

Assay Validation

The OIE will only give an assay international recognition for the diagnosis of a specific disease upon completion of four stages of diagnostic validation (OIE, 2018a). Stage 1 involves the analysis of the performance and repeatability of the assay. Stage 2 includes the determination of diagnostic sensitivity and specificity (OIE, 2018a). The sample size required to validate assays depends on the expected diagnostic sensitivity and specificity, the desired precision and the confidence in the assay. Like prevalence, an estimated sensitivity or specificity of 50% requires a higher sample size and a sensitivity or specificity of 99% requires fewer samples . If the true diagnostic sensitivity (or specificity) is 99% (with 98% precision and 99% confidence, one would need to test 164 known true positive (or true negative) samples (OIE, 2018a).

The OIE specifies that validation occur on a panel of reference samples of animals of known infection status and from the region where the test will be used (OIE, 2018a). Additionally, positive reference samples should not come from experimentally infected or vaccinated animals as these methods can induce a higher or lower antibody response than natural exposure to the virus (OIE, 2018a). Finally, the OIE recommends using latent class Bayesian analysis to determine the sensitivity and specificity of imperfect diagnostic assays for field samples where the exact exposure is unknown (OIE, 2018a).

Virus Neutralization Assays

The current gold standard for RVFV serology is the virus neutralization test (VNT; also called serum neutralization test, SNT, or plaque reduction neutralization test, PRNT). Through 2017, neutralization tests were the only assays approved for use in international trade to identify previous RVFV infection in livestock (OIE, 2014). VNTs use live RVFV, which restricts their use to biosafety level-3 (BSL-3) or higher laboratories. The Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2019 describes a method for conducting a VNT. No estimates of the sensitivity and specificity of VNTs for RVFV are available. In 1986 Swanepoel et al. (1986) compared VNT results for RVFV to four other African phleboviruses (Arumowot virus, Gordil virus, Gabek Forest virus, and Saint-Floris virus) to assess cross-reactivity in sheep (n=16). They found that none of the four phleboviruses elicited a strong immune response in sheep. Thus, there was little, if any, cross-reaction on either a microneutralization or a hemagglutinin inhibition test (Swanepoel et al., 1986). No one has assessed the risk of cross-reaction with the several phleboviruses identified in Africa since 1986.

Enzyme-Linked Immunosorbent Assays

The OIE states that enzyme-linked immunosorbent assays (ELISAs) are reliable and sensitive tests to detect RVFV and recommends several protocols, which will be discussed here (OIE, 2018b). For each ELISA and the species it is used in, the type of assay, sensitivity, specificity, sample size and gold standard are reported (Tables 1-1 and 1-2). Among the seven ELISAs reviewed here, the only sensitivity estimates that achieve the modest sample size required for a 99% sensitivity were among goats and people (Tables 1-1 and 1-2). Therefore, the precision of the sensitivity estimates for the other assays is low. Most of the specificity estimates have achieved and far exceeded the sample sized needed

to make this estimate. In several studies, a portion of the known RVFV positive samples came from vaccinated or experimentally infected animals (Fafetine et al., 2007, Paweska et al., 2003, Paweska et al., 2005b, Williams et al., 2011, van Vuren et al., 2007). Thus, published sensitivities and specificities may therefore be higher than would be expected when used in African field scenarios. It is clear from the tables below that none of the recommended assays have provided sufficient samples required by the OIE to validate an

Table 1-1 The sensitivity (Se) and specificity (Sp) with the total number of sera tested (n) in cattle, sheep and goats for the ELISA recommended in the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2019 (OIE, 2018b). Also included is the gold standard against which the assay was tested and the reference for the test. The assays used by Munyua et al. (2010) are not included in the table as the methodologies could not be confirmed for two of the three assays. ND = not done.

*Biological Diagnostic Supplied Limited (BDSL) closed on December 31, 2014. [†]on days 9-34 post vax or 5-42 post infection. [‡]a specificity of 99.78% was reported, but it is not clear what species that is for. [§]only 18 sera were compared to the gold standard, VNT. [‖]from 24 sheep. [¶]18 seropositive cattle sera were screened with VNT.

	Cattle				Sheep				Goats					
	Se	n	Sp	n	Se	n	Sp	n	Se	n	Sp	n	Gold standard	Reference
Indirect ELISA (recombinant N-based antigen)	100	42	98.3	58	100	51	100	200	99.4/ 100	162/ 197	99.5/ 100	200/ 116	VNT	(Fafetine et al., 2007)
Competitive ELISA Commercial product BDSL*	100	63	100	191	100	0	100	192	100	12	100	100	None	(Cêtre-Sossah et al., 2009) based on(Paweska et al., 2005b)
IgG Sandwich ELISA (whole antigen)	96.3	82	99.67	915	99.1	105	99.1	1216	100	357	99.9	1102	VNT	(Paweska et al., 2003)
IgM capture ELISA (whole antigen)	ND	ND	99.2	915	100[†]	18	99.4	1216	ND	ND	97.4	1102	VNT	(Paweska et al., 2003)
IgG inhibition ELISA (whole antigen)	100	59	99.52	635	100	65	99.3	428	99.56	232	99.7	574	VNT	(Paweska et al., 2005b)
cELISA ID Screen RVF multispecies ELISA (recombinant N-based antigen)	100[§]	40	100	370	ND	ND	100	280	ND	ND	100	70	[¶]	(Comtet et al., 2010)
IgM capture ELISA (recombinant N-based antigen)	ND	ND	ND	148	100	24	[‡]	205[‖]	ND	ND	ND	108	cELISA ID Screen RVF multispecies EILSA	(Williams et al., 2011)

Table 1-2 The sensitivity (Se) and specificity (Sp) with the total number of sera tested (n) in humans for the ELISAs recommended in the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2019 (OIE, 2018b). The gold standard against which the assay was tested and the reference for the test are also given.
*This was in people that were vaccinated for RVFV that had the same ELISA result as VNT (e.g. 5 vaccinated people were negative for antibodies both on VNT and the ELISA).

	Humans					
Assay	Se	n	Sp	n	Gold standard	Reference
IgG indirect ELISA (recombinant N-based antigen)	100	101*	100	5*	VNT	(van Vuren et al., 2007)
IgM capture ELISA polyclonal antigen	NA	349	NA	485	Virus isolation, RT-PCR or immunohistochemistry	(Madani et al., 2003) based on (Ksiazek et al., 1999)
IgG inhibition ELISA (whole antigen)	99.5	189	99.7	1178	VNT	(Paweska et al., 2005b)

assay, though the IgG inhibition assay was sufficiently validated in humans and goats (Paweska et al., 2005b).

It is worth noting that the commercially available cELISA ID Screen RVF multispecies ELISA has been widely used in previous studies (Nanyingi et al., 2017, Rissmann et al., 2017, Moiane et al., 2017), despite the absence of assay validation according to OIE guidelines. The sample size for assay validation was small and they only confirmed 18 of the positive reference sera using VNT (or any other diagnostic method) (Comtet et al., 2010). Additionally, all of the negative reference sera were from countries where RVFV is not endemic (Comtet et al., 2010), where other potentially cross-reacting arboviruses may not occur. A sample size of 18 is able to validate a 99-100% sensitivity with 5% error and 95% precision (OIE, 2018a). This suggests that false positives could account for a seroprevalence estimates up to up to 5%. The performance figures for the cELISA are derived from a study by Kortekaas et al. (2013). They compared the cELISA ID Screen RVF multispecies ELISA, the BDSL Competitive ELISA and IgM assays (no longer available) and the IgM and IgG ELISAs described by Williams et al. (2011). They found a 90% concordance amongst the assays on a total of 71 reference sera, which included samples from animals from an RVFV-free area, an RVFV-endemic area, experimentally infected with RVFV and vaccinated against RVFV) (Kortekaas et al., 2013). For the cELISA ID Screen RVF multispecies ELISA, the five laboratories estimated that the sensitivity was 91-100% and the specificity of 100%. For the Williams et al. (2011) IgG assay, the five laboratories estimated that the sensitivity was 73-100% and the specificity was 79-100% (Kortekaas et al., 2013). These results indicate that further validation is necessary for both assays - a sensitivity of 90% with 98% precision and 99% confidence would require a sample size of 1493 known positive samples for validation (OIE, 2018a).

Serological tests rarely have both 100% sensitivity and 100% specificity. Yet the sensitivities and specificities reported here are nearly all above 99%. Coupled with the evidence that the sensitivity and specificity of these assays are not as high as suggested, doubt remains about our ability to interpret the results of many of these assays. This in turn means that the interpretation of should always be made within the context of the serological assay used.

Rift Valley Fever Virus Vaccines

There are three types of RVFV vaccines commercially available for animals and no vaccines available for use in people. Several new vaccines are currently in development for use in animals and/or people. Here, we will focus on the three commercially available vaccines.

Smithburn Vaccine

In 1949 the first vaccine against RVFV from virus isolated from mosquitoes in the Semliki Forest, Uganda was developed (Smithburn et al., 1948). Smithburn (1949) attenuated the RVFV strain through serial intracerebral passage in mice (67-85 passages). The current Smithburn vaccine (Onderstepoort Biological Products (OBP), Pretoria, South Africa) uses the 103 MB 2BHK (29/1074) strain of RVFV originally isolated from the Semliki forest (Smithburn et al., 1948). OBP maintains the Smithburn strain in baby hamster kidney cells (Pers. Comm. B. Nthangeni (OBP), 2017). This vaccine is the most widely available across Africa (Dungu et al., 2018). However, the Smithburn vaccine can cause abortion in pregnant animals and foetal malformations (Botros et al., 2006). There is also a risk of reversion via reassortment if used during an outbreak (Grobbelaar et al., 2011). One study identified a reassortment between the Smithburn strain and a wild type RVFV in a clinically ill person (Grobbelaar et al., 2011). The Smithburn vaccine must be kept refrigerated. One study demonstrated that there was no detectable viral infectivity in a Smithburn vaccine (90 passages) after five days at 37^{o}C (Mims, 1956). Additionally, once reconstituted the Smithburn vaccine must be used within six hours (Pers. Comm., OBP Representative).

Strains of the Smithburn virus (from which the vaccine is made) induce long-lasting antibody titres in sheep following inoculation (Capstick and Gosden, 1962, Coackley et al., 1967, Barnard and Botha, 1977). In these studies, the animals were immunized with higher doses of virus (e.g. 10^{6}-10^{8} infant mouse lethal dose$_{50}$ [IMLD$_{50}$]) (Capstick and Gosden, 1962, Coackley et al., 1967). As compared to the dose of the commercially available Smithburn vaccine (at least $10^{4.4}$ IMLD$_{50}$) (Pers. Comm. B Nthangeni, 2017). Two studies found that sheep responded to vaccination with the Smithburn strain with either a high or low titre (Capstick and Gosden, 1962, Coackley et al., 1967). They suggested that sheep may have been naturally exposed to a strain of RVFV that did not generate an antibody

titre that could be detected by a neutralizing assay or less likely, that antibodies may wane in sheep (Capstick and Gosden, 1962). A more recent study found that in 10 sheep antibodies induced by a Smithburn vaccine waned from 95% to 50% in nearly five years (Elfadil and Shaheen, 2007). Neutralizing antibodies were rarely detected in cattle vaccinated with a Smithburn strain (Barnard, 1979). However, several challenge studies found that immunity was long lasting following vaccination with Smithburn, even in cattle that no longer had detectable neutralizing antibodies (Coackley et al., 1967, Barnard, 1979).

Clone 13 Vaccine

Clone 13 (OBP, Pretoria, South Africa) is a commercial, modified-live vaccine produced and initially licensed in South Africa in 2010. A strain of RVFV with a partial deletion of the NSs gene was used to make Clone 13 (Muller et al., 1995). Clone 13 provided an apparently non-pathogenic, modified-live vaccine alternative to Smithburn. Clone 13 is available for purchase in a number of African countries (Dungu et al., 2018). One randomized trial investigated the duration of the antibody response. They found that in cattle, sheep and goats, 67%, 84% and 97% responded with neutralizing antibodies following vaccination, respectively. After 12 months, only 44%, 53% and 97% of cattle, sheep and goats retained their neutralizing antibodies, respectively (Njenga et al., 2015).

To examine pathogenicity of Clone 13, two groups of ruminants in their first (n = 87) or second (n = 147) half of pregnancy were vaccinated. Only one goat aborted and histopathological examination of the foetus did not indicate RVFV infection (Njenga et al., 2015). Another study found that calves vaccinated with either Clone 13 or Smithburn (n = 5 for each vaccine) did not have any clinical signs of infection after vaccination or challenge (von Teichman et al., 2011). Finally, 38 ewes at various stages of pregnancy and vaccinated with Clone 13 had no abortions (Dungu et al., 2010). Still births and birth malformations were noted among ewes that were vaccinated with an overdose of Clone 13 (7 $\log_{10}$ median tissue culture infectious $dose_{50}$ [$TCID_{50}$]/ml) (Makoschey et al., 2016). However, no clinical signs were noted in young lambs vaccinated with a slightly higher overdose of Clone 13 (Makoschey et al., 2016). Daouam et al. (2014) found that while lyophilized Clone 13 vaccine was stable at 4^{o}C for one year, at room temperature (22-25^{o}C) the liquid form lost all infectiousness by 11 days and lyophilized form's dose decreased by 1.2 logs within 7 days. At 37^{o}C both the liquid and lyophilized forms of the vaccine lost all infectiousness within seven days (Daouam et al., 2014).

Inactivated Vaccines

Several different inactivated vaccines are available, including one from OBP and ZH501 from Egypt (Dungu et al., 2018). All require a booster approximately 3-4 weeks after the first injection and annually thereafter. Much less research has been conducted on the inactivated livestock vaccines than the modified-live vaccines. Barnard and Botha (1977) vaccinated 18 cattle with a variety of doses of an inactivated vaccine and all cattle produced neutralizing antibodies that persisted for at least nine months. Another study reported that only four of 10 vaccinated cattle responded with neutralizing antibodies after the initial RVFV vaccination (OBP inactivated vaccine). The five cattle that received a booster dose had neutralizing antibodies that persisted at least 12 months (Barnard, 1979). Another inactivated vaccine (not commercialized) was tested at various dilutions in cattle (30), sheep (60) and goats (20) (Yedloutschnig et al., 1979). All animals were vaccinated with either undiluted or diluted inactivated vaccine and received a booster of undiluted inactivated vaccine within 90 days. All sheep and goats and 50% of cattle that received undiluted vaccine produced neutralizing antibodies. Among the animals that received diluted vaccine, all goats, some sheep and 30% of cattle produced neutralizing antibodies. At higher dilutions no antibodies were detected (though they were detected after the booster vaccine). They challenged the sheep with RVFV 75 days following the booster vaccine and none had signs of RVF (Yedloutschnig et al., 1979).

Animal vaccination plays an important role in supporting herd immunity, yet across Africa, it is rarely used. It appears that high doses of Smithburn vaccine induce long-lasting antibodies in sheep. However, the longevity of RVFV antibodies in sheep vaccinated with a currently available commercial product has not been investigated. Despite the research presented here, South African farmers complained that RVFV various vaccines were not efficacious (Pers. Comm. C. Cordel; (FAO, 2015)). A clear gap is understanding why RVFV vaccination is not widely implemented, particularly on the large sheep farms that are at the greatest risk.

EPIDEMIOLOGICAL AND ECOLOGICAL MODELS APPLIED TO RVFV

A variety of analytical techniques and models have been used to answer questions about RVFV ecology and epidemiology (Métras et al., 2011). Here we will discuss what these models have contributed to our understanding of differences in seroprevalence, risk factor

determination, identifying ecological drivers of epizootics, predicting epizootics and understanding the underlying biological mechanisms of RVFV epidemiology and ecology.

Seroprevalence and Risk Factor Studies

A recent review by Clark et al. (2018) identified 124 studies of seroprevalence in livestock, wildlife and people from across Africa (through 2016). The statistical methods used in these studies ranged from comparing seroprevalences or risk factors with chi-square tests (Thonnon et al., 1999, Rissmann et al., 2017) to using Generalized Linear Models (Bett et al., 2018, Nanyingi et al., 2017). For more details, please see the sections above on Animal Risk Factors Associated with RVFV Seroprevalence and Human Risk Factors Associated with RVFV Seroprevalence. In the first of several spatial models discussed below, Clements et al. used a Bayesian, spatial logistic regression model to map high- and low-seroprevalence clusters across Africa (Clements et al., 2007).

Modelling RVF Outbreaks

Several studies have described RVF outbreaks using a variety of methods. In particular, the 2010-2011 outbreak in South Africa has been well characterized because during this outbreak the country made 17 reports to the OIE, providing the relative location, the number of animals affected and the number of susceptible animals (OIE, 2019). The excellent reporting by the Government of South Africa provided one of the richest datasets regarding the spread of RVF during a regional epizootic period. Métras et al. (2012) modelled the spatial movement of the epizootic in central South Africa. Xue et al. (2012) combined an SIR compartmental model with a network analysis of the 2010-2011 RVF outbreak. Additional network and metapopulation patch analyses, which primarily describe the dynamics of RVF outbreaks (Xue et al., 2013, Xiao et al., 2015). Given the importance of the movement bans during RVF outbreaks and the impact that it can have on pastoralist herders (Rich and Wanyoike, 2010), further, detailed network analyses could contribute to our understanding of the socio-economic impacts of RVF outbreaks. Métras et al. (2013) also estimated the farm-to-farm effective reproduction number of RVFV during the 2010-11 outbreak in South Africa, which is the first estimate of the reproductive number for RVFV made from outbreak data, rather than mathematical models.

Bayesian Modelling

Bayesian techniques could be very powerful in working with RVF data, as this methodology allows for the analysis of latent variables and can cope with missing data better than frequentist statistical methods, which lends itself to analysing a system that still has so many questions. Métras et al. (2017) used Bayesian models to investigate the drivers of the RVF outbreak in Mayotte. Olive et al. (2017) also used Bayesian techniques to estimate the force of infection between years based on seroprevalence levels in Madagascar. Though not associated with outbreaks, Bayesian models have also been applied to assessing the molecular diagnostic capabilities of RVFV PCR assays (Odendaal et al., 2014).

Ecological Drivers Associated with RVF Outbreaks

Spatial models that identify ecological/environmental risk factors and predict future RVF outbreaks have also been commonly used. These spatio-temporal models have identified the following environmental factors as associated with the occurrence of RVF outbreaks: livestock density (Olive et al., 2016, Mosomtai et al., 2016), sheep density (Walsh et al., 2017), wild Bovidae species richness (Walsh et al., 2017), low altitude (<500m) (Munyua et al., 2016, Hightower et al., 2012), evapotranspiration (Mosomtai et al., 2016), soil types (Munyua et al., 2016, Hightower et al., 2012, Sindato et al., 2014, Njenga and Bett, 2019), humid environments (Olive et al., 2016), livestock trade (Lancelot et al., 2017, Métras et al., 2017), proximity to intermittent wetlands (pans) (Walsh et al., 2017), dense bush (Hightower et al., 2012), and plains habitat (Hightower et al., 2012). While these factors have been linked to RVF outbreaks, some of the underlying mechanisms have yet to be explained (e.g. soil type). Additionally, these factors have not been assessed regarding their contribution to the probability that an individual farm may or may not experience a severe RVF epizootic.

Predicting RVF Outbreaks

Predicting when large RVF outbreaks might occur and mitigating them requires a mechanistic understanding of environmental and climatic drivers. In the 1980s, above normal precipitation was first associated with RVF outbreaks (Davies et al., 1985, Linthicum et al., 1987). RVF outbreaks were since associated with the occurrence of

complex weather patterns, such as El Niño (East Africa) and La Niña (southern Africa) (Anyamba et al., 2001). Anyamba et al. (2002) developed risk maps for RVF outbreaks using satellite-derived normalized difference vegetation index (NDVI). They predicted the 2006-07 RVF outbreak one month prior to the detection of the index case in the in East Africa (Anyamba et al., 2006). However, their predictions of RVF outbreaks in southern Africa were not as accurate (Anyamba et al., 2010). Anyamba et al. (2019) reported that livestock owners in Kenya, vaccinated an estimated 27% of livestock (Oyas et al., 2018) in response to their prediction of an outbreak during the 2015-2016 El Niño. They claimed that this was sufficient to prevent the outbreak they predicted (Anyamba et al., 2019). However, two years later, in 2018, a large outbreak affecting Kenya, Uganda and Rwanda occurred and was not announced as a prediction before the outbreak started (WHO Regional Office for Africa, 2018). The predictive model described above has clear limitations and requires more data on the ecological and epidemiological drivers of RVFV to incorporate into the system. In particular, herd immunity in the local ruminant populations would be important factor to be included in the predictive algorithms. If the herd immunity threshold is exceeded in a local population of ruminants, the outbreak predictions would not be as accurate because the outbreak would be prevented despite the climatic conditions. This is the reason suggested by Anyamba et al. (2019) that the 2015-2016 outbreak did not occur.

Mechanistic Models of RVFV

Compartmental models (e.g. SIR) are useful tools to better understand the mechanisms that drive viral transmission cycles and host and vector population dynamics. A compartment model involves moving individuals from one compartment to another and are often based on a discrete time-step (e.g. one day) or a continuous time model (based on a set of ordinary differential equations). In epidemiology, compartments most frequently represent health states, though they may also represent development states. The most basic example of an epidemiological compartmental model has three compartments: susceptible (S), infected (I) and recovered (R), often referred to as an SIR model (Keeling and Rohani, 2008). An exposed (E) compartment can be included if there is an incubation period between an individual becoming infected and when they become infectious (Keeling and Rohani, 2008). The model is parameterized by the rates of entry and exit of each compartment, including birth, mortality, transmission, incubation, and recovery rates as well as specific mortality rates associated with the pathogen of interest (Keeling and Rohani, 2008). Compartmental models are powerful tools and have been used to explain

cyclic or seasonal patterns in infectious diseases, the spread of epidemics, the likelihood of a pathogen persisting or going extinct over time and to evaluate potential mitigation efforts (such as vaccination or medical care; Keeling and Rohani, 2008).

Using these models and parameter rates, one can estimate the reproductive number (R_0), which represents the number of secondary cases generated by one typically infected individual in a completely susceptible population (Diekmann et al., 1990). R_0 can indicate whether a pathogen will persist (R_0>1) in the host population, or go extinct (R_0<1). As an example, the R_0value for certain common diseases are: measles: 12-18, mumps: 4-7 and pertussis: 12-17 (Fine, 1993). It is also critical to understanding herd immunity, or the protection of populations from infection which is brought about by the presence of immune individuals (Fine, 1993). The herd immunity threshold is the minimum proportion that must be immunized to drive a pathogen to extinction (Fine, 1993) and is often used to recommend population vaccination targets.

Vector-borne pathogens require a biological vector to be transmitted from one mammalian host to another. To incorporate this into a compartmental model an additional set of equations are required for the mosquitoes to also go through the SIR or SEIR process. In the 1950's, Dr. George MacDonald developed a mathematical model to describe malaria (Macdonald, 1952). He also calculated the reproductive number for vector-borne diseases, which is the square root of the sum of the reproductive number for the vector, R_v, and that for the host, R_h (Macdonald, 1952). His work had significant implications for mosquito population control and became the foundation for future compartmental modelling of vector-borne diseases, including work on Rift Valley fever virus.

In 2016, a review by Danzetta et al. (2016) identified 24 mathematical models describing RVFV and several more have been published since. Many of these models include two vector species: Aedes, which are capable of transovarial transmission and Culex, which can only transmit the virus horizontally (Leedale et al., 2016), though a few models exclude mosquitoes entirely (Métras et al., 2017) or exclude only the transovarial transmitting vectors (Mpeshe et al., 2011). Models of epidemic (Mweya et al., 2014) and inter-epidemic (Iacono et al., 2018) dynamics have been developed. However, questions remain regarding the mechanism of RVFV maintenance and the drivers of the apparent interepizootic-epizootic cycles that have not yet been sufficiently explained. There are differences in the epizootic patterns caused by RVFV in the various regions and countries

affected by the virus. However, most models have been developed to describe population level dynamics across a whole region or country (Cavalerie et al., 2015, Iacono et al., 2018, Métras et al., 2017). These models have all been designed to describe the dynamics of a particular system Thus, several models that have been able to describe a particular system in the specific location/country that was modelled (Cavalerie et al., 2015, Iacono et al., 2018, Métras et al., 2017, Cavalerie et al., 2015, Iacono et al., 2018, Chapter 4).

Temperature and precipitation play vital roles in the rate and success of mosquito development and gonotrophic activity (Rueda et al., 1990). In recognition of these important factors, more and more RVFV models are incorporating climatically forced parameters to drive the vector dynamics (Iacono et al., 2018, Leedale et al., 2016, Chitnis et al., 2013, Xue et al., 2012). By incorporating these drivers some RVFV models now have also included the stages of larval and pupal development or an aquatic stage (Mweya et al., 2014, Iacono et al., 2018, Manore and Beechler, 2015), rather than including only the adult and egg stages that many of the earlier models included (Gaff et al., 2007, Mpeshe et al., 2011). Understanding the vector dynamics are critical to understanding RVFV transmission – both during epizootic and interepizootic periods. Another model applied ordinary differential equations toward understanding vector dynamics, including population dynamics richness assessment, and risk maps (Bicout and Sabatier, 2004). The explicit models of the vector dynamics have primarily been applied to work in West Africa and has not been applied to other regions.

There still remain a number of gaps that mathematical models have not yet been used to address. These include questions regarding the mechanism of RVFV maintenance and the drivers of the apparent interepizootic-epizootic cycles that have not yet been sufficiently explained, can we describe interepidemic RVFV persistence at small scales (e.g. a single pan) and no compartmental models have been applied to all three regions where RVFV causes repeated, large outbreaks (West Africa, East Africa and South Africa).

Comparison to Compartmental Models Used to Understand the Ecology and Epidemiology of Other Arboviruses

Although there are some aspects of RVFV ecology that make the epidemiology of this arbovirus distinctive, modelling RVFV has similarities to approaches used for other vector-borne viruses. The primary similarities involve modelling the vector component, and that is what we will focus on here. All compartmental models of vector-borne diseases need to

support a mechanistic understanding of drivers of vector population dynamics, which includes temperature dependent hatching and development and the estimation of carrying capacities. The integration of climate data to drive vector population dynamics were used in malaria and dengue models before they were commonly being used in RVFV models. While the early models incorporated lifetable data (Focks et al., 1995), later models have directly integrated the climate data into the model (McLennan-Smith and Mercer, 2014, Hoshen and Morse, 2004).

Another factor that is similar across all arbovirus models is the need to cope with the fact that a recently hatched population of vectors will quickly reach population numbers in the hundreds of thousands (Linthicum et al., 1985a), whereas we are often interested in a local host population that may only have a few hundred animals or people. The two most frequently used methods of countering this extreme host-vector ratio is to limit the ratio. One such method is to assume a population level scale for the hosts such that the host-vector ratio remains around 1:1 (Lizarralde-Bejarano et al., 2017, Sardar et al., 2015). Another method that has been employed is to assume that only a portion of the vectors will feed on the target host (the rest are presumed to feed on wildlife or other non-target hosts) (Hoshen and Morse, 2004).

RIFT VALLEY FEVER IN SOUTH AFRICA

History of RVF Epizootics in South Africa

Rift Valley fever was first detected in South Africa during a large outbreak in 1950-51. There have been three widespread outbreaks in South Africa, 1950-1951 (Gear et al., 1951), 1974-1975 (Barnard and Botha, 1977) and 2008-2011 (Pienaar and Thompson, 2013). All three of these RVF outbreaks most severely affected the ruminants on the central plateau, and in the region around Bloemfontein and Bultfontein (Free State Province) and Kimberley (Northern Cape Province).

The 1950-51 outbreak was estimated to have caused 100,000 livestock deaths (Barnard and Botha, 1977) and 500,000 livestock abortions (Swanepoel and Coetzer, 2004). No estimates of the magnitude of animal RVF cases have been made for the 1974-5 outbreak, though it was reported to be far more widespread than the 1950-51 outbreak (Swanepoel and Coetzer, 2004). More than 17,000 cases of RVF in livestock were reported to the OIE between 2009-2011, with over 11,000 mortalities (OIE, 2019).

An apparent trend of increasing severity was reported in people infected with RVFV during these outbreaks. In 1950-1951, many people were reported to have had clinical symptoms of RVF and it was the first time RVFV was implicated in causing ocular disease (Gear et al., 1951); however, no mortalities were reported. In 1975 the first human mortalities attributed to RVF were reported (five deaths) and the first occurrence of RVF manifesting as encephalitis and haemorrhagic disease was described (Van Velden et al., 1977). During the 2008-2011 outbreaks and across South Africa, there were 302 cases of laboratory confirmed cases of RVF, which resulted in 25 deaths (Archer et al., 2013). In South Africa researchers have determined that multiple RVF outbreaks were caused by different strains of RVFV, one only ever detected in South Africa, one detected in East Africa and one previously detected in Namibia (Grobbelaar et al., 2011).

In addition to these three widespread outbreaks, many much smaller outbreaks of RVF have been detected across South Africa (Pienaar and Thompson, 2013). Many of these only affected one farm or a small cluster of farms and isolated outbreaks have occurred in all provinces of South Africa. The most recent RVF outbreak occurred in April and May of 2018 in Jacobsdal, Free State (within the study area described below) (OIE, 2018d). The farmer was expecting 360 ewes to lamb and they were synchronized to lamb in cohorts of 120-150 ewes each week. Of the three cohorts of lambs expected, only 20 lambs survived the abortions and mortality caused by RVF (Pers. Comm., Dr. C. Cordel). While the sheep were severely affected by the outbreak, the feedlot cattle that were also present on the farm did not show any clinical signs. This epizootic only affected the single farm on which the outbreak occurred.

Previous research conducted in South Africa

Much of our understanding about RVFV ecology comes from studies carried out in South Africa, including studies on the clinical course of RVF and RVFV epidemiology in people (Van Velden et al., 1977, Archer et al., 2013, Jansen van Vuren et al., 2018), RVFV vector ecology (Gargan et al., 1988, McIntosh et al., 1983) and transmission rates (McIntosh et al., 1973, Jupp and Cornel, 1988), RVFV vaccine production and development (Barnard, 1979, Barnard and Botha, 1977), diagnostic testing (Paweska et al., 2003, Paweska et al., 2005a, Paweska et al., 2005b, van Vuren et al., 2007), RVFV phylogenetics (Grobbelaar et al., 2011, Maluleke et al., 2019, Jansen van Vuren et al., 2019), seeking an elusive RVFV reservoir in wildlife (Gear et al., 1951, McIntosh, 1961, Davis, 1957) and outbreak

investigations (Gear et al., 1951, Archer et al., 2011). Despite these comprehensive investigations, little work has been done to understand RVFV field epidemiology in the domestic ruminant hosts, specifically on interepizootic transmission and herd immunity.

Two interepizootic serosurveys have been conducted in KwaZulu-Natal (KZN) (Kokernot et al., 1961, van den Bergh et al., 2019) and this Ph.D. contributes to two surveys carried out on the central plateau of South Africa in 2015 (Ngoshe et al., submitted) and 2017 (Chapter 2). In the 1961 study in KZN, a serosurvey in cattle, sheep and goats estimated seroprevalences of 3% (n = 109) in sheep, 11% (n = 104) in goats and 10% (n = 166) in cattle from across mountain, riverine pan and coastal plain ecosystems (Kokernot et al., 1961). In 2016, another serosurvey was conducted in the same region – primarily in the riverine pan ecosystem. This study found the seroprevalence to be 34% in cattle (n = 423) and 31% in goats (n = 104) (van den Bergh et al., 2019); none of the owners reported that they vaccinated these animals against RVFV. The region of KZN in which these two studies were conducted has never reported an outbreak of RVF. It is also the same region where RVFV was isolated out of field caught mosquitoes outside of an outbreak period (Smithburn and De Meillon, 1957), indicating that RVFV is circulating in this region. The findings in KZN indicate that RVFV can circulate in ruminant populations without causing large-scale outbreaks and will be further discussed in Chapter 2. Within our study area (described below), we previously detected a higher seroprevalence in cattle and sheep (Ngoshe et al., submitted). The seroprevalence (adjusted for clustering and herd size) was 42.9% in cattle, 28.0% in sheep and 9.3% in goats (Ngoshe et al., submitted); these data include animals that have been vaccinated.

Prior to the study presented in this thesis, the only other seroconversion incidence study was carried out in KZN in 2016-2018 and reported a seroconversion incidence of 0.59 per animal-year in cattle and 0.41 per animal-year in goats (van den Bergh et al., 2019). No studies have yet reported seroconversion incidence in sheep.

Differences in the ecology of Rift Valley fever virus between South Africa and East Africa

Though Rift Valley fever occurs across Africa there seems to be three primary foci that have had repeated, large, widespread outbreaks, including Mauritania and Senegal in West Africa, Kenya and Tanzania in East Africa and South Africa (Nanyingi et al., 2015). While there are some similarities amongst these regions, e.g. they all have regions with pans

(called ponds and dambos in West and East Africa, respectively (Linthicum et al., 1985b, Soti et al., 2013)), there are some distinct ecological differences. Largescale RVF outbreaks seem to happen more frequently in East Africa (~10 years) as compared to South Africa (~24-35 years) (Nanyingi et al., 2015). Here we will specifically discuss the ecological factors that differ between central South Africa and Garissa District in northeast Kenya, which was reported as the epicentre of the 1996-97, 2006-07 and 2018 RVF outbreaks (Murithi et al., 2011, OIE, 2018c).

Though both Garissa and the central plateau of South Africa (represented by Bloemfontein) have arid climates, Garissa receives an average of 374 mm of annual precipitation (Weather Atlas, 2019), which is similar to the mean in our study area, though portions of our study area receive twice that amount annually (Anyamba, unpublished data). The monthly high temperature in Garissa ranges from 33.6-38.6^{o}C and the low temperatures range from 21.2-24.5^{o}C, whereas Bloemfontein has a temperate climate and the monthly high and low air temperatures vary between 17.4-30.8^{o}C and -1.9-15.3^{o}C during the year, respectively (Weather Atlas, 2019). Thus, Bloemfontein has two climatic factors that limit the mosquito populations – a dry and cold season, whereas in Garissa mosquitoes only need to survive the dry season. Logan et al. (1992) found that *Aedes mcintoshi* eggs that were gathered from the soil survived better when maintained in the lab at 24^{o}C than at 15^{o}C; however, there may be some as of yet unknown factors in the soil that permit the eggs to survive at lower temperatures or general conditioning may be required (Horsfall and Trpiš, 1967) to allow the eggs to successfully hatch despite the lower temperature.

Another significant difference is the herd structure and composition. In Garissa, goats were the most common farmed species, followed by cattle and sheep and average herd sizes were 75, 31 and 25 respectively (Mwanyumba et al., 2015). Whereas in our study area 75% of farms had cattle, 71% had sheep and 26% had goats and average herd sizes were 257, 1,083 and 78 respectively (Chapter 2). It is likely that breeding schedules are also different as many farmers in the study area use a synchronized breeding management system (Pers. Comm. Dr. C. Cordel). Finally, farmers in South Africa frequently use a RVFV vaccine product to protect their sheep (Chapter 3). All of these factors could impact the ecology and epidemiology of the virus in the two regions.

THE UNDERSTANDING RIFT VALLEY FEVER IN THE REPUBLIC OF SOUTH AFRICA PROJECT

This Ph.D. was completed within the framework of a larger project, the Understanding Rift Valley Fever in the Republic of South Africa Project (URVFRSAP). The core research questions the project sought to answer were conceived of during the grant application process in 2013. I am a co-PI on this project and I contributed to the conception of the research, drafted the application and assisted with the submission of the grant application. The grant was awarded by the U.S. Defense Threats Reduction Agency in 2014 to a collaboration led by EcoHealth Alliance (my employer) with the National Institute for Communicable Diseases (South Africa), the Universities Space Research Association/National Aeronautics and Space Administration, the University of Pretoria, South African National Parks (SANParks), the Department of Economic Development, Tourism and Environmental Affairs of the Free State Province and the Republic of South Africa Defence Department. The following research questions were the primary focus of the project:

- What is the current herd immunity status of free-ranging wildlife, farmed wildlife and domestic ruminants in the study area and how does it change with time?
- How does immunity (antibody levels) against RVFV change over time in individual domestic ruminants and how may herd immunity be altered by management approaches (e.g. vaccination)?
- How does mosquito abundance relate to environmental factors in South Africa, what are the factors affecting mosquito succession and what percentage of captured mosquitoes are infected with RVFV?
- What is the current seroprevalence of RVFV antibodies in people in high-risk occupations and is interepidemic infection occurring in people?

As co-PI for the project I managed the execution of the work and the collaboration of the One Health team of experts working on the project. This included veterinarians, entomologists, virologists, geographers, soil and climate scientists, vegetation ecologists, epidemiologists and theoretical ecologists. With the support of EcoHealth Alliance and my collaborators, I applied to and was accepted into the Ph.D. program at the University of Glasgow in 2015. My thesis questions were framed to contribute to the success of the URVFRSA project as well as to the field of Rift Valley fever epidemiology. As it is

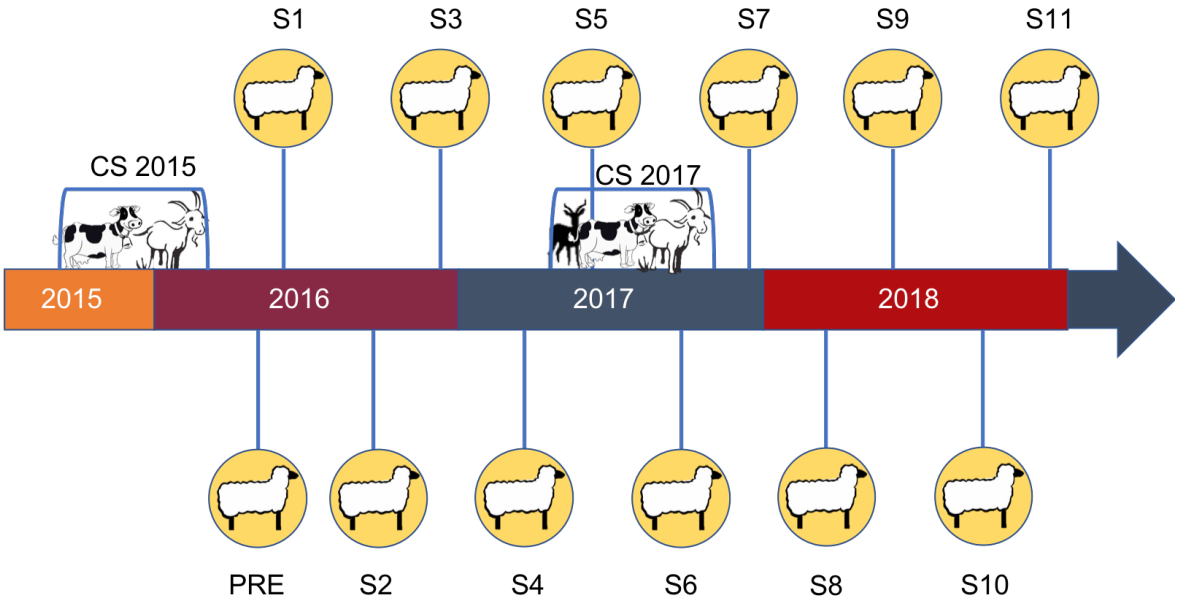


Figure 1-1 Timeline of sampling activities for the herd immunity studies conducted in South Africa. Two cross-sectional (CS) studies were conducted, one from October 2015-February 2016 and the other from May-November 2017. During the 2015 cross-sectional study, sheep, cattle, and goats were sampled. During the 2017 cross-sectional study, we sampled the same sheep, cattle and goats as we had previously, where possible, in addition to nine previously unsampled animals. The longitudinal sheep study started in May 2016. The sheep in the longitudinal study were first sampled to confirm they were seronegative during the sampling point marked "PRE" on the timeline. The duration between PRE and S1 was one month, the interval between the rest of the S sampling periods (S1-S11) was three months. The last set of samples were collected in December 2018.

integrated within a large, collaborative study, I have elaborated on my role in each aspect of the research related to my Ph.D. in Table 1-3. A timeline of the ruminant studies detailed in Chapters 2 and 3 is given in Figure 1-1. As this study had so many components and collaborators, and to prepare for publishing the chapters in the peer-reviewed literature, I will be using the term "we" going forward. In order to distinguish what I specifically did with respect to this thesis please refer to Table 1-3.

The Study Area

The study area is a 200 x 200 Km^2 region stretching roughly from Bloemfontein (Free State Province, South Africa) to Mokala National Park (Northern Cape Province, South Africa) and stretching 100 Km north and south of those points (see Figure 1-2). There are two major rivers that flow into the study area, the Orange River in the southwest corner and the Vaal River in the northwest corner.

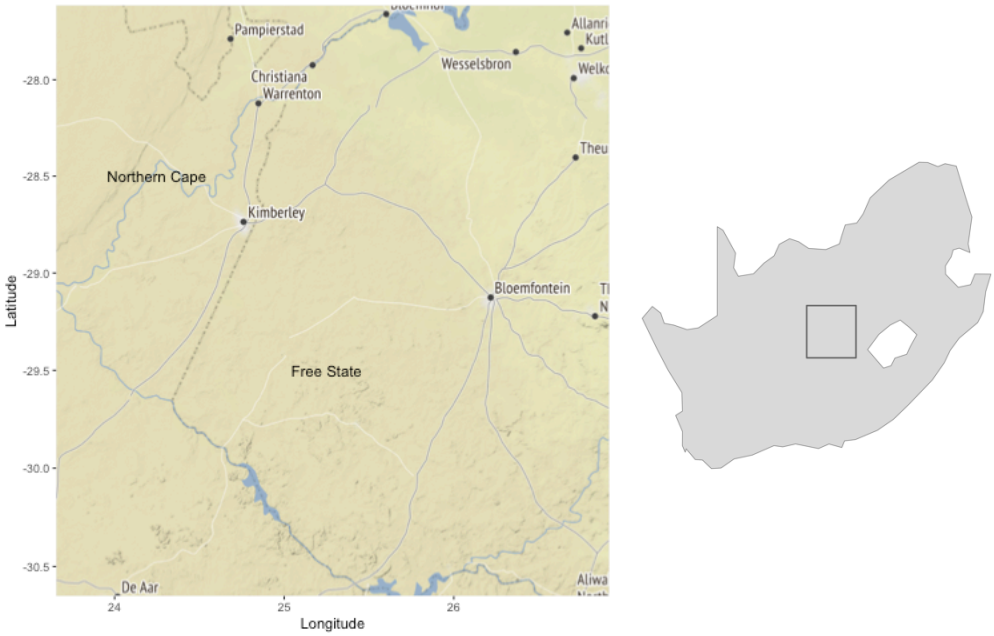


Figure 1-2 The study area is situated in central South Africa, encompassing part of the Northern Cape and Free State Provinces.

The land is relatively flat with many shallow depressions, or pans, that collect water on a seasonal basis. The pans have very little drainage and water usually remains in the pan until it evaporates. The region is at an altitude of ~1,200-1,400 m above sea level (Geldenhuys, 1982). Annual rainfall ranges across the study area from approximately 213.6-603.6 mm with a mean of 385.9 mm (precipitation data from 1983-2018; Anyamba, unpublished data). The driest month of the year is July, when the monthly mean precipitation is 2.3 mm, whereas in January the mean precipitation is 66 mm (Anyamba, unpublished data). The average land surface temperature in January is 40.6ºC and in June, it is 0.04ºC (Anyamba, unpublished data).

This region is characterised by "panveld," which is a South African term for plains that have shallow depressions that fill with water during the rainy season creating a temporary wetland before they dry out via evaporation (Geldenhuys, 1982) (in other regions these are called playas (Briere, 2000) and in East Africa they are called dambos (Linthicum et al., 1985b)). These shallow depressions will be referred to as pans in the rest of this text.

Geldenhuys estimated the number of pans and the area of these pans in the western Free State. While this area does not cover the entire extent of the study area, within the region of our study area, he estimated that there were 174 pans, which have a cumulative area of approximately 501 Km^2 of the area out of the full region that he assessed (17,537 Km^2) (Geldenhuys, 1982).

The study area is well known for its extensive sheep farming, with 31% of the country's commercial sheep farms in the Northern Cape and 23% in the Free State (Cloete and Olivier, 2010). While the dominant agricultural sector in the southern portion of the study area is sheep farming, the northwest and central-east portions of the study area have more cattle farming and in the northeast grains are the predominant sector (Cloete and Olivier, 2010).

THE GAPS THAT WILL BE ADDRESSED BY THIS THESIS

This thesis was designed to address some of the gaps identified in the literature review presented in this chapter and to specifically answer the 1st (relative to the livestock) and 2nd research questions asked by the URVFRSAP, which will be addressed in Chapters 2 and 3.

The understanding of where RVFV is between outbreaks and what is the impact of interepizootic transmission, remains limited. We hypothesize that interepizootic transmission is occurring at a low level and that we can detect it in sheep. Related to this hypothesis, we ask the questions: How does herd immunity in sheep and domestic ruminants change over time? More specifically, is herd immunity, represented by population seroprevalence, increasing, decreasing or remaining stable, when we exclude the vaccinated animals? Is the incidence rate we observe in sheep sufficient to maintain herd immunity at a stable level that matches the estimated seroprevalence or possibly sufficient to increase herd immunity to reach the herd immunity threshold? To answer these questions, we will evaluate changes in herd immunity and seropositivity in individuals using seropositivity as an indicator of immunity. We will use the results of a repeated cross-sectional and a longitudinal study (Chapter 2) to describe the incidence of seroconversion in sheep naturally infected with RVFV during an interepizootic period from October 2015-December 2018. We will also examine how the population-level seroprevalence changed in sheep, cattle and goats between 2015 and 2017.

To further understand the drivers of herd immunity, we sought to test the hypothesis that RVFV antibodies will eventually wane in sheep following vaccination by answering the question how long do antibodies persist following RVFV vaccination with the commercial Smithburn vaccine? We also asked what factors predict whether a farmer will choose to vaccinate their animals? To do this we used the same cross-sectional data described above and included a longitudinal study of vaccinated sheep (Chapter 3). The key to this study is that we will use the same strain of vaccine as the farmers are using by administering a commercially available vaccine as opposed to the previous studies that used different strains with higher viral titres. These data will be used by farmers and modelers alike to mitigate the effect of the next RVF outbreak.

While a number of mathematical models have been developed to describe RVFV dynamics, none have considered the unique ecosystem of central South Africa. In order to examine the biological mechanisms of RVFV persistence in our South African study area, we developed an SEIR model. The model was developed to test the hypothesis that RVFV can persist in a system where the proposed transovarial vectors have a univoltine lifecycle and where both transovarial and horizontal vector population dynamics are forced by climate data in a temperate ecosystem. To test the hypothesis, RVFV can persist in a single pan system and produce dynamic patterns of infection that are representative of the natural conditions we have observed. To do this we asked whether a simulation can reproduce natural conditions of low-level RVFV transmission resulting in a relatively low population seroprevalence while persisting the in host-vector system? We also ask to what model parameters is persistence is most sensitive? Do we see a pattern of a long, apparent, interepizootic period with a large epizootic that approximates the actual timeline of the 2010 RVF outbreak in the study area? And what is the reproduction number for interepizootic transmission of RVFV? To do this I developed a two vector, one host SEIR model of RVFV (Chapter 4) with the aim that the results can also support the herd immunity analysis conducted in Chapter 2.

Finally, while a significant amount of literature seeks to explain and predict large-scale epizootics, very little research has investigated factors that contribute to the occurrence and severity of an epizootic on a single farm. We hypothesized the factors that drive an RVF epizootic on an individual farm will be related to imports and environmental factors that are believed to be associated with endemic transmission of RVFV. We further hypothesize that the severity of the outbreak is driven by herd susceptibility and environmental factors previously associated with RVF epizootics. To do this we asked what factors were

important in estimating the probability that RVF occurred on the farm and further what factors contribute to the severity of the RVF outbreak as measured by the number of deaths and abortions. We built a Bayesian multinomial-hurdle model to estimate the effect size of the parameters selected to be part of the model (Chapter 5). This allowed us to assess the contribution of farm-level factors that have never been included in epizootic RVF prediction maps.

In Chapter 6, we will summarise our results and interpret them with respect to each other and the literature. We also identify potential limitations and directions for future research.

While the current state of research provides evidence for understanding the conditions under which widespread epizootics occur, many fewer studies have investigated the impact of interepizootic transmission and the biological mechanisms that support it. Together with the results of our vaccination study we will improve the understanding of RVFV herd immunity, which can be used to support the prediction of RVFV outbreaks and be used directly by veterinarians and farmers. Our results will also provide evidence to identify factors that put farms at higher risk of RVF epizootics and more severe clinical disease during the epizootic.

Table 1-3 Activities that relate both to the completion of the URVFRSAP and my Ph.D. thesis along with the roles of the people who participated in that activity. The lead researcher is the person or people that developed the concept or actually managed the work that was completed. The people listed as supporting the lead research in concept development contributed significantly to the development of the concept, study design, analytical planning and supported the lead researcher(s). The people listed as implementing the work were the people that actually carried out the field, laboratory or statistical work for the activity listed.

Activity	Lead researcher(s)	Support lead researcher(s) in concept development	Implemented the work
Development of project concept	W.B. Karesh, P. Hosseini, **M.K. Rostal**, J.T. Paweska	Key Personnel listed on the grant proposal	N/A
Write the first draft of the grant proposal	W.B. Karesh, P. Hosseini, **M.K. Rostal**, J.T. Paweska	Key Personnel listed on the grant proposal	**M.K. Rostal**, P Hosseini
Manage the submission of the grant applications	W.B. Karesh, **M.K. Rostal**		W.B. Karesh, **M.K. Rostal**, C. Machalaba
Manage the implementation of the entire project	W.B. Karesh, **M.K. Rostal**, J.T. Paweska	9 work package leaders	**M.K. Rostal** (manager)
Develop questionnaires for *farm* surveys for both the cross-sectional study and longitudinal study	**M.K. Rostal**		**M.K. Rostal**, V. Msimang, C. Cordel, C. Machalaba, University of Pretoria students
Manage animal (3) and human (5) ethical applications and approvals	**M.K. Rostal**, V. Msimang	W.B. Karesh, J.T. Paweska	**M.K. Rostal**, V. Msimang
2015 Livestock cross-sectional surveys and sample collection *PhD program started at the same time as this cross-sectional study	**M.K. Rostal**, C. Cordel	P. Thompson	**M.K. Rostal** (collected samples for the first 5 weeks of field work), C. Cordel, One Health field team, V. Msimang
2015 Livestock cross-sectional diagnostic testing	J.T. Paweska		P. Jansen van Vuren, NICD staff, UP students
2017 Livestock cross-sectional surveys and sample collection (Chapters 2, 3 and 5)	**M.K. Rostal**, C. Cordel		**M.K. Rostal** (collected samples for a few weeks), C. Cordel, One Health field team, V. Msimang
2017 Livestock cross-sectional diagnostic testing (Chapters 2 and 3)	J.T. Paweska		P. Jansen van Vuren, NICD staff
2016-2019 Longitudinal study surveys and sample collection (Chapters 2 and 3)	**M.K. Rostal**, C. Cordel	J.T. Paweska, P. Jansen van Vuren, S. Cleaveland	**M.K. Rostal** (collected samples during 3/12 events), C. Cordel, J.C. Bester, L. van Staden
2016-2019 Longitudinal study diagnostic testing (Chapters 2 and 3)	J.T. Paweska		P. Jansen van Vuren, NICD staff, University of Pretoria student
2016-2017 Wildlife cross-sectional sample collection	**M.K. Rostal**, C. Cordel		C. Cordel, ExecuVet staff
2016-2017 Wildlife cross-sectional diagnostic testing	J.T. Paweska		P. Jansen van Vuren
Data cleaning: 2015 and 2017 livestock cross-sectional studies and longitudinal study data (Chapters 2, 3, 4 and 5)	**M.K. Rostal**		**M.K. Rostal,** W. Leo (2017 field data only) and W. Bagge (2017 field data only)
Data cleaning: wildlife cross-sectional studies (Chapter 2)	**M.K. Rostal**		**M.K. Rostal**, W. Leo
2015 Livestock cross-sectional study data analysis	P. Thompson		University of Pretoria students

Activity	Lead researcher(s)	Support lead researcher(s) in concept development	Implemented the work
2017 Livestock cross-sectional study data analysis (Chapters 2 and 3)	**M.K. Rostal**		**M.K. Rostal**
Longitudinal study data analysis (Chapters 2 and 3)	**M.K. Rostal**	S. Cleaveland, D. Hayden, N. Ross, P. Thompson	**M.K. Rostal**
Development of follow up questionnaire for 2010-2011 outbreak impacts (Chapter 5)	**M.K. Rostal**	D. Hayden, M. Viana, N. Ross, C. Cordel, S. Cleaveland,	**M.K. Rostal**, C. Cordel, ExecuVet Staff
Implement the follow-up questionnaire for the 2010-2011 outbreak impact (Chapter 5)	C. Cordel	**M.K. Rostal**	C. Cordel, L. van Staden
Development of Bayesian model formulation (Chapter 5)	**M.K. Rostal**	N. Ross, D. Hayden, M. Viana	**M.K. Rostal**
Writing the code for Bayesian model in R and Stan (Chapter 5)	N. Ross	M. Viana	N. Ross, M. Viana
Analysing the results of the Bayesian model (Chapter 5)	**M.K. Rostal**	N. Ross, D. Hayden, M. Viana	**M.K. Rostal**
Developing, writing and analysing SEIR model for RVFV (Chapter 4)	**M.K. Rostal**	L. Matthews, J. Prentice, N. Ross, D. Hayden,	**M.K. Rostal**
Conducting R_0 analysis of SEIR model (Chapter 4)	J. Prentice L. Matthews		J. Prentice L. Matthews
Writing thesis chapters	**M.K. Rostal**	S. Cleaveland, L. Matthews, N. Ross, D. Hayden, M. Viana, J. Prentice	**M.K. Rostal**

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Chapter 2: Rift Valley Fever Herd Immunity: Rift Valley Fever Virus Incidence and Seroprevalence in Naturally-Infected Sheep

ABSTRACT

The epidemiology of Rift Valley fever virus (RVFV), a zoonotic arbovirus, is characterized by devastating outbreaks in ruminant livestock populations followed by long interepizootic periods that can span decades. Outbreaks are likely driven by a combination of climate conditions conducive to mosquitoes and low herd immunity in livestock. To better understand RVFV herd immunity in domestic ruminants in central South Africa we investigated changes in population seroprevalence over time in cattle, sheep and goats and the incidence of RVFV seroconversion in unvaccinated sheep. A repeated cross-sectional survey in cattle, sheep and goats was conducted in 2015 and 2017 to assess the seroprevalence in a farmed population, in which routine RVFV vaccination occurred on some farms. Additionally, a 2.5-year longitudinal study was conducted to examine rates of seroconversion in a panel of unvaccinated sheep. Using Generalized Linear Models, the mean incidence of natural RVFV infection in sheep was estimated to be 2.1 (95% CI: 1.0-3.8) and 1.8 (95% CI: 0.3-5.7) infections per 100 animal-years, for the longitudinal and repeated cross-sectional studies, respectively. The estimated herd immunity was 28.2% (95% CI: 21.5-35.6%) in all domestic ruminants assayed in 2017. This estimate represented the seroprevalence at the end of the study, and was adjusted for herd size and sampling design. It included vaccinated (17%; 95% CI: 15-18), unvaccinated animals (40%; 95% CI: 38-42%) and animals of unknown vaccine status (43%; 95% CI: 42-45). These results indicate that the domestic ruminant population in the study area was unlikely to have sufficient herd immunity to prevent an outbreak of RVF should the climate conditions be appropriate. Additionally, the low-level of RVFV transmission detected among sheep is unlikely to contribute significantly to herd immunity.

INTRODUCTION

Rift Valley fever (RVF) is caused by a zoonotic arbovirus and manifests as abortion storms in pregnant livestock with high mortality in very young animals (Swanepoel and Coetzer, 2004). Outbreaks have been reported throughout much of Africa and RVF virus (RVFV) has spread to the Arabian Peninsula, Madagascar and other islands off the east coast of Africa (Woods et al., 2002, Ksiazek et al., 1989, Balkhy et al., 2003, Morvan et al., 1991, Balenghien et al., 2013). The central plateau of South Africa has had three massive outbreaks of RVF in 1950-51, 1974-75, and 2010-2011. The 2010 outbreak resulted in at least 12,638 RVF cases in ruminants (OIE, 2019). Sheep are the most susceptible to RVF, followed by cattle and goats as well as some ruminant wildlife species (Swanepoel and Coetzer, 2004). Outbreaks may be isolated to one farm or may be widespread, impacting an entire region or country (Pienaar and Thompson, 2013). Interepizootic periods with few reported RVFV cases may last from two to over 30 years (Nanyingi et al., 2015). Though clinical RVF is seldom reported between outbreaks, evidence of ongoing low-level transmission has been detected through seroconversions in most places that have suffered recurrent RVF outbreaks (Rostal et al., 2010, Sumaye et al., 2013).

A significant body of work has been dedicated to predicting sporadic RVF outbreaks (Anyamba et al., 2006, Munyua et al., 2016). In particular, some success at predicting outbreaks has been made by monitoring precipitation, normalized vegetation difference index (NDVI), and the El Niño Southern Oscillation (or La Niña). Using these variables, researchers predicted the expansive 2006-07 RVF outbreak in East Africa approximately two months prior to the outbreak (Anyamba et al., 2006). However, the predictions are not always accurate in western and southern Africa. Even in East Africa, a predicted outbreak did not occur in 2015 (which may have been due to vaccination following the warning) and an unpredicted outbreak in 2018 occurred and spread to three countries (Anyamba et al., 2010, Anyamba et al., 2019, OIE, 2018a). Herd immunity, assessed by serosurveillance, has been identified as an important factor that may improve future predictive models (Anyamba et al., 2010). Herd immunity may be induced in domestic ruminants through vaccination or natural infection due to an outbreak or interepizootic transmission.

Here we investigated RVFV herd immunity within a region of South Africa that was impacted by three large outbreaks in 1950-51, 1974-75 and 2010-11 (Pienaar and Thompson, 2013). To determine the contribution of interepizootic transmission to livestock herd immunity, we tracked population-level changes in the seroprevalence in sheep, cattle

and goats over two years and determined the incidence of seroconversion following natural RVFV infection in sheep.

METHODS

Study Area

The study was conducted in a 200 x 200 Km area encompassing part of the Free State and Northern Cape Provinces in South Africa (see Figure 2-1). The study area was bounded by the following geographic positioning coordinates: -28.20, 24.20; -30.20, 24.20; -28.20, 26.40; -30.20, 26.40 (WGS84 decimal degrees). The study area includes regions of three different biomes, Nama-Karoo, Savanna and Grassland (Ngoshe et al., submitted). The climate is semi-arid with annual rainfall varying from 213-603 mm and mean land surface temperatures ranging from 15-52^{o}C (Anyamba, unpublished data). The coldest months are June-August. During the study, most years had lower than average rainfall from June through December (Anyamba, unpublished data). The landscape is characterised by shallow depressions, called pans, that undergo seasonal flooding during the rainy season. Wild antelope roam freely across many livestock farms and are also found on game farms within the study area. Some farms in the study kept and managed both wild and domestic ruminants. The locations of the farms sampled during the repeated cross-sectional (2015

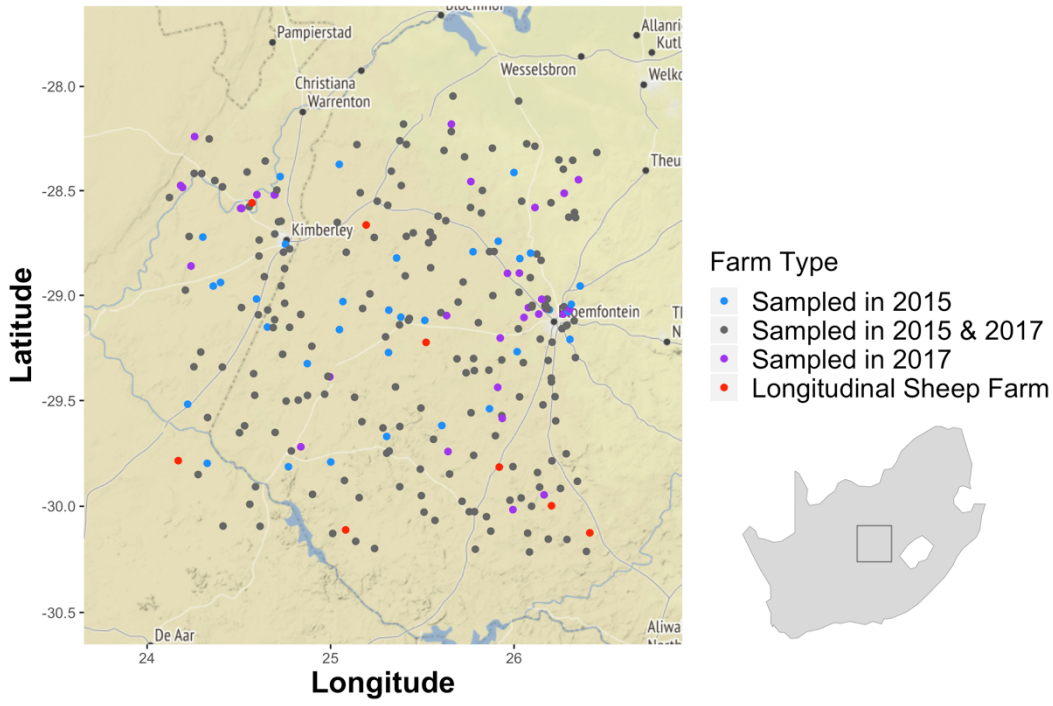


Figure 2-1 A map of farms sampled as part of the cross-sectional studies (either sampled in 2015 [blue], 2017 [purple] or both 2015 and 2017 [black]) or the longitudinal study (red).

and 2017) and a longitudinal study are shown in Figure 2-1 (see timeline in Figure 1-3, Chapter 1).

Ethical approval

Animal ethical approval for the cross-sectional and longitudinal studies was obtained from the University of Pretoria Animal Ethics Committee (t005-16, V013-15, V090-15), Tufts University Institutional Animal Care and Use Committee (G2014-03 and G2016-148), the U.S. Army Medical Research and Material Command Animal Care and Use Review Office (CT-2014-33) and for the longitudinal study the University of Glasgow (Ref 62a/15) (Appendix 1). Permission to conduct research on Rift Valley fever virus in livestock was obtained by DAFF (12/11/1/1/13) (see Appendix 2). Human subjects research approval was obtained for the questionnaires from HummingbirdIRB (2014-25), the University of Witwatersrand (M140306), Northern Cape Provincial Health Research Ethics Committee (NC2015/001), Free State Provincial Department of Health (04/04/2015) and the U.S. Army Medical Research and Materiel Command, Office of Research Protections, Human Research Protection Office (A-20745.1a and A-20745.1b) (Appendix 3). Given the extensive human ethics approvals that were already in place and that the author was specifically named on the protocols, the human ethics committee of the College of Medical, Veterinary and Life Sciences, University of Glasgow waived the requirement of review.

Cross-sectional study

Two cross-sectional serosurveys were conducted among cattle, sheep and goats. The first was carried out in 2015 from October 2015-February 2016, and the second in May-November 2017. For the first cross-sectional study, 351 points were randomly generated based on weights corresponding to the number of livestock-owning households within each census enumeration area (EA; range 0-173 LOHHs; [Lehohla, 2013]). For each EA (large city-centres were excluded), a Poisson distribution with a mean proportional to the number of livestock-owning households (K. Parry, Statistics South Africa, 2014, pers. comm. to P. Thompson) was used to determine the number of points to be randomly generated. The means were scaled to ensure the total number of points within all EAs would be 351. This was done using the “rpoisson” function in Stata 15 (StataCorp, College Station, TX, U.S.A.). The geographic points were generated randomly using the “Create Random Points” command in ArcGIS 10.2 (ESRI, Redlands, CA, U.S.A.). Once the points were

generated, they were then randomly assigned a position on a list. The first 180 points were visited based on their geographic vicinity, the remaining points required to obtain our sample size (235 farms) were visited in the order they appeared on the list. Using a map of the points and when possible, state veterinarians provided the name of the owner who we contacted and invited to join the study. More frequently, we drove to the point, then drove concentrically from that point to identify and invite the nearest livestock-owning household to participate. Participating farmers provided informed consent (Appendix 4) to complete the questionnaire and allow us to collect blood samples from their ruminant livestock.

Up to nine sheep, cattle and goats were sampled (haphazard/convenience sampling) on each farm. The precision of the 2017 crude seroprevalence estimate was calculated by species using the sample size, the number of farms (clusters) on which that species was sampled, and the intra-cluster correlation coefficient (ICC) that was estimated per species based on the 2015 serosurvey ($\rho = 0.26$, $\rho = 0.19$, and $\rho = 0.29$ respectively for cattle, sheep and goats; Ngoshe et al., submitted; Bennett et al., 1991). Farm owners/managers were administered a questionnaire (see Appendix 5) to collect information on the number of animals they owned (including wildlife), their husbandry practices (including vaccination) and biosecurity practices.

For each animal sampled, farmers were asked about its RVFV vaccination status, who administered the RVFV vaccine (if vaccinated) and whether the animal was born on the farm. As records of individual vaccination did not exist on most farms, the farmers provided the last year in which they vaccinated their animals for RVFV. To increase the certainty that we identified unvaccinated sheep for the incidence studies, all animals that were old enough to be alive at that time were considered to be vaccinated. All sheep were ear tagged with a study ID number during the 2015 cross-sectional study, to ensure we could include them in the longitudinal study if they met the enrolment criteria (see below). If the owner reported the sheep were not vaccinated against RVFV, we sampled up to 41 additional sheep to identify farms with naturally seropositive sheep for the longitudinal study. Sheep owners in 2015 were requested to not sell any sheep that we ear tagged for six months, in case we invited them to enrol in the longitudinal study.

For the second cross-sectional study (2017), we revisited the same farms and resampled all of the same cattle, sheep and/or goats where possible. We resampled all sheep that were available from among those that we ear tagged in 2015. For goats and cattle, if the owner

had their own permanent unique identifiers (ear tags) on their livestock we were able to resample the same animals; however, most animals did not have individual identification. We also sampled an additional nine animals that we had not previously sampled. We included these additional nine animals so that we could still estimate the seroprevalence for the population in the study area to compare to 2015. To prevent bias in the comparison between sample years, we excluded all animals resampled in 2017. If a farmer declined to participate during the repeat visit in 2017, we then recruited the nearest farm to the next geographic point on the list produced in 2015. For the cross-sectional sheep that were sampled in both 2015 and 2017 the incidence was calculated as described for the longitudinal study (below) with the exception that the estimated date of seroconversion was randomly selected between the two time points, instead of using the mid-point.

Longitudinal Study

The longitudinal study was conducted from May 2016-December 2018 (see timeline in Figure 1-3; Chapter 1). The longitudinal study involved all eight farms identified on the basis of preliminary laboratory results of the cross-sectional study. The enrolment criteria were: 1) seropositive sheep were identified on the farm; 2) these sheep were born on the farm; and 3) the farmer never vaccinated the sheep before. The sample size for the incidence study indicated we needed 18 sheep per farm to precisely estimate the annual incidence rate based on a presumed interepizootic incidence (λ_a) of at least 7% per year, power of 90 and alpha of 0.05 (Lwanga and Lemeshow, 1991). Thus, we requested to enrol 20 sheep per farm (with a total of 40 sheep sampled on each of the farms). The additional 20 sheep were included in a second longitudinal study in vaccinated sheep that was conducted simultaneously, Chapter 3). Not all farmers permitted us to enrol 40 sheep, therefore we increased the sample size (to maintain the same total number of animals per study (160) on farms that permitted it and initially enrolled between 19-92 sheep per farm (for both studies). All farmers were asked to complete an abbreviated version of the cross-sectional study questionnaire at every sheep sampling event (every three months) (Appendix 6), including March 2019, which was included in the descriptive analysis; however, no samples from this event were tested.

Given the large flock sizes (173-6,040 sheep), nearly all farmers restricted the selection of sheep for the study to a subset of their animals. On most farms this subset was run through a chute system and every other animal that came out of the chute was allocated into the unvaccinated group (animals were also allocated at this time to receive a vaccination, see

Chapter 3). If a farmer did not have a chute system for their sheep, animals were captured haphazardly and every other animal was allocated to the unvaccinated group. At the initial enrolment, all sheep had a blood sample taken, were ear tagged (if they did not already have ear tags in place) and were injected with 1 mL of saline (as a control for the sheep allocated to the vaccinated group; see Chapter 3). After 30 days, the sheep were sampled again (in order to confirm seroconversion in the sheep allocated to the vaccination group; Chapter 3) and after that, blood samples were collected via jugular venepuncture every three months. Sheep that were absent for two or more sampling periods were considered to have died. These sheep were then replaced in the same group (through August 2018) by a lamb (older than six months) that had been born on the farm and never vaccinated. These criteria increased the likelihood that the animal was seronegative at enrolment. As the study continued and where the farmers permitted it, we enrolled more sheep on the farms that initially did not permit us to enrol 40 sheep to reach a target of 40 animals per farm. Throughout the longitudinal study, sheep were owned and managed by the farm owner following the farmer's normal practices.

Box 2-1. Definitions used in this chapter.

Seropositive: An animal that tested positive on an inhibition ELISA.
Seronegative: An animal that tested negative on an inhibition ELISA.
Seroconversion: Seroconversion was reported if a previously seronegative animal tested positive on two consecutive inhibition ELISAs. It was considered to have seroconverted prior to the first consecutive positive result.
Farm enrolment criteria:
1) Seropositive sheep were identified on the farm;
2) These sheep were born on the farm; and
3) The farmer never vaccinated his sheep before.
Crude seroprevalence: The seroprevalence as determined without adjusting for herd size, clustering or diagnostic test.
Adjusted seroprevalence: The estimated seroprevalence adjusted for clustering and herd size.
True seroprevalence: The estimated seroprevalence adjusted to incorporate the sensitivity and specificity of the diagnostic assay used.

Sheep that were seropositive on two consecutive sampling points after collection of the initial seronegative sample were considered to have seroconverted. This convention was used because we could not determine antibody titres, nor could we conduct VNT on all samples. Therefore, we assumed that if two separate, consecutive samples were positive it was unlikely to be a diagnostic false positive caused by the assay. For the longitudinal study sheep that were sampled every three months, the date of seroconversion was estimated as occurring midway between the period when last seronegative sample and the

first seropositive sample were taken (approximately 45 days after the last seronegative sample). Following seroconversion sheep were removed from the study.

Laboratory Analysis

All samples from the 2015 cross-sectional study were initially screened with an N-protein recombinant ELISA (van Vuren et al., 2007) and confirmed with an inhibition ELISA (Paweska et al., 2005), as previously described. The longitudinal study sheep farms were selected based on identifying seropositive sheep (using the two assays in series) amongst the sheep sampled during the 2015 cross-sectional study that met the enrolment criteria. After the longitudinal sheep study was initiated, we determined that the recombinant ELISA performed poorly on the field samples compared to the expected sensitivity and specificity. Thus, we changed the protocol and going forward all cross-sectional samples (2015 and 2017) were retested/tested using only the inhibition ELISA as reported in Ngoshe et al. (submitted). Likewise, even though the longitudinal farms were selected on the basis of the serial tests, all of the longitudinal samples were only tested with the inhibition ELISA to determine seroconversion. Only the results from the inhibition ELISA were used in the statistical analyses (sensitivity: 100%, 100%, 99.56% and specificity: 99.52%, 99.29% and 99.65% in cattle, sheep and goats, respectively (Paweska et al., 2005)).

A subset of samples from the longitudinal study were confirmed by virus neutralisation testing (VNT) (Swanepoel et al., 1986). This was to confirm the serostatus of the subset of pre-injection and 30-day post injection samples (the latter was primarily to confirm seroconversion within the subset of sheep that were vaccinated, described in Chapter 3).

Age was rarely recorded on ear tags or in individual records, requiring the animals to be aged by dentition. Ages were not perfectly identified; however, once an animal was determined to be four years of age they rarely were estimated to be a different age (age cannot be reliably determined by dentition after four years of age). Therefore, to appropriately allocate the ages beyond four a computer program was written to identify the first time an animal was recorded as four, then every four sampling events its age was increased by one year. To ensure continuity, the same algorithm was used to ensure the animal spent the appropriate amount of time (four three-month periods) in each year of age. For each animal, we evaluated the age assigned by the algorithm and manually corrected any mistakes.

Statistical Analysis

Data were analysed using R 3.5.0 (R Core Team, 2019) in Rstudio 1.0.153 (RStudio Team, 2015) and all plots were made using the `ggplot2` (Wickham, 2009), `ggally` (Schloerke et al., 2018), `ggpubr` (Kassambara, 2019) and `egg` (Auguie, 2018) packages. The descriptive statistics were based on the questionnaire data. The mean and standard deviation of all domestic and wild ruminants (kept for commercial purposes) populations on each farm participating in the longitudinal study were estimated. The birth and mortality rates were calculated using the number of animals that were born and died during the entire period of the longitudinal study were divided by the median (3-month) population. The turnover rate for the entire study period was determined using a Poisson intercept model, with the total number of losses (absolute value) and gains per survey as the response variable and the time between surveys as the offset. The demographic data from the cross-sectional study are provided in Chapter 3. For all species, the crude and adjusted seroprevalence were estimated. To determine true crude seroprevalence the sensitivity and specificity of the inhibition ELISA were taken into account using the following formula: $TSeroP = \frac{SeroP+(SP-1)}{SP+(SE-1)}$ (Rogan and Gladen, 1978). Where $TseroP$ = the true seroprevalence, $SeroP$ = the crude seroprevalence, SP = the test specificity and SE = the test sensitivity. The seroprevalence was adjusted for clustering using svyciprop ("likelihood" method) from the `survey` package (Lumley, 2004). The farm-level weights were calculated as $\frac{1}{Probability\ of\ an\ animal\ being\ sampled}$. To calculate the adjusted seroprevalence, svyciprop estimated a generalized linear model based on a complex survey design with (Lumley, 2004). The linear model was run and resulted in the adjustment was used to calculate the estimated proportion of each species that is seropositive within the entire study area. The "likelihood" method calculates the proportion using a chi-squared distribution of binomial data to better estimate the confidence interval for proportions that are near zero.

A chi-square test was used to compare the proportion of seropositive animals in 2015 and 2017 by species (all animals sampled in 2017 that were previously sampled in 2015, were excluded from the 2017 group for the comparative analysis).

Survival analysis was used to analyse the cumulative incidence, instantaneous cumulative hazard and to identify risk factors associated with seroconversion using the `survival` and

`cmprsk` packages (Therneau and Grambsch, 2000, Gray, 2014). The exponential of Nelson's cumulative hazard estimate was calculated using a Cox model fitted using the Nelson-Aalen method (survfit(coxph(x~1), type = 'aalen') on the right censored data. We applied a smooth function to convert the cumulative hazard into the estimated seroconversion hazard function as previously described (Singer and Willett, 2003a, Singer and Willett, 2003b). A Poisson regression of an intercept model with an offset (time in the study) was used to identify the mean incidence of RVFV seroconversion across all farms (Flanders and Kleinbaum, 1995). Natural death was included as a competing interest for RVFV seroconversion. A few sheep were sold for slaughter by the owner despite their agreement to keep the sheep in the study. The sheep that were sold were censored at the last event at which they were present. We calculated the cumulative incidence function for both RVFV seroconversion and death. To identify any factors associated with seroconversion we used both a cause specific cox proportional hazards model and a Fine-Gray subdistribution model (Austin et al., 2016). Age at the start of the study and whether or not the farm reported abortions in sheep were included as predictors of RVFV seroconversion (and the competing risk of death). In the cox model we included the farm as a random effect. The assumption of proportional hazards was confirmed by analysing the Schoenfeld residuals.

RESULTS

Longitudinal Study Animal Demographics

Each participating longitudinal study farm completed 8-11 surveys during the study. Five of eight farms were meat producers, two produced both meat and wool and one was a wool producer. In 16 of 84 surveys farmers reported abortion(s) in one or more sheep on their farm during the preceding 3 months. In 44 of 84 surveys farmers also reported that sheep had access to flooded pan(s) on the farm during the preceding three months. Average livestock population sizes and birth, mortality and turnover rates on the longitudinal study farms were calculated (Table 2-1). Changes in mortality, birth, turnover and vaccination rates on each farm were assessed (Figure 2-2).

Seroprevalence in livestock from the Cross-Sectional Study

In 2017, we surveyed 228 livestock farms within the study region (Figure 2-1). A total of 2,798 animals (1,629 sheep, 758 cattle, 411 goats) were sampled during the 2017 cross-sectional study. The adjusted seroprevalence for all animals (with survey data) was 28.2% (n = 2,477; 95% confidence interval (CI): 21.5-35.6%); by species the adjusted seroprevalences were: 34.7% in cattle (n = 675; 95% CI: 27.1-42.8%), 27.5% in sheep (n = 1,471; 95% CI: 19.3-36.7%) and 4.2% in goats (n = 358; 95% CI: 1.5- 9.1%). The crude seroprevalence estimates from 2017 were similar to those estimated in 2015 (Table 2-2).

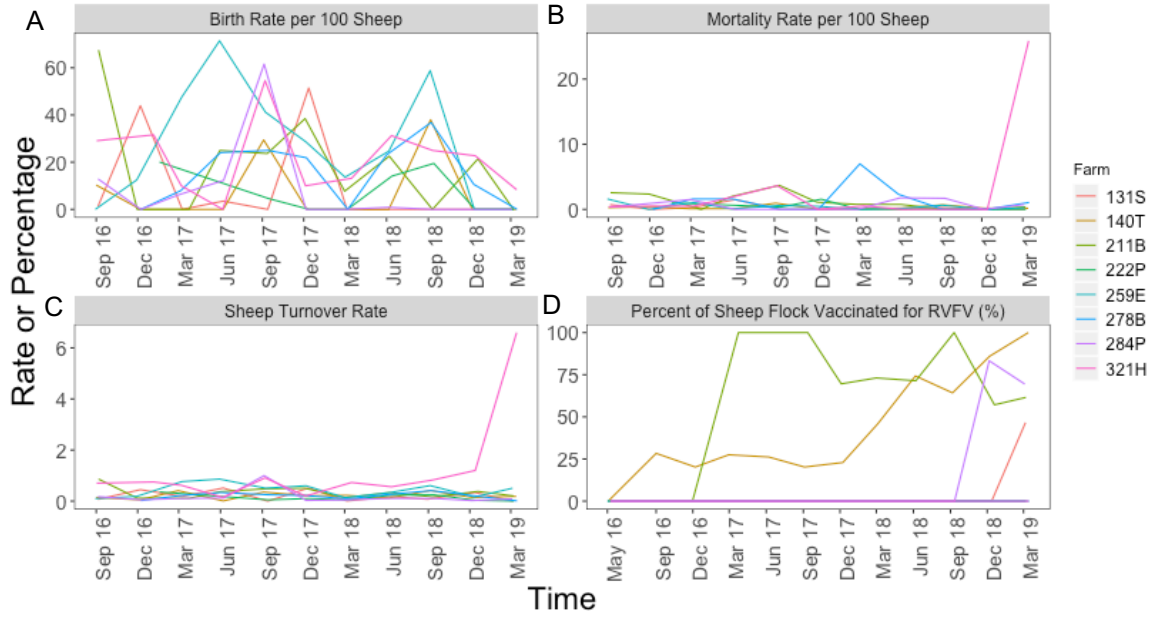


Figure 2-2 The birth, mortality and turnover rates and the percent of the sheep flock that had been vaccinated against RVFV are shown for the eight farms participating the longitudinal study. The A) birth and B) mortality rates are given as the number of births/deaths per 100 sheep. C) The sheep turnover rates are given as the number of gained and lost sheep divided by the total population. D) Indicates the percent of the non-study sheep on the farm that have now been vaccinated by the farmer on his/her own volition. Data for these rates were collected through surveys given every three months (at the time of sheep sampling).

The results of the 2015 cross-sectional survey have been previously published (Ngoshe et al., submitted). The true seroprevalence, accounting for the sensitivity and specificity of the inhibition ELISA, was calculated for each species and found to round to the same estimates as the crude seroprevalences (Table 2-2).

Among reportedly unvaccinated animals (n = 1,011 in 2017 and n = 1,680 in 2015) the crude seroprevalence was calculated for each species was lower than that estimated in 2015 (Table 2-2). However, this decrease in seroprevalence was only significant in cattle (p = 0.002; Table 2-2).

Table 2-1 The demographic characteristics of the livestock populations collected via questionnaire on the eight farms enrolled in the longitudinal study. These represent the mean or rates calculated over the full period of the study that the surveys covered (September 2016-March 2019). The population size for each species is given as the mean (standard deviation). The birth characteristics by species are given as: the number of animals born (the overall birth rate) {95% CI for the birth rate}. The mortality characteristics are given by species as the number of animals that died (the overall mortality rate) {the 95% CI of the mortality rate}. The estimated turnover is also given for each domestic ruminant species.
*The rates for this farm were only calculated based on the eight questionnaires the farmer completed, therefore the rates are for 24 months rather than 33 months. †The rates for this farm were calculated based on 10 questionnaires and therefore the rates are for 30 months. NA indicates the rate was not calculated as that species was not kept on the farm.

Species	131S	140T	211B	222P*	259E	278B†	284P	321H
Number of Surveys	11	11	11	8	11	10	11	11
Population: Mean (SD)								
Cattle	0 (0)	114.5 (18)	348.5 (447.4)	80.4 (21.7)	2.9 (1.7)	192.2 (18.5)	89.7 (128.1)	11.4 (9.8)
Sheep	3495.5 (1018.4)	6040.9 (1018.3)	218.1 (53.7)	2865.8 (176.7)	1975.9 (480)	172.8 (22.8)	226.9 (81.4)	1868.7 (511.6)
Goat	0 (0)	0.4 (0.8)	0 (0)	0 (0)	0 (0)	3.1 (4)	27.5 (18.3)	0 (0)
Springbok	50 (111.8)	37.5 (38)	27 (24.2)	9.4 (13.7)	2.7 (6.1)	0.2 (0.4)	0 (0)	90.5 (32)
Blesbok	1.8 (6)	11.7 (9.6)	13.6 (32.3)	9.4 (6.3)	57.9 (16.7)	0 (0)	0 (0)	12.2 (6.8)
Kudu	0 (0)	0.2 (0.4)	6.3 (10.7)	0 (0)	0 (0)	2.7 (3.5)	0 (0)	0 (0)
Birth Characteristics: Number Born [Birth Rate per 100 Sheep] {95% CI}								
Cattle	NA	100 [95.2] {76.6-113.9}	135 [108] {89.8-126.2}	107 [128.1] {103.9-152.4}	2 [50] {-19.3-119.3}	172 [88] {74.8-101.1}	89 [342.3] {271.2-413.4}	20 [285.7] {160.5-410.9}
Sheep	3710 [106] {102.6-109.4}	5502 [94.9] {92.4-97.4}	419 [196.7] {177.9-215.5}	1631 [57.1] {54.3-59.8}	5390 [269.5] {262.3-276.7}	255 [136] {119.3-152.7}	88 [36.1] {28.5-43.6}	3850 [192.5] {186.4-198.6}
Goat	NA	NA	NA	NA	NA	0	36 [109.1] {73.5-144.7}	NA
Wildlife	NA	8 [21.1] {6.5-35.6}	8 [32] {9.8-54.2}	0	93 [155] {123.5-186.5}	0	NA	137 [144.2] {120.1-168.4}
Mortality Characteristics: Number Died [Mortality Rate per 100 Sheep] {95% CI}								
Cattle	NA	11 [10.5] {4.3-16.7}	13 [10.4] {4.7-16.1}	0	0	10 [5.1] {1.9-8.3}	15 [57.7] {28.5-86.9}	1 [14.3] {-13.7-42.3}
Sheep	73 [2.1] {1.6-2.6}	211 [3.6] {3.1-4.1}	27 [12.7] {7.9-17.5}	92 [3.2] {2.6-3.9}	93 [4.7] {3.7-5.6}	21 [11.2] {6.4-16}	16 [6.6] {3.3-9.8}	721 [36] {33.4-38.7}
Goat	NA	NA	NA	NA	NA	0	0	NA
Wildlife	NA	0	0	0	0	0	NA	0
Estimated Turnover: Mean {95% CI}								
Cattle	NA	2 {1.7-2.2}	0.6 {0.5-0.7}	2.1 {1.8-2.5}	1.7 {0.6-3.7}	1.6 {1.4-1.8}	1.3 {1.1-1.6}	7.9 {6.4-9.7}
Sheep	2.3 {2.3-2.4}	2.3 {2.3-2.4}	3.5 {3.2-3.7}	1.1 {1-1.1}	4.5 {4.4-4.6}	2 {1.8-2.2}	1 {0.9-1.1}	14.8 {14.6-15}
Goat	NA	NA	NA	NA	NA	3.2 {1.6-5.7}	1.3 {0.9-1.8}	NA

Table 2-2 The crude seroprevalence of anti-RVFV antibodies among all (vaccinated and unvaccinated) cattle, sheep and goats together (all) and by species for animals sampled during the 2015 and 2017 cross-sectional studies. Also presented is the crude seroprevalence among unvaccinated animals for all and for each species sampled during the cross-sectional surveys in 2015 and 2017. The precision of the seroprevalence estimate based on the sample size, clustering and ICC is provided for the crude seroprevalence estimates of the combined vaccinated and unvaccinated animals. The p-values of within species chi-square tests comparing the seroprevalence in unvaccinated animals sampled in 2015 vs 2017 are shown. Not shown is the p-value for a comparison between species among animals sampled in 2017. There was no significant difference between sheep and goats, but there was a significant difference between cattle and small ruminants

	2015			2017				
Species	Seroprevalence	Number Sampled	95% Confidence Interval	Seroprevalence (%)	Number Sampled	95% Confidence Interval	Precision of estimate	P-value for temporal comparison
All Animals								
All Species	20.4%**	3070**	[19, 21.9]**	20.6%	2472‡	[19, 22.2]		
Bovine	32%**	987**	[29.1, 35]**	30.5%	740	[27.2, 33.9]	95.5%	
Ovine	16.4%**	1558**	[14.6, 18.3]**	19.9%	1329	[17.8, 22.1]	96.6%	
Caprine	10.7%**	525**	[8.2, 13.5]**	4.7%	402	[2.9, 7.1]	96.5%	
Unvaccinated								
All Species	13.6%	1680	[12, 15.3]	10.2%	1011	[8.4, 12.2]		
Bovine	29.3%	450	[25.2, 33.7]	18.6%†	253	[14.1, 23.7]		0.002*
Ovine	8.7%	901	[6.9, 10.6]	8.5%	517	[6.3, 11.1]		0.925
Caprine	5.5%	329	[3.3, 8.3]	5%	241	[2.7, 8.2]		0.795

*Indicates a p-value < 0.01. †indicates a p-value of 2.2e-16. **The crude seroprevalence for these animals has been reported previously in Ngoshe et al. (submitted).
‡The total sample size for the crude seroprevalence in 2017 does not include any animals that had been previously sampled in 2015.

Seroconversion During an Interepizootic Period

A total of 220 individual sheep were enrolled as unvaccinated sheep in the longitudinal study and sampled during 10 events (Table 2-3). Nine sheep on five farms seroconverted against RVFV over the course of the study (Table 2-3). The cumulative incidence was 0.04 and 0.22 for RVFV seroconversion and death, respectively, across all farms over 31.8 months (Figure 2-3). The hazard function indicated there was very little temporal variation in the rate of the hazard for RVFV seroconversion during the course of the study (Figure 2-3).

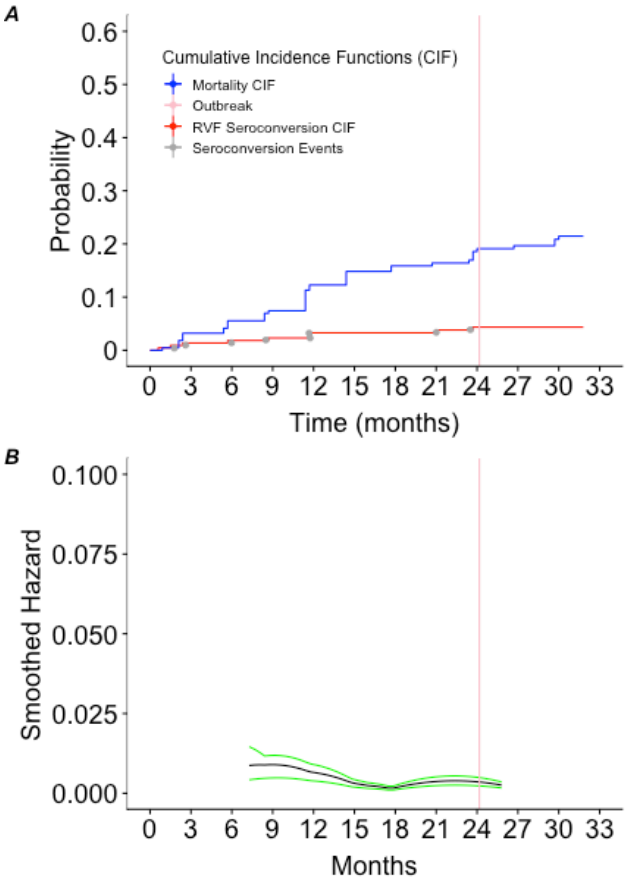


Figure 2-3 A) The estimated cumulative incidence of RVFV seroconversion (red) and mortality (blue) across all farms. The grey points indicate the estimated date of seroconversion for each sheep. The pink line indicates the date of the isolated RVFV outbreak that occurred on a nearby farm in April 2018. B) The instantaneous hazard function indicates that the hazard has remained constant over the duration of the study. There was a dip in the hazard at 18 months after the study started (September-December 2017). The slope of the cumulative hazard indicates that the rate of change in the cumulative hazard was the lowest at 18 months (September-December 2017).

Table 2-3 The percent of animals that seroconverted per month in unvaccinated sheep in the longitudinal study. Data for each sampling event is shown, including the percent of animals that seroconverted, (the total number of animals sampled and tested on that farm) and [the number of sheep enrolled to replace the sheep that died during the previous three months].

Farm ID	June 2016	September 2016	December 2016	March 2017	June 2017	September 2017	December 2017	March 2018	June 2018	September 2018	December 2018
131S	0% (23) [0]	0% (23) [1]	0% (23) [0]	0% (23) [1]	4.3% (23) [0]	0% (23) [5]	0% (28) [0]	0% (27) [0]	0% (27) [0]	0% (27) [0]	0% (23)
140T	0% (23) [0]	0% (23) [0]	0% (24) [0]	0% (24) [0]	4.2% (24) [0]	0% (23) [0]	0% (22) [0]	0% (23) [0]	0% (23) [0]	0% (23) [0]	0% (23)
211B	0% (6) [0]	0% (7) [0]	0% (7) [0]	0% (7) [0]	0% (7) [0]	0% (6) [0]	0% (5) [3]	0% (8) [0]	0% (7) [4]	0% (11) [3]	0% (14)
222P	0% (47) [0]	0% (47) [2]	2.3% (44) [0]	2.2% (45) [0]	0% (41) [0]	0% (42) [0]	0% (42) [0]	2.4% (42) [0]	0% (40) [0]	0% (41) [0]	0% (41)
259E	0% (21) [0]	5.3% (19) [1]	0% (19) [3]	4.5% (22) [0]	0% (19) [2]	0% (23) [0]	0% (22) [2]	0% (25) [0]	4% (25) [0]	0% (24) [0]	0% (22)
278B	0% (17) [0]	0% (13) [0]	0% (15) [0]	0% (14) [0]	0% (13) [0]	0% (10) [0]	0% (9) [0]	0% (7) [0]	0% (7) [0]	0% (7) [0]	0% (7)
284P	0% (11) [7]	0% (11) [0]	5.9% (17) [0]	0% (17) [3]	0% (16) [1]	0% (15) [0]	0% (16) [1]	0% (16) [0]	0% (15) [0]	0% (15) [0]	0% (15)
321H	0% (18) [0]	0% (19) [0]	0% (18) [3]	0% (19) [0]	0% (19) [0]	0% (19) [5]	0% (22) [0]	0% (23) [1]	0% (23) [0]	0% (22) [0]	0% (20)

The mean incidence of seroconversion for all sheep, across all farms was 0.005 seroconversions (95% CI: 0.003-0.009) per 3 animal-months (equivalent to 2.1 sheep per 100 animal-years [95% CI: 1.0-3.8]). The average RVFV seroconversion rate was calculated for each farm, excluding the three farms that did not have any seroconversions (see Figure 2-4). The average incidence of mortality in the longitudinal study sheep across all farms was 9.7 per 100 animal-years (95% CI: 7.1-12.9).

Table 2-4 Mean incidence rate of RVFV seroconversion rate (per 100 animal-years) in sheep during the 2.5-year longitudinal study given by farm. The 95% confidence interval is also given for farms that had a seroconversion rate greater than zero.

Farm ID	Mean Incidence Rate (per 100 Animal-Years)	95% Confidence Interval
131S	1.6	[0.1, 6.9]
140T	1.6	[0.1, 7.2]
211B	0	
222P	2.6	[0.7, 6.8]
259E	5.2	[1.3, 13.4]
278B	0	
284P	2.6	[0.2, 11.6]
321H	0	

The predictors of age and whether or not a farm had reported abortions in sheep during the study were not significantly associated with RVFV seroconversion in neither the subdistribution hazard model nor the cause specific hazard model (Table 2-5). A one-year increase in age increased the relative incidence of overall mortality by 59% and farms that reported abortions also had a 44% relative decrease in the incidence of natural mortality compared to the incidence on farms that did not report abortions. The cause specific hazard of mortality was very similar to the changes observed in relative incidence of mortality. The estimate of variance due to farm via a random effect was very low, 5e-07 in both the cause specific hazard model of seroconversion and for that of mortality (Table 2-5).

The RVFV incidence within the population of unvaccinated cross-sectional study sheep that were sampled in both 2015 and 2017 was 1.8 seroconversions per 100 animal-years (n = 71; 95% CI: 0.3-5.7). Only one unvaccinated cattle and six unvaccinated goats were sampled in both 2015 and 2017, respectively, and none seroconverted during the time period. For all resampled animals the average amount of time between the 2015 and 2017 sampling was 18.7 months.

Table 2-5 Mortality was included as a competing interest in the estimates of relative incidence hazard. Here we present the coefficient estimates and (95% confidence intervals) for the Fine-Gray subdistribution hazard model and cause-specific hazard model (Cox) for the relative incidence and hazard of RVFV seroconversion and natural mortality in the unvaccinated longitudinal study sheep. Both cause specific hazard models included a random effect for farm, though it accounted for very little variance (5e-07).

	Subdistribution Hazard Model		Cause-Specific Hazard Model	
Variable	RVFV Seroconversion	Mortality	RVFV Seroconversion	Mortality
Age at the start of the study	1.01 (0.61-1.67)	1.59 (1.27-2)**	1.04 (0.62-1.73)	1.6 (1.28-2)**
Farm reported abortions in sheep	2.38 (0.28-20.04)	0.56 (0.31-1.03)**	2.23 (0.28-17.88)	0.58 (0.31-1.08)**

** Indicates p-value of <0.001.

DISCUSSION

Here we have demonstrated for the first time that there was a very low level of interepizootic RVFV circulation in sheep the central plateau of South Africa, where three massive outbreaks have occurred since 1950. Levels of RVFV transmission in sheep did not appear to be sufficient to have increased the herd immunity between 2015-17. In cattle, population turnover rates or waning antibodies likely contributed to a significant decrease in the crude seroprevalence over the 18 months of the study (2015-17). Sheep and goats had a much lower seroprevalence than cattle and no significant change in seroprevalence was detected. Assuming that seroprevalence reflects herd immunity, we propose that the current seroprevalence level in livestock is not sufficient to prevent another large RVFV outbreak from occurring.

Seroprevalence

The adjusted 2017 seroprevalence in cattle was 34.7% (95% CI: 27.1-42.8%), in sheep it was 27.5% (95% CI: 18.6-36.7%) and in goats it was 4.2% (95% CI: 1.5-9.3%). These estimates overlapped with the values detected in the same study area in 2015 (Ngoshe et al., submitted) and sheep and goats and was slightly below the estimate for cattle. Estimates from both years are within the range of values reported in the literature. Previous reports of RVFV seroprevalence include 44.2% in sheep in Mozambique; seroprevalences of 26%, 24% and 27% in cattle, sheep and goats respectively, in Kenya; seroprevalences of 15.4% and 3.8% in cattle and small ruminants, respectively, in Mauritania; and seroprevalences of 34% and 32% in cattle and goats, respectively, in Kwa-Zulu Natal, South Africa (Blomström et al., 2016, Bett et al., 2018, Rissmann et al., 2017, van den Bergh et al., 2019). In our study, the seroprevalence in unvaccinated cattle was significantly higher than the combined seroprevalence among unvaccinated sheep and

goats. The difference between cattle and small ruminants has remained significant between the 2015 and 2017 serosurveys (Ngoshe et al., submitted). While the seroprevalence reported here is within the range of that reported in the literature, many farmers in this study reported vaccinating their livestock for RVFV, which is highly unlikely to have occurred in other regions of Africa. For this reason, we also calculated the crude seroprevalence by species within reportedly unvaccinated animals sampled during the 2015 and 2017 surveys. The crude seroprevalences we reported are closer to those reported in regions where RVF outbreaks or disease have not previously been reported (e.g. 6% in cattle in the Democratic Republic of Congo (Georges et al., 2018)).
Comparing seroprevalences between studies can be difficult as very few account for the specificity and sensitivity of the antibody assays – often because RVFV ELISAs are frequently reported at near 100%, which as we noted in our results, gave us the same seroprevalence as the crude seroprevalence that we reported (Table 2-2). While some assays have been extensively validated on samples from regions affected by RVFV, such as the inhibition assay we used (Paweska et al., 2005), most have not been. Kortekaas et al. (2013) compared the cELISA ID Screen RVF multispecies ELISA (Comtet et al., 2010) and a pair of recombinant N-protein IgM and IgG ELISAs (Williams et al., 2011). On 71 reference sera, the five laboratories only had a 90% concordance amongst the laboratories and the sensitivities ranged from 73-100% and specificities from 79-100% (Kortekaas et al., 2013).

Here we identified a significant decrease in the crude seroprevalence in reportedly unvaccinated cattle between the 2015 and 2017 serosurveys. This trend of a decreasing population seroprevalence in cattle following an outbreak has been reported in several countries. In Mayotte, Métras et al. (2016) identified similar patterns of RVFV seroprevalence in cattle. The seroprevalence peaked at 36% in 2008-2009, which they suspected represented an unreported outbreak. After the outbreak, the seroprevalence decreased annually to 6% in 2015, six years after the outbreak. In our study, the unvaccinated cattle still had a seroprevalence of 18.6% seven years after the outbreak. This was most likely because the outbreak in South Africa was much larger and more widespread than the undiagnosed outbreak in Mayotte. Thiongane et al. (1994) also found a rapid decrease in herd immunity following the 1987 RVF outbreak in Senegal. Immediately following the outbreak, they detected a seroprevalence of 80% in domestic ruminants (cattle, sheep and goats), which declined annually to a seroprevalence of 17% in 1990 (n = 1,225 ruminants tested for RVFV neutralizing antibodies). They included sheep and goats in their seroprevalence estimate and the higher turnover rate of small ruminants,

compared to cattle, could explain why they also described a more rapid rate of decline than we found.

Another possible explanation for the decline in the cattle population seroprevalence is that cattle do not respond with antibodies to Smithburn vaccine as well as sheep do. In one study, only 3 of 15 cattle had antibodies following vaccination (Barnard, 1979). Another found that the antibodies in cattle began to wane within 25 weeks (Coackley et al., 1967). It has been reported that even if antibodies wane in cattle following vaccination, immunity may still persist (Barnard, 1979). However, experimental infection with wild type virus induced higher titres of neutralizing antibodies (Barnard, 1979). As antibody persistence has been less studied in cattle following natural RVFV infection, it is not possible to distinguish between antibody waning and turnover as causes for these annual declines in the estimates of seroprevalence in cattle. Alternatively, if naturally induced antibodies do persist in cattle, the annual decline in seroprevalence we observed in cattle but not in small ruminants may have been due to lower mortality rates in cattle as compared to sheep and goats (Table 2-1). Lower turnover rates would mean that the population seroprevalence would decline at a slower rate than in sheep and goats. This could explain why the cattle seroprevalence is still significantly higher than that in small ruminants, while the cattle seroprevalence continues to decline.

In contrast to cattle, the seroprevalence in unvaccinated sheep and goats remained stable between the two surveys. The high rates of turnover on the farms (Table 2-1), particularly in sheep (most are greater than 200% over 33 months), indicated that most if not all animals were replaced during the period of the study. Therefore, after the outbreak the seroprevalence in sheep and goats may have declined more rapidly than we saw in cattle and reached a point where it could be maintained by interepizootic transmission. The high range of the confidence interval of the cross-sectional study sheep incidence and the low range of the seroprevalence estimate in the same sheep population in 2017 nearly overlapped (5.7 vs. 6.3). Thus, a seroconversion rate of 6 infections per 100 animal-years would cause 48 new infections each year in a flock of 800, this is would maintain a 6% seroprevalence. With a high turnover rate (e.g. 2 over 2.75 years), then none of the previously exposed animals on the farm would contribute to the seroprevalence. However, the turnover rates likely overestimated the loss of breeding animals, as it is often the young animals that are born and then sold, potentially inflating the impact of the calculated turnover rate.

Though we did not find a decline in the small ruminant seroprevalence over time, others have described a decline – particularly following an epizootic. Following the 1987 outbreak, Thiongane et al. (1991) identified a decline in seroprevalence in sheep and goats to 24.4% by 1988, and 19.3% in 1989. However, the sample size was quite small. Further work in Senegal by Thonon et al. (1999) expanded this annual surveillance in sheep and goats through 1996 and reported a steady decrease in annual mean seroprevalence. The only exception being an apparent epizootic that occurred in 1993 (the seroprevalence increased to 40% with abortions affecting 50% of the herd; n = 40). This small outbreak was followed by a further decrease in the population seroprevalence to levels that were less than 10%.

The herd immunity threshold (HIT) assesses whether an estimate of herd immunity would be sufficient to drive RVFV to extinction and is based on the calculation of R_0 (Anderson and May, 1982). Using our estimate of R_0 (Chapter 4) and the an estimate from the literature, we estimated the HIT to be 0.31 for animals (Chapter 4) and 0.79 between farms (regionally) (Métras et al., 2013). Our simulation suggests that with 31% of the herd vaccinated the it would take 19 years before no sheep were infected and infected *Aedes* would still be circulating for several years after that (Chapter 4). Thus, higher vaccination rates are recommended. To eliminate the virus from the region, it is likely each farm would need to maintain their ruminant population vaccinated at 31% for >30 years or the overall population of ruminants for the region would need a herd immunity of at least 79%. However, if wild ruminants contribute to viral maintenance (LaBeaud et al., 2011), a higher HIT would be required to drive RVFV to extinction.

RVFV incidence in sheep in central South Africa

We found complementary evidence in both our longitudinal study and cross-sectional studies of ongoing low-level transmission of RVFV in the study area. This adds to a growing body of literature providing evidence for interepizootic circulation of RVFV. Much of the earlier evidence for interepizootic transmission has come from cross-sectional studies that identified RVFV antibodies in livestock or wildlife species that were born after the most recent RVF outbreak in the area, including parts of Kenya, Mozambique, South Africa and Tanzania (Rostal et al., 2010, Moiane et al., 2017, LaBeaud et al., 2011, Sumaye et al., 2013); or regions that have never reported a clinical outbreak of RVF including Mayotte, parts of Mozambique and most recently north-eastern South Africa (KwaZulu-Natal) (Métras et al., 2016, Fafetine et al., 2013, van den Bergh et al., 2019).

Fewer longitudinal studies of RVFV have been conducted and no longitudinal studies have previously been conducted in the outbreak prone central plateau of South Africa. Of the longitudinal studies, most only presented the percent of seroconversions over a rough time period, rather than presenting the incidence rate. In West Africa, Zeller et al. (1997) reported that 1.9% of sheep and goats (n=160) seroconverted over approximately two years ending in 1994. The same site was included in short, four-month longitudinal studies conducted in 2003 and 2004 (Chevalier et al., 2005, Chevalier et al., 2009), where seroconversion rates of 5.3% and 4.1% (n=610 and 201 respectively) were identified in herds of sheep and goats. In Madagascar, two approximately one-year studies were conducted between 2009-2010 (Nicolas et al., 2013) and in 2011 (Nicolas et al., 2014) that identified seroconversion rates of 14% and 7% (n = 516) in cattle, respectively. In Kenya, Lichoti et al. (2014) did not provide an overall seroconversion rate, but at individual sites (over varying periods of time ~ 1-4 months) 0-100% of their sheep and goats (n = 17-139 depending on the site) seroconverted in Ijara (which has been severely affected by multiple RVFV outbreaks). Another study in Kenya identified a seroconversion rate of approximately 5% in sheep and goats over approximately nine months (Mbotha et al., 2018). While all of these studies are important to demonstrate that interepizootic transmission of RVFV occurs, unfortunately, the lack of exact quantification of the time at risk prevents direct comparisons of our estimate of RVFV incidence with the studies described above.

The low rates of infection detected in our longitudinal study were much lower than reported in the only other comparable study (van den Bergh et al., 2019), which was conducted in KwaZulu-Natal (KZN), South Africa and was roughly concurrent (June 2016-June 2018). They identified an incidence of 0.59 infections per animal-year in cattle and 0.41 per animal-year in goats, higher than the levels recorded in this study (0.02 seroconversions per animal-year). This difference was unexpected, particularly given the fact that our study was carried out in an area that has seen massive outbreaks of RVF (Pienaar and Thompson, 2013), whereas the van den Bergh et al. (2019) study was carried out in an area that has never reported RVF (Pienaar and Thompson, 2013).

The differences in seroconversion between our study and the van den Bergh et al. (2019) study could be due to temporal and spatial patchiness of RVFV transmission. Temporal patchiness was demonstrated in KZN, where the seroconversion rates at the same site varied from 0.83 infections per animal-year in the first year to 0.18 in the second year (van

den Bergh et al., 2019). Mathematical models describe mechanisms that support temporal patchiness of RVFV transmission as well (Chapter 4). These increases in transmission may or may not manifest as an outbreak that would be detected. This is supported by the unreported outbreaks reported in Senegal and Mayotte that were identified through surveillance (Thonnon et al., 1999, Métras et al., 2017). Spatial patchiness was demonstrated by the 2018 RVF outbreak that had a severe impact on a single isolated farm in central South Africa (OIE, 2018b) (within 15 Km of one of our longitudinal study sites), but no other farms nearby reported clinical signs associated with RVF and our longitudinal study sheep did not have an increase in seroconversions at that time. A likely contributor to the spatial patchiness of RVF outbreaks is the limited (150m) flight range of *Aedes mcintoshi*, a known transovarial vector of RVFV (Linthicum et al., 1985). Close geographic proximity seems to be a very strong spatial driver, making farms 5-13 Km from an infected farm likely to become infected, with the inter-farm force of infection declining as distance between farms increase (Métras et al., 2013, Chapter 6). Cattle movement and rainfall have been modelled by Favier et al. (2006) and they suggested that these two factors could permit the maintenance of RVFV in a system of multiple pans, despite the virus becoming locally extinct in different pans. While there is clear evidence for the importance of some of the drivers in widespread epizootics (Anyamba et al., 2009), we still do not fully understand what drives RVF outbreaks on individual farms (Chapter 6).

Rather than spatial patchiness, the differences observed in the rates of seroconversion and the size of reported outbreaks within South Africa could suggest that there are different mechanisms of RVF maintenance in different regions. Three hypotheses of potential mechanisms of RVFV maintenance are 1) the maintenance solely or primarily by transovarial transmission in certain floodwater *Aedes* mosquitoes with season, low-level transmission to hosts that is driven by the abundance of specific transovarial transmitting vectors (Linthicum et al., 2016); 2) ongoing medium-to-high level transmission by multiple mosquito species, where seasonality is driven by temperature and precipitation (Cavalerie et al., 2015); and 3) a combination of mechanisms 1 and 2 occur simultaneously or in close enough spatial proximity to maintain regions of higher and lower seroprevalence (Sumaye et al., 2019).

In regions where RVFV depends more on transovarial transmission, seasonal RVFV transmission may be driven by the hatching of specific mosquitoes, causing low-level transmission during periods when the transovarial vector is hatching (Chapter 4, Linthicum et al., 1985). While several studies have investigated the seasonality of the relative

population abundances of RVFV vectors (Ba et al., 2005, Logan et al., 1991), very few have examined the seasonality associated with RVFV transmission as evidenced by seroconversions (van den Bergh et al., 2019). We estimated a very low-level of ongoing RVFV circulation, though we did not identify a seasonal pattern (Figure 2-3); however, we only had nine seroconversions. Our low number of seroconversions may have prevented the identification of a pattern. This maintenance system is likely to be more susceptible to local extinction within a pan and be more prone to large explosive outbreaks. Here we have provided evidence of low incidence rates; we have also developed a mathematical model (Chapter 4) that indicates that this system would be susceptible to extinction. More research is required, but it is likely that wildlife and movement of livestock between pans as proposed in Senegal (Favier et al., 2006) will also support the persistence of RVFV in these regions with transovarial RVFV transmission. The risk of extinction in regions with seasonal transmission maintained by transovarial transmission may contribute to the longer-than-average interepizootic periods between the large outbreaks seen in central South Africa (Pienaar and Thompson, 2013).

The second hypothesis suggests that RVFV is primarily maintained through the horizontal transmission of RVFV by vectors throughout the year, and where infected adult female *Culex* mosquitoes may survive the dry/winter season (Jupp, 1969). In this case, we would expect higher rates of seroconversion, as the amplifying vectors would also serve as the maintenance vector. We would also expect that the seasonality of the seroconversion to more closely match to the seasonality of mosquitoes as driven by temperature and precipitation. We do not have evidence of this in our system in central South Africa. However, van den Bergh et al. (2019) identified a high rate of seroconversions (a mean of 59 per 100 animal-years) in KwaZulu-Natal, South Africa and they observed a seasonal trend in seroconversions that matches the trend in precipitation. This mechanism was also proposed in Madagascar, where 7% and 14% of cattle seroconverted during two different years (Nicolas et al., 2014). Mathematical simulations have indicated that systems that have at least a small population of mosquitoes persisting throughout the year can maintain RVFV in the system without transovarial transmission (Nicolas et al., 2014, Cavalerie et al., 2015).

The third hypothesis would suggest that in some regions transovarial transmission may maintain RVFV for a time during which it may or may not have gone extinct, then it could be reintroduced from a region with ongoing transmission in non-transovarial vectors. If, for example, the virus were to go extinct in pan(s) the central plateau, it could be reintroduced

into the region by wild or domestic ruminant trade or free-ranging wildlife movement. While our results suggest that RVFV can persist in the central South Africa for some amount of time, it is possible that the extraordinarily long interepizootic periods in this region occur due to local RVFV extinction and reintroduction. A situation such as this was modelled in Tanzania (Sumaye et al., 2019).

Several features of the study design may provide a partial explanation for the low RVFV incidence rate we observed in sheep in the study area. We enrolled all of the farms from the cross-sectional study that had seropositive sheep that were born on the farm and the farmer confirmed that the sheep were never vaccinated against RVFV. Due to the relatively high rate of vaccination in the region (approximately 46% of the farmers reported vaccinating their sheep and many more were unsure as to the vaccination status of their animals; Chapter 3) and frequent trade making the vaccine history of many animals unknown, we may have selected farms that were ecologically less likely to have been affected by RVF, which would create a bias against vaccinating for RVFV. In fact, only two of the eight longitudinal study farms previously reported having confirmed RVFV on their farm. Alternatively, many of the farmers that were participating in the longitudinal study decided to vaccinate a majority of their flock after our study started (Figure 2-2D). Some of them have reached and maintained rates that could be sufficient to eventually cause local extinction of RVFV (Chapter 4). While most of them were not vaccinating for the duration of the study, some began vaccinating when the project was initiated. The herd immunity induced by this vaccination could also have contributed to the low seroconversion rate. Finally, effective vaccination rates in the entire study area are high enough that they may dampen the transmission of RVFV.

The low incidence rate also made interpretation of the subdistribution hazard and specific cause hazard analyses difficult. The small number of seroconverted sheep may have reduced our ability to identify a link between age and/or abortion occurring in sheep in the three months prior to sampling. Future work will include a longer study with more animals to better understand factors that increase the risk of seroconversion in the central plateau. It was important to include mortality as a competing interest as we demonstrated that mortality occurs with a higher incidence (9.7 deaths per 100 animal-year) than RVFV infection (2.1 seroconversions per 100 animal-year). As one might expect, older age increased the risk of mortality. Surprisingly, reporting abortion(s) in sheep during the previous three months actually decreased the risk of mortality. This may be because

farmers that notice abortions in their animals while in the field have better management practices than farmers that do not notice abortions in their flocks.

CONCLUSION

This paper examined the current status of herd immunity in the study area in central South Africa, and whether natural seroconversion contribute to it. Among unvaccinated livestock, the crude RVFV seroprevalence decreased significantly between 2015 and 2017 in cattle, but remained relatively constant in small ruminants. The stability of the small ruminant seroprevalence may be due to natural, low-level incidence of RVFV that has been estimated here to be 2 seroconversion per 100 animal-years in sheep. While the observed rate of seroconversion may be sufficient to maintain the seroprevalence observed in unvaccinated sheep (given the high rates of turnover), it would not be sufficient to maintain herd immunity at a high enough level to prevent an RVF outbreak.

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Chapter 3: Rift Valley Fever Herd Immunity: Patterns and Determinants of RVFV Antibody Dynamics in Vaccinated Sheep

ABSTRACT

The epidemiology of Rift Valley fever virus (RVFV), a zoonotic arbovirus, is characterized by devastating outbreaks in ruminant livestock populations followed by long interepizootic periods that can span decades. Outbreaks are likely driven by a combination of climate conditions conducive to mosquito vectors and low livestock herd immunity. To better understand RVFV herd immunity in sheep in central South Africa we investigated the persistence of antibodies to RVFV following vaccination and patterns and predictors of vaccination practices among farmers. A cross-sectional survey in sheep was conducted in 2015 to assess the serostatus of a vaccinated population of sheep and the farm-level factors that may predict whether a farmer chooses to vaccinate against RVFV. Additionally, a 2.5-year longitudinal study was conducted to examine changes in seropositivity in vaccinated sheep using Generalized Linear Models. Seropositivity in sheep vaccinated as part of our study with the Smithburn (modified-live) vaccine was long-lived, with only 0.46 seroreversions per 100 animal-years (95% CI: 0.08-1.4). Seropositivity was much higher in sheep vaccinated in the longitudinal study flocks (97%; 95% CI: 95-99%) compared to those from flocks in the cross-sectional study that were reportedly routinely vaccinated against RVFV (34%; 95% CI: 28-39%), suggesting that routine vaccination procedures are currently sub-optimal. While under current vaccination regimens, herd immunity is low, this study demonstrated that vaccination has the potential to induce and sustain sufficiently high levels of herd immunity to prevent outbreaks. Further investigation is needed to understand where problems might be arising in the storage, distribution and delivery of vaccines to improve the effectiveness of vaccination.

INTRODUCTION

Rift Valley fever (RVF) outbreaks have been reported throughout much of Africa and can be isolated to a single farm or affect multiple nations (Woods et al., 2002, Laughlin et al., 1979, Ksiazek et al., 1989). RVF virus is zoonotic and it causes widespread abortion storms in pregnant livestock and high mortality in new born animals (Swanepoel and Coetzer, 2004). In South Africa there were widespread outbreaks across multiple provinces in 1951, 1974-75 and 2008-2011 (Pienaar and Thompson, 2013). During the 2008-2011 outbreaks 17,611 ruminant cases (15,488 sheep) were reported to the OIE (2019). Sheep are the most susceptible to RVF, while cattle and goats are less susceptible. Ruminant wildlife and people can also be affected by RVF (Swanepoel and Coetzer, 2004).

The three available commercial livestock vaccines against RVF virus (RVFV) include two modified-live vaccines (Smithburn and Clone 13) and an inactivated vaccine. During the 2008-2011 outbreak in South Africa, over 40 million doses of RVFV vaccine (unspecified type) were sold (Dungu et al., 2018). Clone 13 is a modified-live vaccine that is reported to have few side effects at the doses recommended by the manufacturer (Njenga et al., 2015). While it does induce immunity, the antibody levels were reported to wane in sheep after 12 months (Njenga et al., 2015) and it is unclear if immunity persists after the antibodies wane. The inactivated vaccine requires a booster within 3-4 weeks of the first injection, followed by annual vaccination (Barnard and Botha, 1977). It is, however, safe to use in pregnant animals.

The Smithburn vaccine was initially developed in 1949 (Smithburn, 1949) and is still commonly used today. While it is quite efficacious, it can cause abortion if given to a pregnant animal (Onderstepoort Biological Products, 1947). The Smithburn vaccine is believed to stimulate long-lived immunity, though few field studies have been conducted (Capstick and Gosden, 1962, Coackley et al., 1967, Barnard and Botha, 1977). Coackley et al. (1967) reported multiple experiments on the duration of immunity in vaccinated sheep, though they had quite low samples sizes (n = 16, 13 and 8). Most of the above studies immunized the animals with higher doses of the Smithburn strain (e.g. 10^6-10^8 infant mouse lethal dose$_{50}$ [IMLD$_{50}$]) (Capstick and Gosden, 1962, Coackley et al., 1967) as compared to the dose currently used in the commercially available Smithburn vaccine (at least $10^{4.4}$ IMLD$_{50}$; Pers. Comm., B. Nthangeni, 2017). A further study used a Smithburn strain at a commercial dosage (10^4 IMLD$_{50}$) in four sheep, with all producing neutralizing antibodies by 32 days post-vaccination and 3 of 4 had no viremia post-challenge with

RVFV (Barnard and Botha, 1977). The longest duration during which neutralizing antibodies were detected in sheep (and tested for) was three years post-vaccination (Coackley et al., 1967).

Following vaccination with a Smithburn strain, sheep have been reported as producing either very high or low post-vaccination titres (Capstick and Gosden, 1962, Coackley et al., 1967). Three explanations for this have been posited in the literature: (a) sheep that developed higher titres may have become viremic following vaccination, whereas the other sheep did not become viremic with the vaccine strain; (b) the neutralization assay was not sensitive enough to identify previously exposed animals with too low of a titre and that a more sensitive assay is needed (such as an ELISA); (c) the high-titre responders had previously been exposed to RVFV and antibodies waned resulting in no neutralizing titres being detected prior to vaccination (Capstick and Gosden, 1962, Coackley et al., 1967). In comparison to sheep, neutralizing antibodies were often absent in cattle following vaccination with the Smithburn strain though upon challenge, they were protected for up to a year after vaccination (Coackley et al., 1967, Barnard, 1979).

Although the Smithburn vaccination may be an effective method for increasing herd immunity in sheep, several key questions still remain about duration of immunity in sheep and the approaches used to detect the antibody response. For instance, do the antibodies induced by the commercial Smithburn vaccine persist as well as those induced by the strains of Smithburn with higher viral titres in the previous studies? And do antibody levels induced in sheep by Smithburn or natural infection wane, thereby allowing some sheep to respond to a second exposure with much higher titres? Given the limitations of small sample sizes in the previous studies, there is uncertainty as to whether those results can be generalized to large sheep populations. It is important to answer these questions in order to make recommendations regarding RVF vaccination to improve herd immunity. If farmers only need to vaccinate their sheep once in a lifetime, they may be more likely to continue vaccinating their flocks during the long inter-epizootic periods (up to 35 years).

The aim of this study was to provide evidence for veterinarians, farmers and disease ecologists on the waning rate of RVFV antibodies, which can be used to identify ways to support herd immunity through vaccination strategies to minimize the risk of RVF outbreaks as well as to parameterize mathematical models. Here we will evaluate the rates of RVFV antibody waning in sheep vaccinated with Smithburn modified-live vaccine, compare these rates with those calculated from reportedly vaccinated sheep in a cross-

sectional study conducted in the same area and identify determinants of RVFV vaccination at the farm-level to determine what role vaccination should play in maintaining herd immunity.

METHODS

Study Area

The study area is a 40,000 Km^2 region stretching roughly from Bloemfontein (Free State Province, South Africa) to Mokala National Park (Northern Cape Province, South Africa) and stretching 100 Km north and south of those points (Figure 3-1). The study area was bounded by the following geographic positioning coordinates: -28.20, 24.20; -30.20, 24.20; -28.20, 26.40; -30.20, 26.40 (WGS84 decimal degrees, latitude and longitude, respectively). The dominant agricultural activity in the southern portion of the study area is sheep farming, with more cattle and grain farming in the northern part of the study area

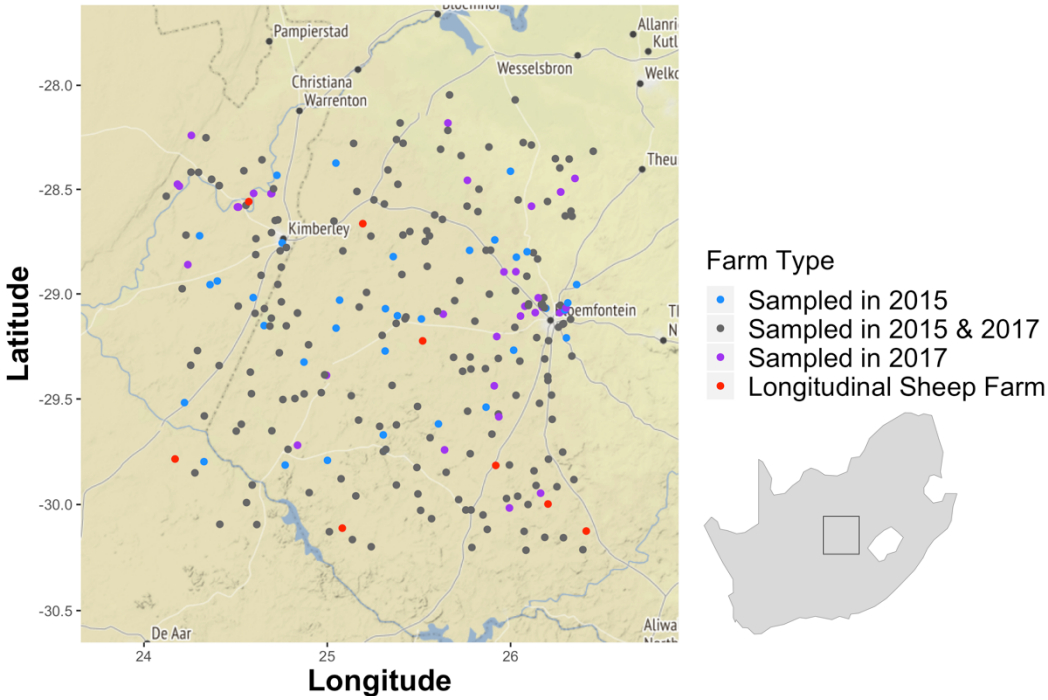


Figure 3-1 A map of farms sampled for the cross-sectional studies (either sampled in 2015 [blue], 2017 [purple] or both 2015 and 2017 [black]) and the longitudinal sheep study (red).

(Cloete and Olivier, 2010). The region has many shallow depressions that temporarily fill with rain water (called pans in South Africa) (Geldenhuys, 1982).

Ethical Approval

Animal ethical approval for the cross-sectional and longitudinal studies was obtained from the University of Pretoria Animal Ethics Committee (t005-16, V013-15, V090-15), Tufts University Institutional Animal Care and Use Committee (G2014-03 and G2016-148), the U.S. Army Medical Research and Material Command Animal Care and Use Review Office (CT-2014-33) and for the longitudinal study the University of Glasgow (Ref 62a/15) (see Appendix 1). Permission to conduct research on Rift Valley fever virus in livestock was obtained by DAFF (12/11/1/1/13) (see Appendix 2). The questionnaire and human subjects research approval was obtained from HummingbirdIRB (2014-25), the University of Witwatersrand (M140306), Northern Cape Provincial Health Research Ethics Committee (NC2015/001), Free State Provincial Department of Health (04/04/2015) and the U.S. Army Medical Research and Materiel Command, Office of Research Protections, Human Research Protection Office (A-20745.1a and A-20745.1b) (see Appendix 3). Given the extensive human ethics approvals that were already in place and that the author was specifically named on the protocols, the human ethics committee of the College of Medical, Veterinary and Life Sciences, University of Glasgow waived the requirement of review.

Cross-Sectional Study

The 2015 cross-sectional study was conducted from October 2015-February 2016 and included a farmed population of sheep reported to be undergoing routine RVFV vaccination carried out by farmers. An additional 37 farms were surveyed in May-November 2017 to replace farmers that elected not to participate in a repeat cross-sectional study that year, these data were primarily collected for use in another study (Chapter 2). The data provided by the additional farms is included in our analysis of the demographic data, but not the serological study presented below. Farms were invited to participate based on their proximity to randomly generated geographic points (previously described in Chapter 2). Livestock owners were informed of the study and were asked to provide informed consent (Appendix 4) to permit us to sample their animals and administer a questionnaire (Appendix 5). The questionnaire asked about farm and animal population sizes, possible risk factors for RVF, history of RVF, RVFV vaccination protocols, general husbandry and biosecurity practices. The survey that was given to all farmers the first time they participated (in either 2015 or 2017), will be referred to as the “full” survey, whereas the “repeat” survey was given to farmers in 2017 that had already completed the full

survey in 2015 and focused on factors that may have changed during the intervening 18 months.

The minimum sample size required to estimate the seroprevalence with 95% precision (s) and 95% confidence for sheep was calculated using a formula for cluster sampling (Bennett et al., 1991): $s = \sqrt{\frac{p(1-p)D}{n}}$, where p = the current estimated prevalence, D = the design effect and n = the total number of animals sampled. The design effect was calculated using $D = 1 + (b - 1)\rho$, where b = the average number of animals per cluster/farm and ρ is the intra-cluster correlation coefficient. For sheep, we used an estimated seroprevalence of 0.25, yielding a required sample size of 752 animals from 84 farms with 9 sheep per farm.

Thus, up to nine sheep were sampled (haphazard/convenience sampling) on each farm (sampling was previously described in Chapter 2). It was determined that the minimum sample size of sheep required to precisely estimate the seroprevalence was 752, as was previously described in Ngoshe et al. (submitted). Briefly, for each sheep sampled, age was determined by dentation, and farmers were asked about the RVFV vaccination status, whether the animal was born on the farm, and if vaccinated, who administered the vaccine. As records of individual vaccination did not exist on most farms, the farmers provided the last year in which they vaccinated their animals for RVFV and all sheep that were old enough to be alive at that time were considered to be vaccinated (see Figure 3-2). Nearly 80% of farmers indicated they either vaccinated all their sheep yearly or all of the young animals yearly, providing support for our assumptions. All sheep had ear tags placed with their study ID number in case we wanted to enrol them into the longitudinal study.

Longitudinal Study

The longitudinal study was conducted May 2016-December 2018. The longitudinal study involved all eight farms from the cross-sectional study that met the inclusion criteria. The inclusion criteria and enrolment procedures are described in Chapter 2.

Sheep allocated to the vaccination group were administered 1 mL of modified-live Smithburn vaccine subcutaneously as per the manufacturer's directions (Onderstepoort Biological Products (OBP), South Africa). OBP produces Smithburn vaccine that has a minimum of $10^{4.4}$ $IMLD_{50}$ (Pers. Comm. B. Nthangeni, 2017). The vaccine was purchased

directly from the manufacturer and cold chain was maintained. A new bottle of vaccine (100 doses) was reconstituted by adding 5 mL of sterile diluent to the freeze-dried vaccine and mixed, on each farm. The vaccine was used within 6-hours of reconstitution. All sheep were bled and then injected with RVFV vaccine 30 days prior to the start of the study. A set of unvaccinated sheep were also bled and injected with saline on the same day and at the same farms to be used in another study, but also provided a control for the vaccinated animals (Chapter 2). After 30 days, the sheep were bled again to confirm seroconversion, this sampling event was considered the start of longitudinal study. Following that, a blood sample was collected from each sheep every three months (see timeline, Figure 1-3, Chapter 1). Sheep that were absent for two consecutive sampling periods were considered to be dead. All sheep that died were replaced in the same group by a young lamb (older than six months) that had been born on the farm and never vaccinated. Sheep were managed following the farmer's normal practices as described in Chapter 2.

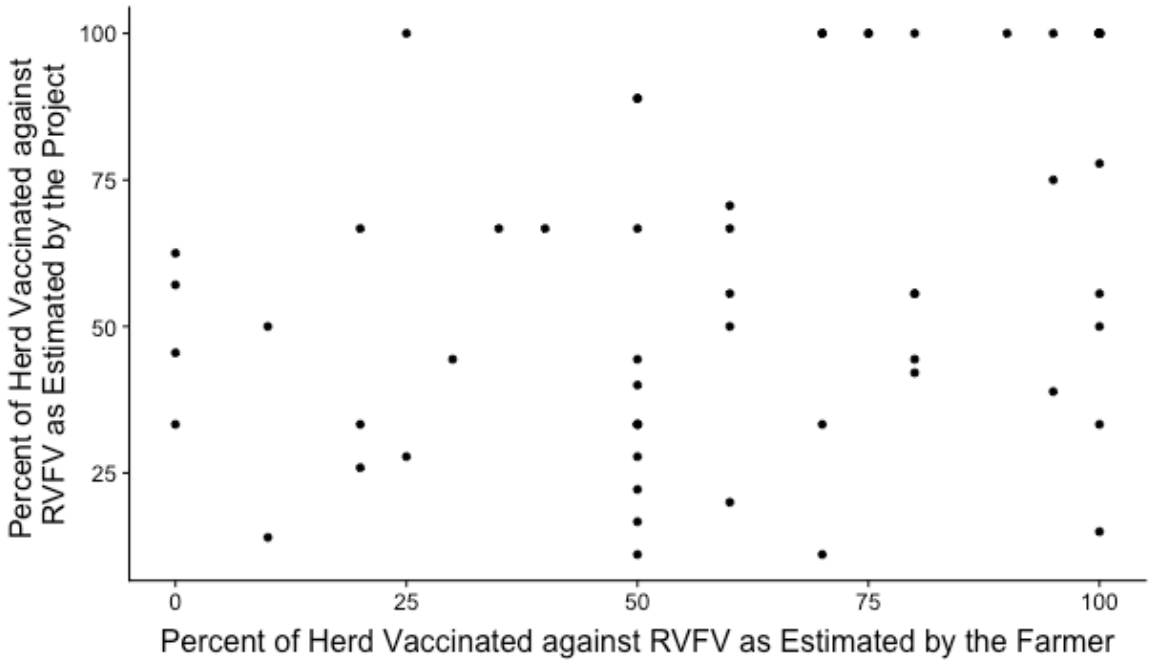


Figure 3-2 The estimate of the percent of the herd vaccinated made by the authors (y-axis) and by the farmer (x-axis). Farmers were asked to estimate the proportion of their entire herd (cattle, sheep and goats) that was vaccinated against RVFV. We estimated whether the individual animal we sampled was vaccinated based on the year of most recent vaccination and the animal's age. Our estimate of the proportion of sheep that were vaccinated was based on the proportion of the sampled sheep that were assumed to be vaccinated.

Sheep that tested seropositive on the "PRE" sample, collected prior to vaccination, were excluded from all analyses because the previous exposure (or vaccination) may impact the rate of antibody decline. Project vaccinated sheep that never tested seropositive on any assay (ELISA or VNT) were excluded from all analyses of the longitudinal study, but were included in the comparison between the cross-sectional and longitudinal results. Ages were not perfectly identified; however, once an animal was determined to be four years of age

they rarely were assigned a different age (age cannot be reliably determined by dentition after four years of age). Therefore, to appropriately allocate the ages beyond four, a computer algorithm was used as described in Chapter 2.

Laboratory Analysis:

Box 3-1. Definitions used in this chapter.

Herd Immunity: The individual protection provided to a susceptible individual animal when a sufficient proportion of the herd is protected from infection to prevent the occurrence of an outbreak (Fine, 1993). The presence of RVFV antibodies has not been equated to protection from RVFV. Thus, here we are representing an estimate of herd immunity with the proportion of animals in which we detected antibodies against RVFV.

Routine vaccination: Vaccination that was given on the farm by the farmer, his/her staff or a veterinarian (not a project member) and was reported by the farm owner or manager. The term applies to the animals in the cross-sectional study.

Seronegative: An animal that tested negative on an RVFV inhibition ELISA.

Seropositive: An animal that tested positive on an RVFV inhibition ELISA.

Seroreversion: An animal that tested negative on RVFV inhibition ELISA after having seroconverted (at least two RVFV seropositive test results) following RVFV vaccination.

Serostatus: The serological status of an animal for RVFV antibodies, either seropositive or seronegative.

The testing protocol to enrol farms into the longitudinal study was described in Chapter 2. All cross-sectional and longitudinal study sheep samples were tested with an inhibition ELISA (Paweska et al., 2005). The reported sensitivity for the inhibition ELISA is 100%, 100%, 99.56% and the specificity is: 99.52%, 99.29% and 99.65% in cattle, sheep and goats, respectively. To confirm the initial serostatus, the pre-injection and the 30-day post-injection samples from the longitudinal study sheep were confirmed by the gold standard of RVFV diagnostics, virus neutralization testing (VNT) (Swanepoel et al., 1986b).

Statistical Analysis:

Data were analysed using R 3.5.0 (R Core Team, 2019) in Rstudio 1.0.153 (RStudio Team, 2015) and all plots were made using the `ggplot2` (Wickham, 2009), `ggpubr` (Kassambara, 2019) and `egg` packages (Auguie, 2018). Prior to analysis, data exploration was undertaken. All continuous variables were visually assessed using a dot plot and histogram. The distributions of the histograms were often left skewed. Collinearity was assessed among the continuous and the categorical variables. For categorical variables, it was assessed using ggpairs() (from the `GGally` package [Schloerke et al., 2018]). ggpairs()

created a set of figures that includes a bar or density graph, a scatter or box plot, and an estimate of the correlation for each pair of variables (Emerson et al., 2013). For continuous variables, collinearity was assessed using ggscatmat() (`GGally` package), which produced a set of figures that include a scatter plot, a density plot and an estimate of the correlation for each pair of variables. If two variables had a correlation of >0.70 then one was excluded from the model. Collinearity was also assessed by examining the variance inflation factor (VIF) values for the continuous variables in the model as described by Zuur et al. (Zuur et al., 2010). A VIF of >3 indicated collinearity. The VIF values were calculated using the corvif() function[1] (Ieno and Zuur, 2015). Residuals were visualized using the `DHARMa` package (Hartig 2019) and a Hosmer-Lemeshow goodness of fit test (hoslem.test() from the `ResourceSelection` package [Lele et al., 2019]) were conducted for all logistic regression analyses.

Descriptive analyses were conducted on selected factors from the questionnaire that we considered as important in assessing the risk of RVFV infection. The full risk factor analysis was conducted by Ngoshe et al. (submitted). The descriptive statistics were calculated separately for farmers that grazed their animals on land they owned (private farmers) vs those that grazed their animals on land they did not own (communal farmers) because the question about the reason they kept the animals was asked with different options for private and communal farmers. The proportion of farms with a given factor were calculated using svymean() from the `Survey` package (Lumley, 2004), and calculated the 95% confidence interval using the Wald method with the confint() command. The variable "water source on farm" combines information collected during the first few weeks of the 2015 survey (presence of a water source on the farm) and that collected subsequently (presence of a water source on the farm and within access of the livestock). The crude seroprevalence in vaccinated sheep was calculated using svyciprop() (using the "likelihood" method) from the `Survey` package. The "likelihood" method calculates the proportion using a chi-squared distribution of binomial data and svyciprop() better estimates the confidence interval for proportions that are near zero.

The mean incidence of RVFV seroreversion was estimated using a Poisson regression with an offset (time in the study/time since vaccination [Flanders and Kleinbaum, 1995]). Given the low rate of seroreversion in the vaccinated longitudinal study sheep, no additional covariates were included to estimate the average rate of seroreversion. Since age cannot be

[1] The function can be sourced from http://highstat.com/Books/BGS/GAMM/RCodeP2/HighstatLibV6.R.

reliably determined by dentition after four years of age, for the cross-sectional study animals all animals older than four were assumed to be four years of age. While the results will be affected by the variation intrinsic in the fact that some of the animals are older than four, we still used age as a continuous term. We justify this based on the high mortality incidence and turnover rates previously described in sheep in this region which suggests that there are not a significant number of very old sheep compared to the younger animals (9.7 per 100 animal-years and 200% (over 30 and 33 months), respectively; Chapter 2).

To assess the probability of a sheep being seropositive following RVFV vaccination, we used a logistic regression. The model for the longitudinal sheep had seropositivity as the outcome and age, vaccine type and time since vaccination as the predictors, we also included random effect terms for farm and for individual sheep. We used the same model in the cross-sectional sheep with the addition of vaccine type (as longitudinal sheep were all vaccinated with Smithburn) and without the additional random effect for individual (as the vaccinated sheep were all only sampled once). This model was applied to the vaccinated, cross-sectional study sheep data using a mixed-effects logistic regression (package `glmmTMB` [Brooks et al., 2017]). All vaccinated sheep in the cross-sectional and longitudinal studies were from privately owned farms; thus, land tenure was not included in this model.

We estimated the range of probability that a previously vaccinated study sheep would be seropositive using the linear predictor equations for each cross-sectional and longitudinal study sheep using the following parameters: immediately after RVFV vaccination (zero time since vaccination) and for a sheep aged one and aged four.

Assuming that the variation in probability of seropositivity that we observed among the cross-sectional study sheep was due to differences in vaccine handling and management, we ran a simulation that used the data from the cross-sectional analysis, but included the intercept term from the longitudinal study sheep. This allowed us to predict the probability that the cross-sectional study sheep would have been seropositive at the time of sampling (in 2015) assuming they had the same initial conditions (seroconversion rate) immediately following vaccination with Smithburn as the longitudinal study sheep. We simulated 1,000 response data points from the model used to analyse the cross-sectional study sheep vaccinated by Smithburn (the outcome was serostatus and the predictors were time since vaccination, age and vaccination with Smithburn) using the `GLMMisc` package (Johnson et al., 2015).

To predict whether a farmer was likely to vaccinate any of their livestock against RVFV (cattle, sheep or goats), we used a multiple logistic regression. All continuous predictors in the model(s) were standardized, such that one unit was equal to one standard deviation, if the range of values for any given continuous predictor in that model exceeded 10. We used a logistic regression to predict whether a farm vaccinated for RVFV, where RVFV vaccination was the outcome factor and with 25 predictors (listed in Table 3-5). The predictors included several biosecurity procedures under the assumption that a farm may be more likely to vaccinate for RVFV if they invest in biosecurity. The RVFV vaccination model was conducted using a 10-fold, cross-validated, logistic regression with a 0.85 to 0.15 split of training vs test data, using the `Caret` package (Kuhn et al., 2018). The prediction capacity of the model was assessed using the area under the curve (AUC) and a receiver operating characteristic (ROC) curve.

RESULTS

Study Area Demographics and Seroprevalence

Cross-sectional Study: We surveyed 271 livestock farms within the study region (Figure 3-1). Farms were grouped based on whether the farmer owned the land the livestock were grazed on (private; n = 236) or whether farmers grazed their animals on public lands (communal; n= 29), and excluding those that gave conflicting responses to this question in 2015 and 2017. For each characteristic, the percent of farms that indicated they have that characteristic and the 95% confidence interval was calculated (Tables 3-1 and 3-2).

RVFV Antibody Persistence in Vaccinated Sheep

We enrolled 242 sheep into the longitudinal group vaccinated against RVFV. Three sheep did not seroconvert after vaccination and four others either seroconverted late or never had two seropositive tests in a row. Thus, the seroconversion rate was 97% (95% CI: 95-99%). Only two sheep seroreverted to being seronegative after having at least two RVFV seropositive test results following RVFV vaccination (0.9%; 95% CI: 0.1-3%; Table 3-3). All other sheep remained consistently seropositive during the study. The average rate of seroreversion among all longitudinal study sheep, across all farms was 0.0012 per 3 animal-months (95% CI: 0.0002-0.004) (which is 0.46 sheep per 100 animal-years [95% CI: 0.08-1.4]). At the current rate of decline, detectable RVFV antibodies will persist for

the life-time of the sheep (the calculated rate of seroreversion is equivalent to RVFV antibodies persisting 216 years (95% CI: 70-1300 years).

Table 3-1 Characteristics of cross-sectional study farms. Farms were classified as either grazing their animals on privately owned or communal land. The sample size for all farmers that responded are given at the top with the farm type. Farms with missing values were excluded. Columns left blank indicate that questions was not asked of farmers that owned that type of farm. 95% CI = 95% confidence interval.

Characteristic	Private Farms (n = 226)		Communal Farms (n = 29)	
	Percent Yes (%)	95% CI	Percent Yes (%)	95% CI
Own multiple ruminant species	62.8	[56.5, 69.1]	48.3	[29.8, 66.8]
Kept in an open corral during the night	40.7	[34.3, 47.1]	65.5	[47.9, 83.1]
Kept in a roofed corral during the night	11.1	[7, 15.2]	34.5	[16.9, 52.1]
Kept in the field during the night	81	[75.8, 86.1]	13.8	[1, 26.6]
Kept in a community corral during the night	0.4	[-0.4, 1.3]	6.9	[-2.5, 16.3]
River(s) present on farm	32.3	[26.2, 38.4]	51.7	[33.2, 70.2]
Pan(s) present on farm	38.1	[31.7, 44.4]	31	[13.9, 48.2]
Dam(s) present on farm	72.6	[66.7, 78.4]	41.4	[23.1, 59.6]
Trough(s) present on farm	97.8	[95.9, 99.7]	79.3	[64.3, 94.3]
Spring(s) present on farm	37.6	[31.3, 43.9]	17.2	[3.2, 31.2]
RVFV previously confirmed on farm	30.5	[24.5, 36.5]	10.3	[-0.9, 21.6]
Previously vaccinated for RVFV	46	[39.5, 52.5]	6.9	[-2.5, 16.3]
Meat is the main industry of the farm	61.5	[55.1, 67.9]	44.8	[26.4, 63.2]
Meat and wool are the main industries of the farm	22.1	[16.7, 27.5]	NA	
Resale is the main industry of the farm	10.2	[6.2, 14.1]	51.7	[33.2, 70.2]
Dairy is the main industry of the farm	3.1	[0.8, 5.4]	13.8	[1, 26.6]
Wool is the main industry of the farm	2.2	[0.3, 4.1]	3.4	[-3.3, 10.2]
Meat and dairy are the main industries of the farm	0.9	[-0.3, 2.1]	NA	
Ceremony is the main reason the animals are kept	NA		10.3	[-0.9, 21.6]
Wealth is the main reason the animals are kept	NA		6.9	[-2.5, 16.3]

NA = not asked.

Of the 2015 cross-sectional study sheep (n = 1589), 16% (n = 262) were reported to be vaccinated. All of the vaccinated sheep from the cross-sectional study were from private farms. The crude seroprevalence among vaccinated sheep was 34% (95% CI: 28-39%) and was 41% (n = 71; 95% CI: 30-53%) among sheep vaccinated with Smithburn. Sheep farmers reported using a modified-live RVFV vaccine product in sheep 69% of the time (27% specified they used the Smithburn vaccine and 22% specified they used Clone 13, and 20% did not specify which modified-live vaccine they used), 13% indicated they used an inactivated vaccine, 6% indicated they used a combination of products and 12% did not know what vaccine product they used. The estimated time since vaccination ranged from 0.1-4.9 years (4.9 years were sheep that were four years of age or older when sampled in October-December 2015 that were vaccinated at some point in 2011 and were included in the study) and sampled. Ages among the cross-sectional study sheep ranged from 0.5 years to 4 years of age or older (aging by dentation is not accurate beyond four years of age).

Table 3-2 Additional characteristics of cross-sectional farms in either 2015 or 2017 and classified as either privately owned or communal.

	Private Farms					Communal Farms				
Variable	Mean	Standard Deviation	Median	Range	Number of Farms	Mean	Standard Deviation	Median	Range	Number of Farms
Number of cattle	256.6	721.9	100	2-8550	179	140.5	212.1	44	4-600	11
Number of sheep	1082.9	1917.7	293	2-13000	169	41.1	56.9	20	1-200	11
Number of goats	77.9	108.2	40	2-550	42	65.5	148	17	2-600	25
Farm size (ha)	5532.3	49019	1200	2-738000	226	2781.4	4300.6	1000	10-13000	25
Number of owners that share communal area						29.1	57	15	1-300	28

Table 3-3 Vaccinated sheep from the longitudinal study by date and farm identification, including the percent of animals that seroconverted, (the total number of vaccinated animals sampled and tested from that farm inclusive of new replacement sheep) and [the number of new sheep enrolled to replace the sheep that died during the previous three months]. Two sheep seroreverted.

Farm ID	June 2016	September 2016	December 2016	March 2017	June 2017	September 2017	December 2017	March 2018	June 2018	September 2018	December 2018
131S	0% (24) [0]	0% (23) [6]	0% (28) [0]	0% (21) [2]	0% (24) [0]	0% (28) [4]	0% (30) [0]	0% (30) [0]	0% (30) [0]	0% (30) [0]	0% (28) [0]
140T	0% (22) [0]	0% (21) [0]	0% (23) [0]	0% (23) [0]	0% (23) [0]	0% (23) [0]	0% (23) [0]	0% (23) [0]	0% (23) [0]	0% (21) [0]	0% (21) [0]
211B	0% (10) [0]	0% (10) [0]	0% (10) [0]	0% (10) [0]	0% (9) [0]	0% (9) [0]	0% (9) [4]	0% (11) [0]	0% (11) [2]	0% (13) [6]	0% (12) [6]
222P	0% (42) [0]	0% (47) [0]	0% (46) [0]	0% (46) [0]	0% (37) [0]	0% (43) [0]	0% (38) [0]	0% (39) [0]	0% (35) [0]	0% (36) [0]	0% (35) [0]
259E	0% (21) [0]	0% (22) [0]	0% (20) [6]	0% (20) [0]	0% (18) [3]	0% (19) [0]	4.8% (21) [3]	0% (22) [0]	0% (19) [0]	0% (22) [0]	0% (21) [0]
278B	0% (18) [0]	0% (13) [0]	0% (13) [0]	0% (13) [0]	0% (13) [0]	0% (10) [0]	0% (8) [0]	0% (6) [0]	0% (7) [0]	0% (6) [0]	0% (6) [0]
284P	0% (10) [8]	0% (10) [0]	0% (16) [0]	0% (15) [0]	0% (10) [2]	0% (16) [0]	6.7% (15) [3]	0% (17) [0]	0% (17) [0]	0% (16) [0]	0% (14) [0]
321H	0% (24) [0]	0% (23) [0]	0% (20) [5]	0% (17) [4]	0% (23) [0]	0% (25) [6]	0% (31) [0]	0% (31) [7]	0% (38) [0]	0% (38) [0]	0% (36) [0]

Of the cross-sectional study sheep that were vaccinated, only 253 were included in the analysis as the estimated age was occasionally missing. Age was positively and significantly associated with probability of seropositivity among the vaccinated cross-sectional study sheep (odds ratio (OR) = 2.64; 95% CI: 1.69-4.12). The time since vaccination was not significantly associated with seropositivity (OR = 0.80; 95% CI: 0.58-1.10). The vaccine type (Smithburn, Clone 13, unspecified, modified-live, inactivated or a using a mixture of products) was not significant (Figure 3-3). The random effect of farm had an estimated variance of 0.96 (standard deviation (SD): 0.981). The estimated probability that a 1-year-old cross-sectional study sheep vaccinated with Smithburn was seropositive was 0.10 and that for a 4-year-old sheep was 0.68. A Hosmer and Lemeshow goodness of fit test was non-significant, indicating that the model fit was sufficient (see Figure S3-1 for analysis of residuals).

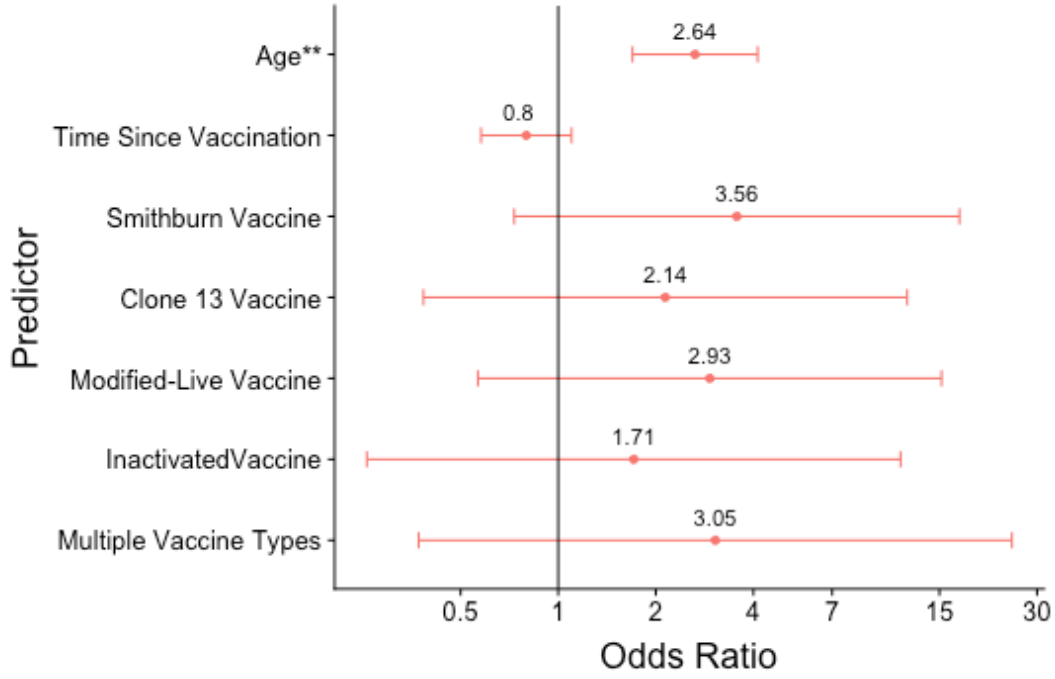


Figure 3-3 The odds ratios of the predictors of RVFV seropositivity in reportedly vaccinated cross-sectional study sheep. The points represent the odds ratios and the lines are the 95% confidence intervals. The random effects of township and farm ID were not included. **indicates the term had a p-value of < 0.01.

In comparison, in the longitudinal study sheep, neither age (OR: 0.78; 95% CI: 0.37-1.62) nor time since vaccination (OR: 0.96, 95% CI: 0.44-2.1) were significantly associated with seropositivity. The variance for the random effect of individual animal was 7.87 (SD: 8.82), whereas the variance estimated by farm was 2.84 e-8 (SD: 0.0002). The estimated probability that a 1-year-old longitudinal study sheep vaccinated with Smithburn was seropositive was 0.99 and that for a 4-year-old sheep was 0.99. A Hosmer and Lemeshow goodness of fit test was non-significant, indicating that the model fit was sufficient.

The mean probability of cross-sectional study sheep that were vaccinated against RVFV being seropositive immediately following vaccination (assuming time to seroconvert, based on the mean random intercept) was estimated to be 0.02. In comparison, the probability of longitudinal study sheep vaccinated with Smithburn being seropositive immediately following vaccination was 0.99. There was more variation in the initial serostatus after vaccination by farm among the cross-sectional study sheep (with the probabilities ranging by farm from 0.005-0.06) than among the longitudinal study sheep (0.99-0.99; Figure 3-4).

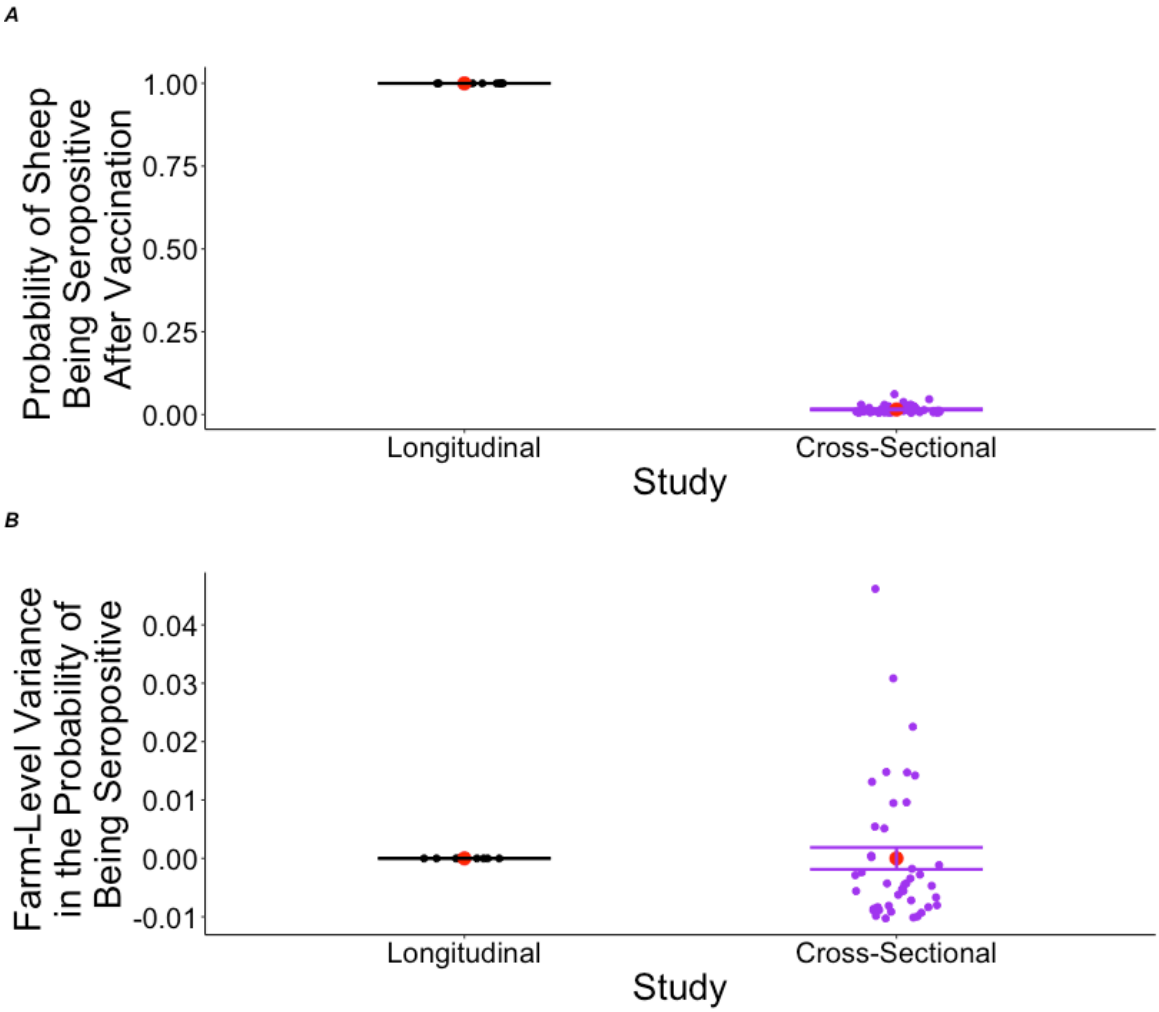


Figure 3-4 A) The probability that the vaccinated sheep on each farm being seropositive immediately (allowing time for initial seroconversion) after vaccination with Smithburn vaccine (based on the predicted random intercepts). B) The individual farm variance from the mean probability of being seropositive immediately after vaccination (variance in random intercepts) between the farms in each study type. The farms enrolled in the longitudinal study are in black and the farms in the cross-sectional study are in purple. The mean of each group is in red, with standard error bars.

By imposing the initial serostatus of the longitudinal study sheep (model intercept) on the cross-sectional study sheep data, we predicted that the seroprevalence at sampling in 2015 in cross-sectional study sheep vaccinated with Smithburn would have been 100%, rather than the 41% observed, if they had been vaccinated as the longitudinal study sheep were.

RVFV Vaccination Usage

Among the 229 private farmers that indicated whether or not they vaccinated against RVFV in the full farm survey, 47% reported having vaccinated against RVFV at some point in the past, compared to 7% of communal farmers (n = 30). Of respondents to the repeat survey (2017), 38% of private farmers (n = 159) and 12% of communal farmers (n = 17) reported having vaccinated against RVFV during the previous 18 months. Overall, 41 farmers indicated that they vaccinated their animals against RVFV during both time periods and 22 farmers vaccinated for the first time during the interim 18 months between surveys. Farmers used a variety of RVFV vaccine types and protocols (Table 3-4). Farmers that have previously used an RVFV vaccine were asked to estimate the percentage of their animals were currently vaccinated (n = 104), the mean estimate was 58% (range 0-100, median 60; Figure 3-2). Farmers that vaccinated their animals before or during the 2010 RVF outbreak were asked if they thought the vaccine protected their animals and 86% (n = 70; 95% CI: 75-92%) indicated that they thought the vaccine they used protected their animals.

Table 3-4 All farmers that indicated they vaccinated their livestock (cattle, sheep and goats) against RVFV were asked what type of vaccine they gave most recently. A majority of them indicated they used a modified live vaccine (Clone 13, Smithburn or unspecified). In 2017, all farmers that indicated they vaccinated against RVFV at any time in the past or within the past 18 months (full and repeat surveys, respectively) were asked what their vaccination strategy was: whether they vaccinated: all of their animals once, when they are young; all animals every year or only vaccinate prior to a predicted outbreak.

	Private		Communal	
Variable	Percent (%)	95% Confidence Interval	Percent (%)	95% Confidence Interval
Vaccine Type	***n = 107***		***n = 2***	
Modified-Live (Unspecified)	23.4	[15.3, 31.4]	0	[0, 0]
Clone 13	16.8	[9.7, 23.9]	0	[0, 0]
Smithburn	15	[8.2, 21.7]	0	[0, 0]
Inactivated	13.1	[6.7, 19.5]	0	[0, 0]
Modified-Live and Inactivated	5.6	[1.2, 10]	0	[0, 0]
Smithburn and Clone 13	0.9	[-0.9, 2.8]	0	[0, 0]
Clone 13 and Inactivated	0.9	[-0.9, 2.8]	0	[0, 0]
Unknown	24.3	[16.1, 32.5]	100	[100, 100]
Vaccination Strategy	***n = 64***		***n = 2***	
Vaccinate Once	43.8	[31.5, 56]	0	[0, 0]
Vaccinate Yearly	35.9	[24.1, 47.8]	50	[-48, 148]
Vaccinate Before an Outbreak	20.3	[10.4, 30.2]	50	[-48, 148]

Farmers that had a history of confirmed RVF on the farm (odds ratio [OR]: 6.6; 95% CI: 2.6-16.5), had a larger population of sheep (OR: 3.7; 95% CI: 1.8-7.9) and vaccinated their animals against brucellosis (OR: 3.2; 95% CI: 1.4-7.3) had a higher odds of vaccinating for RVFV. Whereas those farmers that had higher numbers of pans within access of the animals on the farm (OR: 0.5; 95% CI: 0.31-0.81) and those that employ quarantine as a biosecurity measure (OR: 0.26; 95% CI: 0.09-0.75) had a lower odds of vaccinating their sheep against RVFV (see Figure 3-5 and Table 3-5 for all estimates). A Hosmer and Lemeshow goodness of fit test indicated the fit was sufficient (not significant). The classification accuracy was 0.70 (95% CI: 0.53-0.84) and the area under the ROC curve was 0.7076.

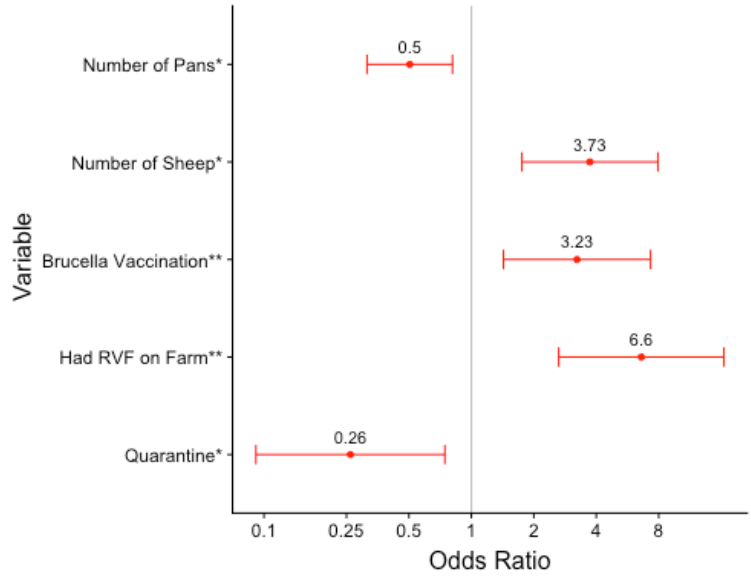


Figure 3-5 The odds ratios for the significant predictors of RVFV vaccination. The two variables, number of sheep and number of pans, are plotted as unstandardized. The points represent the odds ratios and the lines are the 95% confidence intervals. *indicates the term was significantly associated with RVFV vaccination with a p-value of < 0.05. **indicates the term had a p-value of < 0.01.

Table 3-5 The coefficient estimates and 95% confidence intervals for all predictors in the multiple regression RVFV vaccination model. The continuous variables remain standardized to permit the comparison of the estimate size on the same scale as the other variables. The numbers of cattle, sheep, goats and pans, as well as the size of the farm were standardized. *indicates the term was significantly associated with RVFV vaccination with a p-value of < 0.05. **indicates the term had a p-value of < 0.01.

Variable	Estimate	95% Confidence Interval	P-value
Intercept	-5.475	[-8.119, -2.832]	0.000
RVFV previously confirmed on farm	1.887	[0.97, 2.804]	0.000**
Survey year (2015)	1.494	[-0.61, 3.598]	0.164
Farmer vaccinates animals against any pathogen	1.24	[-0.363, 2.843]	0.129
Previously vaccinated for brucellosis	1.173	[0.356, 1.99]	0.005**
Farmer uses tick control	1.073	[-0.104, 2.25]	0.074
Number of sheep (standardized)	0.884	[0.129, 1.639]	0.022*
Farmer feeds/works with/treats sick animals after the healthy ones are fed/worked with	0.709	[-0.199, 1.617]	0.126
Farmer prevents nose-to-nose contact between species	0.633	[-0.216, 1.483]	0.144
Number of ruminants purchased in the past year	0.508	[-0.054, 1.07]	0.076
The land the farm is on is privately owned	0.496	[-1.248, 2.239]	0.577
Farmer uses mosquito control	0.47	[-0.384, 1.324]	0.28
Farmer keeps pregnant animals separate from the rest of the herd	0.406	[-0.4, 1.213]	0.323
Farm size (standardized)	0.393	[-0.871, 1.658]	0.542
Each species has own equipment	0.144	[-0.699, 0.987]	0.738
Farmer exports animals	-0.011	[-1.163, 1.142]	0.985
Number of cattle (standardized)	-0.042	[-0.736, 0.653]	0.907
Keeps species separate	-0.057	[-0.908, 0.794]	0.895
Vaccinates (for an unspecified disease) new animals when brought into the herd	-0.243	[-1.066, 0.58]	0.563
Number of goats (standardized)	-0.272	[-0.674, 0.13]	0.185
Disinfects transport vehicles before and after transport	-0.276	[-1.071, 0.519]	0.496
Dips or uses tick treatment on new animals when brought into the herd	-0.352	[-1.345, 0.641]	0.487
Gives antibiotics to new animals when brought into the herd	-0.398	[-1.674, 0.877]	0.541
Had animals that had abortions in the previous three months	-0.474	[-1.374, 0.425]	0.302
Number of pans within access of the animals	-0.552	[-1.026, -0.077]	0.023*
Farmer implements quarantine for new animals	-1.343	[-2.392, -0.294]	0.012*

DISCUSSION

Here, we have determined that proper vaccination with Smithburn induced anti-RVFV antibodies, which will likely remain for the lifetime of the sheep. Despite this, sheep reported to be vaccinated routinely on farms throughout the study area had quite a low seroprevalence, even those reported to be vaccinated with Smithburn. We estimated what the probable serostatus was in the sheep immediately after they were vaccinated and found that it likely varied between farms. Age remained a significant driver of seropositivity in the vaccinated cross-sectional study sheep. Additionally, several historical, biosecurity and environmental characteristics on the farm can be used to predict whether a farmer is likely to vaccinate for RVFV and can be used to provide targeted advice. These results indicate that improved vaccine maintenance and practices along the chain of custody would likely

result in a higher population seroprevalence in the study area and presumably improve herd immunity in the region.

RVFV Antibody Longevity in Vaccinated Sheep

Vaccine-Induced Antibody Longevity in Longitudinal Study Sheep

The results of our longitudinal study on antibody longevity, measured by inhibition ELISA, demonstrated that almost all vaccinated sheep seroconverted after vaccination with Smithburn vaccine and remained seropositive throughout the course of the study after this single dose of vaccination. While this has long been reported anecdotally, many epidemiological models still include a term to represent waning immunity (Sumaye et al., 2019, Chamchod et al., 2014, Bicout and Sabatier, 2004). This study adds substantially to the literature in providing clear evidence of long-lived antibody responses in sheep following vaccination.

While the relationship between antibody response and immunity is not fully understood, several studies indicate that antibodies are protective against challenge infection (Swanepoel et al., 1986a, Coackley et al., 1967), and immune serum has been shown to protect lambs against experimental infection (Bennett Jr et al., 1964). The results of this study therefore suggest that that existing commercial vaccines would have the potential to induce high enough levels of herd immunity to prevent outbreaks if a sufficient proportion of the population was vaccinated.

In our study, only two sheep seroreverted (two or more seronegative results), while the remainder of the sheep that seroconverted following vaccine remained seropositive for the 30-month duration of the study. This is consistent with previous reports. Sheep immunized with Smithburn strains, 10^6 $IMLD_{50}$ and 10^8 $IMLD_{50}$ retained serum neutralizing antibodies 15 months later or withstood challenged 19 months later, respectively (Pini, 1973). However, Pini's (1973) study and the studies described in the introduction (Capstick and Gosden, 1962, Coackley et al., 1967) used strains of Smithburn that had higher infectious doses than we inoculated the sheep with as we used the commercial Smithburn product available to the farmers ($10^{4.4}$ $IMLD_{50}$). Thus, our study demonstrated the dynamics of antibody persistence under normal farm conditions. Our results indicate that once a sheep seroconverts following vaccination with the Smithburn RVFV vaccine, it will likely

maintain those antibodies for the duration of its lifespan. Therefore, if antibody presence does correlate to protection from RVF, these animals should continue to be protected.

In our study three sheep did not seroconvert following vaccination and four additional animals seroconverted late. The late seroconversions may indicate that they seroconverted in response to natural infection rather than due to vaccination. These results suggest that we would only expect a very small proportion of sheep (0.03) to be seronegative following vaccination with Smithburn.

Vaccine-Induced Antibody Longevity in Cross-Sectional Study Sheep

Given the high rate of seroconversion in the longitudinal study sheep following vaccination by our project staff, we were surprised to find such a low seroprevalence (34% 95% CI: 28-39%) among cross-sectional study sheep reportedly vaccinated by farmers. This indicates that there may be much lower herd immunity than suggested by vaccination coverage/use and aligns with the finding of Mdlulwa and Ngwane (2017) that there was no significant association between the number of sheep lost during the 2010 RVF outbreak in South Africa and whether the farmer vaccinated their sheep against RVFV.

A positive association between seropositivity and age was highly significant. While age is certainly an important contributor to RVFV seropositivity in nearly every livestock and human risk factor analysis (Bett et al., 2018, Matiko et al., 2018, Nanyingi et al., 2017, Ngoshe et al. submitted), it was expected that seropositivity associated with vaccination would be high enough to mask to effect of age, as was seen in the longitudinal study sheep. However, due to the apparent suboptimal vaccine management practices along the supply chain from the manufacturer to final injection into the sheep, the seroprevalence was low enough that age was the most significant predictor.

Time since reported vaccination did not have a significant association with seropositivity. This result is consistent with the finding of long-lived antibody following vaccination in our longitudinal study.

It was expected that the type of RVFV vaccine product would predict seropositivity, specifically that the modified-live vaccines would induce antibodies for a longer duration than the inactivated vaccines. Assuming that all modified-live vaccines are likely to produce relatively long-lasting antibody persistence (though Clone 13 induced antibodies

do not appear to persist as long as those induced by Smithburn) and inactivated vaccines require annual booster injections, then the variance in the serostatus at the time of sampling in 2015 could be expected to be driven by the type of vaccine used. Our hypothesis that the type of RVFV vaccine would predict seropositivity was based on the following evidence: (a) the duration of post-Smithburn vaccination antibody responses to in sheep reported in the literature (Pini, 1973); (b) the extremely low seroreversion rate in sheep vaccinated with Smithburn; (c) a clinical trial found that of 91% of sheep (n = 77) that seroconverted after vaccination with Clone 13, antibodies persisted in 53% of sheep after a year (Njenga et al., 2015), and (d) that the inactivated vaccine requires an annual booster dose. Despite the evidence of RVFV antibody persistence in sheep from (a) and (b), the estimated seroprevalence in cross-sectional study sheep reportedly vaccinated with Smithburn had only a slightly higher seroprevalence (41%, 95% CI: 30-53%) than all vaccinated sheep in the study regardless of vaccine product (34%, 95% CI: 28-39%).

We were able to retrospectively predict the initial serostatus immediately following Smithburn vaccination in sheep from the cross-sectional study. The initial seroprevalence varied by farm (Figure 3-4), but the effect was tempered by the age. As age is a continuous variable, at the intercept it is set to zero and therefore the intercept is estimated to be extremely low. For this reason, we also examined the wide variance in the probability of a one- or four-year-old animal being seropositive. Even with the effect of age on the intercept value, the variance within the probability of seropositivity in the cross-sectional study sheep was much higher than that in the cross-sectional sheep (where there was no variation between farms). Our results suggest that the using optimal vaccination protocols, as our research team did, could prevent the low seroprevalence seen in vaccinated sheep. Our simulation used the predicted initial serostatus of the longitudinal study sheep in the model with the cross-sectional study sheep parameters. The results suggested that under these conditions the cross-sectional study sheep vaccinated with Smithburn would have a seroprevalence of 100%, rather than 41%. Given the evidence we presented above, we suspect that the low seroprevalence detected in reportedly vaccinated cross-sectional study sheep were influenced by suboptimal vaccination practices/management.

Suboptimal vaccination practices/management could include issues with maintaining cold chain, use of expired vaccine, and incorrect administration of the vaccine. Both Clone 13 and Smithburn vaccines have been shown to be inactivated at approximately 37^{o}C after only a few days, and Clone 13 was also inactivated at room temperature after 9-10 days (Daouam et al., 2014). On the label of the Smithburn vaccine the manufacturer states that

once reconstituted, “The vaccine is now ready for use and must be injected without delay,” (Onderstepoort Biological Products, 1947). On clarification, the manufacturer indicated that this meant the vaccine should be given within six hours of reconstitution. Anecdotally, it is common for farmers to keep open medications or vaccines in the refrigerator until the next time they need to use it. If they were to use the Smithburn vaccine in this fashion it is likely that the next time they used the vaccine, it would no longer be efficacious.

We recommend that an education campaign be initiated to improve vaccine practices. This could include educating retailers, transportation companies, farmers, and farm managers on the proper maintenance, use and lifespan of reconstituted Smithburn vaccine. We recommend further analysis to examine vaccine management policies on the farms to identify potential issues that could contribute to the low seroconversion rate. We also recommend that, farmers who use Smithburn vaccine, at a minimum, vaccinate their young lambs with Smithburn at least once (after the age of six months and before chance of pregnancy) and during the winter/dry season, if they cannot vaccinate all of their animals yearly. This would minimize the side effects and maximize the benefits given the long duration of antibody presence that we have detected here. Finally, we recommend that farmers request their veterinarians to collect blood samples following vaccination from a subset of lambs to confirm seroconversion via laboratory analysis.

Perceptions of RVFV Vaccination

We found that most farmers (85%) think that RVFV vaccines worked to protect their animals during the 2010 outbreak. During that same outbreak, rumours of poor efficacy of Clone 13 were reported in South Africa. However, studies reported by the U.N. Food and Agriculture Organisation (FAO) and from other parts of Africa indicated that Clone 13 was indeed efficacious in the field. The reported “poor efficacy” was attributed to improper use of the vaccine or interruption of the cold chain (FAO, 2015). Similarly, our longitudinal study results have demonstrated that Smithburn vaccine produces long-lasting antibodies, and significant variation in the antibody response of owner-vaccinated sheep across the farms sampled in our study.

Our results suggest there is likely a discrepancy in the farmers’ perception of how protected their vaccinated sheep are from RVFV and the proportion of vaccinated sheep that actually have antibodies to protect them from RVFV. The median estimate made by farmers regarding the proportion of their herd that was vaccinated was 60%. More than 50

farmers estimated that 100% of their herd had been vaccinated. These farmers are investing in an RVFV vaccination program, but likely their animals are not receiving the protection that the farmer expects. While it has been shown that vaccinated cattle whose antibodies waned or were never induced were still immune to RVFV (Barnard, 1979), we cannot assume this would be true for these sheep as the initial vaccination did not appear to be efficacious and we have shown that sheep have high seroconversion rates and antibody persistence is long-lived following vaccination with Smithburn. We recommend that farmers work with their veterinarian to have a subset of their animals tested for the presence of RVFV antibodies following vaccination.

Limitations

Our method of estimating whether an animal was vaccinated was based on the year the farmer vaccinated and the age on the animal. It is possible that we over estimated the number of vaccinated animals and that this contributed to the low seroprevalence rate detected. Our estimate of the proportion of animals vaccinated on the farm based on the animals we sampled did not align perfectly with the farmer's estimation of the percent that was vaccinated (Figure 3-2). However, 79% (95% CI: 69-89%) of farmers either vaccinated all sheep yearly or all young at least once against RVFV. This suggests that for these 79% of farms, the sheep we assumed were vaccinated would likely have been vaccinated at some point in their lives and we have shown that the antibodies likely persist for the lifetime of the sheep. Thus, we propose that at least 80% of the sheep that we assumed were vaccinated were in fact likely to have been vaccinated. This does imply that our estimates of the time since vaccination are likely biased toward a shorter time period as older animals may have only been vaccinated when they were lambs, rather than in the most recent year that the farmer vaccinated.

When we converted our assumption of the vaccine status of individual sheep that were sampled to an estimate of the proportion of vaccinated animals on the farm (Figure 3-2), we saw there was a discrepancy between our estimate and the farmer's. The discrepancy between the two estimates of the proportion of animals vaccinated on the farm may not be as large as it seems. The estimates were likely affected by recall bias, as most farms did not provide individual records on the animals that were sampled. Additionally, if animals were brought in from other farms they may have mistakenly been classified as vaccinated (or were vaccinated previously and misclassified as not vaccinated).

Finally, we collected samples from the animals based on age stratification (three animals per age group: < 2 years of age, 2-3.9 years of age and 4 years and older), thus our estimate of the proportion of animals on the farm that were vaccinated would necessarily reflect this stratification rather than the actual age structure of the population on that farm. If, for example, we sampled three sheep that were four years of age or older and the farmer reported that he vaccinated in 2011 (and the survey was conducted in 2015), then we would assume those three sheep were vaccinated and the other six sheep we sampled were younger and assumed not to be vaccinated. This would mean our farm level estimate would be 30%, but if the farmer sells most animals before they become too old (e.g. a meat producer) and sheep aged four years and older constitute only 15% of the herd, the farmer's estimate of vaccinated sheep would only be 15%. Though our estimate of the proportion of sheep on the farm would be incorrect (30%), our assumption of which individual sheep were vaccinated would be correct. All of these factors surely contributed to some of the variation that was observed in the serostatus of vaccinated animals.

Predictors of RVFV Vaccination

Some of the predictors of RVFV vaccination identified in this study were not surprising, such as whether the farm had previously suffered a confirmed RVF outbreak. Consistent with other studies (Mdlulwa and Ngwane, 2017), we also found that flock size was a significant positive predictor of vaccination status. However, this does not necessarily reflect socio-economic status as owning land (the primary indicator of high socio-economic status in this analysis) was not associated with vaccinating against RVFV.

We might have expected farmers with high numbers of pans on the farm to be more likely to vaccinate because of the association between pans and RVFV infection risk (Ngoshe et al., submitted). However, paradoxically, vaccinating for RVFV was negatively associated with the number of pans animals could access on the farm. While we do not have a clear explanation for this finding, but the results do indicate that farmers may benefit from further information about the ecological risks associated with pans, and the patterns of low-level RVFV transmission in sheep in the region (Chapter 2). While vaccination of animals in general (with any vaccine) was not a significant predictor, as was also reported following the 2010 outbreak in South Africa by Mdlulwa and Ngwane (2017), farmers that vaccinated for brucellosis were more likely to vaccinate against RVFV. This may be because those farmers that vaccinate for brucellosis are more aware of the importance and concerned with the prevention of controlled (brucellosis) and reportable (RVFV) diseases.

It was surprising that owning the land (the primary indicator of higher socio-economic status in this analysis) was not associated with vaccinating their animals against RVFV. One explanation may be that during the 2010 outbreak, state veterinarians vaccinated animals against RVFV for free (Mdlulwa and Ngwane, 2017). During our study, we asked about RVFV vaccination at any time in the past, including during the RVFV outbreak, and this likely included periods when vaccine was delivered free of charge to communal farmers.

We hypothesized that farmers that invest more in biosecurity would be more likely to invest in RVFV prevention. However, except quarantining new animals, none of the factors relating to biosecurity were significantly associated with RVFV vaccination. Surprisingly keeping new animals in quarantine before introducing them into the herd was negatively associated with RVFV vaccination. It is possible that these farmers feel like they are reducing the likelihood of introducing a RVFV into their herd by quarantining the new animals and given the short incubation time do not need to vaccinate against RVFV. For farmer decision-making, it may be helpful to provide information about mosquito transmission of RVF and advise that the quarantined animals would need to be screened off and protected from mosquitoes to prevent the introduction of RVFV from importation. As with those farmers with more pans on their farm, they should also be reminded that RVFV can also be maintained on the farm without being introduced from another source. Finally, Mdlulwa and Ngwane (2017) identified a correlation between farm size (ha) and RVFV vaccination, whereas our study did not. This may be due to a difference in methodology as their study used chi-square analysis with 2x2 contingency tables, which would not account for confounding by the other variables. The multiple regression that we conducted would account for confounding. Indeed, if we use a Mann-Whitney U test to look for a correlation between farm size and vaccination, we find there is a significant difference (p-value 0.0007). This demonstrates that farm size is likely a confounder, perhaps with the number of sheep on the farm size or other predictor.

Understanding the predictors of RVFV vaccination can help target directed education materials to farmers who are not vaccinating, particularly those with many accessible pans on the farms. Métras et al. (2013) estimated the farm-to-farm R_0 to be 4.3 during the 2010 outbreaks, indicating that the regional herd immunity threshold would need to be 77% to prevent another large outbreak. Unless a substantial number of farms add RVFV

vaccination to their management plan the region will continue to be at risk of RVF outbreaks, even if vaccine use and maintenance is improved.

CONCLUSION

This paper examined the current vaccination practices and the efficacy of these practices in inducing antibodies against RVFV in the study area in central South Africa. We determined that antibodies following Smithburn vaccination, when administered appropriately, are long lasting and likely are maintained for the lifespan of a normal sheep. Despite this, only 33% of reportedly vaccinated animals have RVFV antibodies. Our results indicate that there is variation in seroprevalence following vaccination at the farm level, suggesting that suboptimal vaccine handling provides a likely explanation for low seroprevalence in animals vaccinated by farmers. Further advice on vaccine handling and administration of RVFV vaccines would likely be beneficial. Our analysis of determinants of RVF vaccination indicates that some at-risk farms may also benefit from information about ecology and interepizootic transmission of RVFV. Properly maintained and administered Smithburn vaccination should induce long-lasting antibodies and would be an important contributor to raising the population level herd immunity and preventing the next large outbreak of RVFV.

SUPPLEMENTAL FIGURES

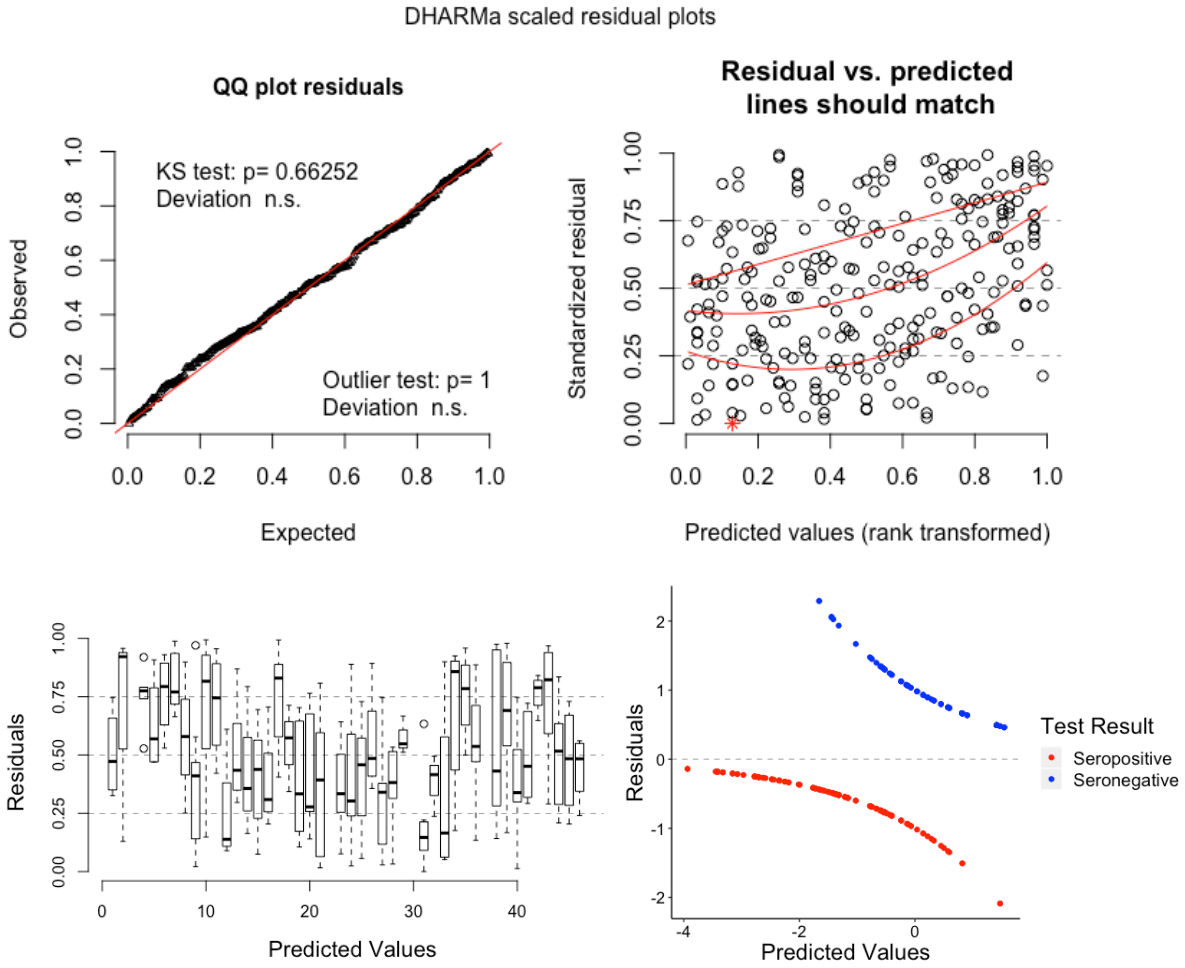


Figure S3-1. Residual analysis of the multiple logistic regression conducted on the data from the reportedly vaccinated cross-sectional study sheep. A) A normal quantile-quantile (Q-Q) plot shows the quantiles of the observed residuals vs those of the expected distribution. The Kolmogorov-Smirnov test and outlier test were automatically conducted by DHARMa and both suggest a non-significant deviation. B) The standardized residuals vs predicted values, indicate a slight pattern, but are sufficient for our analysis. C) The residuals are plotted against the random effect of farm ID. D) Unstandardized residuals vs the predicted values demonstrate the typical pattern of a logistic regression.

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Chapter 4: Interepizootic Persistence of Rift Valley Fever Virus in South Africa

ABSTRACT

The complex ecology of Rift Valley fever virus (RVFV) has yet to be fully understood, particularly as there is mounting evidence that RVFV circulates at extremely low levels between outbreaks. In order to understand RVFV persistence, we developed a deterministic, ordinary differential equation (ODE), susceptible-exposed-infected-recovered (SEIR) model to ask whether persistence is possible in a single pan. The simulation demonstrated that persistence is possible whilst also capturing the known patterns of outbreaks in central South Africa. Crucially, persistence was only possible in a single pan over a narrow range of parameters. One critical parameter was the transovarial transmission rate, which was estimated to be higher than often assumed; lower values would necessitate large annual outbreaks to maintain infection in the mosquitoes, which would then drive the median seroprevalence to levels higher than what is observed in nature. Thus, persistence was balanced between extinction and a system with high seroprevalence. This was reflected in an R_0 that was close to 1 in this interepizootic system, making it very sensitive to initial conditions. Relatively low rates of vaccination (~30%) could drive RVFV to local extinction in a pan; however, overcoming the high rate of transovarial transmission would require maintenance of the vaccination coverage for years to drive the virus to extinction. Finally, we found that while the parameters that support interepizootic persistence can result in periodic, large epizootics as expected, the outbreaks last much longer than observed historically. This could be due to a shift in parameter values, such as vector bite rates and host-vector ratios, that changes the dynamics during an epizootic and increases R_0. This suggests that different mechanisms may drive epizootic and interepizootic RVFV transmission and require different management schemes to control the number of infections caused by each.

INTRODUCTION

Rift Valley fever (RVF) is caused by a zoonotic arbovirus. Outbreaks of RVF can be expansive and are followed by periods of apparent quiescence (Linthicum et al., 2016). RVF epizootics can cause massive losses among ruminant livestock: in 2008-2011 there were 18,767 individual cases in livestock and 302 cases of RVF in people in South Africa (Glancey et al., 2015). Outbreaks across Africa occur every 5-15 years (Davies et al., 1985), though in South Africa, large RVF outbreaks tend to occur every 23-35 years (1951, 1974-75, 2009-10) (Pienaar and Thompson, 2013). Smaller epizootics, sometimes isolated on a single farm, have occurred across South Africa during the period between the large epizootics (Pienaar and Thompson, 2013). Specifically, between 1983-2017, 13 outbreaks/clusters of outbreaks of RVF were reported in South Africa. The western Free State/eastern Northern Cape was/could have been affected by five of these: in 1984-85 (Kroonstad), in 1987 and in 1989 (unknown location, may have affected the area of interest), 2010 (an expansive outbreak across all of the Free State), and 2011 (eastern Northern Cape) (Pienaar and Thompson, 2013). Even when no cases of RVF were reported between the epizootics, significant empirical evidence for interepizootic transmission of RVF virus (RVFV) has been reported in various ruminant populations (Rostal et al., 2010, LaBeaud et al., 2011, Chevalier et al., 2009, Chapter 2).

RVFV is transmitted between animals by mosquitoes and certain floodwater *Aedes* spp. are capable of transovarial transmission (Linthicum et al., 1985b). Floodwater *Aedes* (referred to as simply *Aedes* going forward) hatch at the beginning of the season (Linthicum et al., 1985b, Logan et al., 1991, Linthicum et al., 1985a). It is believed that these *Aedes* have a univoltine (hatch only once per season) life cycle (Crans, 2004, Linthicum et al., 1985a) that often starts upon the initial seasonal inundation of shallow depressions in the grasslands (called pans). When infected floodwater *Aedes* eggs hatch and become adults, they can transmit RVFV to ruminants (including cattle, sheep and goats as well as wildlife). The *Aedes* then lay their eggs at the edge of a pan; the eggs remain in a state of aestivation until they are flooded again (Linthicum et al., 1984). Once the epizootic is initiated by the infected *Aedes*, some *Culex* spp. and other mosquitoes can transmit the virus horizontally and amplify the virus to cause a large epizootic (Linthicum et al., 2016). An outbreak on a single, large farm may last 2-3 months, cause abortion in up to 100% of the pregnant ruminants and cause mortality in nearly all of the very young ruminants (Gear et al., 1951).

There have been several mathematical models developed to explain RVFV dynamics in various tropical locations, including Madagascar (Nicolas et al., 2014), Mayotte (Cavalerie et al., 2015), Senegal (Favier et al., 2006), and East Africa (Iacono et al., 2018). Aside from models of RVFV spreading to the United States or Europe (Fischer et al., 2013, Niu et al., 2012), which are focused on whether the virus can become established rather than persistence at a site, no models have simulated RVFV dynamics in a temperate region. This is despite the fact that RVFV is endemic in temperate, central South Africa.

Temperature and precipitation have been clearly linked to mosquito hatching and development (Rueda et al., 1990). Several mathematical models have incorporated these data as drivers of mosquito population dynamics (Iacono et al., 2018, Leedale et al., 2016). The importance of these drivers of mosquito abundance is such that it is critical to include them to create realistic simulations. In 1984, Linthicum et al. (1984) determined that *Aedes* spp. begin hatching within two days of a pan flooding Kenya. Specifically, *Aedes mcintoshi,* a known RVFV transovarial vector, hatched between days 5-13, and the *Culex* were collected on days 7-22. Since then the difference in peak populations has been confirmed through adult mosquito captures (Ba et al., 2005) and by a recent study of larval succession in South Africa (Kemp et al. in prep). Yet as far as the author is aware, only Gachohi et al. (2016) incorporated this succession into a modelled system, in which they had *Culex* start hatching 36 days after *Aedes*.

Finally, a majority of models have simulated RVFV dynamics over multiple sites, a region or country (Iacono et al., 2018, Sumaye et al., 2019, Nicolas et al., 2014), very few have focused on the dynamics within a single pan or site. Others simply do not provide the spatial scale at which they are working or indicate that the mosquito and host populations are the same size, which would indicate that their model was not developed to represent the dynamics in a single pan (Mpeshe et al., 2011, Gaff et al., 2011).

To examine whether and under what conditions RVFV persistence occurs, specifically at the smallest epidemiological scale, the single pan, we developed an SEIR model. Specifically, we aimed to simulate a system in a temperate ecosystem using a climate dataset for a 34-year interepizootic period that results in low-levels of interepizootic transmission such that the virus persists and the median seroprevalence in the host population is between 15-40% (Rostal et al., 2010, Bett et al., 2018, Nanyingi et al., 2017, Ngoshe et al. submitted). This is the first RVFV model in which the *Aedes* are simulated

using a univoltine life cycle. Finally, we will evaluate whether the timeline of outbreaks approximates that recorded for the western Free State, South Africa.

METHODS

We developed a deterministic, ordinary differential equation (ODE)-based model with two mosquito vectors and a single host driven by realistic climate forcing. Specifically, to drive the vector population dynamics and simulate the RVFV dynamics during a 34-year period, we used precipitation and temperature data (described below) from a representative farm in central South Africa. The vector-borne component is conceptually based on the MacDonald malaria model (Macdonald, 1952) with the addition of transovarial transmission in one vector species, more development stages and climate forcing to drive the population dynamics of both vector species. A single, large sheep flock situated within a single pan in central South Africa was selected as an appropriate epidemiological unit. The transovarial *Aedes* vector was modelled with a univoltine life cycle and the amplifying *Culex* vector hatching shortly after the *Aedes*. We used a susceptible (S)-exposed (E)-infected (I)- and recovered (R) model in one host species with two life stages: sheep (S) and lambs (L) and two vector species: *Aedes* mosquitoes (A) and *Culex* mosquitoes (C) with multiple life stages (see Table 4-1 for all state variable definitions; see Figure 4-1). *Aedes* transmitted RVFV both transovarially and horizontally, while *Culex* could only transmit RVFV horizontally.

Climate-Driven Hatching and Development Rates

The hatching of both *Aedes* and *Culex* were triggered by precipitation once the temperature exceeded a threshold of 20^{o}C. To simulate the univoltine life cycle (Crans, 2004) triggered early in the rainy season (Linthicum et al., 1985b, Logan et al., 1991, Linthicum et al., 1985a), seasonal hatching of *Aedes* was initiated once 38 mm cumulative precipitation had fallen during seven days, while the mean daily temperature was greater than 20^{o}C. *Aedes* eggs hatched for nine days (Table 4-2). These parameters were estimated based on preliminary simulation to represent sufficient rainfall to allow the larvae to hatch following flooding in the *Aedes*, and to ensure that some amount of standing water remains in containers or natural water sources for *Culex* to continue hatching.

The delay (H) in the hatching of Culex eggs after the Aedes start hatching was set at 7 days (Linthicum et al., 1984, Ba et al., 2005), provided the temperature is greater than 20^{o}C and

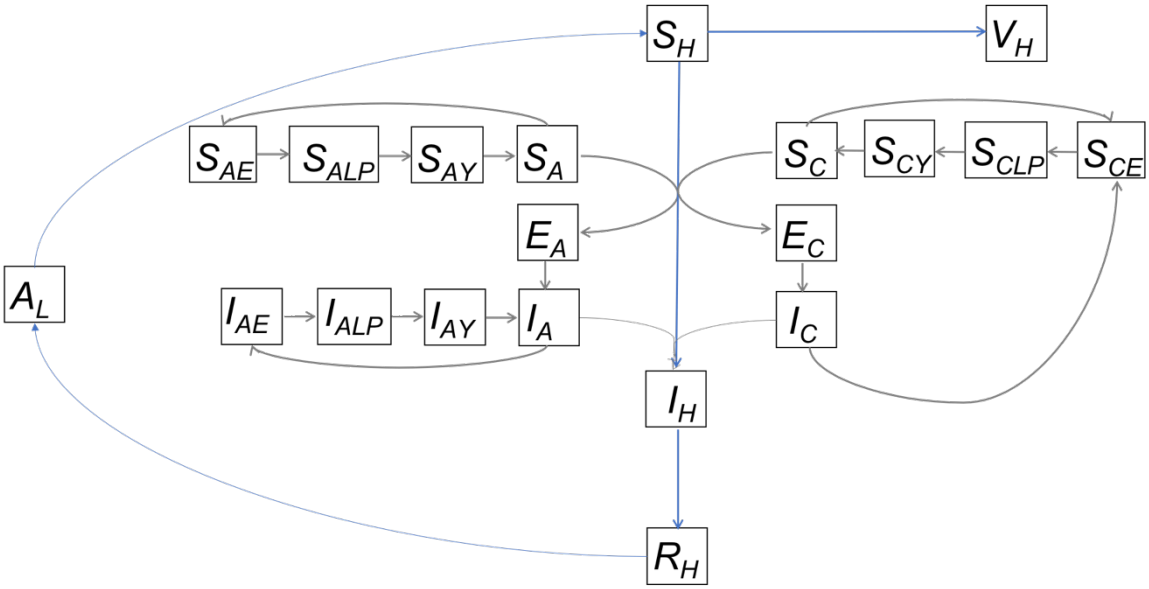


Figure 4-1 A diagram of the mathematical model. The infectious states of Susceptible (***S***), Exposed (***E***), Infected (***I***), Maternal Antibodies (***MA***) and Vaccinated (***V***) are represented for the host (***H***), *Aedes* vectors (***A***) and *Culex* vectors (***C***). All of the life stages of are represented for the two mosquito populations, including susceptible and infected *Aedes* eggs ($\boldsymbol{S_{AE}}$, $\boldsymbol{I_{AE}}$), larvae/pupae ($\boldsymbol{S_{ALP}}$, $\boldsymbol{I_{ALP}}$), young adults ($\boldsymbol{S_{AY}}$, $\boldsymbol{I_{AY}}$) and adult *Aedes* ($\boldsymbol{S_A}$, $\boldsymbol{I_A}$) as well as susceptible *Culex* eggs ($\boldsymbol{S_{CE}}$), larvae/pupae ($\boldsymbol{S_{CLP}}$), young adults ($\boldsymbol{S_{CY}}$) and adult *Culex* ($\boldsymbol{S_C}$). H represents either life stage in sheep, (adult sheep or lambs) while ***L*** only represents lambs as only lambs can have maternal antibodies.

there has been greater than 2 mm of cumulative rainfall over three days (to ensure there are some containers with standing water). We simulated the process of Culex reproduction throughout the entire year provided the temperature and precipitation thresholds continued to be met. Summaries of the number of animals or mosquitoes infected were calculated over a yearly timeframe that represents the hatching season (September 1 – August 30), rather than the calendar year, though if a specific date or year is referred to, it represents the calendar year. To simulate the temporal changes in standing water level on which Culex can lay their eggs, we used the proportion:

$$\frac{\text{10 day cumulative rainfall}}{\text{the maximum 10 day cumulative rainfall for that particular year}} \quad (1)$$

The daily development rates of both *Aedes* and *Culex* larvae and pupae (dev_{LP}) were modelled as temperature-dependent and were calculated based on the equation (2) and the species dependent parameters HA, HH and TH given in Rueda et al. (1990) (Table 4-3):

$$\mathrm{dev}_{LP} = \frac{\rho_{025} \cdot \frac{K}{298.15} \cdot \exp\left(\frac{HA}{1.987} \cdot \left(\frac{1}{298.15} - \frac{1}{K}\right)\right)}{1 + \exp\left(\frac{HH}{1.987} \cdot \left(\frac{1}{TH} - \frac{1}{K}\right)\right)} \quad (2)$$

Table 4-1 Definition of the state variables used in the model.

Variable	Population represented
Sheep and Lambs	
AL	Lambs with temporary maternal immunity
SL	Susceptible Lambs
IL	Infected Lambs
RL	Recovered Lambs
VL	Vaccinated Lambs
SS	Susceptible Sheep
IS	Infected Sheep
RS	Recovered Sheep
VS	Vaccinated Sheep
Aedes Mosquitoes	
SA	Susceptible Adult *Aedes*
EA	Exposed Adult *Aedes*
IA	Infected Adult *Aedes*
SAY	Susceptible Young *Aedes* that have not had their first blood meal
IAY	Infected Young *Aedes* that have not had their first blood meal
SALP	Susceptible *Aedes* Larvae/Pupae
IALP	Infected *Aedes* Larvae/Pupae
SAE	Susceptible *Aedes* Eggs
IAE	Infected *Aedes* Eggs
Culex Mosquitoes	
SC	Susceptible Adult *Culex*
EC	Exposed Adult *Culex*
IC	Infected Adult *Culex*
SCY	Susceptible Young *Culex* that have not had their first blood meal
SCLP	Susceptible *Culex* Larvae/Pupae
SCE	Susceptible *Culex* eggs

The rate formula developed by Rueda *et al*. (1990) was based on data from *Aedes aegypti* and *Culex quinquefasciatus*. To adjust for a difference in species development time, the development rate (dev_{ALP}) for *Aedes mcintoshi* was increased by a factor of 1.392704 as that species develops at a faster rate (Linthicum et al., 1984). Similarly, the development rate (dev_{CLP}) for *Culex pipiens* is slower than that for *C. quinquefasciatus* and therefore it was multiplied by 0.7650069 (Gomez et al., 1977).

The larval/pupal mortality rates for *Aedes* and *Culex* (μ_{LP}) were derived from the following equation (parameters are defined in Table 4-4):

$$\phi = \frac{\mathrm{dev}_{\mathrm{LP}}}{\mathrm{dev}_{\mathrm{LP}} + \mu_{\mathrm{LP}}} \quad (3)$$

using the species-specific development rate and survival fractions (ϕ) known from the literature (Table 4-4).

Table 4-2 Parameter values for the climate forcing to drive the hatching and development of the mosquitoes as described in the text.

Description	Value	Unit	Reference
Number of days that *Aedes* eggs can hatch	9	days	(Linthicum et al., 1984),
Number of cumulative days of rainfall needed to initiate hatching of *Aedes* eggs	7	days	See main text
Minimum amount of rainfall over 7 days, above which *Aedes* can hatch	38	mm	See main text
Minimum temperature above which both *Aedes* and *Culex* eggs can hatch	20	Celsius	(Rueda et al., 1990)
Number of cumulative days of rainfall needed to initiate and maintain hatching of *Culex* eggs	3	days	See main text
Minimum amount of rainfall over the 3 days, above which *Culex* can hatch	2	mm	See main text
Number of days after the *Aedes* eggs start hatching that the *Culex* can start hatching (H)	7	days	(Linthicum et al., 1984)

The following assumptions were made about the sheep population:

- No sheep were imported into the flock.
- Rams were sold upon reaching adulthood such that all adult sheep in the flock were female.
- There was no specific lambing season as farmers in South Africa often manage their sheep to lamb two or even three times a year (Cordel pers. comm., Chapter 2).
- Lambs born to recovered ewes had maternal immunity that waned before they mature into sheep.
- No exposed compartment was included among the sheep populations as the incubation period of RVFV in lambs is only 12-20 hours (Easterday et al., 1962).

The following assumptions were made about the *Aedes* and *Culex* mosquito populations:

- The carrying capacity acts on the number of eggs laid as eggs must be laid on the periphery of the flooded pan, which is a limited area and therefore acts on a constraint on the number of eggs successfully laid (Iacono et al., 2018).
- Hatching and development were driven by precipitation and temperature (see above, [Rueda et al., 1990]).
- Larvae and pupae stages were modelled together as one stage.
- Young adult mosquitoes did not take their first blood meal for the first three days.

Table 4-3 Parameter values for calculating the larval development rates (Eq. 2). Rueda et al. (1990) confirmed the Sharpe & DeMichele model (Sharpe and DeMichele, 1977) with high temperature inhibition with laboratory raised *Aedes* and *Culex* larvae. Their model was built to estimate the development rate per day as the temperature changed (Eq. 2).

Variable	Value	Description	Reference
ρ_{025_A}	0.1546	Effect of temperature on *Aedes* larval development estimated by a linear regression	(Rueda et al., 1990)
HA_A	33255.57	Effect of temperature on *Aedes* larval development estimated by a linear regression	(Rueda et al., 1990)
TH_A	301.67	Effect of temperature on *Aedes* larval development estimated by a linear regression	(Rueda et al., 1990)
HH_A	50543.49	Effect of temperature on *Aedes* larval development estimated by a linear regression	(Rueda et al., 1990)
ρ_{025_C}	0.21945	Effect of temperature on *Culex* larval development estimated by a linear regression	(Rueda et al., 1990)
HA_C	28049.98	Effect of temperature on *Culex* larval development estimated by a linear regression	(Rueda et al., 1990)
TH_C	298.6	Effect of temperature on *Culex* larval development estimated by a linear regression	(Rueda et al., 1990)
HH_C	35362.18	Effect of temperature on *Culex* larval development estimated by a linear regression	(Rueda et al., 1990)

The parameters for the model are given in Table 4-4. The equations describing the sheep and lamb populations are as follows:

$$\frac{dSS}{dt} = g \cdot SL - \text{sold}_S \cdot SS - \theta_{SLC} \cdot \text{SS} \cdot IC - \theta_{SLA} \cdot SS \cdot IA - \mu_s \cdot SS \quad (4)$$

$$\frac{dIS}{dt} = g \cdot IL - \text{sold}_S \cdot IS + \theta_{SLC} \cdot SS \cdot IC + \theta_{SLA} \cdot SS \cdot IA - \mu_S \cdot IS - \sigma \cdot IS - \rho_S \cdot IS \quad (5)$$

$$\frac{dRS}{dt} = \sigma \cdot IS + g \cdot RL - \text{sold}_S \cdot RS - \mu_S \cdot RS \quad (6)$$

$$\frac{dVS}{dt} = g \cdot VL - \text{sold}_S \cdot VS - \mu_S \cdot VS \quad (7)$$

$$\frac{dSL}{dt} = b_L \cdot \left(1 - \frac{NS}{NL_{max}}\right) \cdot SS + \omega_{MA} \cdot AL + \text{buy}_L \cdot SL - g \cdot SL - \text{vax} \cdot SL - \theta_{SLC} \cdot SL \cdot IC - \theta_{SLA} \cdot SL \cdot IA - \mu_L \cdot SL \quad (8)$$

$$\frac{dIL}{dt} = \theta_{SLC} \cdot SL \cdot IC + \theta_{SLA} \cdot SL \cdot IA - g \cdot IL - \mu_L \cdot IL - \sigma \cdot IL - \rho_L \cdot IL \quad (9)$$

$$\frac{dRL}{dt} = \sigma \cdot IL - g \cdot RL - \mu_L \cdot RL \quad (10)$$

$$\frac{dAL}{dt} = b_L \cdot \left(1 - \frac{NS}{NL_{max}}\right) \cdot (RS + ((1 - \text{Abort}) \cdot IS)) - \omega_{MA} \cdot AL - \mu_L \cdot AL \quad (11)$$

$$\frac{dVL}{dt} = vax \cdot SL - g \cdot VL - \mu_L \cdot VL \quad (12)$$

The equations for the *Aedes* free-living stages are given, followed by the equations for the egg stages.

$$\frac{dSALP}{dt} = bh_A \cdot \text{End}_A \cdot SAE - \text{dev}_{ALP} \cdot SALP - \mu_{ALP} \cdot SALP \quad (13)$$

$$\frac{dSAY}{dt} = \text{dev}_{ALP} \cdot SALP - \text{wait}_A \cdot SAY - \mu_A \cdot SAY \quad (14)$$

$$\frac{dSA}{dt} = \text{wait}_A \cdot SAY - \theta_{ASL} \cdot SA \cdot (IL + IS) - \mu_A \cdot SA \quad (15)$$

$$\frac{dEA}{dt} = \theta_{ASL} \cdot SA \cdot (IL + IS) - \varepsilon \cdot EA - \mu_A \cdot EA \quad (16)$$

$$\frac{dIALP}{dt} = bh_A \cdot \text{End}_A \cdot IAE - \text{dev}_{ALP} \cdot IALP - \mu_{ALP} \cdot IALP \quad (17)$$

$$\frac{dIAY}{dt} = \text{dev}_{ALP} \cdot IALP - \text{wait}_A \cdot IAY - \mu_A \cdot IAY \quad (18)$$

$$\frac{dIA}{dt} = wait_A \cdot IAY + \varepsilon \cdot EA - \mu_A \cdot IA \quad (19)$$

$$\frac{dSAE}{dt} = Eg_{AE} \cdot EgN_{AE} \cdot (SA + (1-q) \cdot IA) \cdot \left(1 - \frac{NAE}{NAE_{max}}\right) - bh_A \cdot \text{End}_A \cdot SA - \mu_{AE} \cdot SAE \quad (20)$$

$$\frac{dIAE}{dt} = Eg_{AE} \cdot EgN_{AE} \cdot q \cdot IA \cdot \left(1 - \frac{NAE}{NAE_{max}}\right) - bh_A \cdot \text{End}_A \cdot IAE - \mu_{AE} \cdot IAE \quad (21)$$

The equations for the *Culex* free-living stages are given, followed by the equations for the egg stages.

$$\frac{dSCLP}{dt} = bh_C \cdot \text{Hatch}_C \cdot SCE - \text{dev}_{CLP} \cdot SCLP - \mu_{CLP} \cdot SCLP \quad (22)$$

$$\frac{dSCY}{dt} = \text{dev}_{CLP} \cdot SCLP - \text{wait}_C \cdot SCY - \mu_C \cdot SCY \quad (23)$$

$$\frac{dSC}{dt} = \text{wait}_C \cdot SCY - \theta_{CSL} \cdot SC \cdot (IL + IS) - \mu_C \cdot SC \quad (24)$$

$$\frac{dEC}{dt} = \theta_{CSL} \cdot SC \cdot (IL + IS) - \epsilon \cdot EC - \mu_C \cdot EC \quad (25)$$

$$\frac{dIC}{dt} = \epsilon \cdot EC - \mu_C \cdot IC \quad (26)$$

$$\frac{dSCE}{dt} = Eg_{CE} \cdot EgN_{CE} \cdot SC \cdot \left(1 - \frac{SCE}{NCE_{max}}\right) + \delta \cdot Eg_{CE} \cdot EgN_{CE} \cdot IC - bh_C \cdot \text{Hatch}_C \cdot SCE - \mu_{CE} \cdot SCE \quad (27)$$

The transmission terms were calculated as follows:

$$\theta_{SLA} = \frac{\text{bite}_A}{NS+NL} \cdot \beta_{SLA} \quad (28)$$

$$\theta_{ASL} = \frac{\text{bite}_A}{NS+NL} \cdot \beta_{ASL} \quad (29)$$

$$\theta_{SLC} = \frac{\text{bite}_C}{NS+NL} \cdot \beta_{SLC} \quad (30)$$

$$\theta_{CSL} = \frac{\text{bite}_C}{NS+NL} \cdot \beta_{CSL} \quad (31)$$

The initial conditions for each population were: SS(0) = 584, RS(0) = 16, SL(0) = 200, SAE(0) = 955,00, IAE(0) =500, SCE(0) = 1000 and all other initial populations were zero.

The values used for the model parameters were primarily obtained from the literature (Table 4-4). For those that were not available in the literature we conducted preliminary

simulations to identify a reasonable value. For the transovarial transmission rate, our simulations suggest that the parameter value(s) based on the current expert opinion were unable successfully simulate persistence. We also used the preliminary simulation to estimate parameters for which there was no prevailing recommendation for the value (e.g. the carrying capacity for the mosquitoes). Thus, we selected a value with which we were able to simulate realistic dynamics. This simulation was to select the exact values used for the transovarial transmission rate, the bite rates and the carrying capacities in the simulation.

Table 4-4 Parameter values for the SEIR model of mosquitoes, sheep and lambs (Eqs. 4-31).

Parameter	Value	Description	Unit	Reference
σ	0.25	Recovery rate	days^{-1}	(Wichgers Schreur et al., 2016)
g	0.005	Maturation rate of lambs to sheep	days^{-1}	(Kilborn et al., 2002)
μ_S	0.00014	Sheep mortality rate	days^{-1}	Rostal *et al* (Chapter 2)
sold_S	0.002	Percent of sheep sold	days^{-1}	Set to maintain sheep population constant
ρ_S	0.063	RVF specific mortality rate in sheep	days^{-1}	(Swanepoel and Coetzer, 2004)
b_L	0.004	Birth rate in sheep	days^{-1}	(Cloete and Olivier, 2010)
μ_L	4x10^{-4}	Lamb mortality rate	days^{-1}	Rostal *et al* (Chapter 2)
ρ_L	0.751	RVFV specific mortality rate in lambs	days^{-1}	(Easterday, 1965)
Abort	0.9	Abortion fraction among pregnant ewes infected with RVF	No units	(Swanepoel and Coetzer, 2004)
ω_{MA}	0.008	Waning of maternal antibodies	days^{-1}	(Weiss, 1962)
vax.prop	0	Proportion of the sheep and lamb populations that should be vaccinated	No units	Set by user as needed
vax	0	$\frac{\text{vax.prop} \cdot (mu_L + g)}{(1 - \text{vax.prop})}$	days^{-1}	Vaccination rate to give the vaccinated proportion of vax.prop when at disease-free equilibrium
NL_{max}	800	Herd size maintained by the carrying capacity acting on lambs	No units	Rostal *et al* (Chapter 2)
Eg_{AE}	0.333	Egg laying Rate for *Aedes circumluteolus*	days^{-1}	(Zavortink, 1990)
EgN_{AE}	34.583	Number of female eggs per adult Asian *Aedes lineatopennis*	No units	(Jitpakdi et al., 2005)
μ_A	0.07	*Aedes* mortality rate	days^{-1}	(Arum et al., 2016, Ba et al., 2005)
μ_{AE}	0.001	*Aedes* egg mortality rate	days^{-1}	See main text above for estimation process
NAE_{max}	1.9 x10^5	Carrying capacity acting on the number of *Aedes* eggs that can be laid on the available habitat	No units	See main text above for estimation process
ϕ_A	0.36	Fraction of *Aedes mcintoshi* larvae/pupae that survive	No units	(Linthicum et al., 1985a)
bh_A	0.23	Rate that *Aedes* eggs hatch based the approximately 5-day interval between flooding and initial larval detection in the pan.	days^{-1}	(Linthicum et al., 1984)
dev_{ALP}	0.068	Mean development rate of Aedes - calculated daily based on ambient temperature (Eq. 2)	days^{-1}	(Rueda et al., 1990)
wait_A	0.333	Reciprocal of the number of days (3) it takes before a newly emerged *Aedes* takes its first bite (delay due to mating, host-seeking etc.)	days^{-1}	(Chapman and Woodard, 1965, Dziem and Cupp, 1983) Subra, 1980
μ_{ALP}	0.12	Mean mortality rate in *Aedes* larvae/pupae on days when there was at least 1 susceptible or infected *Aedes* larvae/pupae - calculated daily based on ambient temperature (Eq. 3)	days^{-1}	Estimated from dev_{ALP}

Parameter	Value	Description	Unit	Reference
bite_A	0.15	*Aedes* bite rate on the flock of sheep	days^{-1}	See main text above for estimation process
ϵ	0.083	Extrinsic incubation rate in mosquitoes	days^{-1}	(Gad et al., 1987)
β_{ASL}	0.41	Transmission probability to *Aedes lineatopennis/mcintoshi* from the host	No units	(McIntosh et al., 1980, McIntosh et al., 1973)
β_{SLA}	0.16	Transmission probability to the host from *Aedes lineatopennis/mcintoshi*	No units	(McIntosh et al., 1980)
q	0.85	Transovarial transmission fraction	No units	See main text above for estimation process
End_A	[1,0]	When the value is one, *Aedes* eggs hatch; represents the univoltine hatching of *Aedes*	No units	Estimated by climate thresholds
Eg_{CE}	0.333	Egg laying rate of *Culex*	days-1	(Gad et al., 1987)
EgN_{CE}	118	Mean number of female eggs laid by *Culex*	No units	(Gomez et al., 1977)
δ	0.8	Fraction of RVFV infected *Culex* that lay eggs	No units	(Turell et al., 1985)
ϕ_C	0.291	Fraction of *Culex* that survive larval/pupal stages	No units	(Gomez et al., 1977)
bh_C	0.667	Reciprocal of the *Culex* hatching time	days^{-1}	(Gomez et al., 1977)
dev_{CLP}	0.06	Mean development time for *Culex* pupae/larvae - calculated daily based on ambient temperature (Eq. 2)	days^{-1}	(Rueda et al., 1990)
wait_C	0.333	Reciprocal of the number of days (3) it takes before a newly emerged *Culex* takes its first bite (delay due to mating, host-seeking etc.)	days^{-1}	(Chapman and Woodard, 1965, Dziem and Cupp, 1983)
μ_C	0.063	Adult *Culex* mortality rate	days^{-1}	(Ba et al., 2005, Gad et al., 1987, Dohm et al., 1991)
μ_{CLP}	0.147	Mean mortality rate in *Culex* larvae/pupae - calculated daily based on ambient temperature (Eq. 3)	days^{-1}	Estimated from dev_{CLP}
μ_{CE}	0.146	*Culex* egg mortality rate - calculated using $\mu_{CE} = bh_C \cdot \frac{1-\psi_C}{\psi_C}$	days^{-1}	Estimated as per the equation
ψ_C	0.82	Fraction of *Culex* eggs that hatch in the lab	no units	(Gomez et al., 1977)
NC_{max}	650000	Carrying capacity acting on the number of *Culex* eggs laid on the available habitat	No units	See main text above for estimation process
β_{CSL}	0.71	Transmission probability to *Culex* from the host	No units	(Jupp et al., 2002, McIntosh et al., 1973, McIntosh et al., 1980, Gargan et al., 1983, Jupp and Cornel, 1988, McIntosh et al., 1983)
β_{SLC}	0.31	Transmission probability to the host from *Culex*	No units	(McIntosh et al., 1983, McIntosh et al., 1973, McIntosh et al., 1980)
bite_C	0.035	*Culex* bite rate on the sheep	days^{-1}	See main text above for estimation process
Hatch_C	0-1	When the value is greater than zero, *Culex* eggs hatch at the specified proportion	No units	Estimated by climate data

Sensitivity Analysis

Three types of sensitivity analyses were conducted. First, we varied two parameters at a time and evaluated persistence and median seroprevalence across the 34-year model for the *Aedes* and *Culex* system and the *Aedes* only system. The parameters included in this first analysis were the transovarial transmission, bite rate(s), host-vector ratio, the mortality rate of *Aedes* eggs and proportion of the flock vaccinated. For the *Aedes* and *Culex* comparisons that included bite rate, the given bite rate was used for both vector species. As the host-vector ratio changes throughout the simulation (as the mosquito populations start near zero, reach their maximum and then decline each year), the host-vector ratios represent the limit that the ratio reached during the simulation.

Second, we varied two or three parameters at a time to evaluate epizootic size and length over a one-year outbreak (September 2008-March 2009) in a system with both *Aedes* and *Culex* vectors. Paired combinations of the following parameters were evaluated: bite rates, extrinsic incubation period, host-vector ratio, and transovarial transmission. In another analysis we varied three parameters (bite rate, extrinsic incubation period and host-vector ratio) and visualized the number of infected sheep and lambs over the timeframe of the outbreak. As indicated above, the same vector bite rate was used for both *Aedes* and *Culex* during these simulations.

Third, we used Latin hypercube sampling to explore the model parameter space (Marino et al., 2008, Drake and Rohani, 2019). Nine parameters were included in the sensitivity analysis: H, θ_{asl}, θ_{sla}, θ_{csl}, θ_{slc}, bite_A, bite_C, μ_{AE} and q. The parameters were varied across a wide range (see Table 4-5). The sensitivity analysis consisted of 4000 runs. Each parameter was evaluated based on its effect on six outcomes: mean epizootic length, mean epizootic size, the number of outbreaks in which more than 50 animals were infected, median annual seroprevalence and the final recovered population of sheep and lambs. These outcomes were calculated for the full 34-year simulation interval. The results were then analysed using a partial coefficient correlation analysis (Marino et al., 2008).

Table 4-5 Ranges of variables used in the Latin hypercube and R_0 sensitivity analyses.

Variable	Range used in Latin hypercube
bite_A	0.025-2
β_{asl}	0.01-1.0
β_{sla}	0.01-1.0
μ_{AE}*	0.00027-.01
q	0.01-1.0
β_{csl}	0.01-1.0
β_{slc}	0.01-1.0
bite_C	0.025-2
H*	1-20
Host-Vector Ratio†	1:1, 1:10 , 1:100, 1:1000, 1:10000

*This parameter was only used in the Latin hypercube sensitivity analysis; †This parameter was only used in the R_0 analysis

Estimation of the Basic Reproduction Number, R_0

R_0 was calculated using the next generation matrix (NGM) approach (Diekmann et al., 2010). Briefly, to solve for R_0, we first calculated the disease-free equilibrium (DFE) of the system to determine the birth rates and fix the population sizes. Once we had the DFE for each state variable we solved for the system's birth rate, for example by setting the sum of the equations for SS, VS, SL and VL equal to the value of the sum of NS and NL (see Eq. 32; Appendix 7 and 8). The same process was used to calculate a constant birth rate for the *Aedes* and *Culex* populations. This process was necessary as the carrying capacity term in the *Aedes* and *Culex* equations allowed for rapid population changes. Also, as R_0 is close to unity in this system it was very sensitive to changes in initial population sizes, which for the vectors, would change by orders of magnitude. This allowed us to solve for R_0 at the simulated, constant susceptible populations into which RVFV was introduced. An example of the birth rate (birth_{NSL}) calculation for the host population is given in equation 32.

$$\text{birth}_{NSL} = \frac{(NS + NL) \cdot (\mu_L + \text{vax} + g) \cdot (\mu_S + g) \cdot (\mu_S + \text{sold}_S)}{(\mu_S + \text{sold}_S + g) \cdot (\mu_S + \text{vax} + g)} \qquad (32)$$

The vaccinated lamb and sheep populations were set to 0 for the R_0 calculation. We then linearized the infectious subsystem using the birth rates calculated as described above. We calculated the Jacobian matrix and decomposed it into the T matrix (the transmission terms that describes the production of new infections) and Σ matrix (the development part that describes changes in state) (Diekmann et al., 2010). The NGM with large domain $K_L =$

$-T\Sigma^{-1}$ was calculated and we used the NGM with small domain K_S (using only the rows and columns of K_L belonging to new states-at-infection) to calculate R_0. We then solved R_0 numerically using the set population sizes (Appendix 7).

We analysed the sensitivity of R_0 by varying a single parameter while holding the rest of the parameters constant. Eight parameters were included in the sensitivity analysis, including θ_{ASL}, θ_{CSL}, θ_{SLA}, θ_{SLC}, bite_A, bite_C, q and Host-Vector Ratio (Table 4-5).

The herd immunity threshold (HIT) was calculated using Eq. 34:

$$HIT = 1 - \frac{1}{R_0} \tag{33}$$

To confirm the HIT, we simulated that vaccination rate to observe whether the virus went extinct. To confirm the extinction timeframe indicated by the HIT simulation we ran an additional simulation to evaluate the system when the only RVFV transmission pathway available was transovarial transmission and observed the changes in the population size of infected *Aedes* eggs.

Data and Analysis

We used daily precipitation data from January 1, 1983 to April 30, 2017 from a representative farm in the western Free State (latitude: -28.40, longitude: 26.26; rounded for privacy) from the African Rainfall Climatology (ARC) dataset from the NOAA-CPC archives (Love et al., 2004, Novella and Thiaw, 2016). The ARC data were produced using a combination of rainfall gauge measurements and METEOSAT satellite data to produce gridded rainfall estimates at a 0.1° x 0.1° spatial resolution from 1983 (see Figure S4-1A). The temperature data were collected by the nearest local weather stations maintained under the South African Weather Service (SAWS) at the Glenn College site in Bloemfontein, Free State (latitude: -28.95, longitude: 26.33). The stations (Glen College AWS and Glen College AGR) provided the maximum and minimum temperatures in degrees Celsius. As it was a more complete data set, the Glen College AGR station data were used and the Glen College AWS data were only used if data were missing from the AGR station (SAWS unpublished data, 2017). Temperature data were available from April 1, 1915 to August 2017; however, the dates used were restricted to match the timeframe of the precipitation data (see Figure S4-1B).

Data analysis was completed in `R` version 3.6.0 (R Core Team, 2019) using `Rstudio` (RStudio Team, 2015). The package `zoo` 1.8.6 (Zeileis and Grothendieck, 2005) was used to calculate the cumulative precipitation data, the ode solver was used from the `deSolve` 1.21 package (Soetaert et al., 2010), the climate data and other daily data (e.g. the hatching triggers) were introduced into the simulation through the use of the approxfun() function (`deSolve`) and the figures were made using `ggplot2` 3.2.0 (Wickham, 2009). The `lhs` 1.0.1 package (Carnell, 2018) was used to produce the Latin hypercube for the sensitivity analysis. The resultant data were analysed by calculating the partial correlation coefficients using the pcc() function from the `sensitivity` 1.15.2 package (Iooss et al., 2018). `wxMaxima` 5.43.0 (Maxima.sourceforge.net, 2014) was used to conduct the R_0 analysis (see the `wxMaxima` code is presented in Appendix 8).

RESULTS

The simulation demonstrated that RVFV can persist for over thirty years within a single pan, in a system that includes two vectors, one with a univoltine life cycle and capable of transovarial transmission, and with one host species. We will first present the dynamics of the simulated system with only the transovarial vector, followed by the full model with both vectors. Then we will discuss the specific outbreak dynamics, followed by the sensitivity and R_0 analyses. The tables are embedded in the text and the figures are presented in order at the end of the results section. The supplemental figures are presented before the references.

Interepizootic persistence and transmission

The simulation with only *Aedes* vectors, generated small annual outbreaks that infected an average of 53 sheep/lambs per year (Figure 4-2A) and lasted an average of 58 days. The mean annual incidence of seroconversion generated was 7.2 sheep/lambs per 100 animal-years. This resulted in an estimated annual median seroprevalence of 12% (range 8-21%) in the host population. The population size of infected *Aedes* and infected *Aedes* eggs appeared to be stable (not declining) at the end of the simulation (Figure 4-1B and 4-1C, respectively). No large epizootics were simulated under these conditions within the simulation.

When *Culex* and *Aedes* were simulated together RVFV continued to persist in the system (Figure 4-3). The simulation generated annual RVF epizootics that were variable in size and length (Figure 4-3A). The median annual seroprevalence was estimated to be 18% (range 9-75%). In this simulation the estimated infection incidence was 9.1 per 100 animal-years. The total number of infected *Aedes* eggs declined throughout the simulation (Figure 4-3C), though through the sensitivity analyses we noticed that this could be reversed by a slight increase in the *Aedes* bite rate. The mean proportion of infected *Aedes* eggs was 0.003 (Table 4-6). We showed that the timing of the mosquito populations fit our expectation that *Aedes* would proceed *Culex* (Figure S4-2). The simulation generated relatively larger RVF epizootics in 1987 and 1999 and the largest epizootic in the simulation occurred in the spring of 2009 (Figure 4-3A).

Table 4-6 Mean annual maximum host-vector ratio, mean annual seroprevalence and the mean proportion of infected mosquitoes and eggs for the full simulation with *Aedes* and *Culex* (Figure 4-3).

Factor	Mean (Range)
Mean Annual Maximum Host-Vector Ratio	1:205 (1:34-1:839)
Mean Annual Proportion of Infected Aedes Eggs	0.003 (0.001-0.005)
Mean Annual Proportion of Infected Adult Aedes	0.018 (0.0011-0.14)
Mean Annual Proportion of Infected Adult Culex	0.0034 (2.1e-06-0.0265)
Median Annual Seroprevalence	17.5% (2-74.4%)

Epizootic dynamics

Outbreak dynamics, specifically the size and length of an outbreak, during the single year of the largest outbreak (September 2008-August 2009) were further investigated (Figure 4-4). Under the parameters selected to simulate interepizootic RVFV transmission (Table 4-4) 366 sheep and lambs were infected and the outbreak lasted for 212 days. The epizootic resulted in a seroprevalence of 75% at the end of the outbreak. The outbreak was simulated using different bite rates (Figure 4-4, and Figure S4-3, which only depict the dynamics while infected hosts are present). If the *Aedes* and *Culex* bite rates were increased to 2 bites per day, then the simulated outbreak occurred in September/October of 2008 (instead of peaking in March 2009). At the higher bite rate, 404 sheep and lambs were predicted to be infected and the outbreak only lasted 29 days.

As an example, we were able to reproduce the historical outbreak in 2010 by preventing *Culex* from transmitting RVFV during the 2009 season (Figure S4-4). This placed the largest predicted outbreak within the correct historical timeframe within the Free State, South Africa (Pienaar and Thompson, 2013).

Sensitivity analysis

Individual Parameter Analyses

The median RVFV seroprevalence over the 34-year simulation was sensitive to very small changes (in either direction) in the rate of transovarial transmission and/or *Aedes* and *Culex* bite rates (Figure 4-5A). For each value of transovarial transmission or bite rates explored there was just a narrow range of parameter values that supported a median seroprevalence within a biologically plausible range (5-40% (Clark et al., 2018)). Small changes rapidly led to either very high seroprevalence with viral maintenance or to viral extinction (Figure 4-5A), this is evidenced by the steep slopes in Figure 4-5A. Similar sensitivities were seen in regards to viral persistence when small changes were made in the *Aedes* bite rate, percent of vaccinated hosts and the host-vector ratio (Figure 4-5). Persistence mostly increased or was static with increased transovarial transmission and the *Aedes* egg longevity; however, at intermediate rates of transovarial transmission (e.g. 0.75), this effect plateaued and persistence began to decline as egg survival surpassed 2-5 years (Figure 4-5E), likely due to the carrying capacity.

Many of the sensitivities of persistence and seroprevalence described for the *Aedes* and *Culex* model were the same in the full 34-year, single-vector system (Figure S4-5). Comparing the one- and two-vector models, a higher bite rate was required in the *Aedes* and *Culex* simulation (Figure 4-5A) to support persistence at the same rate of transovarial transmission as in the *Aedes* only simulation (Figure S4-3A). Additionally, the slopes were greater, indicating that the *Aedes* and *Culex* model is more sensitive to small changes in bite rate or transovarial transmission than the *Aedes* only model. Likewise, higher bite rates were needed to maintain RVFV persistence for the same host-vector ratio in the *Aedes* and *Culex* model then that which was described by the *Aedes* only model (Figures 4-5B and S4-3B).

Epizootic length was not sensitive to the length of the extrinsic incubation period as compared to its sensitivity to bite rates or host-vector ratios in the single year epizootic simulations (Figures 4-6A and 4-6B). However, at the host-vector ratio of 1:1000, the outbreak length has a steep decline as extrinsic incubation period is lengthened. At very low bite rates no outbreak occurred irrespective of the extrinsic incubation period (e.g. *Aedes* and *Culex* bite rates at 0.05 bites per day; Figure 4-6A). In contrast, once the bite rate was high enough to initiate an outbreak, lower bite rates were generally associated

with longer outbreaks regardless of which other variable they were changed with (incubation period, host-vector ratio or transovarial transmission; Figures 4-6A, C, E). Given this, the most variation in outbreak length was seen as bite rate and host-vector ratio were varied (Figure 4-6C). Lower host-vector ratios required higher bite rates to initiate an outbreak. In all simulations of the host-vector ratio, a ratio of 1:1 never resulted in an outbreak, even with a bite rate of 3 bites per day (Figures 4-6B, C, D). In nearly all of the simulations (Figure 4-6), the outbreak size and length plateau around 400 animals infected and 30 days in length.

We generated 162 simulated outbreaks using different combinations of bite rates, vector-host ratios and extrinsic incubation period (Figure S4-6). Only 11 simulated outbreaks that lasted 50-70 days (red curves, Figure 4-6F), which approximates the 60-day timeframe historically reported (Gear et al., 1951). All 11 outbreaks were a combination of a host-vector ratio of 1:1000, *Aedes* and *Culex* bite rates of 0.15 and an extrinsic incubation rate of 9-29 days.

Latin Hypercube Analysis

The sensitivity of each outcome factor to each parameter was examined individually (see example in Figure 4-7) and in combination with the other parameters (see Table 4-7). The four *Aedes* transmission terms/bite rates were positively and significantly associated with each of the six factors examined, except that the transmission rates to/from *Aedes* from/to ruminants was not correlated with mean outbreak size. The horizontal *Aedes* transmission terms and bite rate had the greatest effect on the persistence of the virus, whereas, transovarial transmission had the greatest effect on the number of outbreaks infecting greater than 50 animals. The *Aedes* egg mortality rate was significantly and positively associated with the mean outbreak size, not associated with the final population of recovered animals and negatively associated with all other outcome factors.

The delay (in days) between the start of the *Aedes* hatching and the *Culex* hatching was negatively associated with mean outbreak length and positively associated with the size of epizootics, the median seroprevalence and the number of outbreaks with more than 50 infected animals. The *Culex* transmission term from mosquito to ruminants and bite rate were only positively associated with mean outbreak length and were negatively associated with all other outcome factors. The transmission term from ruminants to *Culex* followed the same trend, but was not significantly associated with the median seroprevalence, the

final recovered sheep/lamb population, the persistence of RVFV, nor outbreak size. All three *Culex* transmission and bite rates had the largest absolute effect (negative) on the size of the number of outbreaks with more than 50 animals infected (Table 4-4).

As a further indicator of the delicate balance between persistence and mean seroprevalence in this system, in 21% of the 4,000 sensitivity analysis runs RVFV did not persist for the duration of the 34-year simulation (Figure S4-7A). Additionally, 79% of the runs had a median annual seroprevalence of greater than 40% (Figure S4-7B). Only 0.125% of the runs had both persistence and a median seroprevalence of less than 40%.

Table 4-7 Partial coefficient correlation estimates and 99% confidence interval for selected transmission parameters in the model for each outcome factor of interest. Est. = Estimate; CI = 99% Confidence interval. *Indicates a significant factor where the confidence interval does not include zero.

	Number of Outbreaks Infecting more than 50 Animals		Size of Mean Epizootic		Mean Epizootic Length	
Factor	Est.	CI	Est.	CI	Est.	CI
Transovarial Transmission	0.3	[0.26, 0.344]*	0.269	[0.227, 0.318]*	0.132	[0.086, 0.18]*
Aedes Bite Rate	0.412	[0.373, 0.462]*	0.14	[0.086, 0.187]*	0.252	[0.202, 0.304]*
Transmission *Aedes* to Ruminants	0.198	[0.147, 0.249]*	0.021	[-0.026, 0.072]	0.139	[0.091, 0.184]*
Transmission Ruminants to *Aedes*	0.168	[0.124, 0.215]*	0.018	[-0.027, 0.061]	0.165	[0.119, 0.213]*
Aedes Egg Mortality Rate	-0.109	[-0.148, -0.061]*	0.067	[0.021, 0.111]*	-0.217	[-0.267, -0.169]*
Delay in *Culex* Hatching	0.12	[0.077, 0.161]*	0.106	[0.06, 0.155]*	-0.108	[-0.162, -0.063]*
Culex Bite Rate	-0.235	[-0.275, -0.195]*	-0.136	[-0.19, -0.088]*	0.064	[0.016, 0.111]*
Transmission *Culex* to Ruminants	-0.148	[-0.192, -0.111]*	-0.088	[-0.135, -0.036]*	0.062	[0.017, 0.116]*
Transmission Ruminants to *Culex*	-0.112	[-0.163, -0.063]*	-0.046	[-0.097, 0.002]	0.062	[0.013, 0.106]*

	Final Population of Recovered Animals		Persistence		Median Seroprevalence Across All Years	
Factor	Est.	CI	Est.	CI	Est.	CI
Transovarial Transmission	0.191	[0.144, 0.235]*	0.161	[0.114, 0.209]*	0.272	[0.223, 0.319]*
Aedes Bite Rate	0.205	[0.161, 0.248]*	0.48	[0.439, 0.523]*	0.325	[0.284, 0.367]*
Transmission *Aedes* to Ruminants	0.065	[0.021, 0.111]*	0.304	[0.252, 0.354]*	0.119	[0.064, 0.17]*
Transmission Ruminants to *Aedes*	0.07	[0.02, 0.118]*	0.248	[0.199, 0.297]*	0.109	[0.065, 0.152]*
Aedes Egg Mortality Rate	0.025	[-0.023, 0.072]	-0.331	[-0.374, -0.284]*	-0.054	[-0.099, -0.007]*
Delay in *Culex* Hatching	0.015	[-0.027, 0.056]	-0.04	[-0.089, 0.006]	0.053	[0.008, 0.095]*
Culex Bite Rate	-0.096	[-0.141, -0.053]*	-0.078	[-0.121, -0.034]*	-0.063	[-0.104, -0.02]*
Transmission *Culex* to Ruminants	-0.068	[-0.115, -0.026]*	-0.056	[-0.104, -0.015]*	-0.065	[-0.11, -0.016]*
Transmission Ruminants to *Culex*	-0.019	[-0.067, 0.033]	-0.026	[-0.07, 0.023]	-0.029	[-0.075, 0.019]

Analysis of the Reproduction Number

The R_0 was numerically calculated to be 1.44 (range: 0.97-2.08 depending on the size of the susceptible mosquito population). R_0 was sensitive to all variables assessed (Figure 4-8). R_0 was most sensitive to the *Culex* bite rate with R_0 exceeding 10 before the bite rate reached 0.5. Similarly, it was very sensitive to the *Aedes* bite rate, exceeding seven as the bite rate approached two. As the host-vector ratio increased above approximately 1:500, R_0 exceeded one and then rapidly began to increase, reaching 5.25 when the ratio is 1:1000.

For all other parameters R_0 rarely exceeded two across the whole range of values. R_0 was least sensitive to transovarial transmission.

The herd immunity threshold was estimated to be 31%. While this rate of vaccination will cause extinction of the virus, our simulation indicates that it requires 19 years of vaccinating at this rate to reduce the number of infected *Aedes* sufficiently that no sheep or lambs are infected (Figure S4-8). Our simulation also suggested that it takes decades for RVFV to go extinct within the *Aedes* population when RVFV is maintained solely by transovarial transmission with no transmission to or from sheep and lambs permitted (Figure S4-9).

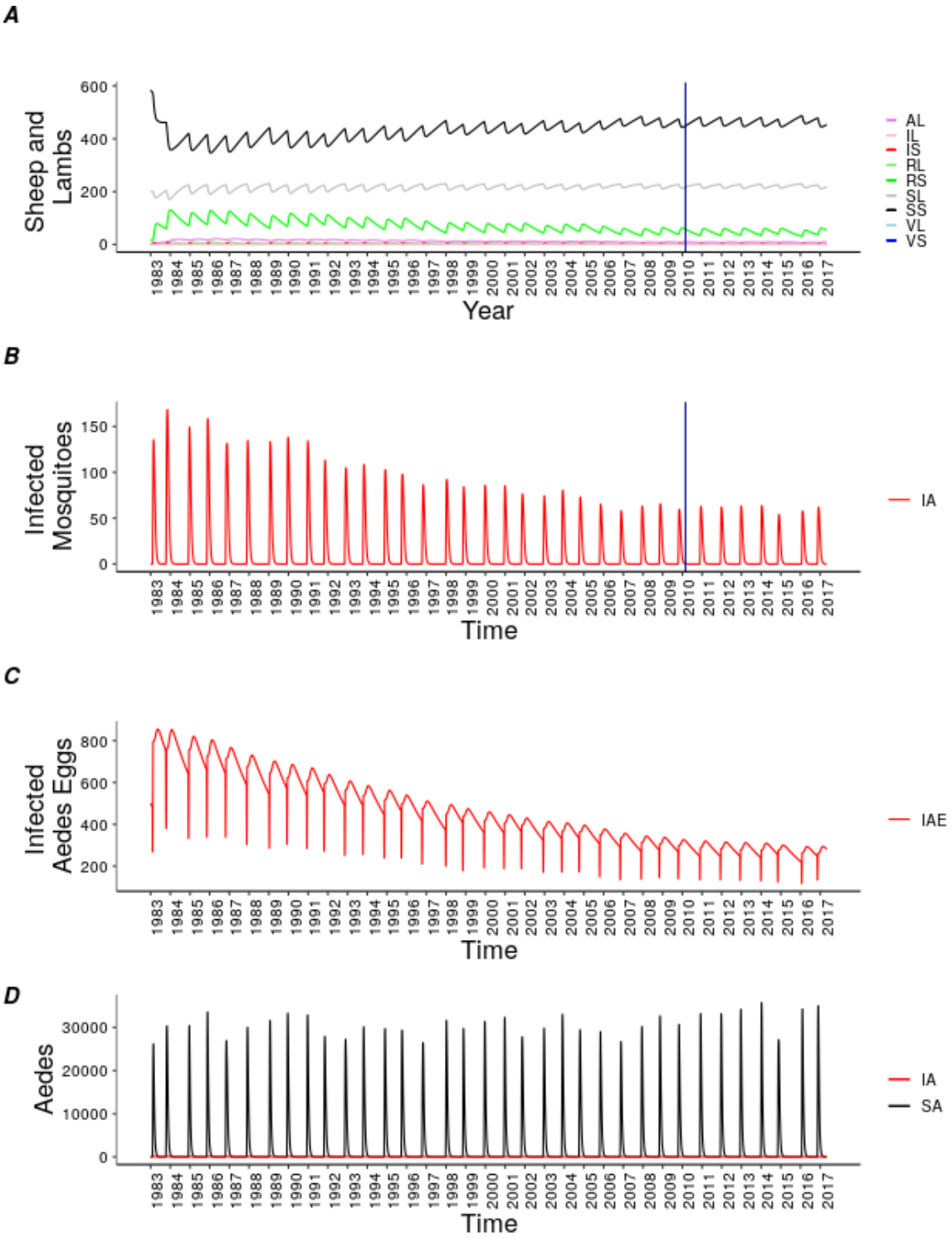


Figure 4-2 The full simulation of RVFV infection dynamics in a single host, single vector (*Aedes*) system run for 34 years. A) The susceptible (black/grey), infected (red/pink) and recovered (green/light green) populations of sheep and lambs are shown, respectively. The lambs also have a compartment for animals with maternal antibodies, which is shown in purple. A compartment for vaccinated sheep is present (blue/light blue); however, no animals were vaccinated for this simulation. The approximate time of the historical 2010 outbreak is indicated by the dark blue vertical line. B) The population of infected *Aedes* mosquitoes is shown in red and the 2010 outbreak is marked by the dark blue line. C) The number of infected *Aedes* eggs in the environment is indicated in red over time. D) The total populations of susceptible (black) and infected (red) *Aedes* mosquitoes are shown over time. AL = lambs with maternal antibodies; IL, IS, IA, IAE = infected lambs, sheep, *Aedes*, and *Aedes* eggs, respectively; RL & RS = recovered lambs and sheep, respectively; SL, SS and SA = susceptible lambs, sheep and adult *Aedes*, respectively. VL and VS = vaccinated lambs and sheep, respectively.

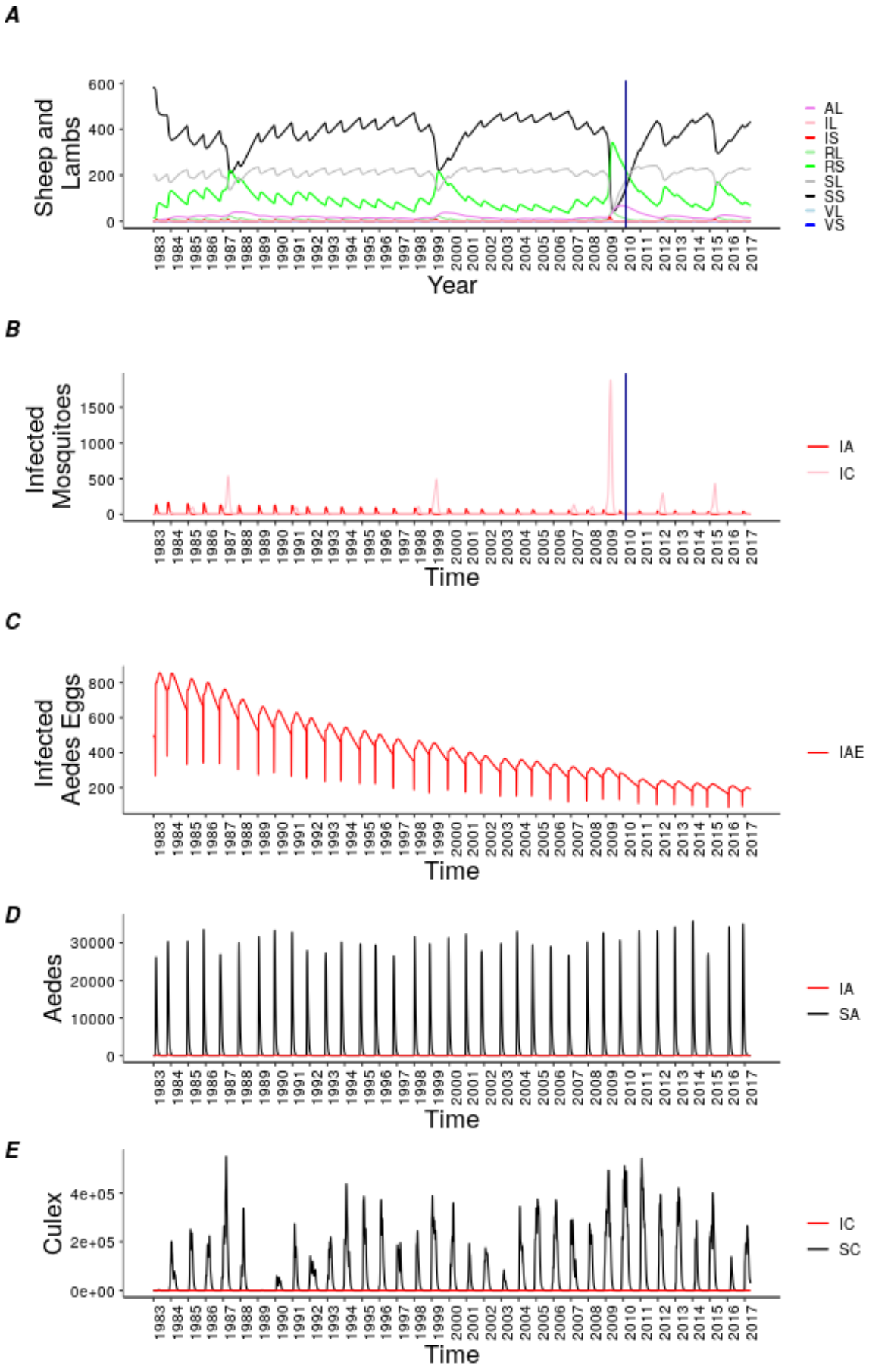

A
Sheep and Lambs
600
400
200
0
AL
IL
IS
RL
RS
SL
SS
VL
VS
1983 1984 1985 1986 1987 1988 1989 1990 1991 1992 1993 1994 1995 1996 1997 1998 1999 2000 2001 2002 2003 2004 2005 2006 2007 2008 2009 2010 2011 2012 2013 2014 2015 2016 2017
Year
B
Infected Mosquitoes
1500
1000
500
0
IA
IC
Time
C
Infected Aedes Eggs
800
600
400
200
IAE
Time
D
Aedes
30000
20000
10000
0
IA
SA
Time
E
Culex
4e+05
2e+05
0e+00
IC
SC
Time

Figure 4-3 The full simulation of RVFV infection dynamics with two vectors (*Aedes* and *Culex*) in a single host system run for 34 years. Several small epizootics occurred and a very large outbreak occurred in 2009. A) The susceptible (black/grey), infected (red/pink) and recovered (green/light green) populations of sheep. The lambs also have a compartment for animals with maternal antibodies, which is shown in purple. A compartment for vaccinated sheep/lambs is in blue/light blue; however, no vaccinated animals were included in the simulation. The dark blue line indicates the approximate time of the 2010 outbreak. B) The population of infected *Aedes* (red) and *Culex* (pink) mosquitoes is shown and the 2010 outbreak is marked by the dark blue line. C) The number of infected *Aedes* eggs in the environment is indicated in red over time. D) The total populations of susceptible (black) and infected (red) *Aedes* mosquitoes are shown over time. E) The total populations of susceptible (black) and infected (red) *Culex* mosquitoes are shown over time. AL = lambs with maternal antibodies; IL, IS, IA, IC, and IAE = infected lambs, sheep, *Aedes*, *Culex* and *Aedes* eggs, respectively; RL and RS = recovered lambs and sheep, respectively; SL, SS, SA and SC = susceptible lambs, sheep, adult *Aedes* and adult *Culex*, respectively. VL and VS = vaccinated lambs and sheep, respectively.

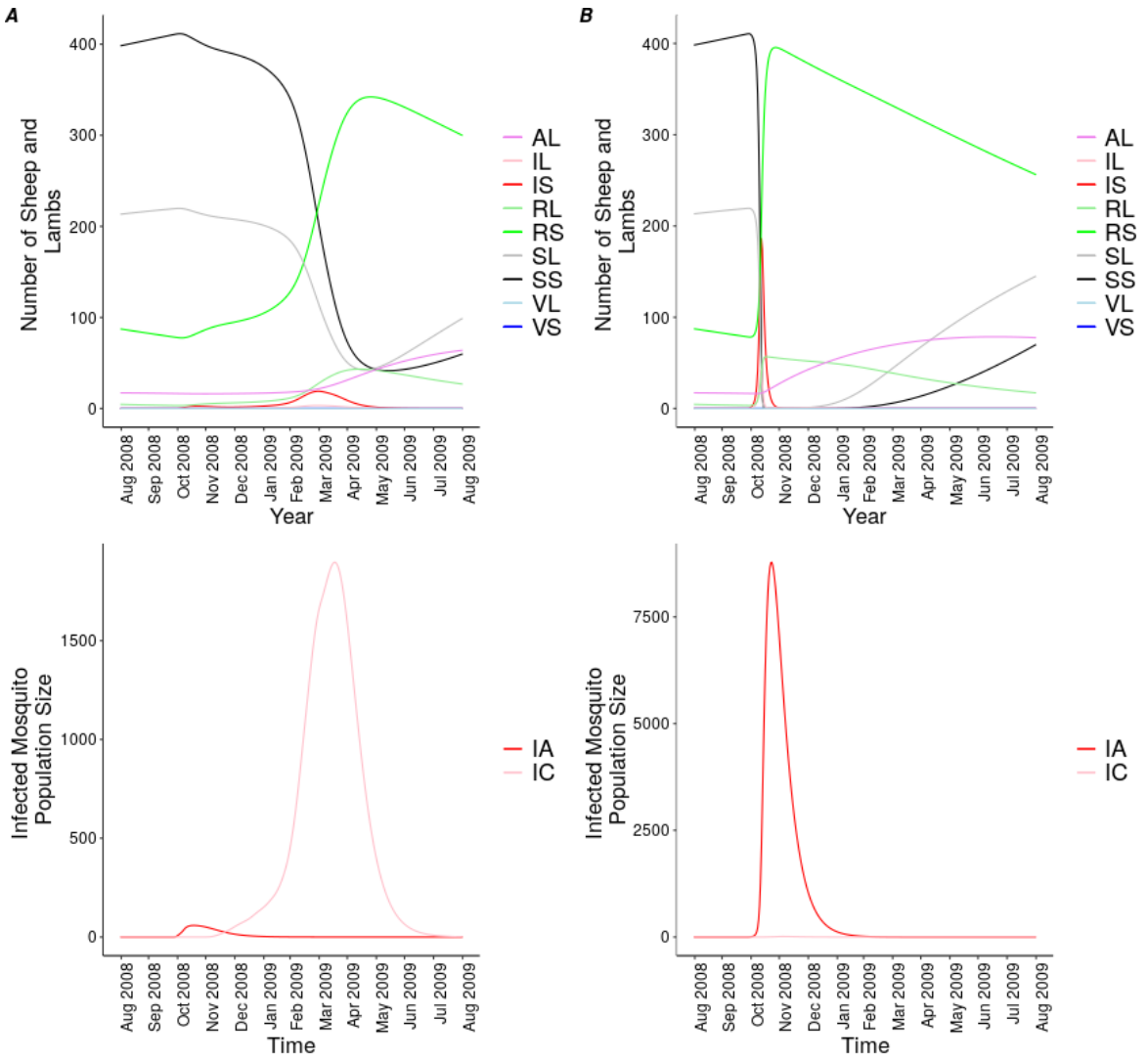


Figure 4-4 Simulated outbreak dynamics between August 2008 and August 2009. A) Depicts the outbreak as simulated in Figure 4-2, with the bite rates set as indicated in Table 4-4. In this scenario the outbreak occurs between January-May 2009. B) A simulation of the outbreak with *Aedes* and *Culex* bite rates set at 2. In this scenario, the outbreak occurs between October -December 2008. For both simulations the sheep and lamb populations are presented on the top, where the susceptible (black/grey), infected (red/pink), recovered (green/light green) and vaccinated (blue/light blue) populations of sheep and lambs are shown, respectively, and the lambs that have maternal antibodies are shown in purple. The infected *Aedes* (red) and *Culex* (pink) mosquito populations are shown on the bottom. AL = lambs with maternal antibodies; IL, IS, IA, IC, and IAE = infected lambs, sheep, *Aedes*, *Culex* and *Aedes* eggs, respectively; RL and RS = recovered lambs and sheep, respectively; SL and SS = susceptible lambs and sheep, respectively. VL and VS = vaccinated lambs and sheep, respectively (no animals were vaccinated during this scenario).

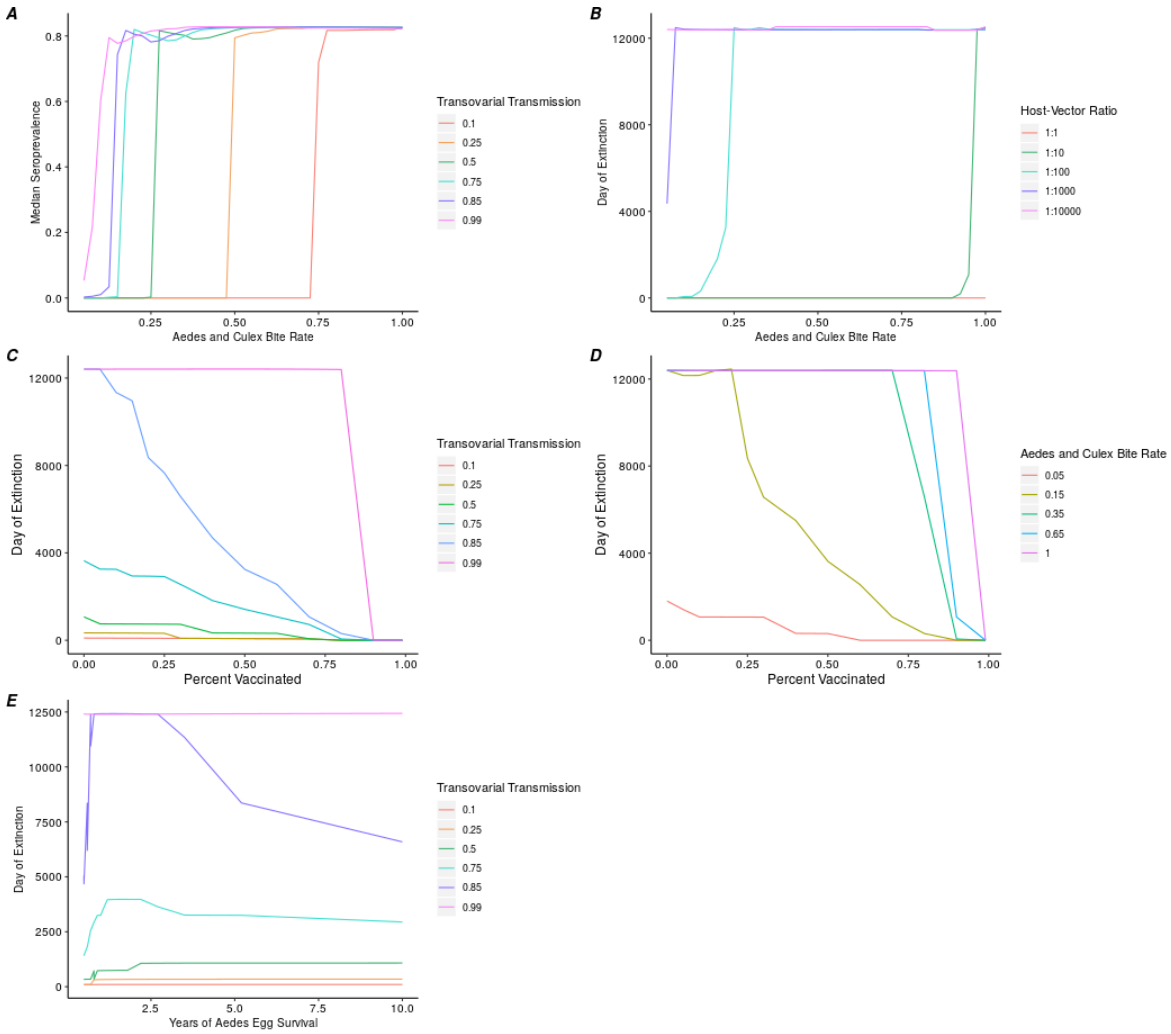


Figure 4-5 RVFV seroprevalence and persistence with respect to key parameters based on simulations with both *Aedes* and *Culex* vectors. For each scenario shown, the *Culex* and *Aedes* bite rates were set to be the same as the *Aedes* bite rates. A) The relationship between the median annual seroprevalence and *Aedes* and *Culex* bite rates and transovarial transmission is shown. B) Demonstrates the increase in persistence as the *Aedes* and *Culex* bite rates and the host-vector ratio increases. C) Viral persistence decreases as the percent of hosts vaccinated increases. High rates of transovarial transmission improve RVFV persistence. D) Indicates that RVFV persistence decreases as the proportion of vaccinated sheep and lambs increases. Higher *Aedes* and *Culex* bite rates require higher rates of vaccination to prevent persistence. E) Persistence either remains the same as *Aedes* egg survival increases or it has a pattern of increasing, reaching a plateau and decreasing again, depending on the transovarial transmission rate.

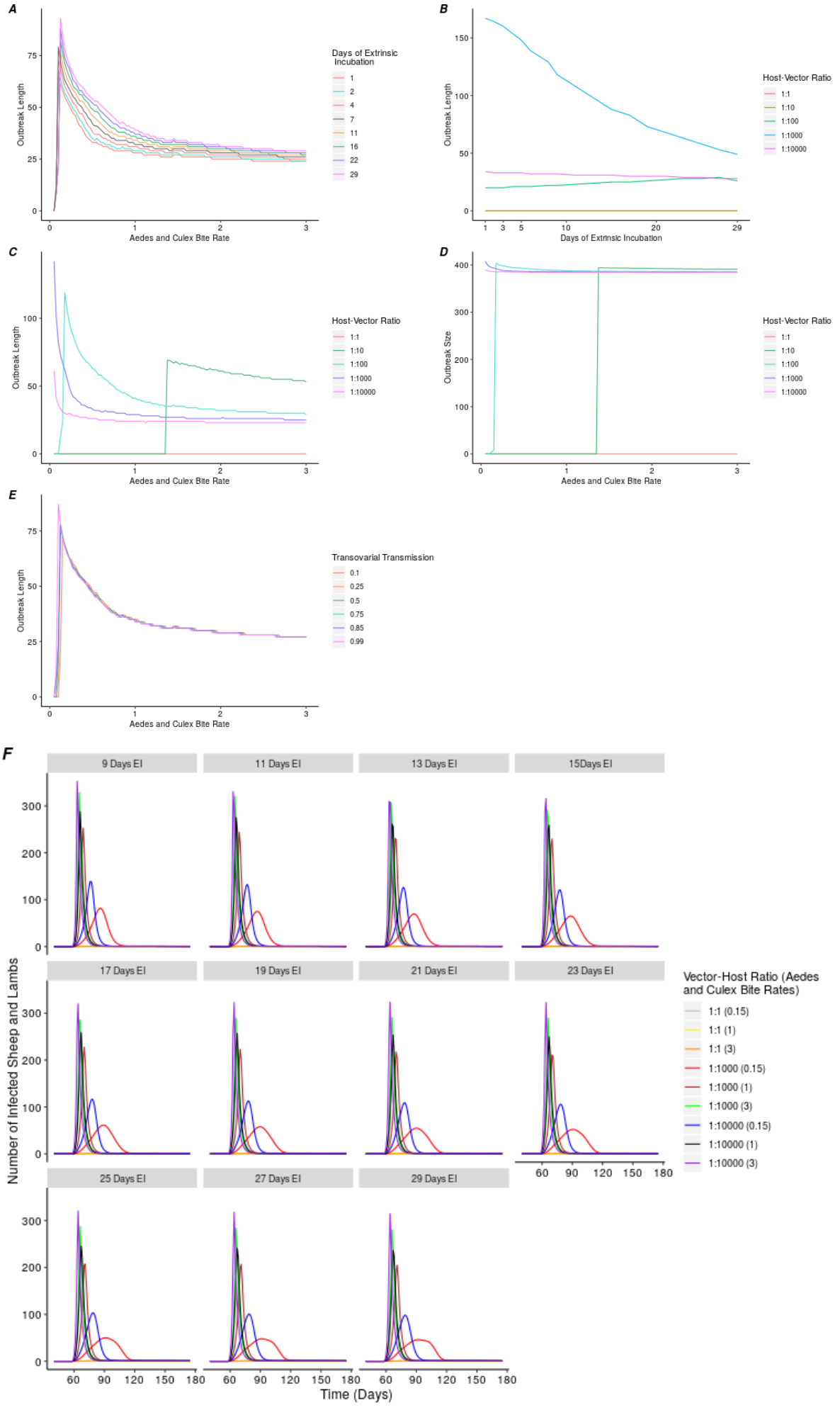

A
Outbreak Length
Aedes and Culex Bite Rate
Days of Extrinsic Incubation
1
2
4
7
11
16
22
29
B
Outbreak Length
Days of Extrinsic Incubation
Host-Vector Ratio
1:1
1:10
1:100
1:1000
1:10000
C
Outbreak Length
Aedes and Culex Bite Rate
Host-Vector Ratio
1:1
1:10
1:100
1:1000
1:10000
D
Outbreak Size
Aedes and Culex Bite Rate
Host-Vector Ratio
1:1
1:10
1:100
1:1000
1:10000
E
Outbreak Length
Aedes and Culex Bite Rate
Transovarial Transmission
0.1
0.25
0.5
0.75
0.85
0.99
F
9 Days EI
11 Days EI
13 Days EI
15Days EI
17 Days EI
19 Days EI
21 Days EI
23 Days EI
25 Days EI
27 Days EI
29 Days EI
Number of Infected Sheep and Lambs
Time (Days)
Vector-Host Ratio (Aedes and Culex Bite Rates)
1:1 (0.15)
1:1 (1)
1:1 (3)
1:1000 (0.15)
1:1000 (1)
1:1000 (3)
1:10000 (0.15)
1:10000 (1)
1:10000 (3)

Figure 4-6 Simulations to assess the impacts of bite rate and host-vector ratios on outbreak size and length for the simulation between August 2008 and August 2009. A) Indicates how outbreak length changes relative to the *Aedes* and *Culex* bite rates and the extrinsic incubation period of RVFV in mosquitoes. B) Shows how outbreak length changes with respect to the vector-host ratio and the extrinsic incubation period. C) Demonstrates how outbreak length changes with relation to *Aedes* and *Culex* bite rate and host-vector ratio. D) Indicates how outbreak size changes with *Aedes* and *Culex* bite rates and host-vector ratio. E) Illustrates how outbreak length changes with *Aedes* and *Culex* bite rates and the transovarial transmission rate. F) Eleven simulations produced outbreaks of 50-70 days. All of the outbreaks of 60 days length were produced at varying lengths of extrinsic incubation and at a host-vector ratio of 1:1000 with a bite rate of 0.15 (these particular scenarios are represented by the red curves). For all simulations both *Aedes* and *Culex* mosquitoes have the same bite rate. The host-vector ratio indicates the limit that the ratio reached between hosts and the combined vectors during the 1-year simulation.

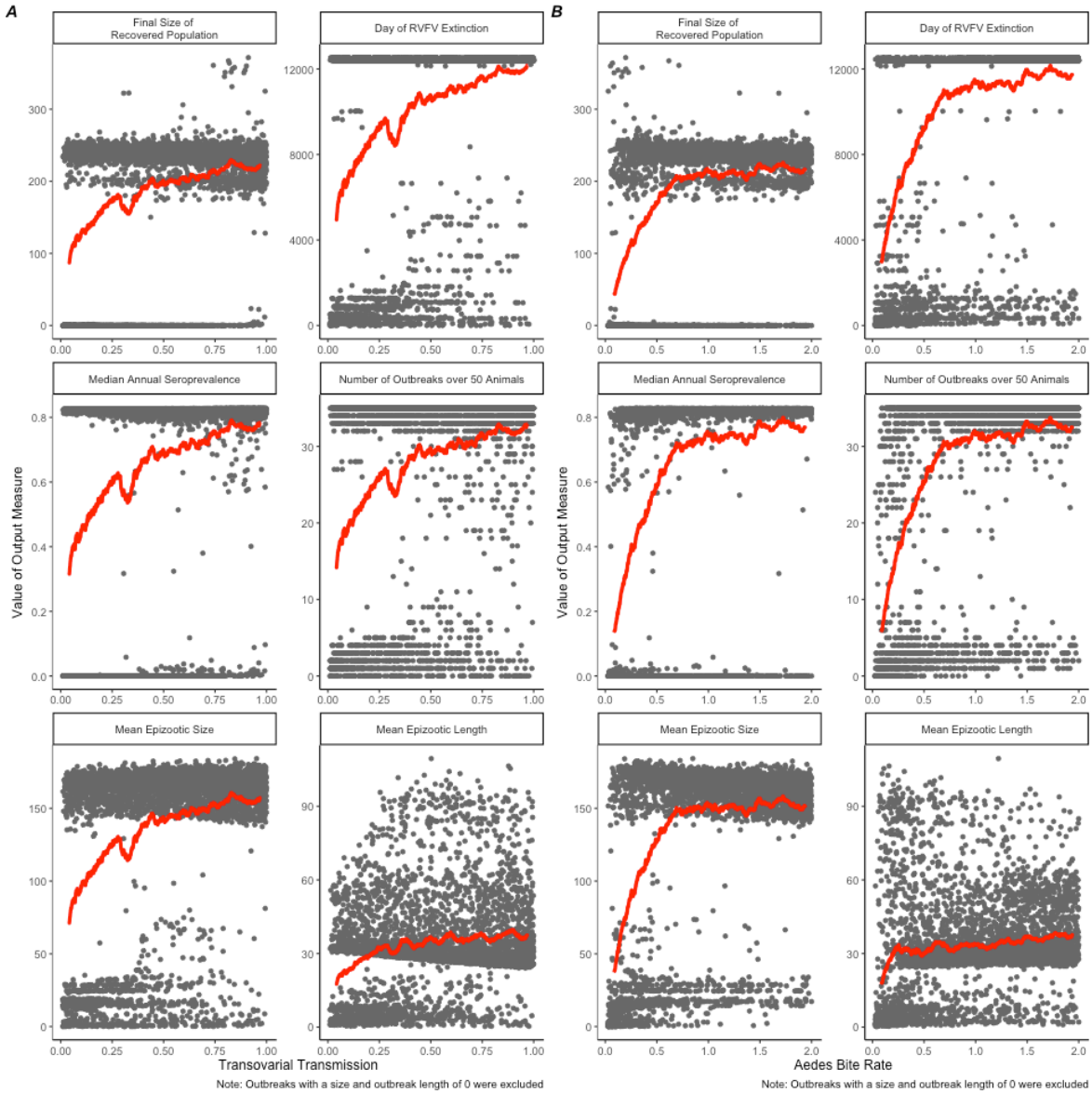


Figure 4-7 The results of 4000 simulations of the model varying the A) transovarial transmission rate and B) the *Aedes* bite rate by outbreak related outcome factors. The grey points show the actual value for each simulation and the red line indicates the moving average of the values.

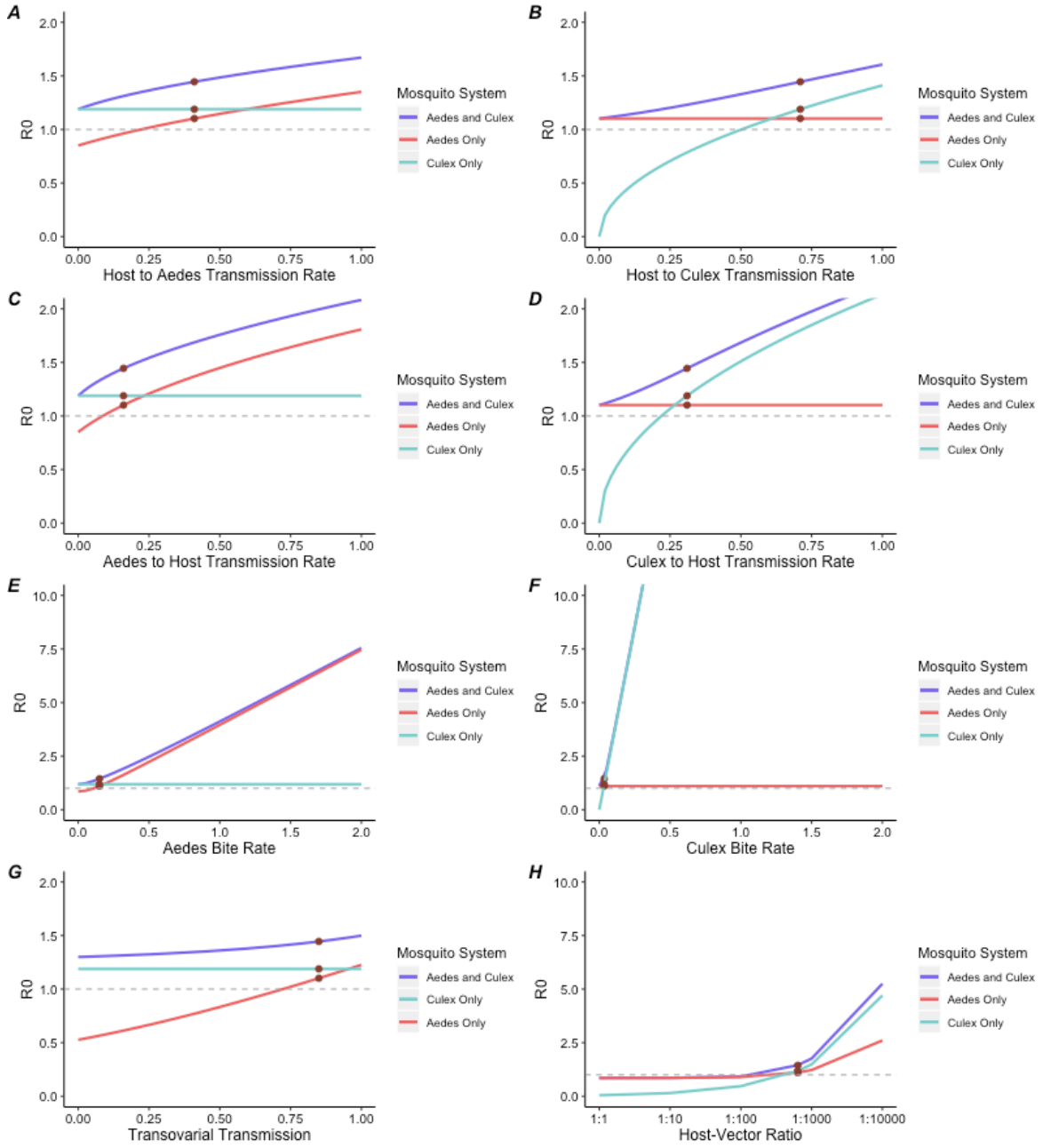


Figure 4-8 Sensitivity analysis of R_0 illustrate how R_0 changes in relation to A) the host-*Aedes* transmission rate (β_{ASL}); B) host-*Culex* transmission rate (β_{CSL}); C) *Aedes*-host transmission rate (β_{SLA}); D) *Culex*-host transmission rate (β_{SLC}); E) *Aedes* bite rate; F) *Culex* bite rate; G) transovarial transmission rate and H) host-vector ratio. The red line represents the system with *Aedes* only, the teal line represents the system with *Culex* mosquitoes only and the purple line represents the system with both *Aedes* and *Culex*. The brown dots indicate the R_0 at the parameter value used in our simulation.

DISCUSSION

To address the question of whether RVFV can persist in a single pan system we developed a mathematical model that indicates that RVFV persistence is possible over a 34-year period. While persistence is possible, it is precariously balanced between extinction and high circulation rates that would lead to unnaturally high levels of seroprevalence. By incorporating a univoltine life cycle in the transovarial vector and temperate climatic forcing provided by rainfall and temperature data from a representative farm in central South Africa, we simulated realistic patterns of RVFV activity over the 34-year period. We show that while only a moderate portion of the herd on a farm would need to be vaccinated to drive RVFV to extinction, this level of vaccine coverage would likely need to be maintained for years to cause local RVFV extinction.

RVFV persistence and R_0 were particularly sensitive to bite rate, the host-vector ratio and transovarial transmission. Small changes in these parameters cause large changes in both RVFV persistence and annual median seroprevalence. Interestingly, a system with only *Aedes* mosquitoes seem to support persistence in a state of quasiequilibrium. In the simulation with both *Aedes* and *Culex*, *Aedes* bite rate was significantly and positively associated with mean outbreak size and a similar pattern was seen with the other *Aedes* transmission terms. Contrastingly, the *Culex* bite rates were negatively associated with the mean outbreak size over the 34-year simulation, though the *Culex* bite rate supports larger individual outbreaks. While the interepizootic transmission dynamics were well modelled by this simulation, outbreak dynamics are less so. The duration of the largest predicted epizootic lasted 212 days. While this timescale is appropriate for a regional outbreak period, it is far too long for the time course of an outbreak on a single farm. This suggests a different set of parameters may govern epizootic dynamics.

Enzootic transmission

The simulations supported persistence, while maintaining median annual seroprevalence levels between 12% and 17%. The median seroprevalence was key to assessing whether the model produced a simulation of biologically plausible RVFV dynamics. These values are within the range of observed seroprevalences reported in the literature (2-40%) (Moiane et al., 2017, Sumaye et al., 2013, Roger et al., 2011, Ngoshe et al. submitted). Additionally, the mean incidences estimated by the simulations (7 and 9 infections per 100

sheep) are near the estimates of incidence by a repeat cross-sectional study conducted in the same area (95% CI ranged up to 6.3 seroconversions per 100 animal-years; Chapter 2).

Our simulation generated a pattern of temporal variation in the annual epizootic size. The simulation produced many small annual outbreaks interspersed with medium-sized outbreaks and a very large outbreak in 2009. This simulated temporal variation is consistent with changes observed in the rate of incidence in KwaZulu-Natal, South Africa (van den Bergh et al., 2019). Pedro et al. (2014) suggested that *Aedes* oviposition rates/transovarial transmission played a key role in the irregular behaviour of the system that led to outbreaks. However, by simulating both a system with *Aedes* only and *Aedes* with *Culex,* we were able to compare the dynamics. The system with *Aedes* only rapidly approached a quasiequilibrium of small seasonal outbreaks. Our results may have been different than Pedro et al.'s (Pedro et al., 2014) findings because we simulated a univoltine life cycle in the transovarial vector.

When *Culex* were included in the simulation, larger epizootics occurred interspersed among the smaller outbreaks. We suggest two reasons for this change, first R_0 was very sensitive to increases in the *Culex* transmission terms and bite rate, which suggests that *Culex* mosquitoes support individual outbreaks of a larger size. The second is that climate forcing likely had a more significant effect on the *Culex* populations than on the *Aedes*. We suggest that climate forcing had a different effect on *Aedes* and *Culex* populations on the basis for the difference is the amount of time each species' eggs were hatching and therefore how long the larval and pupal stages were present during the simulated year. The *Culex* eggs continued hatching and larvae/pupae developing for the duration of the season, whereas the *Aedes* larvae and pupae were only present following the few days they hatched each year. The short timeframe when the *Aedes* developmental stages were present likely dampened the effect of climate forcing on their population. It is well known that climate forcing can dampen or intensify transmission dynamics through seasonal and multi-annual variation (Metcalf et al., 2017).

The fact that all *Aedes* transmission terms (including bite rate), except one, were significantly and positively correlated with all of the outcome factors examined indicates that the *Aedes* dynamics are the primary driver of persistence and seroprevalence, rather than the *Culex* dynamics. RVFV persistence is most sensitive to the *Aedes* bite rate (Figures 4-5 and S4-5). The univoltine life cycle and its biological implications could make RVFV persistence more dependent on *Aedes* transmission terms (horizontal and vertical).

The *Culex* transmission terms (bite and transmission rates) were only significantly and positively associated with mean outbreak length. As amplification vectors we expected that the *Culex* would contribute to outbreak length and size. Though we were initially surprised by the finding that they were negatively associated with outbreak size, we were able to provide a biological explanation for it, which is discussed below.

Our simulation captures the general historical pattern of RVF outbreaks. The largest predicted epizootic occurred in the spring of 2009, only one year earlier than the historical epizootic of 2010 (Pienaar and Thompson, 2013). Within a single pan system, if sheep were not be present when the conditions for an RVF outbreak were right, the mosquitoes would need to feed on other wildlife that may not be amplifying hosts (e.g. birds, lizards, rodents etc.). The simulation also generated smaller epizootics in 1987 and 1999. Historically, RVF outbreaks were reported in South Africa in October 1987 (no location was provided) and in 1999, which affected African buffalo (*Syncerus caffer*) near Kruger National Park (Pienaar and Thompson, 2013).

Rift Valley Fever Virus Persistence

We found that Rift Valley fever virus persistence was possible in a single pan system, though it appears to be balanced on a knife edge, with slight changes to parameter values shifting the system toward extinction or high rates of seroprevalence (Figure S4-5). Our sensitivity analysis indicated that only 0.125% of 4,000 simulations demonstrated persistence within a biologically observed range (5-40% [Clark et al., 2018]). This knife edge is illustrated by the steep slope from high seroprevalence to extinction as we varied two parameters, such as bite rates and vector-host ratio (Figures 4-5 and S4-5).

We propose that RVFV persistence is so sensitive to the parameters investigated because the R_0 was calculated to be 1.44. When R_0 is close to unity, disease systems are very sensitive to initial conditions. Iacono et al. (2018) also noted that as their RVFV system neared non-persistent conditions, it became highly sensitive to initial values of infection prevalence. The R_0 we estimated is similar to previously reported estimates made using other mathematical models, most of which range from 0.798-2.28 (Chitnis et al., 2013). Métras et al. (2012) estimated a higher effective reproduction number (4.3) during the 2010-2011 RVFV outbreak in South Africa. However, their estimate was based on farm-to-farm transmission, rather than the animal-to-animal transmission that we estimated.

Transovarial Transmission

We estimated that that transovarial transmission rate would need to be high (0.85) to support RVFV persistence. While persistence and R_0 were positively associated with transovarial transmission, on the Latin hypercube analysis persistence was more associated with the horizontal transmission terms and the *Aedes* bite rate (Table 4-7 and Figure 4-8). We propose that the transovarial transmission rate was critical in the balance between persistence and high seroprevalence within the ecosystem simulated. As the bite rate decreased, the transovarial transmission term had to be increased to maintain the seroprevalence. Transovarial transmission would likely be critical to consider in plans to locally eliminate RVFV using vaccination. Higher rates of transovarial transmission require higher proportions of the herd to be vaccinated for longer periods of time (Figures 4-5 and S4-8). The use of vaccination in this system is discussed further below.

We are not the only researchers to suggest that the transovarial transmission rate must be high to support persistence. Sumaye et al. (2019) also used a high transovarial transmission rate to allow RVFV to persist. This challenges the common perception that RVFV transovarial transmission rates are low, approximately 1%, as described for dengue virus (Shroyer, 1990). There are viruses that have higher rates of transovarial transmission, such as *Culex* flavivirus infections (Saiyasombat et al., 2011), indicating that it is biologically plausible. In our study we found that if the transovarial transmission rate was low, the bite rate needed to be increased to maintain RVFV persistence. High vector bite rates generated an unrealistically high seroprevalence that was maintained for the entire simulation period (Figure S4-7).

The question of what is the transovarial transmission rate continues to be debated. While there is field evidence to support its occurrence (Linthicum et al., 1985b, Romoser et al., 2011), no laboratories have yet confirmed its occurrence (Turell et al., 2008, McIntosh et al., 1980, Gargan et al., 1988). A low transovarial transmission rate for RVFV was suggested based minimum field infection rates that were estimated at 1/820 (Gargan et al., 1988) and 1/1000 (McIntosh et al., 1980) in *Aedes juppi* and *Culex zombaensis*, respectively. Our simulations suggest that even with the high transovarial transmission rate, the estimated mean proportion of infected *Aedes* eggs to be 0.0032 (range: 0.0019-0.0054; Table 4-6).

Transovarial transmission rates of RVFV may vary across ecosystems that have different evolutionary pressures based on the length of the dry/cold season. Iacono et al. (2018) simulated low-levels of interepizootic transmission of RVFV over a region where the temperature was rarely lower than 20ºC and water was always present using a transovarial transmission rate of 0.007. Both Nicolas et al. (2014) and Cavalerie et al. (2015) simulated ongoing RVFV transmission in the complete absence of transovarial transmission in Madagascar and Mayotte, respectively. The single exception was the scenario that had a longer dry season, which required transovarial transmission (Cavalerie et al., 2015). Manore and Beechler (Manore and Beechler, 2015) also suggested transovarial transmission was needed for persistence in systems with prolonged dry seasons. Our model had distinct seasonal periods when the simulated mosquitoes were not hatching, thus requiring a higher transovarial transmission rate.

Aedes and *Culex* Bite Rates

R_0 was extremely sensitive to vector bite rates and persistence was the most sensitive to the *Aedes* bite rate. We assumed that vector bite rates were low on the basis of: 1) zoophilic mosquito vectors (Meegan and Bailey, 1989) will also feed on wildlife present in the pan; and 2) given that the average farm size was 22 Km^2 in the western Free State (Chapter 3), the sheep will not always be within the 150 m dispersal range of floodwater mosquitoes (Linthicum et al., 1985a). *Culex* were assumed to have an even lower bite rate even though they have a larger range (e.g. 550 m – 9.7 Km (Verdonschot and Besse-Lototskaya, 2014)), as we assume there will be larger numbers of wildlife in the larger area, which should decrease the proportion of bites on the sheep. It is quite common for mathematical models to use a low bite rate when simulating RVFV epidemiology. Iacono et al. (2018) assumed that only 1% of the vectors seeking a bloodmeal fed on the simulated livestock. Alternatively, Sumaye et al. (2019) incorporated a wildlife term that the vectors could bite, reducing the bite rate on livestock.

Interestingly, our sensitivity analysis indicates that *Aedes* bite rates were positively associated with persistence, but *Culex* bite rates were negatively associated with persistence. The *Culex* bite rate was also negatively associated with epizootic size. The reason for these findings are likely related. Our R_0 and epizootic sensitivity analyses are supported by the literature in suggesting that the *Culex* bite rate is associated with larger individual outbreaks during a single year and a higher R_0, which would result in a larger outbreak (Fischer et al., 2013, Gachohi et al, 2016, Chitnis et al., 2013, Pedro et al., 2016).

R_0 was the most sensitive to the *Culex* bite rate compared to all other parameters examined, with R_0 exceeding 10 for bite rates approaching 1. This aligns with the perception that the *Culex* mosquitoes are the amplifying vectors of the outbreaks (Linthicum et al., 2016). However, over 34 years, it is possible that the individual, large outbreaks amplified by *Culex* could result in an overall smaller mean outbreak size and lower persistence. This is because there would be fewer susceptible animals available to the infected vectors the following years, resulting in smaller outbreak sizes and possibly extinction.

Host-Vector Ratios

Our analysis indicates that host-vector ratios are very important drivers of persistence and R_0. Host-vector ratios of a greater magnitude than 1:100 supported RVFV persistence even at low vector bite rates. We found that after bite rates, R_0 was the most sensitive to the host-vector ratio, this finding has been supported by other analyses (Fischer et al., 2013, Barker et al., 2013). Most mosquito species, particularly floodwater mosquitoes, do not have well-defined carrying capacities. Yet, our interest in a relatively small population of sheep on a single pan made mosquito carrying capacity estimates particularly important.

As would be expected, several other models have suggested that host-vector ratios are important for RVFV persistence (Pedro et al., 2016, Cavalerie et al., 2015, Chitnis et al., 2013). McMahon et al.'s (McMahon et al., 2014) work suggested that decreasing the mosquito population by a factor of 2.5 would reduce the number of RVFV-specific livestock deaths by 10-fold during an outbreak.

The Effect of the Single-Pan Scale

Our simulation indicated that RVFV can persist in a single pan system for 34 years while capturing the expected pattern of epizootics. However, we have also provided evidence that slight changes in parameters can force the system toward extinction or maintaining high seroprevalence. Additionally, we see that the number of simulated *Aedes* eggs were decreasing over time (Figure 4-3), this may indicate that RVFV may not continue to persist in the system beyond our timeframe. Given the high risk of extinction, it is important to consider other mechanisms of persistence. Favier et al. (2006) used a meta-population approach to suggest that RVFV could be maintained within a network of local pans by the

movement of livestock between seasonally inundated pans. They suggested that RVFV tended to go extinct within each pond system over varying periods of time, but was maintained in the system through long interepizootic periods.

Most other studies focus on a region or system of pans as Favier et al. (2006) did rather than at the level of a single pan. At the regional level RVFV transmission has often been modelled at a lower host-vector scale because the dynamics occur at the meta-population level. The larger area also permits more water availability, even if the size of the water changes seasonally (Iacono et al., 2018). Alternatively, several models use movement between regions/pans reduce livestock-mosquito contact, which effectively reduces the host-vector ratio. Sumaye et al. (2019) integrated livestock movement between a region with high-levels of RVFV circulation and regions with little interepizootic transmission. Nicolas et al. (2014) also used a spatially explicit model to simulate ongoing low-level transmission of RVFV (without transovarial transmission) in Madagascar. They suggested that RVFV is maintained either by movement of *Culex* mosquitoes between villages or by farmers that move their animals back and forth between villages.

Epizootic Dynamics

Our model was designed to investigate interepizootic RVFV transmission and maintenance. However, it did predict a large epizootic in the spring of 2009. The simulated outbreak lasted for 212 days. This is not a realistic timeframe for an RVF epizootic on a single farm. Xue et al. (2012) modelled the 2010 outbreak across three provinces in South Africa and suggested that the whole series of individual epizootics across all farms took up to 200 days. An early report of an RVF epizootic on a large farm in South Africa stated the outbreak started in March and was waning at the end of April. This suggests that a reasonable timeframe for an outbreak on a single large farm would be approximately 60 days (Gear et al., 1951). Daubney and Hudson (Daubney et al., 1931) also reported an outbreak of RVF that lasted two months, although they stated that the farmer moved the sheep to higher altitude, which was likely out of the range of the infected mosquitoes. Given these timeframes, our model of long-term circulation does not appear to depict realistic epizootic conditions as well. Alternatively, it is possible that RVFV does circulate on farms longer than has been recorded. Our simulation indicates that the 2008-09 outbreak started in October 2008, but the number of sheep affected per day was very low until the outbreak started taking off with the *Culex* amplifying the outbreak in January (Figure 4-4). There is sufficient evidence that RVFV can circulate on farms without

detection of clinical signs by the farmer (Chapter 2), thus it is possible that epizootics start slowly smouldering for some time before the *Culex* are able to amplify the outbreak sufficiently for outbreak detection.

To better understand the drivers of outbreak size and length, we explored the contribution of several parameters that could potentially change during an epizootic. Specifically, the abnormally high precipitation during an epizootic (Glancey et al., 2015) would drive the mosquito populations to explode, increasing the host-vector ratio. If the sheep are unable to move away from the flooded pan, the bite rate targeted at the sheep may increase as well. Finally, depending on the number of different mosquito species contributing to horizontal RVFV transmission, the extrinsic incubation period may vary significantly. Given the same initial conditions going into the outbreak year (August 2008), the epizootic length of the 160 outbreak simulations ranged significantly. It is surprising that all of the simulations that resulted in an outbreak that was between 50-70 days long (Figure 4-8F) had a bite rate of 0.15 (*Aedes* and *Culex*) and a host-vector ratio was 1:1,000. This might indicate that these values are biologically relevant if outbreaks usually 60 days in length. While our analysis did not identify many outcome factors that were sensitive to extrinsic incubation period, other studies have suggested that it is associated with outbreak size (McMahon et al., 2014, Fischer et al., 2013).

We found that as the host-vector ratio increased, so did epizootic size and length, especially at higher bite rates (Figure 4-8C and 4-8D). Few studies have evaluated outbreak length as an important outcome as we did, Mweya et al. (2014) suggested that epizootic length was sensitive to host incubation period. Several models have identified factors associated with outbreak size that were similar to what we found. Chitnis et al. (2013) reported that the propensity for large outbreaks in their system was sensitive to the host-vector ratio, vector bite rates and the probability of transmission. Cavalerie et al. (2015) found that outbreak size was most sensitive to increasing the mosquito hatching rates (thereby likely increasing the host-vector ratio).

We also found that decreasing the delay between the hatching of *Aedes* and *Culex* increased the outbreak size, but also decreased the outbreak length. This is likely because the earlier the *Culex* contact infected hosts, the larger the outbreak would be and therefore it would burn through the susceptible population faster, resulting in a large but short outbreak. Several studies identified parameters to which the size of the epizootic was sensitive that we did not include in our sensitivity analysis. These include: infectious

period (Mweya et al., 2014, Gachohi and Bett, 2016), infectivity in hosts and vectors (Gachohi and Bett, 2016), vector death rates (Mpeshe et al., 2011, Fischer et al., 2013) (or specifically *Culex* death rates [Gachohi and Bett, 2016]) and the recruitment of susceptible hosts (Mpeshe et al., 2011).

RVFV Vaccination

The R_0 calculation suggests that the herd immunity threshold (proportion that need to be vaccinated to prevent an outbreak) is 31%. However, our simulations indicate that vaccination at this level would need to be maintained for 19 years to force RVFV to extinction (Figures 4-5 and S4-8). The most likely explanation for this behaviour is the high transovarial transmission rate we used. In a system with no sheep-to-vector transmission, 85% of the next generation of *Aedes* eggs laid by infected *Aedes* adult mosquitoes will be infected through transovarial transmission. This can continue to produce one or two infected *Aedes* mosquitoes for decades (Figure S4-9). Additionally, it is not clear whether low level vaccination (e.g. 31%) could drive RVFV to extinction in the presence of wildlife or other unvaccinated ruminant hosts. Vaccinating a higher proportion of animals in the flock could reduce the likelihood of RVFV persisting in other ruminants and drive the virus to local extinction faster. Adongo et al. (2013) developed one of the first mathematical models to include RVFV vaccination as a mitigation strategy. By implementing an optimal control technique, that varied periods of maximum vaccination followed by reduced levels of vaccination, they found that a high vaccination rate (maximum of 85%) would be required to drive RVFV to extinction. They assumed the vaccination rate would vary with time, but that the virus would go extinct in approximately 10 years (Adongo et al., 2013).

Gachohi et al. (2016) examined three scenarios, 1) vaccination implemented prior to an outbreak; 2) vaccination at six month intervals over two years and 3) vaccinating one of two host species. They suggested that pulse vaccination (biannual, annual or prior to an outbreak) would be quite effective (given sufficient time before an outbreak for the vaccine to be efficacious). They estimated that with a perfect vaccine even 20-30% vaccination coverage would decrease the incidence of RVFV to zero. Their estimate of the proportion of animals that need to be vaccinated is similar to ours, though they have a much shorter timeframe. This is not surprising given they excluded transovarial transmission from their model (Gachohi et al., 2016). A third study suggested that, even at relatively low rates, vaccination can prevent infection and lead to a 30-fold reduction in RVFV-associated

mortality (McMahon et al., 2014). They suggested vaccination would be even more efficacious if given over a two-week period. Our model simulates a daily vaccination rate, it is likely that mass vaccination before the vector season would be more effective at driving RVFV to extinction at lower vaccination rates.

CONCLUSIONS

This is the first mathematical model to indicate that RVFV persistence in a single pan is possible over a 34-year period. Our simulation generated plausible dynamics when compared to the historical record of RVF in the western Free State, South Africa. While persistence was maintained, it was only possible for within a small range of key parameters. Small changes in vector bite rate, host-vector ratio and transovarial transmission rapidly shifted the system to either extinction or maintenance with a higher seroprevalence than is observed in nature. Our results suggest that different dynamics might drive interepizootic and epizootic behaviour. In addition, although the low R_0 means that the dynamics are sensitive to initial conditions and suggested that while a low herd immunity threshold would be necessary to drive RVFV to extinction, the high transovarial transmission rate means that the proportion of animals vaccinated would need to be maintained for well over a decade to drive the virus to extinction with a local pan.

SUPPLEMENTARY FIGURES:

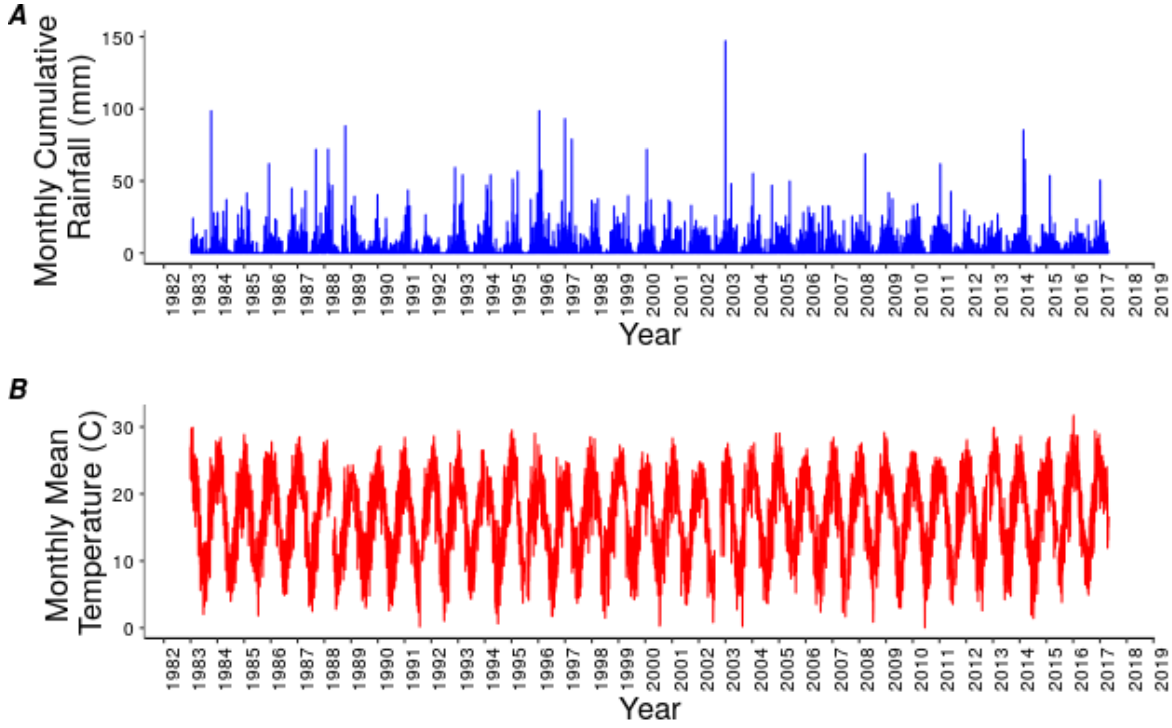


Figure S4-1 Monthly A) cumulative rainfall and B) mean temperature by year from 1983-2017.

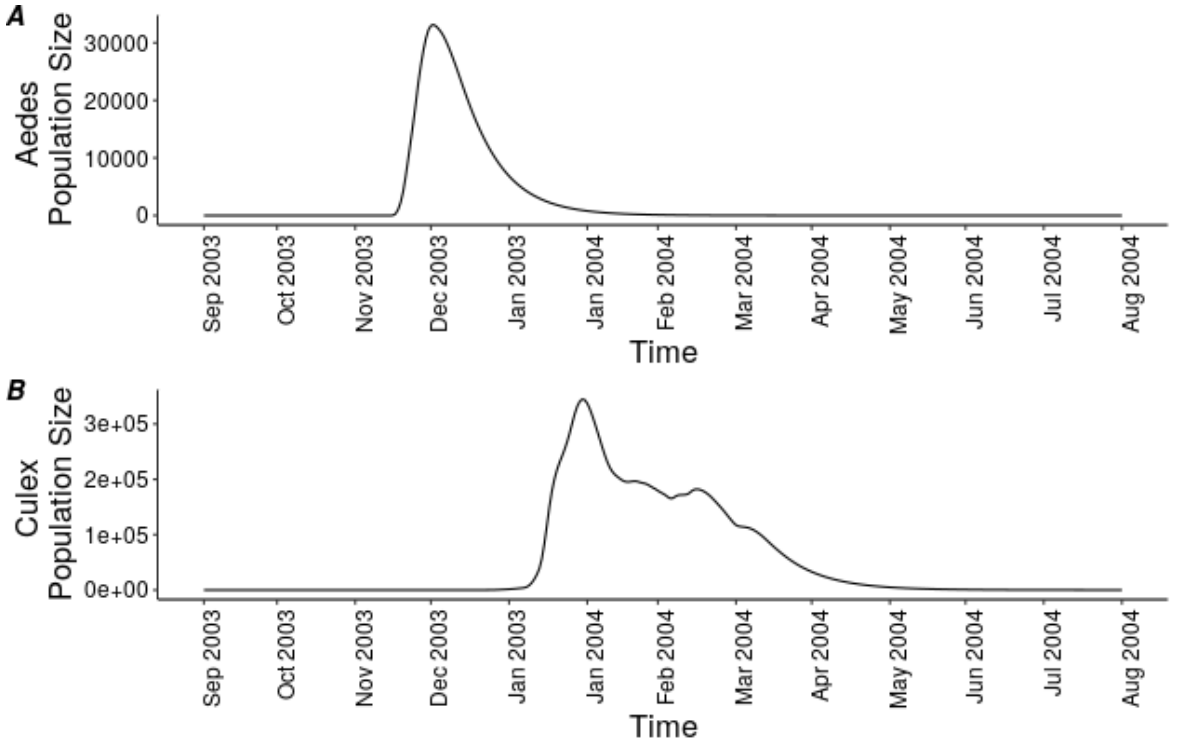


Figure S4-2 The total population of adult A) *Aedes* and B) *Culex* mosquitoes over a randomly selected year (September 2003- August 2004).

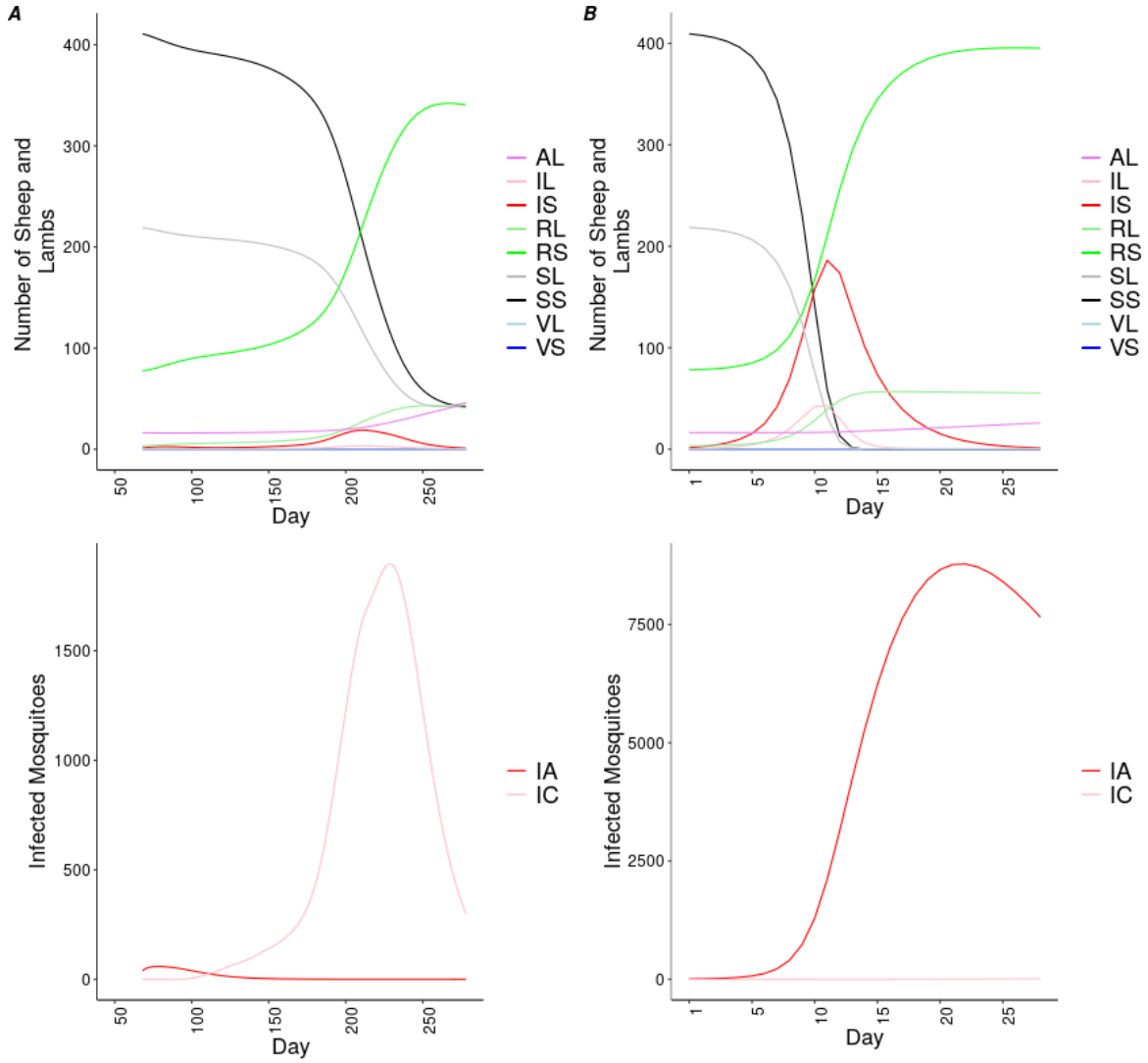


Figure S4-3 A closer look at the outbreak dynamics shown in Figure 4-3. For both panels the top figure is the sheep and lamb dynamics and the bottom panel are the mosquito dynamics. A) An outbreak simulated with the parameters from Table 4-4. B) An outbreak simulated when the *Aedes* and *Culex* bite rates are set to 2. Now the outbreak is very short and occurs much earlier in the year than outbreak A). AL = lambs with maternal antibodies, IL, IS, IA and IC = infected lambs, sheep, *Aedes* and *Culex*, respectively; RL and RS = recovered lambs and sheep, respectively; SL and SS = susceptible lambs and sheep, respectively; VL and VS = vaccinated lambs and sheep, respectively (note there were no vaccinated animals in this scenario).

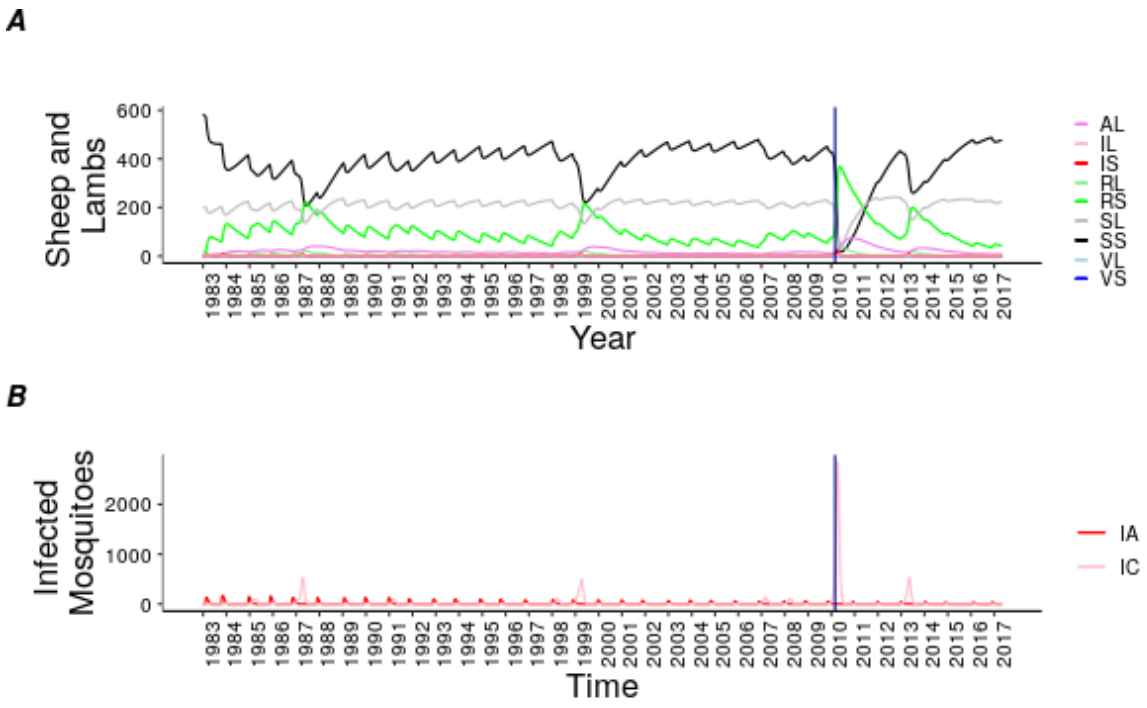


Figure S4-4 The full simulation of RVFV infection dynamics with two vectors in a single host system run for 34 years. This simulation indicates that if the *Culex* mosquitoes were prevented from transmitting the virus during 2009, the largest outbreak of the system occurs in the spring of 2010, matching the historical record. A) The susceptible (black/grey), infected (red/pink), recovered (green/light green) and vaccinated (blue/light blue) populations of sheep and lambs are shown, respectively, and the lambs that have maternal antibodies are shown in purple. The dark blue line indicates the approximate time of the 2010 outbreak. B) The population of infected *Aedes* (red) and *Culex* (pink) mosquitoes is shown. The 2010 outbreak is marked by the dark blue line. AL = lambs with maternal antibodies; IL, IS, IA, IC, and IAE = infected lambs, sheep, *Aedes*, *Culex* and *Aedes* eggs, respectively; RL and RS = recovered lambs and sheep, respectively; SL and SS = susceptible lambs and sheep, respectively. VL and VS = vaccinated lambs and sheep, respectively (no animals were vaccinated during this scenario).

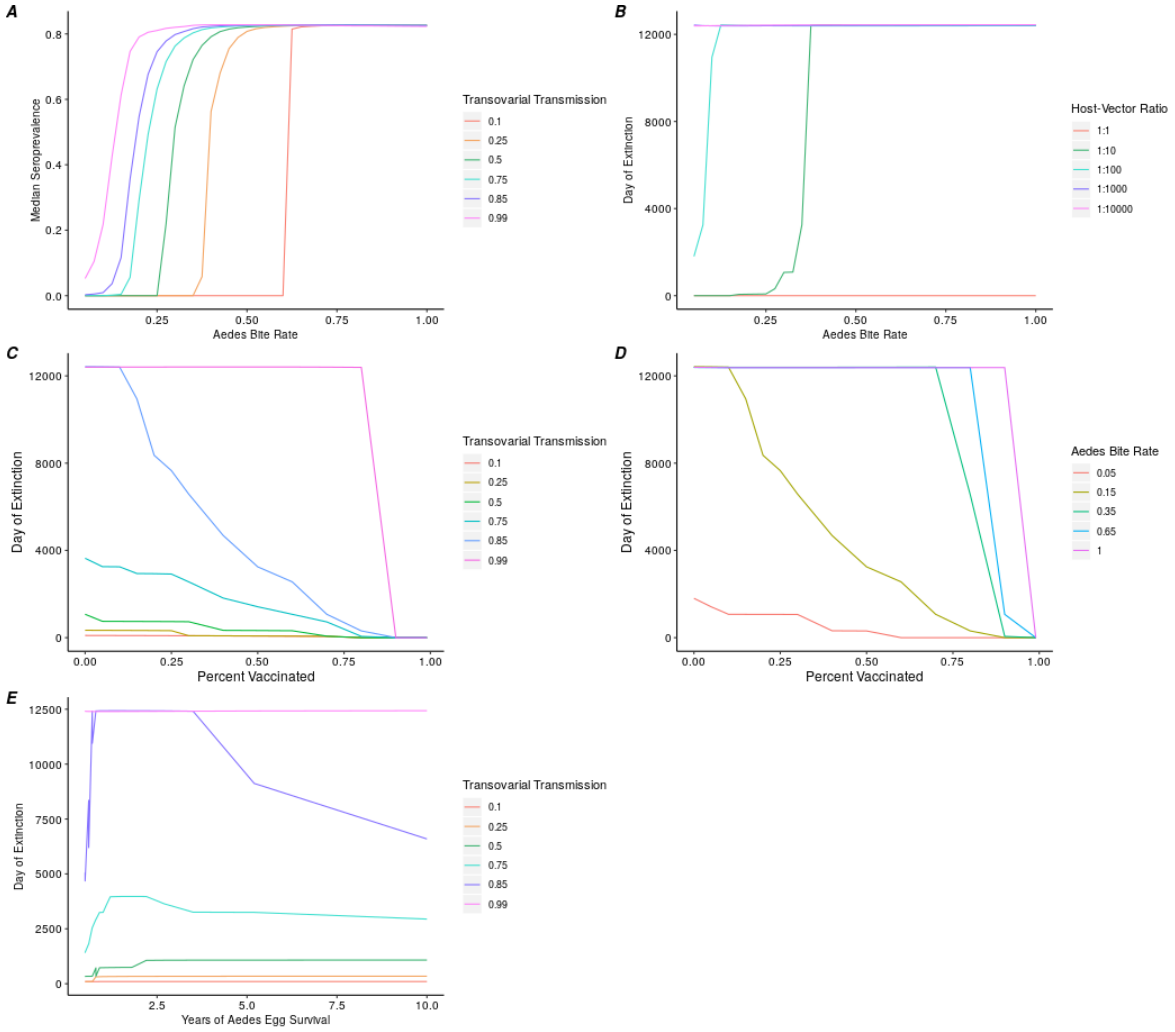


Figure S4-5 RVFV seroprevalence and persistence with respect to key parameters based on simulations with only *Aedes* vectors. A) The relationship between median seroprevalence and bite rate and transovarial transmission is shown. The slopes of the curves indicate the sensitivity of seroprevalence to these parameters within our target seroprevalence of 5-40%. B) Demonstrates the increase in persistence as the *Aedes* bite rate and the host-vector ratio increases. C) Viral persistence decreases as the percent of hosts vaccinated increases, though high rates of transovarial transmission improve RVFV persistence. D) Indicates that a very high proportion of hosts must be vaccinated to reduce persistence at very high bite rates. E) Persistence either remains the same as *Aedes* egg survival increases or it has a pattern of increasing, reaching a plateau and decreasing again, depending on the transovarial transmission rate.

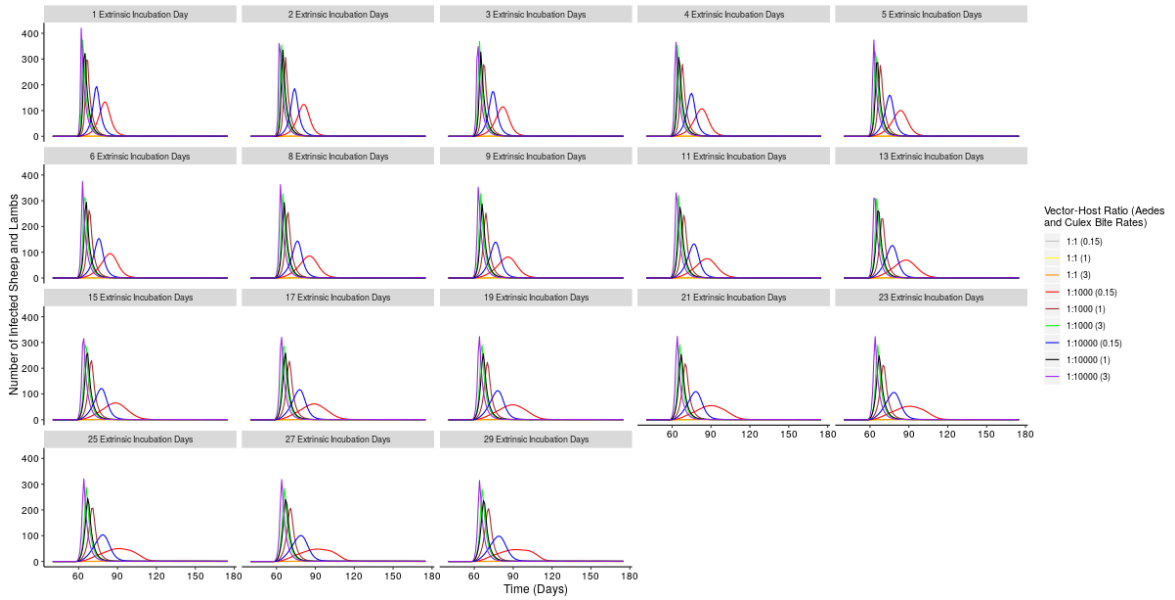


Figure S4-6 The full set of epidemic curves with all combinations. On the y-axis is the number of infected sheep and lambs on a given day, on the x-axis is time in days. The various colours represent a combination of a host-vector ratio and bite rate. The days of extrinsic incubation in the mosquito is on the top of each plot.

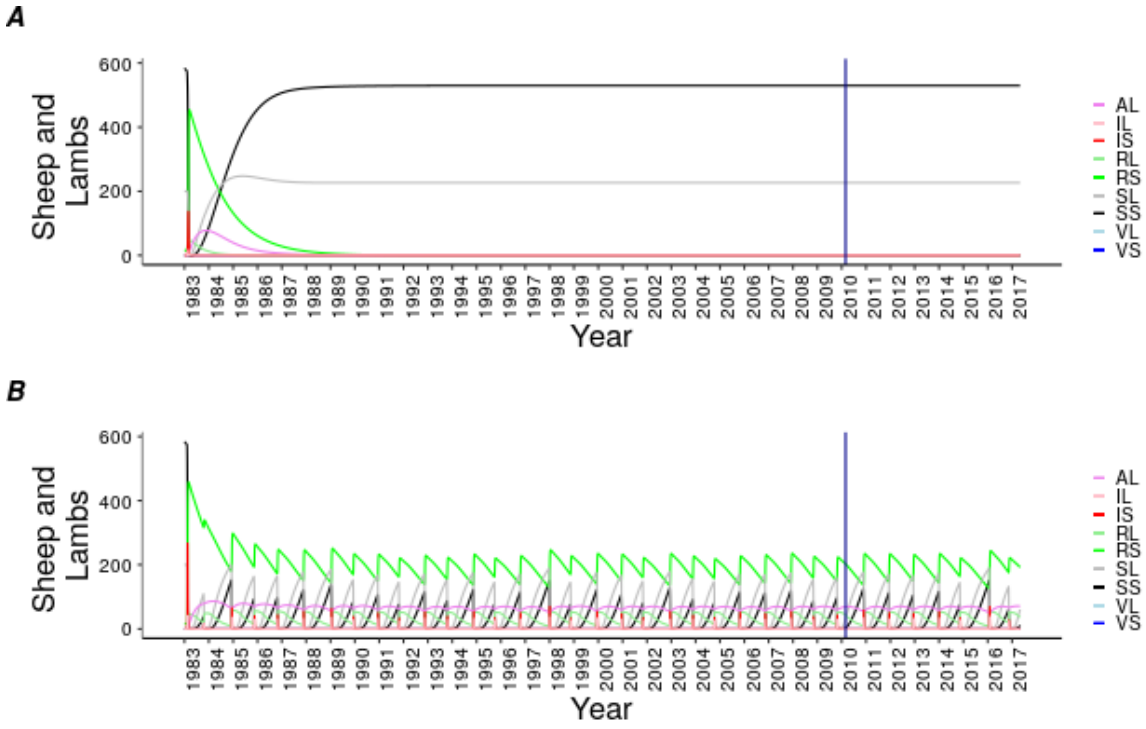


Figure S4-7 Simulations of randomly selected scenarios where RVFV A) is driven to extinction or B) circulates at a high level to induce a high seroprevalence each year. The dark blue line indicates the historical 2010 RVF outbreak. AL = lambs with maternal antibodies; IL and IS = infected lambs and sheep, respectively; RL and RS = recovered lambs and sheep, respectively; SL and SS = susceptible lambs and sheep, respectively. VL and VS = vaccinated lambs and sheep (no animals were vaccinated during this scenario).

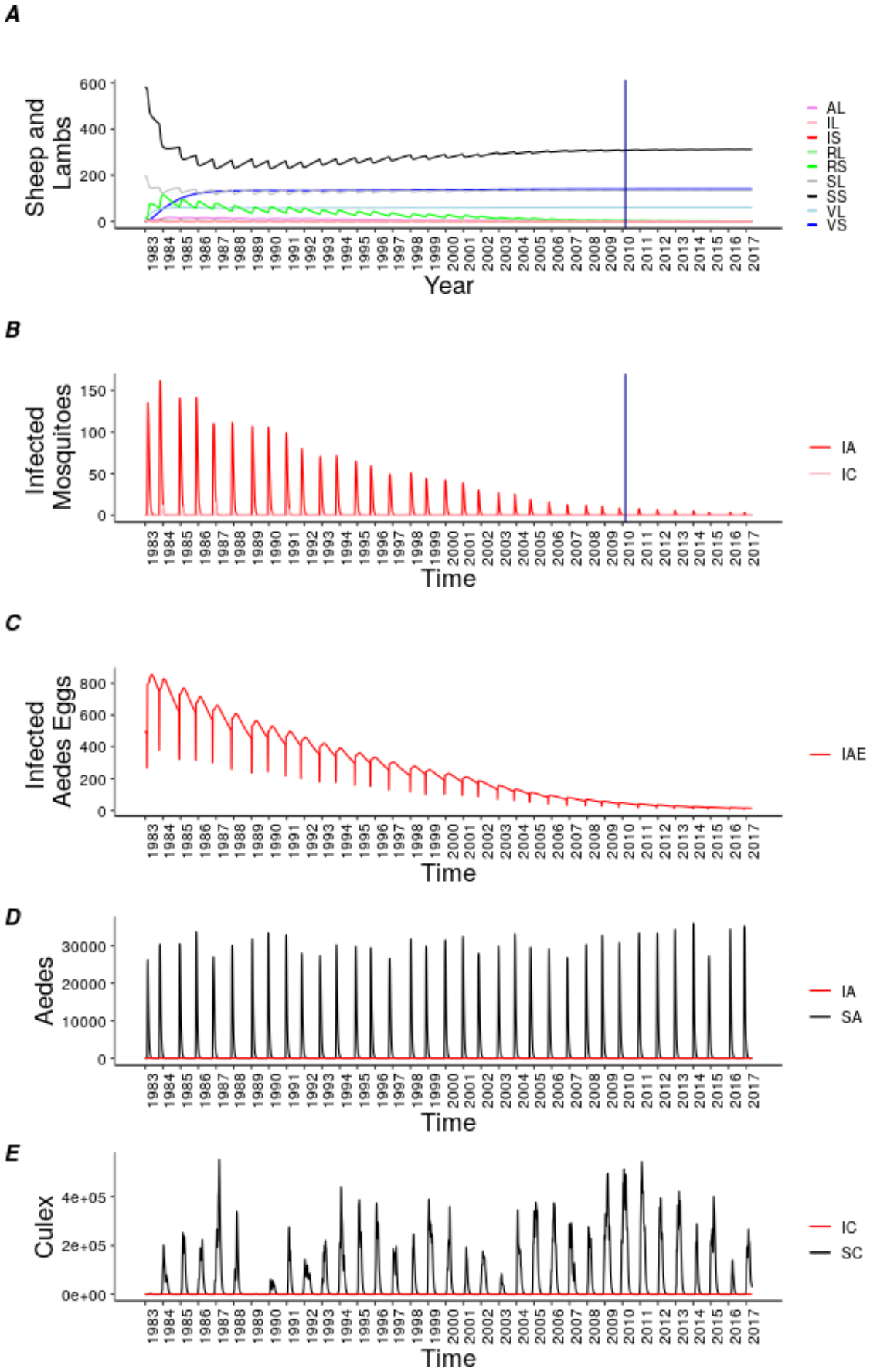


Figure S4-8 A simulation of the full system when the lambs are vaccinated at a rate to ensure that 31% of the flock is vaccinated. A) The dynamics of the virus in the sheep and lambs in the presence of vaccinated (blue) animals. At least one sheep (red) or lamb (pink) is infected each year through 2000. B) The number of infected *Aedes* (red) and *Culex* (pink) mosquitoes throughout the simulation. C) The number of infected *Aedes* eggs (red). D) The number of susceptible (black) and infected (red) *Aedes* mosquitoes. E) The number of susceptible (black) and infected (red) *Culex* mosquitoes. AL = lambs with maternal antibodies; IL, IS, IAE, IA, IC = infected lambs, sheep, *Aedes* eggs, adult *Aedes* and adult *Culex,* respectively; RL and RS = recovered lambs and sheep, respectively; SL, SS, SA, SC = susceptible lambs, sheep, *Aedes* and *Culex*, respectively. VL and VS = vaccinated lambs and sheep, respectively.

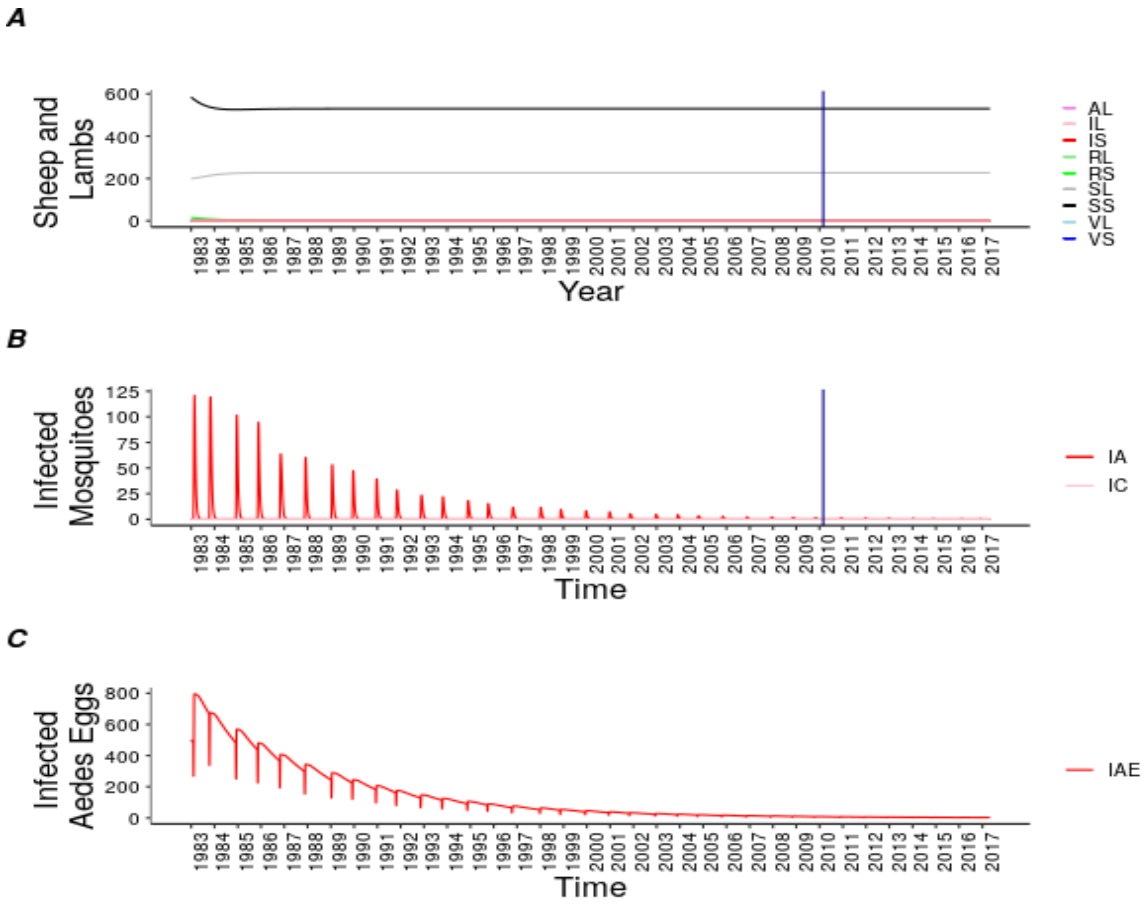


Figure S4-9 A simulation of the full system when RVFV transmission is restricted to transovarial transmission only. A) The population dynamics of the sheep and lambs in the system in the absence of infection with RVFV. B) The number of infected *Aedes* (red) and *Culex* (pink) mosquitoes. C) The number of infected *Aedes* eggs. AL = lambs with maternal antibodies; IL, IS, IAE, IA, IC = infected lambs, sheep, *Aedes* eggs, adult *Aedes* and adult *Culex,* respectively; RL and RS = recovered lambs and sheep, respectively; SL and SS = susceptible lambs and sheep, respectively; VL and VS = vaccinated lambs and sheep, respectively.

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Chapter 5: Preliminary Predictors of Livestock Mortalities and Abortions During the 2010 Rift Valley Fever Outbreak in Central South Africa

ABSTRACT

Rift Valley fever virus (RVFV) can cause expansive outbreaks impacting tens of thousands of domestic ruminants. The primary clinical signs of Rift Valley fever (RVF) are abortion in pregnant animals and mortality. Mortality rates can reach up to 90% in very young ruminants, while in adults the mortality rate is much lower (10-20%). Despite the significant amount of research focused on the pathogenesis in animals infected by RVFV, very little, if any, research has been conducted on farm-level drivers of mortality and abortion. Here we analysed survey data indicating the severity of RVF outbreaks on farms during the 2010 outbreak of RVF in central South Africa using a Bayesian multinomial hurdle model. We hypothesized that farm characteristics that support endemic RVFV circulation or importation of RVFV onto the farm would drive the occurrence of RVF on the farm. We further hypothesized that factors involved in vector amplification and host susceptibility would drive the number of abortions and deaths that occurred during the RVF outbreak. We found strong support for all of hypothesized drivers of RVF-associated death and abortions. Contrastingly, all of the factors driving RVF occurrence had weak or negligible support for the association. However, we did find that short-distance spatial correlation amongst farms was a driver of RVF on the farm, indicating the importance of local spatial processes in the occurrence of RVF outbreaks.

INTRODUCTION

Rift Valley fever (RVF) is caused by a zoonotic arbovirus that can cause massive outbreaks interspersed with periods with very few clinical cases of RVF. RVF virus (RVFV) has spread across Africa, the islands off the east coast of Africa and the Arabian Peninsula (Nanyingi et al., 2015). RVF outbreaks can cause high mortality rates in young lambs (~90%), and lower mortality in adult sheep (~20%), cattle and goats (~10%), and can cause abortion in up to 100% of pregnant domestic ruminants (Easterday, 1965, Swanepoel and Coetzer, 2004). Despite the abundance of work on pathological and clinical effects of RVFV infection in livestock, our epidemiological understanding of RVFV in

farmed ruminants remains lacking. On the farm incidence records over the course of the outbreak (epidemic curves) have not been produced for ruminants on the farm level. At a larger scale, researchers have used spatio-temporal analyses with OIE records to identify ecological risk factors, regions at higher risk for RVF outbreaks (Anyamba et al., 2009), specific ecological factors that contribute to regional RVF outbreaks (Glancey et al., 2015, Lancelot et al., 2017), and the pattern of how RVFV moves between farms (Métras et al., 2013).

RVFV is transmitted to livestock by mosquito vectors (Linthicum et al., 2016). There is evidence that RVFV transmission continues at a low level during the interepizootic period (Chapter 2, Rostal et al., 2010, van den Bergh et al., 2019). However, clinical cases are rarely reported outside of outbreaks, indicating that the occasional abortion or death due to RVFV are not distinguished from other common causes. Interepizootic transmission is maintained by vectors capable of transovarial transmission and/or horizontal transmission (Linthicum et al., 2016). The known transovarial vectors lay their eggs in grassland depressions (called pans) that flood following annual precipitation, which triggers the mosquito eggs to hatch (Linthicum et al., 1984). It has been suggested that interepizootic transmission can support RVFV persistence with transmission between livestock and mosquitoes (Chapter 4; (Njenga and Bett, 2019, Favier et al., 2006)). The role wild ruminants play in RVFV maintenance is unknown; however, many species are susceptible (Rostal et al., 2017). This process likely supports endemic transmission on a farm and is likely magnified by larger farms that can support more pans.

It is thought that interepizootic transmission can shift into an epizootic if the environmental conditions support RVFV amplification by non-transovarial transmitting vectors. After RVFV is transmitted to domestic ruminants by the infected transovarial vectors, it can be amplified by other vectors that can spread the virus horizontally among livestock (Linthicum et al., 2016). These amplification vectors (e.g. some species of *Culex* mosquitoes) generally lay their eggs directly on standing water and can continue hatching throughout the rainy season (Clements, 1992). Thus, abnormally high rainfall events that flood natural water sources, including pans, are associated with RVFV epizootics (Anyamba et al., 2002, Anyamba et al., 2001) and are important for supporting the amplifying vectors.

Several risk factor analyses have suggested that trade can play a role in elevated seroprevalence levels (Nicolas et al., 2014, Chevalier et al., 2011). Further, it is believed

that RVFV spread to the Arabian Peninsula and the east African islands through legal and illegal animal trade with East Africa (Balkhy et al., 2003, Métras et al., 2017). Finally, phylogenetic analyses of RVFV from a number of outbreaks have demonstrated that multiple strains of RVFV can circulate in the same outbreak and some can be from distant regions in Africa (Bird et al., 2007). All of this evidence indicates that trade and importation of RVFV could be an important risk factor for an outbreak.

As domestic ruminants are quite susceptible to RVF, especially sheep, it is important to note that there are vaccines available for animals. There is evidence that the Smithburn modified-live vaccine induces antibodies that persist for the lifespan of sheep (Chapter 3); however, if given to pregnant ruminants it can cause abortion and foetal malformations (Onderstepoort Biological Products, 1947b). The antibodies induced by the Clone 13 (also modified-live) vaccine declined such that only 53% of the vaccinated sheep retained them after the first year (Njenga et al., 2015). An inactivated vaccine is also available; it requires an initial booster as well as annual revaccination (Onderstepoort Biological Products, 1947a). It has been proposed that herd immunity, supported by vaccination, may play an important role in whether RVF outbreaks could occur (Anyamba et al., 2010).

A few studies of RVFV have made use of Bayesian statistical methods to understand the force of infection in livestock (Olive et al., 2017) or spread of RVFV following introduction to a new region (Métras et al., 2017). Though both of these studies included a spatial component, neither included a Gaussian process. Gaussian processes were initially developed for spatial analyses and given that we know spatial patchiness has been described as being associated with RVFV outbreaks (Métras et al., 2013) and seroprevalence studies (LaBeaud et al., 2007), it would be an appropriate method to capture localized spatial variance. A Gaussian process is a collection of random variables, any subset of which will have a Gaussian distribution (Rasmussen and Williams, 2006). The Gaussian process describes a distribution over functions. It is specified by functions that describe the mean and covariance. The covariance function is specified by the covariance between the data points of interest, e.g. locations. The Gaussian process takes a length-scale and signal variance as priors. The Gaussian process acts as a linear smoother and can remove extra noise from the system (Rasmussen and Williams, 2006).

Given the risk factors that have been reported for regional epizootics and what is known about the ecology of RVFV, we proposed to use this knowledge to develop a Bayesian multinomial-hurdle model to estimate the contributions that these various risk factors have

on RVF occurrence and severity at the farm level. We hypothesized that factors associated with RVFV endemicity and importation would drive the risk of RVF occurring on the farm. Further we hypothesized that factors associated with vector amplification and host susceptibility would be drivers of the number of RVF-associated abortions and deaths during an outbreak.

METHODS

Study Area

This study was conducted in central South Africa. Specifically, we worked in a 200 x 200 Km area between approximately Bloemfontein on the east, Mokala National Park on the west and 100 Km north and south of those locations (Figure 5-1). The WGS84 decimal degree geographic coordinates of the study area corners were: -28.20, 24.20; -30.20, 24.20; -28.20, 26.40; -30.20, 26.40). The region is temperate and fairly flat with many pans that temporarily fill with water following precipitation (Geldenhuys, 1982). The southern part of the study area is primarily used for sheep ranching and the northern portion has more crop farming (east) or cattle ranching (west) (Cloete and Olivier, 2010). The study area has previously been described in detail (Chapter 1).

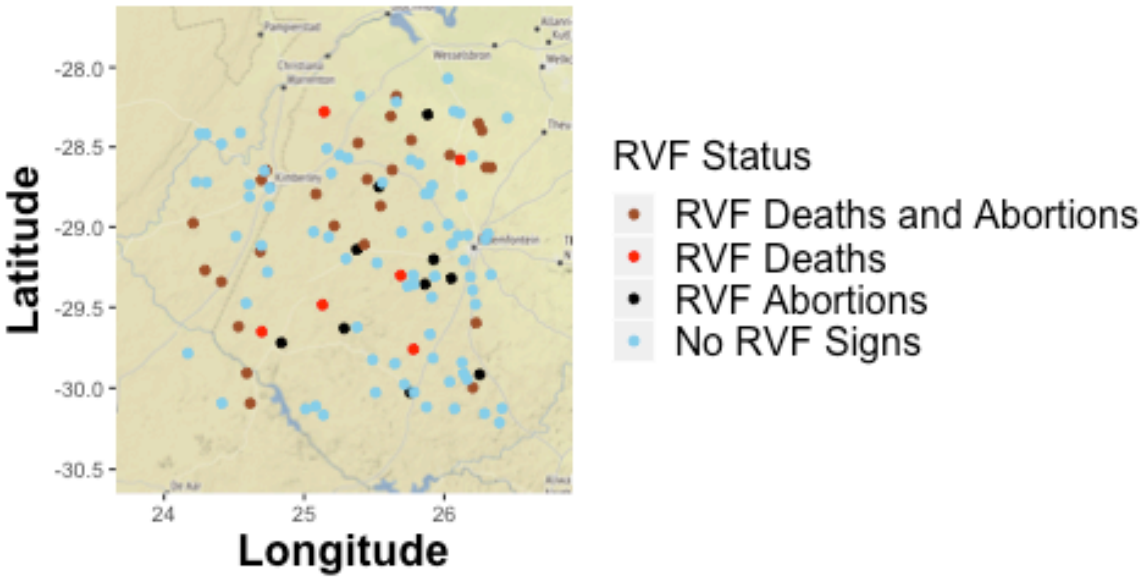


Figure 5-1 Map of the study area with the participating farms. Farms that reported both deaths and abortions during the 2010 RVF outbreak are in brown. Farms that reported only deaths are in red. Farms that reported only abortions are in black and farms that reported no suspected clinical signs associated with the RVF outbreak are in light blue.

Ethical Approvals

This study received ethical approval to conduct human subjects research using questionnaires (Appendix 5) approved by HummingbirdIRB (2014-25), the University of Witswatersrand (M140306), Northern Cape Provincial Health Research Ethics Committee (NC2015/001), Free State Provincial Department of Health (04/04/2015) and the U.S. Army Medical Research and Materiel Command, Office of Research Protections, Human Research Protection Office (A-20745.1a and A-20745.1b) (Appendix 3). A follow-up study was conducted via phone and with verbal consent (Appendix 9) to ask a few additional clarification questions (Appendix 10). This follow up was also approved as an amendment. Given the extensive number of approvals that were already in place, the University of Glasgow did not require additional ethical approval.

Survey Data Collection

The collection of the survey data has been previously described (Ngoshe et al., submitted, Chapter 2, Chapter 3). Briefly, geographic points were randomly selected using ARC-GIS 10.2 (ESRI, Redlands, CA, U.S.A.). The nearest ruminant owning farm/household to the geographic point was identified. The farmer/owner was invited to participate, we explained the study to them and requested them to sign a consent form. The farmer manager or owner was asked to complete a questionnaire regarding the history of RVF outbreaks on the farm, RVFV vaccination, animal demographics and farm management and biosecurity practices. These data were collected in 2015 and 2017 and have already been analysed for their intended purposes (Ngoshe et al., submitted, Chapter 2, Chapter 3).

We later decided to investigate factors associated with the number of abortions and mortalities believed to be caused by RVFV during the 2010 outbreak in the Free State. As the original surveys were not designed to completely answer this question we developed a brief follow-up questionnaire to determine if the farmer suspected they had any RVF cases in 2010-2011, what the size of the ruminant population was by species at that time and how many domestic ruminants died or aborted during the outbreak. During the follow-up survey we confirmed that they did not count an abortion as the death of a lamb or vice versa. Thus, in 2019 we called all of the farm owners that had previously allowed us to sample their animals to administer the follow-up questionnaire. While we were able to obtain estimates of the total population size by species that the farmer had just prior to the outbreak in 2010, we did not obtain data on the sex ratio or age structure of the animals owned in 2010.

The Data

Daily precipitation data were extracted from the African Rainfall Climatology dataset from the NOAA-CPC archives from January 1, 2009 through December 31, 2017. The data were a combination of rainfall gauge readings and METEOSAT satellite data used to produce a gridded layer of precipitation across Africa (Novella and Thiaw, 2013). The resolution was approximately 10 Km^2. From these data the 8-year cumulative average rainfall was calculated for each farm for a two-month period from January 15-March 15 of each year. The two-month cumulative rainfall and the percent of days with any precipitation were calculated for the period of January 15, 2010-March 15, 2010. All variables were assessed for co-linearity.

The Model

We used a Bayesian multinomial hurdle model to estimate the effect various risk factors had on the number of RVF-associated deaths and abortions during the regional outbreak period (Figure 5-2). The model had two components that provided a joint estimate of the results. One component of the model used a binomial regression as a hurdle that estimated the latent variable representing the probability of RVF being on the farm. The other component was a multinomial logit that estimated the proportion (then converted into the number) of animals on the farm that had no clinical signs of RVF, had RVF-associated abortions or had RVF-associated deaths. We assumed that the three outcomes (number of animals with no clinical signs, death and abortion) were mutually exclusive at the individual animal level.

The predictors used in the model represent four processes that we hypothesized were associated with the occurrence and severity of RVF on a farm (Figure 5-2). We hypothesized that whether RVF occurred on the farm would be driven by whether RVF was already endemic on the farm during the interepizootic period and whether RVF was imported onto the farm. Thus, for the hurdle portion of the model, the predictors were: farm size, the number of pans within access of the animals, whether the animals mix with wildlife and the 8-year average cumulative rainfall between January 15-March 15, the distance from which ruminants are purchased, the production class of the farm (small-holder (reference), semi-commercial or commercial) and the number of ruminants purchased each year. We hypothesized that the severity of the clinical signs seen on the farm during the RVF outbreak would depend on whether large populations of vectors

could be supported and on the susceptibility of the host population present. The predictors selected to represent these processes were: the proportion of days with rain and the cumulative precipitation between January 15-March 15, 2010; the number of natural water sources within access of the animals; the proportion of the farm's ruminant population that were sheep; the total number of domestic ruminants on the farm and whether the farmer vaccinated any animals against RVFV before the outbreak started. The same six predictors were used to estimate the probability of RVF-associated abortion and death.

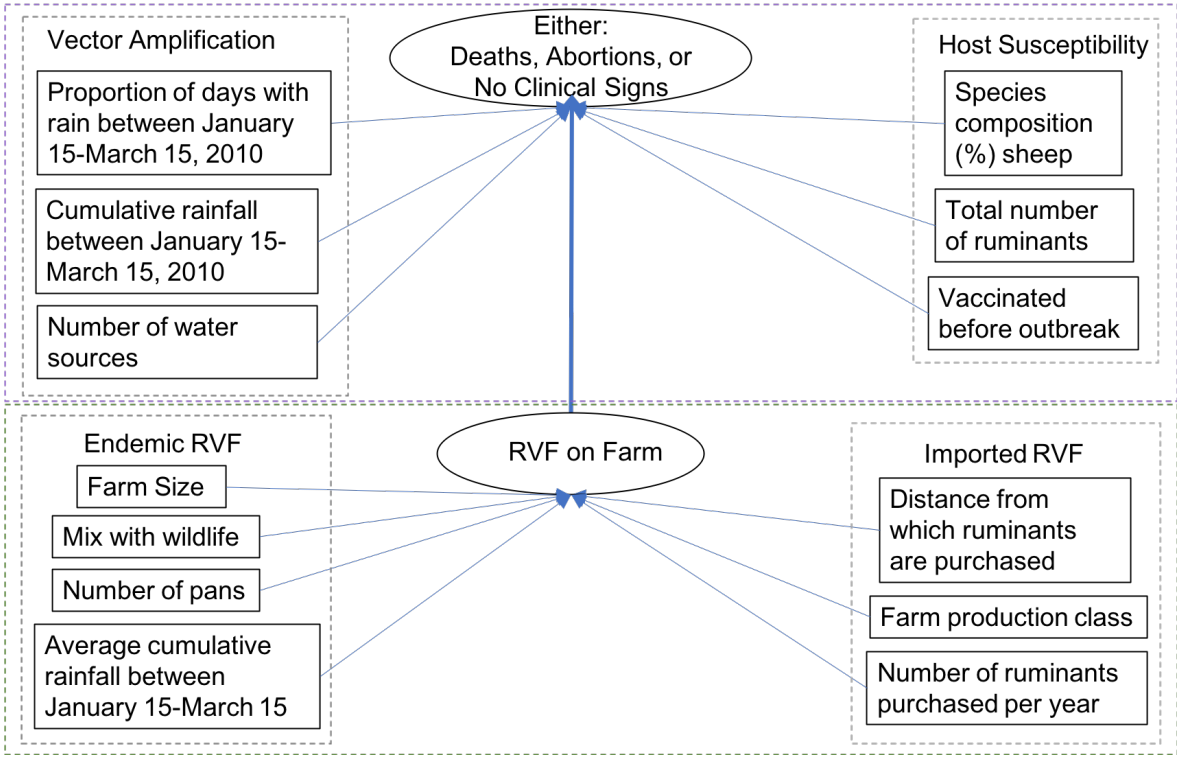


Figure 5-2 Diagram of model used in analysis. The boxes represent variables in the model for which we have data. The circles represent the latent state variables. The binomial hurdle portion of the model is below (light green box) and represents the variables in the linear predictor that estimates the latent variable of whether RVF occurred on a farm. Two types of factors were hypothesized to contribute to whether an RVF outbreak occurred on the farm: those that contribute to a risk of endemic RVF (e.g. RVF is being maintained on the farm through transovarial transmission in mosquitoes) and those that contribute to the risk of importing RVF (e.g. RVF is brought onto the farm through the import of an infected animal). Above, the light purple box surrounds the predictors included in the two linear predictors the compose the multinomial logit part of the model. We hypothesized that predictors that support vector amplification on the farm or are related to host susceptibility would be important in determining severity. The two linear predictors representing the multinomial part of the model use the same set of six predictors.

Two models were developed and compared to see which one had better predictive power. The only difference in the models were the mechanism by which we accounted for the effect of spatial variance. In one, we included a random effect term for township within the linear predictor of the hurdle (Eq. 2). In the second model we included an estimate from a spatial latent Gaussian process (Eq. 3). The covariance function was specified by the distance between farms. The priors for the random effects and Gaussian process were non-informative.

The hurdle portion of the model estimates h, the probability of RVF occurring on a farm. It is defined through a logit transformation:

$$h = \frac{e^{\overline{H}}}{1+ e^{\overline{H}}} \quad (34)$$

where $\overline{H}$ is estimated by the linear predictor, either with random effects (RE):

$$\overline{H} = \eta_0 + \eta_1 G + \eta_2 K + \eta_3 C + \eta_4 N + \eta_5 Z + \eta_6 M + \eta_7 L + RE \quad (35)$$

or with a Gaussian process (GP):

$$\overline{H} = \eta_0 + \eta_1 G + \eta_2 K + \eta_3 C + \eta_4 N + \eta_5 Z + \eta_6 M + \eta_7 L + GP \quad (36)$$

The variables and the parameters for all equations are defined (Table 5-1 and Table 5-2, respectively). H is the predicted outcome, whether RVF occurred on the farm, and is a realization of a Bernoulli process:

$$H \sim Bernoulli(h). \quad (37)$$

Uninformative priors were used for all of the parameters (Table 5-3).

A spatial, latent variable Gaussian process (GP), represented in the equation for $\overline{H}$ as GP, was used to account for localized clustering of outbreaks as is often seen with RVF. The geographic positioning coordinates of each farm were converted into Universal Transverse Mercator (UTM) coordinates and scaled by the furthest distance between any two farms. The GP had a mean function that described a curve that transects each location data point. The covariance function was defined using the scaled distance between each pair of UTM coordinates as the finite covariates. Uninformative priors were used: the length scale (ρ), the marginal standard deviation (α_{GP}) and the latent GP value (σ_{GP}) (Table 5-3). We used an exponentiated quadratic kernel to generate a covariance matrix from the covariance function defined by the data, and the hyperparameters, σ and ρ. The strength of the variation in the covariance function output is controlled by σ and ρ controls the speed at which the variate output varies with respect to the distance between the covariates. A small amount of noise was also incorporated (1^{-12}) into the matrix. Then the covariance matrix was used in a Cholesky decomposition function, which was then multiplied by a vector of standard normal variables (σ_{GP}). This effect was incorporated as a vector to be added to the linear predictor for $\overline{H}$.

The multinomial portion of the model estimated three proportions that sum to one. These include the proportion of ruminants that did not have clinical signs of RVF (N) the proportion that died (D) and the proportion that aborted (A). Here, $\bar{N}$ was used as the reference category and $\bar{D}$ and $\bar{A}$ are estimated by linear predictors (see Tables 5-1 and 5-2 for definitions), given by:

$$\bar{D} = \delta_0 + \delta_1 S + \delta_2 T + \delta_3 V + \delta_4 W + \delta_5 R + \delta_6 P \quad (38)$$

$$\bar{A} = \alpha_0 + \alpha_1 S + \alpha_2 T + \alpha_3 V + \alpha_4 W + \alpha_5 R + \alpha_6 P \quad (39)$$

N, D and A represent the elements of the vector θ, which is a realization of a multinomial process that uses a normalized exponential function (accessed by calling the softmax function in Stan, and referred to below as softmax) and takes the outputs of the linear predictors (Eqs. 5, 6) and generates three proportions that sum to one, where:

$$\theta \sim Multinomial\big(softmax(\bar{N}, \bar{D}, \bar{A})\big). \quad (40)$$

The expected value was defined by the probability mass function for the hurdle model:

$$p(y|h, \bar{N}, \bar{D}, \bar{A}) = \begin{cases} (1-h) + \Big(h \cdot Multinomial\big(y|Softmax(\bar{N}, \bar{D}, \bar{A})\big)\Big) & if\ y = 0, and \\ h \cdot Multinomial\big(y|Softmax(\bar{N}, \bar{D}, \bar{A})\big) & if\ y = 1. \end{cases} \quad (41)$$

We predicted the number of animals (X) that died, aborted or had no clinical signs during the outbreak each infected farm, an example of the equations is:

$$X_{death} = rbinom(1, T, \theta_{death}) \cdot rBernoulli(1, h)$$

Programming and Analysis

The model was written in `Stan` (Carpenter et al., 2017) and implemented in `R 3.6.0` (R Core Team, 2019) through `Rstudio 1.2.1572` (RStudio Team, 2015) using the package `rstan 2.18.2` (Stan Development Team, 2018a). Analyses and diagnostics were completed using `ggmcmc 1.2` (Fernández i Marín, 2016), `coda 0.19.2` (Plummer et al., 2006), `pROC 115.0`, (Robin et al., 2011) `tidybayes 1.1.0` (Kay, 2019) and `bayesplot 1.7.0` (Gabry and Mahr, 2019) packages. Plots were made using `ggplot2 3.2.0` (Wickham, 2009). All continuous data were standardized such that the mean equalled zero. The model was run for 5,000 iterations, with a burn in of 2,500, the thin command was set to 1 and there were four chains. The residuals and log-likelihoods were saved by adding them to the generated quantities block of the Stan code.

Table 5-1 The list of variables used in the model and their definitions.

Variable	Definition
h	The probability of RVF occurring on a farm
H	The latent variable representing whether RVF occurred on the farm (0 or 1)
G	The number of ruminants purchased each year (data)
K	The distance from which ruminants were purchased (data)
C	The production class of the farm (small-holder [reference], semi-commercial or commercial) (data)
N	The number of pans within access of the ruminants (data)
Z	The farm size (km^2) (data)
M	Whether the ruminants mix with wildlife (data)
L	The 8-year average cumulative rainfall between January 15-March 15 (data)
N	The estimated proportion of ruminants with no clinical signs of RVF
D	The estimated proportion of ruminants that died from RVF
A	The estimated proportion of ruminants that aborted from RVF
X_x	The predicted number of animals on the farm with no clinical signs of RVF (X_N), RVF-associated deaths (X_D) and RVF-associated abortions (X_A)
S	The proportion of ruminants that were sheep (data)
T	The total number of ruminants on the farm in 2010 (data)
V	Whether the farmer vaccinated any ruminants against RVFV before the RVF outbreak started (data)
W	The number of natural water sources within access of the ruminants (data)
R	The cumulative precipitation between January 15-March 15, 2010 (data)
P	The proportion of days with rain between January 15-March 15, 2010 (data)
θ	A vector that contains the three proportions N, D and A, which all sum to one.

Table 5-2 The list of parameters used in the model and their definitions.

Parameter	Definition
η_X	A vector of parameters for the binomial hurdle linear predictor of RVF on the farm
GP	The Gaussian process effect
RE	The random effect of township
δ_X	A vector of parameters for the multinomial linear predictor for RVF deaths
α_X	A vector of parameters for the multinomial linear predictor for RVF abortions

Table 5-3 Table of parameters and their prior distributions with their values and the definition of each variable.

Parameter	Distribution	Prior values	Definition
η_X	$\sim StudentT(\upsilon, \mu, \sigma)$	υ =3 μ =0 σ =10	A vector of parameters for the hurdle binomial portion of the model.
δ_X	$\sim StudentT(\upsilon, \mu, \sigma)$	υ =3 μ =0 σ =10	A vector of parameters for the linear predictor of death due to RVF
α_X	$\sim StudentT(\upsilon, \mu, \sigma)$	υ =3 μ =0 σ =10	A vector of parameters for the linear predictor of abortion due to RVF
ω	$\sim HalfStudentT(\upsilon, \mu, \sigma)$	υ =3 μ =0 σ =10	A normalized vector of the farm-specific random effect
σ_{RE}	$\sim Normal(\mu, \sigma)$	$\mu = 0$ $\sigma = 1$	Standard deviation of the random effect term
ρ	$\sim InverseGamma(\alpha, \beta)$	α = 1.839943 β = 0.114937	The length scale - the scale at which distances are measured among inputs (Stan Development Team, 2018b)
α_{GP}	$\sim HalfStudentT(\upsilon, \mu, \sigma)$	υ =3 μ =0 σ =10	The marginal standard deviation corresponding to how much of the variation is explained by the regression function (Stan Development Team, 2018b)
σ_{GP}	$\sim Normal(\mu, \sigma)$	$\mu = 0$ $\sigma = 1$	The latent GP, which is used to generate a multivariate normal vector (Stan Development Team, 2018b)

The convergence of the overall model was assessed by visually inspecting the trace plots for all parameters, evaluating the potential scale reduction factor ($\hat{R}$) and the effective sample size. The residuals of all three linear predictors ($\overline{H}$, $\overline{D}$ and $\overline{A}$) were visually evaluated for clustering. Posterior predictive checks were evaluated for each set of outcomes. The median, 66% and 95% credible intervals (CIs) of all parameter estimates were plotted. Where the credible intervals contained zero the proportion of the posterior that was above or below zero was calculated. Unlike binomial models, the sign and effect size of the parameter estimate of multinomial models are not sufficient alone to assess the relationship as the sign can change across the range of the predictor values. The changing sign is dependent on the relationship between the predictor and other two dependent variables (Bowen and Wiersema, 2004). To confirm that sign of the estimates for the continuous parameters did not change with the predictor, we calculated the predicted probabilities. This was done by calculating the expected probability for each of the three

multinomial outcomes while one variable was input per the original data and all other variables held to their means or reference values.

The random effects model was compared to the model with the spatial Gaussian process using the Watanable-Akaike Information Criterion (WAIC) to compare predictive accuracy. The model with the lower WAIC was selected. As a secondary measure, the amount of deviance for each model and a null model (intercept only) was calculated using the mean estimate of the log likelihood posterior distribution ($Deviance = -2 \cdot loglikelihood$). Then the proportion the deviance of the null model was reduced by was calculated. The model that reduced the deviance the most was used to support our assertion that the model with the lowest WAIC is the best model. The distance that explained 99% of the covariance was calculated for the Gaussian process by estimating the covariance decay rate using $Covariance = e^{-(\frac{scaled.distance^2}{2 \cdot \rho^2})}$, where $scaled.distance$ was a vector containing the distance between each pair of UTM coordinates where the farms were. We identified the distance at which the covariance dropped below 1% and unscaled the distance at that point.

RESULTS

One-hundred twenty farmers completed both the original and follow-up surveys. The median number of owned domestic ruminants reported by participating farms was 365 (range: 5-6,500). Forty-four (37%) farmers reported that an RVF outbreak occurred on their farm in 2010 (Tables 5-4 and 5-5). This included confirmed and unconfirmed reports. On those farms affected, the median farm-level estimate of the percent of ruminants that had an abortion was 4% (range: 0-71%) and the median percent that died due to RVF was 3% (range: 0-60%) (Figure 5-3). Among farms that reported that sheep (adults and lambs) were affected, the median number of sheep on a farm that aborted during the RVF outbreak was 15 (range: 0-468) and the median number of sheep and lambs that died was 10 (range: 0-150).

The estimates of all parameters converged and that $\hat{R} = 1$) for all parameters. We found that the model with the Gaussian process had a lower WAIC (7227; standard deviation (SD): 1471) than the model with the random effect of township (7419; SD: 1496). Similarly, we found that the model with the Gaussian process reduced the mean deviance

by 47% vs 46% for the model with the random effect. Therefore, the rest of the results will focus on the results of the model with the Gaussian Process.

Without the spatial Gaussian process the predictive power of the model is quite low (Table 5-6). When the Gaussian process is included in the predictions, the predictive power dramatically increases, increasing both the accuracy of the hurdle (Figure 5-4, 5-5 and 5-6) and multinomial portions of the model.

Table 5-4 The median, sample size and range of the parameters used in the model. The species-specific population sizes are also included. For variables that represent counts, only the farms that reported having that species or farm feature were included in the estimate.

Variable	Median	Number of Farms Reporting	Range
Cattle in 2010	70	99	[2, 2500]
Sheep in 2010	300	89	[5, 6000]
Goats in 2010	50	16	[6, 400]
Total Ruminants in 2010	365	120	[5, 6500]
Percent of Sheep in 2010 (%)	92.3	89	[11, 100]
Number of Ruminants Purchased	55	6	[1, 200]
Distance from which Ruminants were Purchased	120	5	[12.5, 600]
Number of Pans Ruminants Could Access	1.5	51	[0.5, 7]
Number of Water Sources Ruminants Could Access	6	110	[0.5, 56.5]
Farm Size (Km^2)	1200	120	[0, 15000]
Percent of Days with Rain between January 15-March 15 (%)	50.8	120	[36.7, 58.3]
2-Month Cumulative Precipitation between January 15-March 152010 (mm)	287.4	120	[202.8, 359.8]
8-year Average Cumulative Precipitation between January 15-March 15 (mm)	269.8	120	[217.2, 381.7]

We evaluated the full and partial effects of the model and compared the model predictions to the data reported by the farmers (Figures 5-4 and 5-5). If the hurdle linear predictor was not included, there was much more variation between the number of animals estimated to have had an abortion or death due to RVF and the number reported by the farmer.

Table 5-5 The percent of farmers reporting the following variables, with the total number of reporting farms and the 95% confidence interval.

Variable	Percent	Number of Farms Reporting	95% Confidence Interval
Wildlife Mix with Livestock	49.2	120	[40.1, 58.2]
Vaccinated Before RVF Outbreak	25.8	120	[18.4, 34.3]
Vaccinated During RVF Outbreak	50.8	120	[41.8, 59.9]
Affected by RVF Outbreak	36.7	120	[28.2, 45.7]
Any Reported Deaths from RVF	10.8	120	[6, 17.3]
Any Reported Abortions from RVF	8.3	120	[4.2, 14.3]
Small Holder Production	15	120	[8.6, 21.4]
Semi-Commercial Production	13.3	120	[7.3, 19.4]
Commercial Production	71.7	120	[63.6, 79.7]

Table 5-6 Measures of the predictive power of the model with and without the Gaussian process included, presented by the three linear predictors. The area under the [receiver operator] curve (AUC), sensitivity and specificity were calculated related to whether the model corrected predicted if RVF was on the farm (Hurdle), if there were any deaths (Deaths) or if there were any abortions (Abortions).

Model	Linear Predictor	AUC	Se	Sp
No Gaussian process	Hurdle	0.547	0.159	0.934
	Deaths	0.556	0.171	0.941
	Abortions	0.545	0.162	0.928
Gaussian process	Hurdle	1	1	1
	Deaths	0.556	1	0.059
	Abortions	0.947	1	0.894

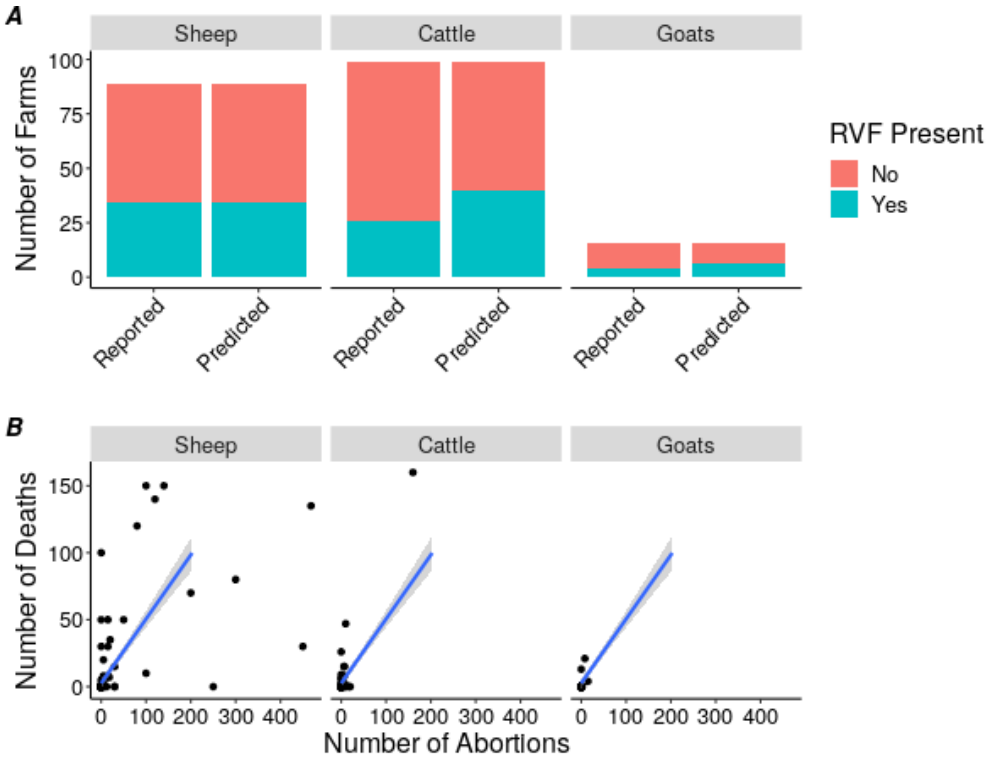


Figure 5-3 A) The number of farms that reported RVF occurred on the farm and the number that the model (with the Gaussian process) predicted would have RVF given by species. B) The number of species-specific deaths and abortions associated with RVF as reported by farmers is represented as black dots. The blue line demonstrates the relationship between the number of deaths and abortions predicted by the model for all species (this is not a species-specific estimate) and the grey ribbon is the confidence interval.

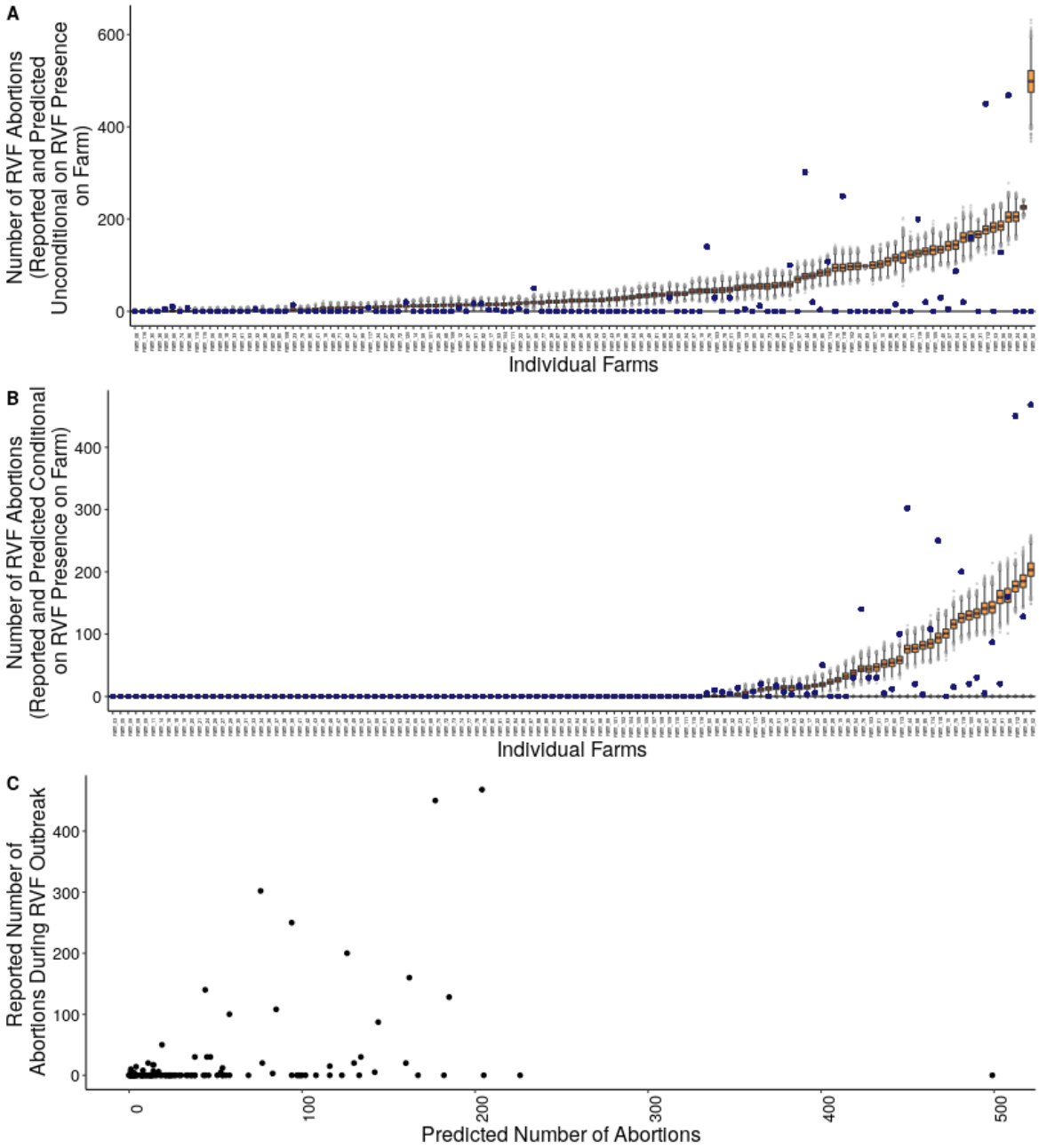


Figure 5-4 The posterior predictive checks of the number of RVF abortions predicted by the model (boxplots in A and B) and the number reported by the farmer (blue points in A and B). A) The predicted (orange) and reported (blue) number of abortions that occurred during the 2010 RVF outbreak and were unconditional on whether the model predicted if RVF would be on the farm (e.g. the hurdle portion was not incorporated). B) The predicted (orange) and reported (blue) number of abortions that occurred during the 2010 RVF outbreak and were conditional on whether the model predicted if RVF would be on the farm (the hurdle portion was incorporated). For both A and B, the orange box plots indicate the distributions of posterior predictions, with the outliers in grey. The blue points are the actual number of abortions reported by the farmer. C) The number of abortions reported by the farmer against the number of abortions predicted by the model for each farm.

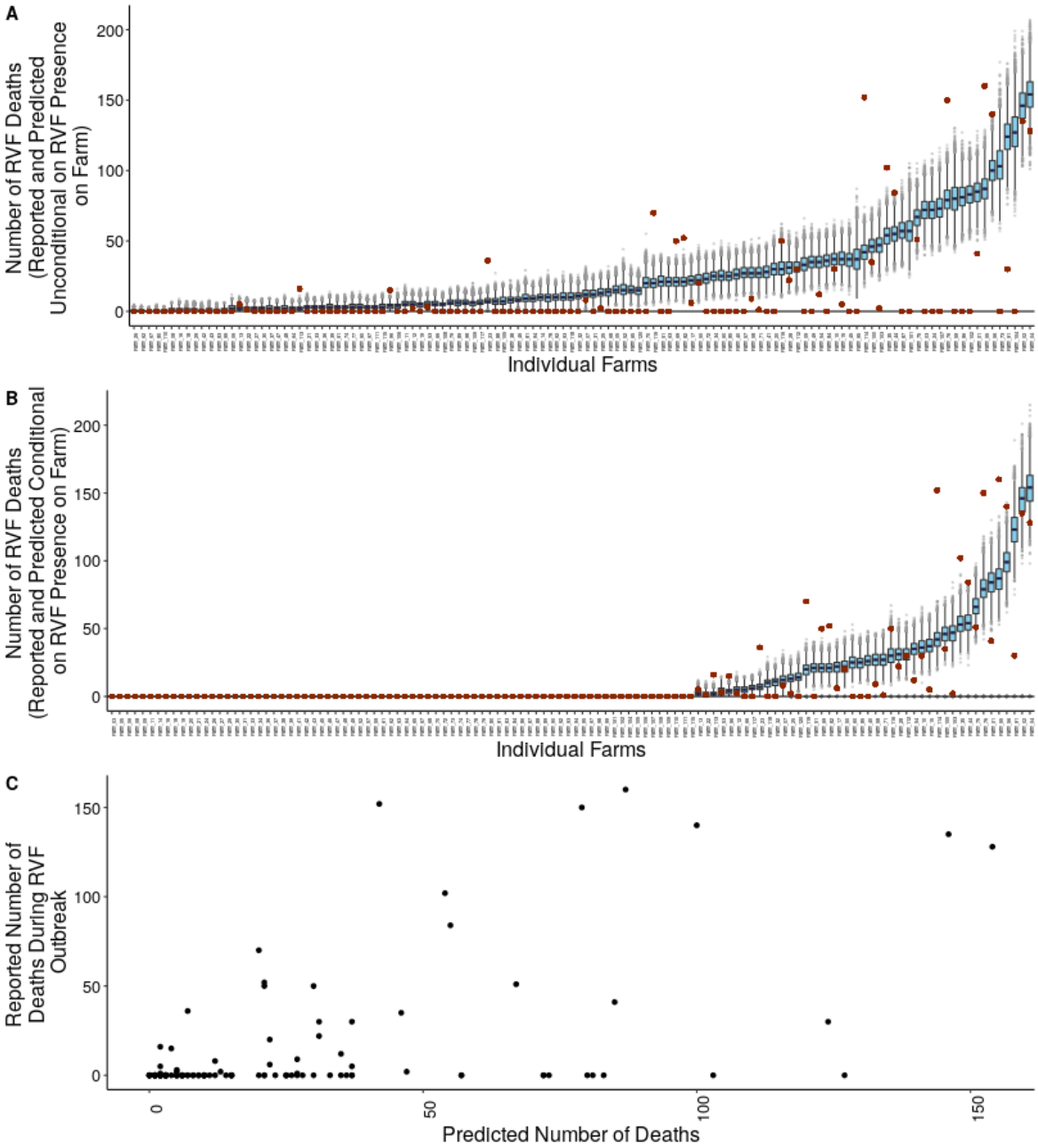


Figure 5-5 The posterior predictive checks of the number of RVF deaths predicted by the model (boxplots in A and B) and the number reported by the farmer (orange points in A and B). A) The predicted (light blue) and reported (orange) number of deaths that occurred during the 2010 RVF outbreak and were unconditional on whether the model predicted if RVF would be on the farm (the hurdle portion was not incorporated). B) The predicted (light blue) and reported (orange) number of deaths that occurred during the 2010 RVF outbreak and were conditional on whether the model predicted if RVF would be on the farm (the hurdle portion was incorporated). For both A and B, the light blue box plots indicate the distributions of posterior predictions, with the outliers in grey. The orange points are the actual values reported by the farmer. C) The number of deaths reported by the farmer against the number of deaths predicted by the model for each farm.

The estimates of all of the parameters in the hurdle linear predictor (η_X) (indicating whether RVF was predicted to occur on the farm) had very wide credible intervals (Figure 5-6 (standardized) and Table 5-7 (unstandardized)). The proportion of the credible intervals that were above zero were: ruminants purchased: 0.04, purchase distance: 0.93, commercial farm: 0.84, semi-commercial farm: 0.84, accessible pans: 0.93, farm size: 0.30, wildlife: 0.22, and average rainfall: 0.31. The parameters of the linear predictors for both abortion and death, had much narrower posterior distributions. The parameter estimates for the percent of days within the past two months with precipitation and the number of water sources on the farm were positively associated with the outcome for both deaths and abortions. In contrast, the two-month cumulative precipitation, the farmer having vaccinated the ruminants before the outbreak and the total number of ruminants on the farm were negatively associated with both abortions and deaths (Figure 5-6). The effect size for the parameters for vaccinating before the outbreak and the two-month cumulative rainfall were of larger magnitude in the linear predictor for RVF deaths than for RVF abortions. The rest of the parameter estimates from the linear predictor of RVF abortions had an effect size of a larger magnitude (regardless of sign) than the effect size of the same parameter in the linear predictor of RVF deaths.

The parameter for the variable, proportion of sheep on the farm, had a positive association with the number of RVF deaths on the farm (Figure 5-6). However, the predicted probabilities indicated that as the proportion of sheep on the farm increased, the probability of death decreased slightly compared to the probability of having an abortion or not having any clinical RVF signs (Figure 5-7A). The direction of the change in probability calculated by the predicted probabilities matched the sign of the parameter estimate calculated by the multinomial linear predictor for the rest of the parameters (Figures 5-6 and 5-7). Except for the parameter associated with the proportion of sheep on the farm, the probability of abortion remained greater than or equal to the probability of death across the range of values used to calculate the predicted probabilities. All other continuous parameters predicting RVF outcomes on farms had the same direction of effects when predicting both deaths and abortions (Figures 5-6 and 5-7).

In our system, 99% of the covariance in the model occurred at distances <5% (13.6 km) of the maximum distance between farms (287 km; Figure 5-8). As the mean distance between farms was 115 km, only 1% of pairwise distances among the 120 farms (7,140 distances) were less than 13.6 km.

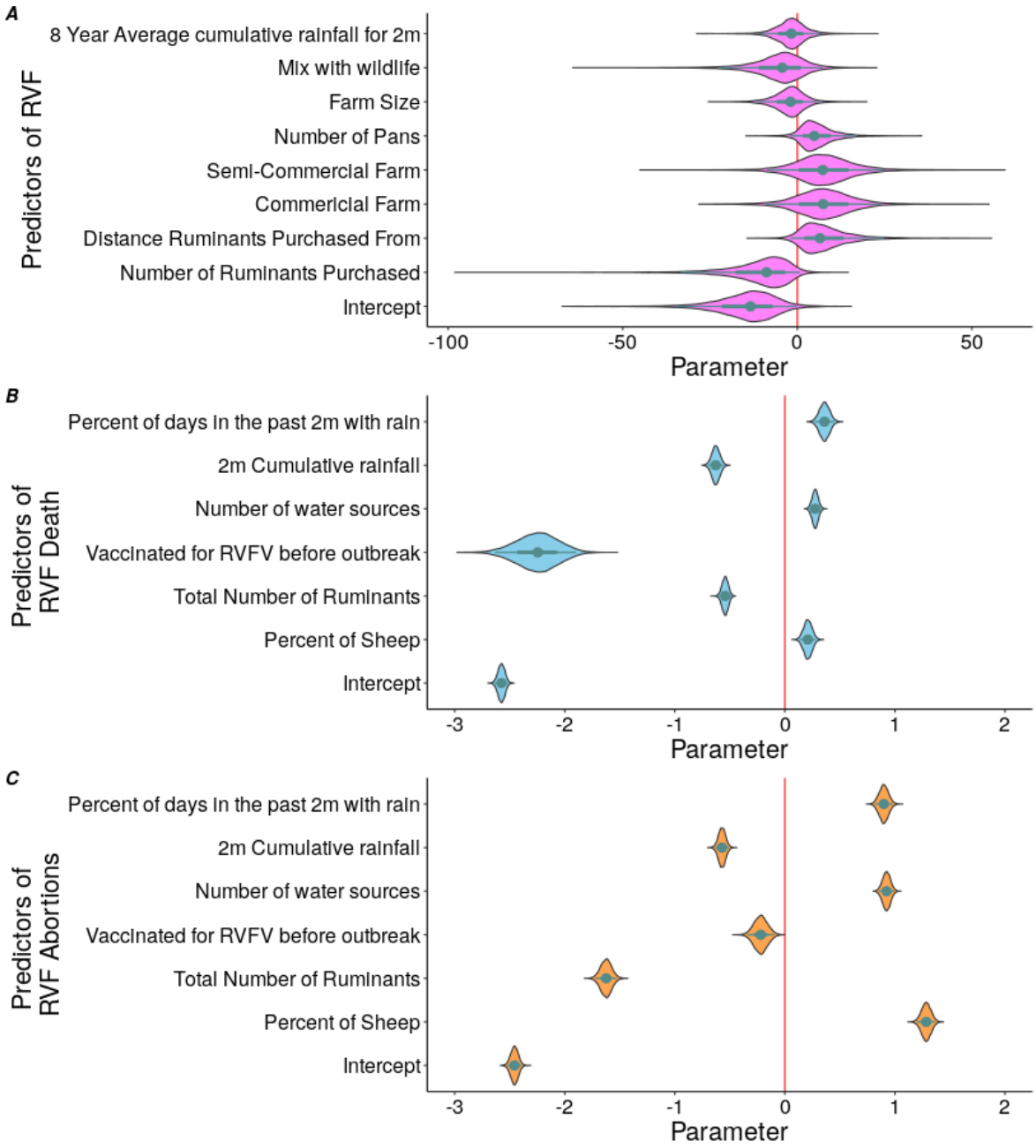


Figure 5-6 The standardized model parameters estimated as part of the A) hurdle linear predictor, B) linear predictor for RVF-associated deaths and C) the linear predictor of RVF-associated abortions. The point is the median estimate of the posterior with the 66% (thicker) and 95% (thinner) credible intervals indicated by the line. The violin indicates the shape of the distribution. A vertical line in red indicates an estimate of zero.

Table 5-7 All of the estimates and 95% credible intervals (95% CI) for all parameters in the model. The continuous parameters are unstandardized and are in their original units. *Only applies to the model run with random effects. †Only applies to the model with the Gaussian process.

Variable	Unstandardized Median Estimate	Unstandardized 95% CI
RVF on the Farm Predictors (Hurdle)		
Intercept (R)	-13.47	[-32.72,-0.35]
Ruminants Purchased (R)	-239.18	[-1018.59,29.23]
Purchase Distance (R)	399.16	[-97.84,1539.12]
Commercial Farm (R)	7.42	[-9.96,23.87]
Semi-Commercial Farm (R)	7.1	[-9.38,24.45]
Accessible Pans (R)	8.6	[-2.79,29.3]
Farm Size (R)	-3659.21	[-37162.93,24185.12]
Wildlife (R)	-4.22	[-21.59,7.75]
Average Rainfall (R)	224.96	[-78.04,481.54]
GP Length Scale (R) †	0.02	[0.01,0.02]
GP Variance (R) †	1034	[135.88,11206.71]
Random Effect Variance (R)*	0.12	[0,1.57]
Abortion Predictors		
Intercept (A)	-2.46	[-2.52,-2.39]
Percent Sheep (A)	1.12	[1.08,1.15]
Total Ruminants (A)	-1014.05	[-1121.63,-909.75]
RVFV Vaccinated (A)	-0.22	[-0.35,-0.1]
Number water sources (A)	17.65	[16.92,18.33]
2m Cumulative rainfall (A)	268.72	[266.54,270.88]
Percent Rainy Days (A)	0.54	[0.54,0.55]
Death Predictors		
Intercept (D)	-2.58	[-2.64,-2.51]
Percent Sheep (D)	0.67	[0.64,0.7]
Total Ruminants (D)	182.12	[118.26,242.25]
RVFV Vaccinated (D)	-2.25	[-2.63,-1.91]
Number water sources (D)	11.04	[10.48,11.59]
2m Cumulative rainfall (D)	266.8	[264.34,269.18]
Percent Rainy Days (D)	0.52	[0.51,0.52]

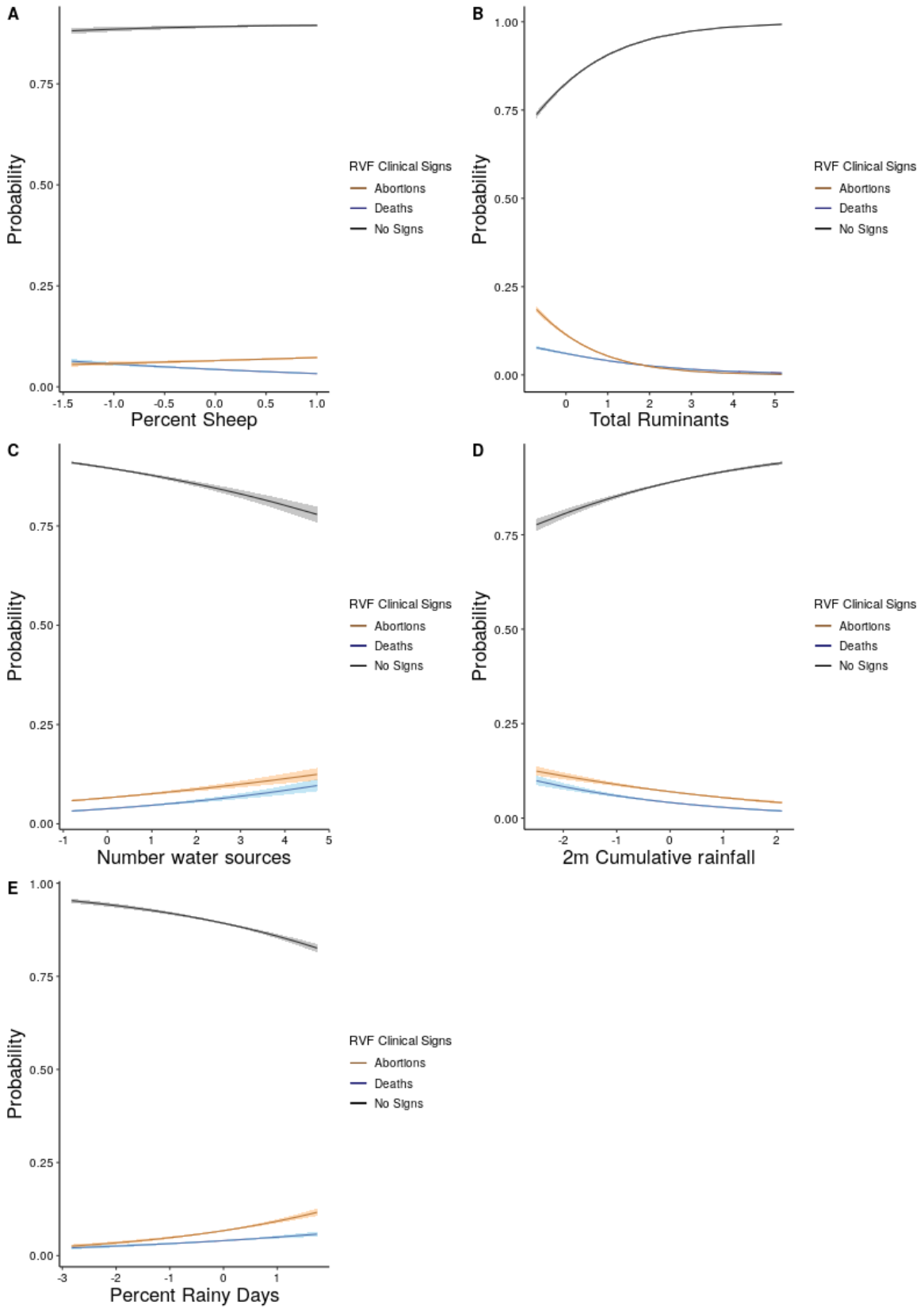


Figure 5-7 The predicted probabilities and 95% credible interval for each of the three outcomes of the model (no RVF clinical signs, RVF death or RVF abortion) for the five continuous predictors in the model. The scaled parameters included A) the percent of the flock that are sheep, B) the total number of ruminants on the farm, C) the number of water sources available to the ruminants, D) the 2-month cumulative precipitation between January 15-March 15, 2010 and E) the percent of days between January 15-March 15, 2010 during which there was some amount of precipitation.

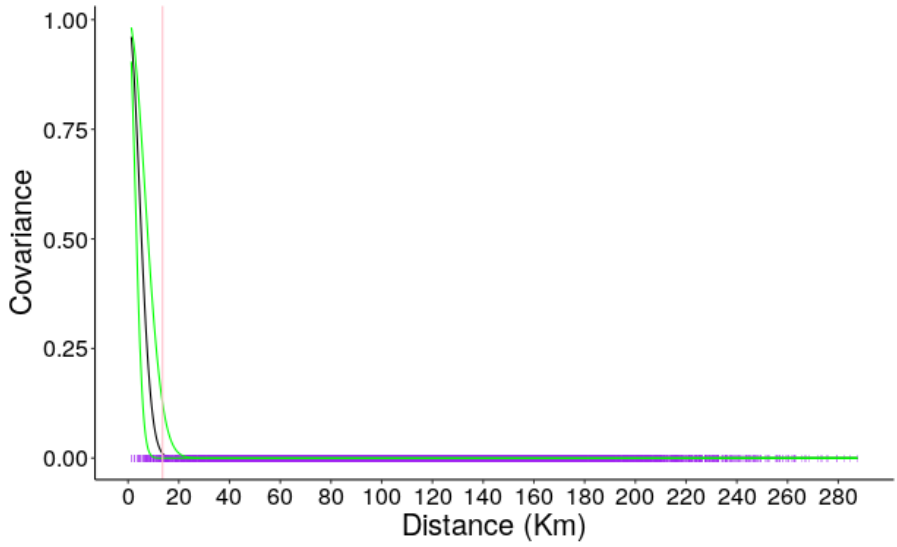


Figure 5-8 The covariance decay curve as a function of distance between farms. The black line represents the estimated covariance (and 95% Credible Interval is shown in green). The pink line indicates the distance at which 99% of the covariance is explained (13.58 Km). The short vertical purple lines represent the distances between each pair of farms in the study.

DISCUSSION

Here we tested the hypothesis that four processes that are important to the circulation of RVFV are associated with RVF occurrence on a farm and the number of deaths and abortions attributed to RVF during an outbreak. We found that most of the farmers reported lower rates of deaths and abortions than expected from the literature. The wide credible intervals for the parameters of the predictors of the latent variable of whether RVFV occurred on the farm indicate that important factors may be missing from the model specification. The narrow interval of the parameter posterior distributions estimated from the linear predictors for the proportion of animals affected by RVF-associated abortion and death indicate that this part of the model is better specified. The predictive power of the model is low, which is likely related to the predictive power of the hurdle portion of the model given that the addition of a spatial Gaussian process increases the predictive power dramatically. The Gaussian process indicated the importance of including a spatial component that can account for small-scale spatial variation when assessing the likelihood of RVF occurring on a farm.

Impact of RVF Outbreaks on Domestic Ruminants

Here we provide a survey-based assessment of the impact of RVF outbreaks on individual farms. The median proportion of animals on a single farm affected by RVF-associated mortality and abortion was 3% and 4%, respectively. These proportions are extremely low

given the high rates of mortality in young lambs and abortion in pregnant ruminants associated with RVFV infection (Swanepoel and Coetzer, 2004). Most historical accounts have been of one farm and are likely biased toward farms that had larger losses during an RVF outbreak (Daubney et al., 1931, Gear et al., 1951). Experimental infection has made a significant contribution to our understanding of the proportion of animals affected by RVFV infection (Easterday et al., 1962, Daubney et al., 1931, Ikegami and Makino, 2011). Some farmers did report losing up to 60% of their ruminants and that up to 71% of their domestic ruminants aborted, which are more in line with the historical records and the results of experimental infection. Our study was almost certainly affected by recall bias as the follow-up survey took place nine years after the outbreak. However, given the community trauma that RVF caused during the 2010 RVF outbreaks in South Africa, with over 14,000 cases in livestock and several human deaths, we believed that the farmers would still remember the deaths and abortions that occurred on their farm during that outbreak, as compared to years when no community trauma of such significance occurred. This was supported by the fact that in our initial survey 85% of farm owners and managers knew what RVF was (Msimang, unpublished data). However, the social trauma of the outbreak may have been significant enough that the farmers considered any deaths or abortions that occurred during the outbreak to have been due to RVF. This may explain why so many farms had a small percentage of affected animals.

Without widespread surveillance before and during an outbreak we cannot discern whether some very small outbreaks occur and go unnoticed on farms during these large regional outbreaks. This would fit with the pattern of spatial and temporal heterogeneity that has been reported in the literature (van den Bergh et al., 2019, Thiongane et al., 1991, LaBeaud et al., 2007) and that is supported by mathematical modelling (Chapter 4). Alternatively, our estimates of abortion rates may have been skewed as we calculated them using the total farm-level population for that species, including male and young animals. We felt that given the risk of recall bias over the nine years, that the total per species population estimates would be more accurate than if we requested them to recall age- or sex-specific estimates.

The range of our per farm abortion and mortality rates encompass the rates of two other survey-based estimates of the impacts of RVF outbreaks. Mdlulwa and Ngawne (2017) report an overall loss of 22% of sheep flocks affected by RVF. They conducted 39 surveys within our study area in the Free State (of 64), and eight surveys within our study area in the Northern Cape (of 39; Mdlulwa, unpublished). While they do not provide the farm

level details, they do report the loss of 970 pregnant ewes/cows, 17 non-pregnant animals, 2,114 suckling animals and 4,550 abortions on the <32 farms that were surveyed and reported RVF in 2010.

Nabeth et al. (2001) also conducted surveys and reported total farm abortion rates ranging from 47% in cattle and goats to 10% in sheep and peri-natal mortality rates from 47% in goats to 5% in cattle during the 1998 outbreak in Mauritania. Neither study provided per farm rates as we did, which are important for understanding the farm-level impacts of RVF. Using data reported to the World Animal Health Organsiation (OIE), Métras et al. (2011) estimated that the mean all-species, on-farm morbidity in South Africa in 2010 was 7-9% and case fatality rates ranged from 66-79%. For each species they determined that the four-year (2008-2011) RVF outbreak mean farm morbidity and case fatality were 8% and 74% on cattle farms, 10% and 81% on small ruminant farms and 7% and 67% on farms with both cattle and small ruminants (Métras et al., 2011). Unfortunately, the OIE data does not specifically track abortions, only the number of animals affected by RVF and the number of mortalities.

Model Selection

The WAIC comparison indicated that the model with the Gaussian process had more predictive power than the model with the random effect for township. Both the Gaussian process and the random effect were used as methods to account for spatial variation. The median estimate of the length-scale was 0.02 Km. This indicates that the Gaussian process is acting at a very small spatial scale. The farms we surveyed were located within 20 townships, given that the study area is 40,000 Km^2, that suggests the spatial scale of the random effect is about 2,000 Km^2. This is far larger than the scale the Gaussian process is acting on and much larger than the mean distance between farms (115 Km).

Métras et al. (2012) found excess RVF risk in farms that were 5 km and 15 km apart during the 2010 and 2011 outbreaks in South Africa, respectively. While further study indicated that as the regional RVF outbreaks continued the distance at which the maximum excess risk occurred increased (Métras et al., 2013), they suggested this was because the nearest neighbouring farms were already infected earlier in the outbreak. Our analysis of the Gaussian process estimate indicated that most of the covariance within the Gaussian process was amongst farms that were between 0-13 Km apart. This implies that spatial correlation occurs at a very fine scale during an outbreak. Although 13 Km is short in

comparison to the longest pairwise distance in our dataset (287 km), it is in line with Métras et al.'s (2012, 2013) results.

Factors Associated with Detecting RVF in Ruminants

First, we discuss the hurdle portion of the model, which predicts the latent probability of RVF occurring on the farm. We will then discuss the results of the multinomial component, estimating the number of RVF-associated abortions or mortalities in the following section.

The estimates of the parameters from the hurdle portion of the model all had wide credible intervals (Figure 5-6, Table 5-7) and they all weakly or negligibly supported an association with the occurrence of RVF. The wide credible intervals for the factors associated with importing RVF were not surprising given that only six farms reported that they purchased ruminants. Thus, the sample sizes for the number of ruminants purchased and the distance from which the ruminants were purchased were very small. Additionally, it is possible that the number *of times* ruminants are purchased, the number of farms/auctions from which they purchased ruminants and the distance from which they come would be more important than the number of ruminants purchased. For example, if a farm bought hundreds of livestock but they were a were purchased from the same site and arrived at the same time, there could be less risk than if they purchased fewer animals from multiple sites at various time points. Chevalier et al. (2011) identified livestock trade as a significant risk factor for ruminant seropositivity in Madagascar. In Mayotte, models that did not include livestock trade had a poorer fit (Métras et al., 2017), indicating that trade played a role.

One reason that self-classified production type may have had large credible intervals is that a variety of livestock business could consider themselves commercial, but have different levels of risk for RVF. In our study, feedlots were included in the commercial category. RVF is probably less likely to be detected on a feedlot as most of the ruminants are not pregnant and are old enough that they would not suffer from high rates of mortality. In contrast, a commercial dairy would be much more likely to notice RVF as they likely would have had a large number of pregnant animals and very young animals during the spring outbreak in 2010. The dairies may have also noticed a decline in milk production, making them more likely to be vigilant to other clinical signs as well. Given that the risk of clinical signs likely differ dramatically between these two business that are classified in the

same category, it could be more difficult to tease out the risk of RVF occurring on each farm. Mdlulwa and Ngwane (2017) found that commercial farmers were more likely to be affected by RVF than small holders. They attributed this to the fact that the state vaccinated the ruminants on many of the small holder farms, whereas the farmers themselves handled the vaccination of their animals on the semi-commercial and commercial farms.

The parameter for the 8-year average precipitation for the two months between January 15-March 15 for each farm also had very wide intervals. It could be that our precipitation data was not at a fine enough scale to produce a narrower credible interval. There were some farms that were within the same grid cell (10 Km^2), though the average distance between farms was 115 Km. Also, the effect of the Gaussian process indicates that the spatial scale of interest is very small, on the order of 13 Km. Farm size also had wide intervals, while this was not colinear with the variables for population size and number of pans/water sources etc., it is possible that the effect of farm size is affected by the presence of these other factors that could be related. Ngoshe et al. (submitted) also did not find a significant association between farm size and seropositivity in the same population of livestock.

Parameters for farms on which the livestock and wildlife mixed and the number of pans within access to the animals also had very wide credible intervals. There could be two opposing forces posed by the presence of wildlife. On the one hand, higher numbers of wildlife will reduce the proportion of all host species composed of domestic animals and thereby reduce the force of infection directed toward domestic ruminants. On the other hand, higher numbers of hosts can support larger vector populations, which may actually increase the likelihood of contact with livestock. The density of pans on the farm may have supported narrower credible intervals. Unfortunately, though we have the farm size, the question was asked about the number of pans within access of the animals of the farm. Therefore, any estimate of pan density would be unreliable as the animals may not have access to all of the pans on the farm.

Some of the variables warrant further attention, in particular those with limited data. Three of the variables had estimates with 90% of the posterior distribution greater than or less than zero, indicating there may be some weak support for those predictors and that they should be further explored. These were the distance ruminants were purchased from and the number of pans on the farm (both had >90% of the distribution above zero). In contrast, >90% of the posterior distribution for the parameter for the number of ruminants

purchased was below zero. The variables related to import had very low sample sizes so no inferences can be made, but as mentioned above, other studies have indicated that trade can increase the risk of RVF (Chevalier et al., 2011, Métras et al., 2017). Proximity to wetlands or pans have also previously been associated with RVF seroconversions (Ngoshe et al., submitted, Chevalier et al., 2011).

Factors Associated with RVF Morbidity in Domestic Ruminants

The posterior distributions of all of the parameters in the linear predictors for RVF-associated deaths and abortions were all very narrow, providing strong support for an association between the predictors and the response variables. The sign of one parameter changed upon examination of the predicted probabilities (Figure 5-7). This indicates that there was in fact a negative association between the proportion of sheep on the farm and death due to RVFV. This was surprising as young lambs are extremely susceptible to RVFV and adult sheep are more susceptible than other domestic ruminants (Easterday et al., 1962). However, the relationship between the predictors and the probability of the outcomes is nonlinear (Wulff, 2015) and the sign/direction of the probability may change as another probability becomes more likely (Bowen and Wiersema, 2004). In this case it seemed that the probability of no RVF signs (the reference response variable) increased slightly as the proportion of sheep increased, while the probability of abortion remained the same.

For all other continuous parameters, the sign of the parameter remained the same as indicated by the parameter (Figure 5-7). Both the parameter estimate and the predicted probability indicated that for all variables except vaccination and the proportion of sheep, the probability of abortion was always higher than or equal to the probability of death. This is expected given that mortality is low in adult animals (10-20%) compared to the abortion rate which is often close to 90% of the flock during an outbreak (Swanepoel and Coetzer, 2004).

We were surprised that the 2-month cumulative rainfall was negatively associated with both abortions and death as other studies have associated abnormally high precipitation for 60 days or greater as being positively associated with the risk of RVF outbreaks (Glancey et al., 2015). Perhaps if we used the rainfall anomaly rather than the cumulative precipitation the effect would be different. The use of rainfall anomaly may also be supported by the fact that as the percent of days with precipitation in the past two months

increased, so did the probability of both RVF-associated abortions and deaths. Unsurprisingly, the number of water sources on the farm was positively associated with RVF related abortion and mortality (Chevalier et al., 2011). These water sources support the mosquito vectors that amplify the virus into a large outbreak.

Among the factors selected to represent individual susceptibility, it was surprising that larger herd sizes were negatively associated with the probability of RVF-associated abortions and deaths as this has previously been associated with greater risk of seroprevalence (Bett et al., 2018). It could be that larger herds are more spread out across the large farms, which might put some ruminants far enough away from the wetlands where the vectors are amplifying the virus to prevent transmission. Farms that had a higher proportion sheep in their herd had higher probability of RVF-associated abortion. This is consistent with previous studies that have noted that sheep were associated with significantly higher seroprevalences (Moiane et al., 2017, Blomström et al., 2016) and that adult sheep are more susceptible to RVF than cattle or goats (Swanepoel and Coetzer, 2004).

The use of an RVF vaccine prior to the outbreak was also negatively associated with the probability of both abortion and deaths. This is not in itself surprising; however, there is evidence from this same population of farms that RVFV vaccination has not been effectively implemented as it was not positively associated with ruminant seropositivity, unless vaccine was given in the past year (Ngoshe et al., submitted, Chapter 3, FAO, 2015). Also, shortly after the 2010 outbreak Mdlulwa and Ngwane (2017) did not identify an association between the number of sheep lost during the 2010 RVF outbreak and whether the farmer vaccinated their sheep against RVFV. It was also very interesting that there was quite a difference in the effect sizes of the parameters for vaccination between each of the two outcomes (death and abortion) and the reference outcome (no RVF clinical signs). This indicates that vaccination may be more associated with reducing the probability of death due to RVF compared to abortion. As far as the author knows, this has not been reported before. Most challenge studies have been conducted in non-pregnant animals and are evaluated by whether the vaccinated animal becomes viremic (Capstick and Gosden, 1962, Coackley et al., 1967). It is possible that this is an artificial finding. While farmers were asked whether they had vaccinated against RVFV before they heard about the 2010/2011 outbreak, if they had actually vaccinated just before or during the outbreak (and seven farmers stated they vaccinated both before and during the outbreak)

with Smithburn vaccine, they may have induced abortions in their animals by administering that modified-live vaccine to pregnant ruminants.

The predictive power of the model was low (AUC: 0.55-0.56) and very few of the posterior estimates of the number of ruminants affected by abortions or deaths on each farm contained the value reported by the farmer (Figures 5-4 and 5-5, respectively). The poor demonstration of posterior predictive power indicates that we may be missing one or more important predictors. Certainly, among the predictors of individual susceptibility the number of pregnant and young ruminants and the herd immunity in the ruminants due to natural infection would all likely contribute to the severity of an outbreak on a farm. The altitude of the farm may also play a role as might factors related to the vegetation and soil types on the farm (Brand et al., 2018, Hightower et al., 2012, Munyua et al., 2016). If we can identify factors that put farms at higher risk of having severe RVF, we will be better able to target mitigation activities. These farm-level epidemiological data are critical to our ability to prevent large outbreaks. In addition to farm-level risk factors as has been discussed here, evaluating on the farm epidemic curves for animals and estimating R_0 from field data are extremely important next steps.

CONCLUSION

Here we estimated that the proportion of animals that farmers reported losing/aborting during the 2010 RVF regional outbreak in South Africa was lower than is typically reported in the literature. Using these data, we specified a Bayesian multinomial-hurdle model to identify drivers of the number of RVF-associated deaths and abortions that occurred during an outbreak. Our results indicate that the percent of days with precipitation within two months of the peak of the outbreak and the number of natural water sources were positively associated with the predicted probability of both RVF-associated death and abortions. In contrast, vaccinating before the outbreak and the total number of domestic ruminants were both negatively associated with the predicted probability of deaths and abortions. The proportion of domestic ruminants that were sheep was positively associated with the predicted probability of abortions. The effect sizes of vaccination before the outbreak indicate that it is associated with a much lower probability of RVF-associated death than abortions. We did not identify strong support for any drivers of RVF occurrence on the farm, although, localized spatial correlation seems to have a strong effect on the occurrence of RVF, suggesting that short-distance transmission of RVFV between farms is more important than long distance transmission.

SUPPLEMENTARY FIGURES

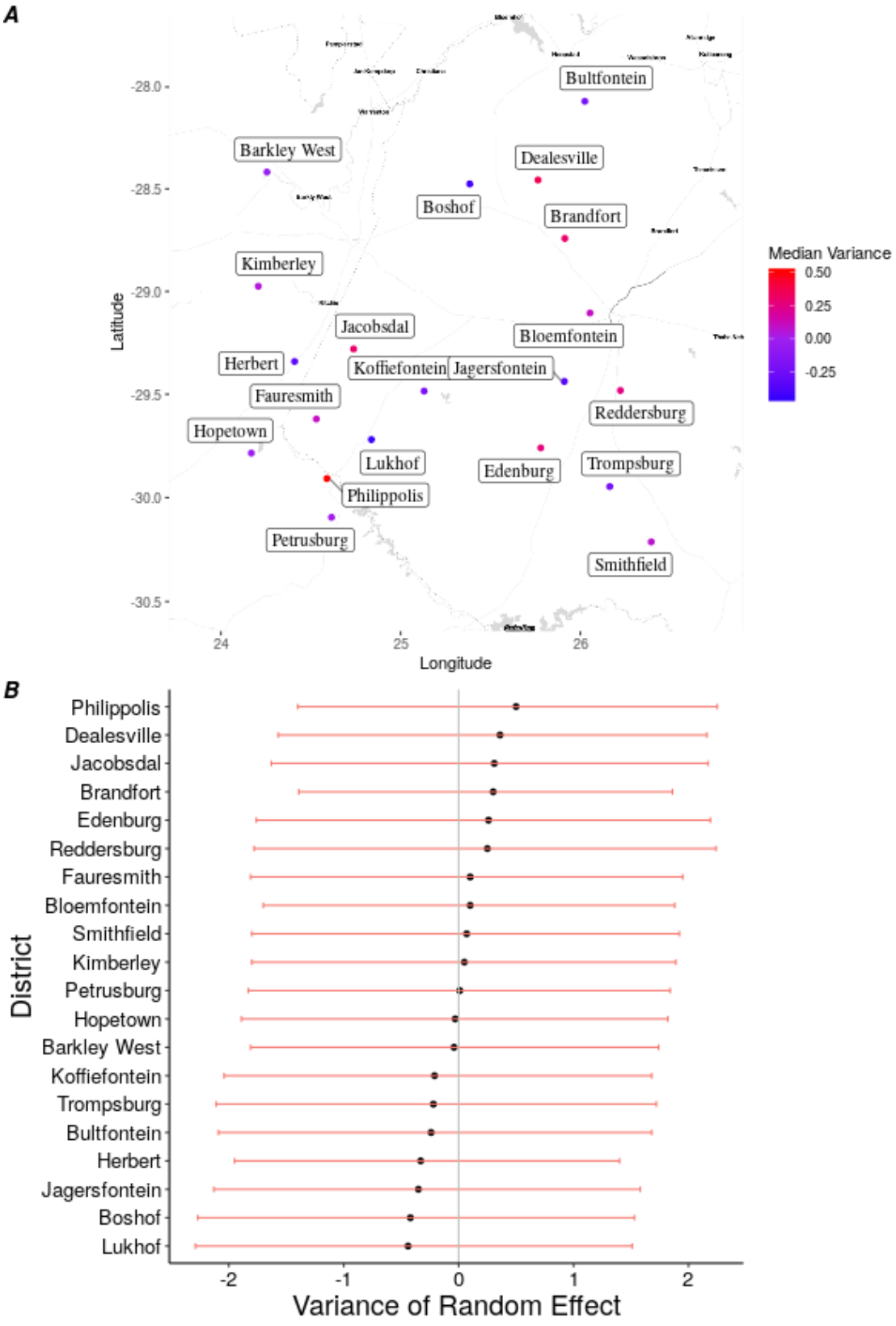


Figure S5-12 A) Map of the median random effect estimated by township. Red indicates the effect was positive, whereas blue indicates the effect was negative for that township. B) A dot plot of the median estimate and 95% credible interval of the random intercept for each township in the random effects model.

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Chapter 6: Conclusion

INTRODUCTION

Rift Valley fever virus (RVFV) is a zoonotic arbovirus that is considered by the World Health Organization to have high potential to cause a public health emergency. They emphasized the need for accelerated research and development to improve our understanding of RVFV epidemiology (WHO, 2018). Rift Valley fever (RVF) was listed as a priority due to the sporadic, widespread, multinational nature of outbreaks that have caused death and abortion in tens of thousands of livestock and illness in thousands of people (Glancey et al., 2015, Breiman et al., 2008). In between these outbreaks, very few clinical cases are reported, though evidence from East and West Africa indicate that there is interepidemic transmission of RVFV in animals and people (Njenga and Bett, 2019, Chevalier et al., 2009). An outbreak can also be isolated to a single farm, where it can cause devastating losses to that farmer, without spreading to the surrounding farms (OIE, 2018). A significant amount of research has been conducted on RVFV. Much of it has focused on virological properties and vaccine development, the seroprevalence of RVFV antibodies in people and livestock across Africa, spatial analyses to associate outbreaks with environmental factors and predicting the next outbreak (Easterday et al., 1962, Grobbelaar et al., 2011, Dungu et al., 2018, Rostal et al., 2010, LaBeaud et al., 2008, Soti et al., 2013, Anyamba et al., 2009).

Despite this work, there are still significant gaps in our understanding of RVFV dynamics. In particular, questions remain regarding how many cases of RVF occur during the interepizootic periods? How well are our current mitigation methods working and how can they be improved? Can RVFV persist within a single pan during an interepizootic period? What drives small and large outbreaks? How long do outbreaks in livestock on a farm actually last? Do the same factors that drive large regional RVF outbreaks also drive outbreaks on a particular farm? In this thesis, we began to answer these questions: We presented RVFV seroconversion rates in sheep, changes in herd immunity represented by seroprevalence over time, the longevity of antibodies induced by Smithburn vaccination in sheep, the mechanisms thought to support the persistence of RVFV during long interepizootic periods, potential differences in interepizootic transmission and epizootic transmission of RVFV and the factors that may affect the probability of the ruminants on a farm succumbing to abortion or mortality should an RVF outbreak occur. We conducted

the field work in South Africa and used models to illustrate mechanistic links with the epidemiology and ecology of RVFV in domestic ruminants there. Here we will present a summary of the significant findings from the research presented in the thesis, discuss the implications and recommendations based on our results, suggest future directions for research and finally discuss potential limitations of the work presented here.

SUMMARY OF RESEARCH FINDINGS

To better understand how herd immunity in sheep and more generally in domestic ruminants may change over time during an interepizootic period we conducted three analyses. First, we evaluated changes in seroprevalence over the post-2010 RVF outbreak period in cattle, sheep and goats by conducting a repeated cross-sectional serosurvey. We found that while in unvaccinated cattle seroprevalence decreased significantly, in unvaccinated sheep and goats it remained stable at a lower level than in cattle. This suggests that cattle populations, with slightly lower turnover rates, are still losing herd immunity following the large RVF outbreak in 2010, whereas in sheep and goats the virus may have already reached a relative equilibrium. Thus, the overall herd immunity in unvaccinated domestic ruminants may continue to decrease until cattle reach their equilibrium point or until more vaccination is implemented. We have evidence that the equilibrium seroprevalence in sheep may be maintained by the low incidence rate we estimated in sheep in two separate studies, (a) over a 2.5-year longitudinal study and (b) through the repeated cross-sectional study wherein we resampled the same sheep in both 2015 and 2017. Our study is a starting point as there is only one other incidence study to which to compare it.

To maintain herd immunity over the interepizootic period, vaccination will need to be implemented. Our 2.5-year longitudinal study indicated that RVFV antibodies likely remain throughout the life of sheep properly vaccinated with Smithburn. This has important implications for farm management, as it provides evidence that antibody waning in sheep is unlikely. Given these results, we were surprised to find that reportedly vaccinated cross-sectional study sheep only had a seroprevalence of only 33% (95% CI: 27-38%). Upon analysis we found that age, as expected, had a significantly positive association with seropositivity. However, surprisingly, vaccine type did not have a significant association with seropositivity. This was unexpected as differences in antibody longevity in livestock between inactivated, Clone 13 and Smithburn vaccines are known to occur. Our results suggest that post-vaccination seroprevalence varied between farms, even

amongst those that vaccinated with Smithburn. In comparison to the sheep from the longitudinal study vaccinated by our research staff, the cross-sectional study sheep seemed to have different rates of initial seroconversion after vaccination that varied by farm. This suggests that some farms may be using suboptimal practices in managing and administrating RVFV vaccine. These are important results that should be used to provide recommendations to the farmers and supply chain managers regarding the maintenance and use of RVFV vaccines. Increasing the seropositivity in vaccinated ruminants would be an important first step to increasing the herd immunity in the study area.

To better understand the probable biological mechanisms that support the persistence of low-level interepizootic transmission of RVFV within a single pan, we developed a mathematical susceptible-exposed-infected-recovered (SEIR) model. The model used a two-vector, one-host system that used real biological drivers of a temperate climate (precipitation and temperature) and a univoltine life cycle in the vector capable of transovarial transmission. While we were able to simulate the persistence of RVFV for 34 years under the realistic biological conditions described above, the system was very sensitive to a small amount of variation within several important parameters. Small changes would either force the virus to local extinction or lead to unnatural dynamics where a very high seroprevalence is maintained. We found that at the scale of a single pan, in order to support persistence and capture the patterns of outbreaks seen historically, the transovarial transmission rate needed to be much higher than is often assumed (0.85 vs 0.01). This emphasizes the importance of obtaining empirical estimates of the transovarial transmission rate. R_0 was estimated to be very close to one (1.44), which makes the virus very sensitive to initial conditions. R_0 was very sensitive to both bite rate and the host-vector ratio suggesting that these parameters may be critical to epizootics. We also found that the parameters that best explained low-level interepizootic RVFV transmission did not support realistic epizootic dynamics (our simulated epizootics lasted over 200 days, rather than approximately 60 days). It is likely that during large epizootics some of the parameters would naturally change. For example, if the mosquito populations were to significantly increase, the host-vector ratio and the bite rates could naturally change as well. By modifying these two factors we are able to simulate biologically realistic epizootic lengths. While the low R_0suggests that only 31% of a herd would need be vaccinated to drive RVFV to extinction, the timeframe that this would need to be maintained spanned nearly two decades. This is likely due to the high transovarial transmission rate.

Finally, to further our understanding of drivers of epizootics, we conducted the first farm-level analysis of drivers of RVF-associated abortion and mortality rates in domestic livestock populations during an RVF outbreak. Through retrospective surveys farmers provided the number of animals they lost to mortality and abortion during the 2010 RVF outbreak. They also provided information on potential risk factors. We found that the per farm median proportion of the livestock that suffered RVF-associated mortality and abortions was much lower than what has frequently been reported (3% and 4% in our study and 10-20% and up to 90% in the literature, respectively). Our Bayesian multinomial-hurdle model did not detect a strong association between our hypothesized drivers and the latent variable, whether RVF occurred on the farm. However, we found that there was quite a significant spatial correlation at a local level (<13 Km between farms). Conditional on RVF occurring on the farm, we found several variables were predictors of the severity of the outcome. We found that the number of water sources and the percent of days with precipitation between January 15-March 15, 2010 were positively associated with the probability of both abortions and deaths. The proportion of sheep on the farm was positively associated with the probability of abortions. The cumulative precipitation in the two months prior to the peak of the outbreak, the farmer having vaccinated their animals prior to the outbreak and the total number of domestic ruminants on the farm were negatively associated with abortions and deaths. Interestingly, vaccination prior to the outbreak had a stronger effect on preventing RVF-associated deaths than abortions. Our application of the Bayesian hurdle model allowed us to describe farm-level drivers of RVF-associated abortions and deaths for the first time.

IMPLICATIONS AND RECOMMENDATIONS

Interepizootic Transmission

We confirmed low-level, ongoing RVFV circulation in central South Africa. This has multiple implications. First, it indicates that RVF is not simply a disease to be concerned about every 30 years. Interepizootic RVFV infection may be causing a small amount of undiagnosed abortions, juvenile mortality and reduced milk production in livestock. It is possible that undiagnosed infection in livestock may put farm workers and veterinarians at a higher risk of contracting RVFV than previously thought. Second, veterinarians should consider including RVF as a differential diagnosis for abortion in livestock even if it is not presenting as an abortion storm. Third, even though the research presented in this thesis estimated that the force of RVFV infection has been low-level and relatively constant, our

simulations indicate that it is likely to vary year by year over the long term. The dynamics we simulated peaked in a large epizootic after 26-27 years, which was aligned with historical records. The results of the simulation also support the first two implications, as we were able to demonstrate persistence of RVFV while maintaining a low, but varying, seroprevalence in sheep.

The simulation of our SEIR model also implies that within a single pan, RVFV persistence is sensitive to a number of factors. This suggests that vaccination or changes that affect the vector bite rate and host-vector ratio may push the virus toward extinction. If our simulation results are accurate, then only 31% of sheep would need to be vaccinated to drive the virus to extinction; however, this rate would need to be maintained for decades. This is very important to communicate to the farmers, in conjunction with the fact that Smithburn vaccine induces long-lasting antibodies, so that they can adjust their management and vaccination protocols accordingly. It also reinforces the importance of better understanding transovarial transmission rates in mosquitoes as we suggest that is what drives the persistence of the virus in the face of vaccination.

The apparent need for ongoing vaccination to prevent viral persistence suggested by the simulation indicates that our finding that only 33% of reportedly vaccinated sheep were seropositive in our cross-sectional study is even more important. If a farmer improperly manages the RVFV vaccine or the vaccine was mismanaged at some point before the farmer purchased the vaccine, the vaccine will lose its efficacy. The partially vaccinated flock would not achieve and maintain the herd immunity threshold. Our research reported here indicates that antibodies following vaccination with Smithburn are long-lived and that the vaccine can be effective under field conditions. Therefore, we emphasize the importance of advising farmers and retailers regarding the handling and use of RVFV vaccines.

Epizootic Transmission

We found that drivers that have predicted RVF at regional scales were not predictive at the smaller-scale farm-level. The only driver of RVF on the farm that we were able to identify was small-scale spatial correlation (being within 13 Km of another farm). The difference in drivers between regional RVF outbreaks and individual farm outbreaks was emphasized when we were unable to identify differences between farms that had RVF outbreaks compared to farms that did not have RVF outbreaks. This highlights the lack of knowledge

about other potential mitigation measures that farmers might be able to take, besides vaccination. We were able to identify drivers associated with the number of deaths and abortions. Although we found that vaccination was associated with lower probabilities of both abortion and death, the probability of death was much lower than that of abortion. This has not previously been reported before and should be investigated to determine if this is a real finding or if it was because farmers were vaccinating pregnant animals with Smithburn and assuming the abortions that resulted were caused by natural RVFV infection. Our findings in this study imply that more epidemiological research needs to be conducted at the farm level during epizootics.

Our simulation suggests that the biological mechanisms that support interepizootic transmission may be different than those that support epizootic transmission. This may be as simple as higher vector populations causing host-vector ratio to decrease. If there are more vectors present and the same number of hosts, then logically the vector bite rate per animal will increase as well. However, it might also imply that there are additional differences in the drivers for interepizootic and epizootic RVF, which need to be better characterized as discussed in the next section. The differences seen between drivers of interepizootic and epizootic RVF suggests that the term interepizootic or interepidemic RVFV may not be appropriate, but rather enzootic or endemic RVFV would more accurate.

FUTURE RESEARCH

Enzootic Transmission

While a significant amount of research has be conducted on understanding the environmental drivers of epizootic RVF, very little work has focused on investigating the drivers of enzootic RVF. The drivers of enzootic transmission likely require dedicated, long-term research in a region that is prone to large outbreaks. Larger scale longitudinal studies of unvaccinated sheep maintained on farms that are not vaccinating their other animals would be advantageous as they would require less time to identify more seroconversions. We recommend using time-to-event analyses or a case-control study nested within a cohort study to identify risk factors associated with seroconversion in studies with higher sample sizes. These analyses can be much more targeted than seroprevalence risk factor analyses. Simultaneous vector studies are also recommended as

seroconversions can be correlated directly with relative vector abundance rather than the environmental predictors of vector abundance.

We also need to better understand the role livestock play in maintaining RVFV in this ecosystem versus wildlife. The blesbok and springbok in the same region had a very low seroprevalence compared to livestock, while the buffalo and kudu had seroprevalence rates that were similar to the unvaccinated small ruminants. It has been hypothesized that livestock themselves are sufficient maintenance hosts for RVFV even without wild hosts being present (Linthicum et al., 2016, Njenga and Bett, 2019). Longitudinal studies in wildlife will be critical to determining the role of wildlife, to date, only one such study by Beechler et al., (2015) has been conducted. Additionally, as RVFV has such a complex ecology, most mathematical models have not included wildlife. Wildlife may support persistence and make the system more robust to small variations in important parameters or wildlife may increase the frequency of larger epizootics or even extinction.

The vaccine study should be repeated with other vaccines that are available, including Clone 13 and the inactivated vaccine. These vaccines reduce the risk of side effects (abortion and foetal abnormality) and are increasingly being used when outbreaks are predicted. We need to better understand how long antibodies persist and if immunity is maintained.

Epizootic Transmission

Currently all estimates of the basic reproduction number for RVFV in livestock come from mathematical models, though there is one study that estimated the farm-to-farm effective reproduction number during the 2010-11 RVF outbreaks in South Africa (Métras et al., 2013). We recommend that several thorough veterinary epidemiological investigations be conducted during RVF epizootics to compare findings from RVF outbreaks that are isolated on farms vs outbreaks that are part of the massive, regional or international epizootics. Here again we may find different drivers for the different types of outbreaks and different patterns in the epidemic curves. This would require collecting samples prior to an epizootic, throughout the epizootic and after the epizootic. Given the length of the interepizootic/epidemic periods, this type of research requires a long-term funding commitment. This will likely need to be conducted by research teams that are outside of the veterinary departments that manage outbreak response, especially as regional RVF outbreaks can easily overwhelm veterinary diagnostic laboratories. Additionally, RVFV is

a select agent that requires a biosafety-level three laboratory or higher to isolate or work with samples that have a high probability of contamination, which makes further research difficult if a laboratory is in a low-resource region in Africa.

Our research indicates that while we have strong evidence for predictors of RVF outbreaks across a country or region, we still do not fully understand what drives epizootics on specific farms. These data could be collected by following up on farms that are affected by an RVF outbreak and the unaffected neighbouring farms to assess important risk factors. These include questionnaire data (which has often been neglected) as well as vector infection rates and abundance, and environmental factors. Additionally, this follow up can be used to repeat our analysis on RVF mortalities and abortions in a setting where outbreaks can be confirmed via serology and recall bias will be limited as all of the survey responses can be collected at the time of the outbreak, rather than 5-10 years later.

POTENTIAL LIMITATIONS

There are several limitations to the studies reported here. The unvaccinated longitudinal study was limited by the sample size and the number of sheep that seroconverted. The low number of seroconversions prevented the detection of any associations between seroconversion and risk factors. The study of the reportedly vaccinated cross-sectional study sheep was dependent on the farmers' memory as to whether they had vaccinated. Several farmers that indicated they had vaccinated against RVFV, were unable to name the vaccine they used and others gave the names of vaccines used against other pathogens (e.g. bluetongue). Additionally, as the sheep were not individually identified we had to assume the vaccination status based on whether they were old enough to have been vaccinated. We could not guarantee that those sheep actually were vaccinated.

In our longitudinal study on the vaccinated sheep, we evaluated the presence of antibodies, rather than actual immunity. Additionally, rather than using a single ELISA assay we would have used antibody titres to obtain a more accurate estimate of the rate of antibody waning. We also recommend that a subset of sheep from the vaccine trial be challenged with RVFV to assess immunity to RVFV for various antibody titres. While a challenge study is costly as it requires BSL-3 conditions, immunity to RVFV in livestock is usually measured in adult sheep by whether the animal becomes viremic and pyretic, rather than in terms of death and abortion. While death is a possible outcome for an adult sheep infected

with RVFV, it is rare (10-20%) and has not been a common outcome in challenge studies in the past (Capstick and Gosden, 1962).

Our mathematical model was based on several assumptions. In particular, in order to simplify the model, we did not vary all of the mosquito parameters with precipitation and temperature that may be affected by the climate variables. Other models incorporated climate forcing into the gonotrophic rate (rate of egg development), blood meal seeking behaviour and egg laying rates (Leedale et al., 2016). We also chose to use carrying capacities to keep the vector population sizes from becoming larger than we expected. However, the true carrying capacity or average population sizes for these mosquito species are unknown.

Finally, our study of farm-level predictors of RVF outbreaks had a number of limitations – some of which have already been discussed. First, the data were collected up to nine years after the 2010 outbreak, which created a significant potential for recall bias. This also meant we could only ask about total populations, rather than asking about the size of subpopulations (e.g. pregnant females, juveniles etc.). The number of pregnant ruminants would certainly have a direct effect on the number of abortions that could occur. While we asked about the population sizes and vaccine status prior to the 2010 outbreak, we did assume that other factors did not change between 2010 and 2015 or 2017 when the cross-sectional study surveys were administered. This includes the data on the number of animals traded and how far they were traded. All of these limitations could have impacted the model's ability to estimate the parameter coefficients.

CONCLUSION

Rift Valley fever virus can likely persist through the long interepizootic periods in South Africa. Here we have provided evidence of low-level, ongoing enzootic transmission and simulated the underlying biological mechanisms that support this transmission. We recommend that herd immunity be boosted by RVFV vaccination, which we demonstrated could effectively induce long-lasting antibodies in sheep when properly administered. We suggest that the drivers of the low-level, interepizootic RVFV transmission that we observed may be different than those associated with regional, epizootic RVF. We further suggest and provide evidence that the drivers of on-the-farm, RVFV-induced abortions and mortalities may also be different than those used to predict regional, epizootic RVF.

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Appendix 1

Animal Ethics Approvals: Note all documents in appendix were modified to fit the appropriate formatting

Institutional Animal Care and Use Committee
Tufts University & Tufts Medical Center
136 Harrison Avenue
Fax: (617) 636-8354
Boston, Massachusetts 02111

Phone: (617) 636-4109

April 4, 2014

Dr. Jonathan Epstein
Conservation Medicine
Eco Health Alliance

Re: Verification of Approval by the Institutional Animal Care and Use Committee for Research Involving Animals

Dear Dr. Epstein,

The Institutional Animal Care and Use Committee reviewed your CBEP proposal "Understanding Rift Valley Fever in Republic of South Africa". The animal work in this proposal is congruent with the animal protocol #G2014-03 entitled "Understanding Rift Valley Fever in Republic of South Africa".

#G2014-03 was approved on ***February 14, 2014.***

Tufts University Cummings School of Veterinary Medicine has an Animal Welfare Assurance on file with the Office of Laboratory Animal Welfare at the National Institutes of Health. The Assurance number is **A4059-01.**

If you have any questions, please call me at 508-887-4639.

Ann Holm, B.S., CPIA
IACUC Coordinator
Tufts University
T: 508-887-4639
ann.holm@tufts.edu

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Animal Ethics Committee

PROJECT TITLE	**Understanding Rift Valley Fever in the Republic of South Africa**
PROJECT NUMBER	**T008-14 (Amendment 1)**
RESEARCHER/PRINCIPAL INVESTIGATOR	**Prof. JT Paweska**

STUDENT NUMBER (where applicable)	**----------**
DISSERTATION/THESIS SUBMITTED FOR	**Academic /contract**

Additional numbers of springbok and sheep

ANIMAL SPECIES	**Cattle, goats, sheep, kudu, Buffalo** **Wildebeest**	**Blue wildebeest, Springbok, Blesbok,**
NUMBER OF ANIMALS	**Various numbers – to report**	**Various numbers – to report**
Approval period to use animals for research/testing purposes		**April 2015-April 2016**
SUPERVISOR	**Prof. JT Paweska**	

KINDLY NOTE:

Should there be a change in the species or number of animal/s required, or the experimental procedure/s - please submit an amendment form to the UP Animal Ethics Committee for approval before commencing with the experiment

APPROVED	Date 20 April 2015
CHAIRMAN: UP Animal Ethics Committee	Signature

REPLY TO
ATTENTION OF

DEPARTMENT OF THE ARMY
HEADQUARTERS, US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
810 SCHREIDER STREET
FORT DETRICK, MD 21702-5000

October 07, 2015

Director, Office of Research Protections
Animal Care and Use Review Office

Subject: Review of USAMRMC Proposal Number CB-2014-33, Award Number HDTRA1-14-1-0029 entitled, "Understanding Rift Valley Fever in Republic of South Africa "

Principal Investigator William Karesh
Ecohealth Alliance Inc.
New York, NY

Dear Dr. Karesh:

Reference: (a) DOD Instruction 3216.01, "Use of Animals in DOD Programs"
(b) US Army Regulation 40-33, "The Care and Use of Laboratory Animals in DOD Programs"
(c) Animal Welfare Regulations (CFR Title 9, Chapter 1, Subchapter A, Parts 1-3)

In accordance with the above references, protocol CB-2014-33 entitled, "Understanding Rift Valley Fever in Republic of South Africa," IACUC protocol number T008-14, Protocol Principal Investigator Janusz Paweska, is approved by the USAMRMC Animal Care and Use Review Office (ACURO) as of 07-OCT-2015 for the use of cattle, goats, sheep and other speicies described in the IACUC approved protocol and will remain so until its modification, expiration or cancellation. This protocol was approved by the Universiteit Van Pretoria IACUC on 01-SEP-2015.

Required Actions: When updates or changes occur, documentation of the following action or events must be forwarded immediately to ACURO:

- IACUC-approved modifications, suspensions, and triennial reviews of the protocol (All amendments or modifications to previously authorized animal studies must be reviewed and approved by the ACURO prior to initiation.)

- IACUC actions involving this protocol regarding
  a. any noncompliance;
  b. any deviation from the provisions of the Guide for the Care and Use of Laboratory Animals; or
  c. any suspension of this activity by the IACUC

- USDA or OLAW regulatory noncompliance evaluations of the animal facility or program

- AAALAC, International status change (gain or loss of accreditation only)

Throughout the life of the award, the awardee is required to submit animal usage data for inclusion in the DOD Annual Report on Animal Use. Please ensure that the following animal usage information is maintained for submission:

- Species used (must be approved by this office)
- Number of each species used
- USDA Pain Category for all animals used

For further assistance, please contact the Director, Animal Care and Use Review Office at (301) 619-2283, FAX (301) 619-4165, or via e-mail: usarmy.detrick.medcom-usamrmc.other.acuro@mail.mil.

NOTE: Do not construe this correspondence as approval for any contract funding. Only the Contracting Officer or Grant Officer can authorize expenditure of funds. It is recommended that you contact the appropriate Contract Specialist or Contracting Officer regarding the expenditure of funds for your project.

Sincerely,

For

Bryan K. Ketzenberger, DVM, DACLAM
Colonel, US Army
Director, Animal Care and Use
Review Office

Copies Furnished:
Mr. Alfred S. Graziano, Jr., Defense Threat Reduction Agency (DTRA)
Dr. Jean Richards, Defense Threat Reduction Agency (DTRA)
Dr. Janusz T. Paweska, Universiteit Van Pretoria
Dr. Daan Verwoerd, Ecohealth Alliance Inc.
Dr. Melinda Rostal, Ecohealth Alliance Inc.

University of Glasgow | School of Veterinary Medicine

17 December 2015

Dr Melinda Rostal
C/o Sarah Cleaveland
Institute of Biodiversity Animal Health and Comparative Medicine
R314C Level 3
Graham Kerr Building
Glasgow G12 8QQ

Dear Melinda

Clinical Research involving animal subjects, material or data

Your application entitled "*Effects of Ruminant Herd Immunity on Rift Valley Fever Outbreaks in South Africa*" (Ref 62a/15) has been approved by the Ethics & Welfare Committee.

Please find enclosed a signed copy for your records.

Yours sincerely,

Dr Timothy Parkin
Convenor
Ethics & Welfare Committee

School of Veterinary Medicine
464 Bearsden Road
Glasgow G61 1QH
The University of Glasgow, charity number SC004401

Appendix 2
Animal Sampling Permit

agriculture,
forestry & fisheries

Department:
Agriculture, Forestry and Fisheries
REPUBLIC OF SOUTH AFRICA

Directorate Animal Health, Department of Agriculture, Forestry and Fisheries
Private Bag X138, Pretoria 0001

Enquiries: Mr Herry Gololo • Tel: +27 12 319 7532 • Fax: +27 12 319 7470 • E-mail: HerryG@daff.gov.za
Reference: 12/11/1/1/13

Prof Janusz Paweska
Centre for emerging and Zoonotic Diseases
NICD
2131 Sandringham
1 Modderfontein RD
Gauteng

Dear Prof Paweska

RE: Permission to do research in terms of Section 20 of the ANIMAL DISEASES ACT, 1984 (ACT NO. 35 of 1984)

Your fax / memo / letter/ Email dated 25 August 2015, requesting permission under Section 20 of the Animal Disease Act, 1984 (Act No. 35 of 1984) to perform a research project or study, refers.

I am pleased to inform you that permission is hereby granted to perform the following research/study, with the following conditions:

Conditions:

1. This permission does not relieve the researcher of any responsibility which may be placed on him by any other act of the Republic of South Africa;
2. All potentially infectious material utilised or collected during the study is to be destroyed at the completion of the study. Records must be kept for five years for audit purposes. A dispensation application may be made to the Director Animal Health in the event that any of the above is to be stored or distributed;
3. This Section 20 approval does not serve as an instruction for any person to part take in this study;
4. Compliance with the Veterinary and Para-Veterinary Professions Act, 1982 (Act No 19 of 1982);
5. Compliance with the Non-Proliferation of Weapons of Mass Destruction Act, 1993 (Act No 87 of 1993);
6. A separate Section 20 application must be submitted for study 3 on cattle/buffalo;

7. If any diagnostic tests other than what has been specified within the Section 20 application are to be performed, an application for an amendment to this Section 20 approval must be submitted;
8. No sampling may be conducted within areas under state veterinary restriction;
9. Any publications or reports must be pre-approved by the Director Animal Health.

Title of research/study: Understanding Rift Valley in Republic of South Africa- Study 1. Ruminant baseline serosurvey.
Researcher (s): Prof Janusz Paweska
Institution: Centre for emerging and Zoonotic Diseases, NICD.
Your Ref./ Project Number:
Our ref Number: 12/11/1/1/13

Kind regards,

DR. MPHO MAJA

DIRECTOR OF ANIMAL HEALTH

Date: 2015 -09- 07

agriculture,
forestry & fisheries

Department:
Agriculture, Forestry and Fisheries
REPUBLIC OF SOUTH AFRICA

Directorate Animal Health, Department of Agriculture, Forestry and Fisheries
Private Bag X138, Pretoria 0001

Enquiries: Mr Herry Gololo • Tel: +27 12 319 7532 • Fax: +27 12 319 7470 • E-mail: HerryG@daff.gov.za
Reference: 12/11/1/1/13

Prof Janusz Paweska
Centre for emerging and Zoonotic Diseases
NICD
2131 Sandringham
1 Modderfontein RD
Gauteng

Dear Prof Paweska

RE: Permission to do research in terms of Section 20 of the ANIMAL DISEASES ACT, 1984 (ACT NO. 35 of 1984)

Your fax / memo / letter/ Email dated 25 August 2015, requesting permission under Section 20 of the Animal Disease Act, 1984 (Act No. 35 of 1984) to perform a research project or study, refers.

I am pleased to inform you that permission is hereby granted to perform the following research/study, with the following conditions:

Conditions:

1. This permission does not relieve the researcher of any responsibility which may be placed on him by any other act of the Republic of South Africa;
2. All potentially infectious material utilised or collected during the study is to be destroyed at the completion of the study. Records must be kept for five years for audit purposes. A dispensation application may be made to the Director Animal Health in the event that any of the above is to be stored or distributed;
3. This Section 20 approval does not serve as an instruction for any person to part take in this study;
4. Compliance with the Veterinary and Para-Veterinary Professions Act, 1982 (Act No 19 of 1982);
5. Compliance with the Non-Proliferation of Weapons of Mass Destruction Act, 1993 (Act No 87 of 1993);
6. A separate Section 20 application must be submitted for study 3 on cattle/buffalo;

7. If any diagnostic tests other than what has been specified within the Section 20 application are to be performed, an application for an amendment to this Section 20 approval must be submitted;
8. No sampling may be conducted within areas under state veterinary restriction;
9. Any publications or reports must be pre-approved by the Director Animal Health;
10. Only sheep may be vaccinated & must be vaccinated with registered vaccines.

Title of research/study: Understanding Rift Valley in Republic of South Africa- Study 2. Sheep cohort study.
Researcher (s): Prof Janusz Paweska
Institution: Centre for emerging and Zoonotic Diseases, NICD.
Your Ref./ Project Number:
Our ref Number: 12/11/1/1/13

Kind regards,

DR. MPHO MAJA

DIRECTOR OF ANIMAL HEALTH

Date: 2015 -09- 07

Appendix 3:
Human Ethics Approvals

November 21, 2014
Corrected November 24, 2014

William B. Karesh D.V.M.
EcoHealth Alliance
460 West 34th St., 17th Floor
New York, NY 10001-2320

Protocol Title:	Towards a Better Understanding of Rift Valley Fever and Brucellosis in the Republic of South Africa
Hummingbird IRB #:	2014-25
Sponsor:	U.S. Defense Threat Reduction Agency
Approval Period:	November 21, 2014 – November 20, 2015

Dear Dr. Karesh:

Hummingbird IRB approved the above referenced study for one year. The following documents are approved for this study:

1. Protocol dated: November 18, 2014
2. Participant, Farmer Questionnaires and Human SeroSurvey, with accompanying data sheets- Not Dated
3. Consent forms-Employer, assent and participant consent -Version 1 (English)
4. Advertisement for local farming magazines and newspapers
5. Peoples Survey 2015:Mosquito & Livestock Disease Exposure

We wish to acknowledge the approval from the School of Pathology, University of Witwatersrand, which approved the study's human subject intervention in South Africa. Hummingbird IRB's approval extends to the work that will be undertaken by the U.S. based investigators both in the field in South Africa and the data analysis.

Any changes made to the protocol, consents or questionnaires and surveys must be submitted to Hummingbird IRB. Approval from Hummingbird IRB must be secured prior to initiation of the revision(s). You will receive a reminder to renew approval of the study approximately 3 months prior to the end of the approval period.

Attached, you will find a summary of investigator commitments with which the Board requires each investigator to adhere to during the approval period.

Sincerely,

David Rosenthal, M.D.
Co-Chairman, Hummingbird IRB

Attachment
cc: Melinda Rostal, EcoHealth Alliance
Hummingbird IRB File

Investigator Commitments

All Hummingbird IRB (HIRB) approved investigators are required to fulfill these commitments.

In granting approval to the investigator for the conduct of an investigational study, Hummingbird IRB requires the investigator to understand and agree to these commitments:

1. The investigator will conduct the study(ies) in accordance with the relevant, current protocol(s) and will only make changes in a protocol when necessary to protect the safety, rights, or welfare of subjects.

2. The investigator will personally conduct or supervise the described investigation(s).

3. The investigator will delegate tasks to only trained, experienced and appropriately credentialed individuals who are familiar with the protocol and understand the tasks required to conduct the study and protect human subjects during screening and while enrolled.

4. The investigator is obligated to inform Hummingbird IRB of any financial conflicts of interest which may exist through submitting appropriate forms on an annual basis. Should a conflict arise during the course of the study, this conflict will be promptly reported to the IRB.

5. The investigator will inform any patients involved in a study involving drugs, devices or biologics, or any persons used as controls, that the drugs, devices or biologics are being used for investigational purposes and will ensure that the requirements relating to obtaining informed consent in 21 CFR Part 50 and institutional review board (IRB) review and approval in 21 CFR Part 56 are met.

6. The investigator will report to the sponsor and Hummingbird IRB (when applicable) adverse and unanticipated problems that occur in the course of the investigation(s). If after the study has concluded, new information is made available that is relevant to ongoing health or safety, the investigator will inform subjects of these results.

7. When applicable, the investigator will read and understand the information in the investigator's brochure, device manual and other scientific background that describes the potential risks and side effects of the drug, procedure or device.

8. The investigator will ensure that all associates, colleagues, and employees assisting in the conduct of the study(ies) are informed about their obligations in meeting the commitments outlined in this document.

9. The investigator will maintain adequate and accurate records and make those records available for inspection.

10. The investigator will promptly report Hummingbird IRB all changes in the research activity and all unanticipated problems involving risks to human subjects or others. Additionally, the investigator will not make any changes in the research without Hummingbird IRB approval, except where necessary to eliminate apparent immediate hazards to human subjects.

11. The investigator will have in place at his or her site, a process by which the HIRB approved consent form is compared to the executed contract to ensure that consistency exists between documents in terms of procedures, study visits, payment to subjects and compensation for injury as well as other conditions effecting human subjects. The investigator and sponsor will resolve any difference and notify HIRB of any changes impacting the consent.

12. The investigator will provide referrals to any subject for whom a condition or potentially adverse information is uncovered during the study. This may include, for example, learning of suicidality or a previously unknown disease. This does not pertain to results of genetic testing unless sharing this information is part of the protocol.

R14/49 Prof JT Paweska et al

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M140306

NAME: (Principal Investigator)	Prof JT Paweska et al
DEPARTMENT:	School of Pathology Mokala National Park, Free State and Bloemfotein
PROJECT TITLE:	Towards a Better Understanding of Rift Valley Fever and Brucellosis Epidemiology in the Republic of South Africa
DATE CONSIDERED:	28/03/2014
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	
APPROVED BY:	Professor PE Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	30/04/2014

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University.
I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**.

Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

DEPARTMENT OF HEALTH
LEFAPHA LA BOITEKANELO
ISEBE LEZEMPILO
DEPARTEMENT VAN GESONDHEID

Department of Health
Private Bag X5049
KIMBERLEY
8301

Enquiries : Dr. Eshetu Worku
Dipatlisiso :
Imibuzo :
Navrae :

Date : 09 February 2015
Letlha :
Umhla :
Datum :

Reference :
Tshupelo :
Isalathiso :
Verwysings :

Tel: 053 830 2134/22
Fax: 086 541 7122

Prof JT Paweska
HOD Centre for Emerging and Zoonotic Diseases
National Institute of Communicable Diseases
1 Modderfontein, Sandringham, 2131

Dear Prof JT Paweska

TITLE: **Towards a Better Understanding of the Rift Valley Fever and Brucellosis in the Republic of South Africa.**

NC PHREC Reference Number: NC2015/001

The application to conduct the study was received and has been reviewed by the Provincial Health Research and Ethics Committee (PHREC)

Approval is hereby granted to conduct the above-mentioned study in the Northern Cape Province

Please note: This approval is valid for a period of one year from the date of approval.

The following conditions have to be noted:

1. The research project shall be conducted at no cost to the Northern Cape Department of Health.
2. The approval is limited to the research proposal as submitted in the application.
3. Variation or modification on the research must be notified formally to PHREC for further consideration.
4. The PHREC may monitor the project at any time.
5. At the completion of your study a copy of the final report must be submitted to the Research and Development Directorate.

6. The Northern Cape Senior Management Committee will be briefed on the outcome of the study prior to publishing.

Furthermore, after the completion of you project, you may be requested to do a presentation on the final findings of your study.

The committee wishes you success on your study

Yours Faithfully

09/02/2015

Dr. Eshetu Worku
Chairperson: PHREC
E-mail: eworku@ncpg.gov.za
Tel: 053 830 2122/34
Cell:

Date

health
Department of Health
FREE STATE PROVINCE

4 April 2015

Prof JT Paweska
School of Pathology
Mokala National Park, Free State and Bloemfotein

Dear Prof JT Pawaska

Subject: Towards a better understanding of Rift Valley Fever and Brucellosis Epidemiology in the Republic of South Africa.

The above mentioned correspondence bears reference.

- Permission is hereby granted for the above – mentioned research on the following conditions:
- Participation in the study must be voluntary.
- A written consent by each participant must be obtained.
- Ascertain that your data collection exercise neither interferes with the day to day running of the health facilities nor the performance of duties by the respondents.
- Serious Adverse events to be reported to the Free State department of health and/ or termination of the study.
- Confidentiality of information will be ensured and no names will be used.
- Research results and a complete report should be made available to the Free State Department of Health on completion of the study (a hard copy plus a soft copy).
- Progress report must be presented not later than one year after approval of the project to the Ethics Committee of the University of Witwatersrand and to Free State Department of Health.
- Any amendments, extension or other modifications to the protocol or investigators must be submitted to the Ethics Committee of the University of Witwatersrand and to Free State Department of Health.
- Conditions stated in your Ethical Approval letter should be adhered to and a final copy of the Ethics Approval should be submitted to khusemj@fshealth.gov.za or sebeelats@fshealth.gov.za before you commence with the study
- No financial liability will be placed on the Free State Department of Health
- Please discuss your study with the institution manager on commencement for logistical arrangements
- Department of Health to be fully indemnified from any harm that participants and staff experiences in the study
- Researchers will be required to enter in to a formal agreement with the Free State department of health regulating and formalizing the research relationship (document will follow)
- You are encouraged to present your study findings/results at the Free State Provincial health research day
- Future research will only be granted permission if correct procedures are followed see http://nhrd.hst.org.za

Trust you find the above in order.

Dr D Motau
HEAD: HEALTH
Date: 11/04/2015

Head : Health
PO Box 227, Bloemfotein, 9300
4th Floor, Executive Suite, Bophelo House, cnr Maitland and, Harvey Road, Bloemfotein
Tel: (051) 408 1646 Fax: (051) 408 1556 e-mail:khusemj@fshealth.gov.za@fshealth.gov.za/chikobvup@fshealth.gov.za

Date: January 27, 2015 at 3:43:24 PM EST
To: Richards, Jean

Dear Dr. Richards,

The DTRA Research Oversight Board (ROB) has reviewed and approved human research for the project titled " Understanding Rift Valley Fever in Republic of South Africa." The principal investigator is Dr. William Karesh of EcoHealth Alliance. The University of the Witwatersrand, Johannesburg IRB approved this effort. This IRB is registered with DHHS under DHHS Assurance, FWA00000715, expiring July 14, 2017. The effort is minimal risk.

Our tracking number is CT-2014-33. Any proposed modifications or amendments to the approved protocol or consent forms must be submitted to the IRB and the DTRA ROB for review and approval.

Examples of modifications/amendments to the protocol include but are not limited to:
1) a change of the Principal Investigator
2) changes in duration or intensity of exposure to some stimulus or agent
3) changes in the information requested of volunteers, or changes to the use of specimens or

data collected
4) changes in perceived or measured risks or benefits to volunteers that require changes to the study

Research pursuant to such modifications or amendments shall not be initiated without IRB and ROB approval except when necessary to eliminate apparent and immediate hazards to the subject(s).

Research projects lasting more than one year require IRB review at least annually, or more frequently as required by the responsible IRB. ROB review and approval is required annually. The contractor or subcontractor must provide documentation of continued IRB review of protocols for ROB review and approval in accordance with the Contract Data Requirements List. Research must not continue without renewed ROB approval unless necessary to eliminate apparent and immediate hazards to the subject(s).

Please contact me if an Unanticipated Problem Involving Risk to Subjects or Others (UPIRTSO) occurs during the conduct of this effort. A UPIRTSO is any incident, experience, or outcome that meets ALL three of the following
conditions:

Is unexpected (in terms of nature, severity, or frequency) given the procedures described in the research protocol documents (e.g., the IRB-approved research protocol and informed consent document) and the characteristics of the human subject population being studied.

Is related or possibly related to participation in the research (in this Instruction, possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research).

Suggests that the research places human subjects or others at a greater risk of harm (including physical, psychological, economic, or social
harm) than was previously known or recognized, even if no harm has actually occurred.

Al

Alfred S. Graziano Jr. MS, GS-14, CIP
Executive Secretary,
Research Oversight Board (Human and Animal) Defense Threat Reduction Agency
8725 Kingman Rd, Stop 6201
Ft Belvoir VA 22060-6201
Phone 703 767-3360 FAX 767-1892
E-mail: al.graziano@dtra.mil

Classification: UNCLASSIFIED
Caveats: NONE

Farm Name: _____ Farm No.: _____

Appendix 4:
Informed Consent forms

National Institute for Communicable Diseases
Centre for Emerging and Zoonotic Diseases
1 Modderfontein Road, Sandringham, 2131
Tel: +27 (0)11 386 6428

EMPLOYER CONSENT

Project Title:

Towards a better understanding of Rift Valley Fever and Brucellosis Epidemiology in the Republic of South Africa

Introduction
Hello my name is _____. I am from the National Institute for Communicable Diseases (NICD) / Department of Health. We are conducting a project that involves a questionnaire and the collection of blood from people residing or working on farms in the Free State. We are doing this to look for exposure and risks of exposure to diseases that affect both people and livestock. These include: Rift Valley fever or other viruses (chikungunya, West Nile, Sindbis fever, Crimean-Congo haemorrhagic fever) and bacterial diseases (brucellosis, leptospirosis, Q fever, and rickettsioses).

With your permission, we will approach your employees or residents on your farm (up to 10-15 per farm) for the study and ask if they wish to participate. Each employee or resident will receive a consent form (attached) and can decide whether or not to participate in the study.

As outlined in the attached farm employee consent form, employees will have the option to volunteer to take part in the study. They will be able to choose whether or not to provide a blood sample and/or take a questionnaire. The blood sample and questionnaire will help determine prior exposure to viral and bacterial diseases. The long-term goal of the study is to better understand risk factors for viral and bacterial diseases and promote animal and public health in South Africa.

There is no cost to you or your employees to participate in the study. Participants can leave the study at any point. Participation is expected to take at most a total of 20 minutes (about 10-15 minutes for the questionnaire, and about 5 minutes to take the blood specimen). We will approach people on the farm that are over the age of 11 years. While you will not receive individual results for the participants, we will provide summary information to you about the region and it will be useful for you to look at with the animal exposure information we are also collecting.

Farm specific information: If you agree to have your farm be part of this project, we will also ask you a number of questions. The questions are not personal, and are focused describing the situation of your animals on this farm.

Employer permission declaration
I have read the information, or it has been read to me. I have had the opportunity to ask questions about it and my questions have been answered to my satisfaction. I understand what the study involves and its purpose and have voluntarily selected the following option:

Farm Name: _____ Farm No.: _____

NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES
Division in the National Health Laboratory Service

National Institute for Communicable Diseases
Centre for Emerging and Zoonotic Diseases
1 Modderfontein Road, Sandringham, 2131
Tel: +27 (0)11 386 6428

Option 1

I voluntarily give my permission for my farm employees to take part in this study (interview) and for their blood samples to be collected for the purpose indicated above, if they also agree to participate in the study (on an individual basis). I voluntarily choose to answer questions about my farm and animals.

Name of Employer:

_____

Postal Address:

_____

Email of Employer

_____

Phone Number of Employer

_____

Employer Signature: _____

Date (Day/Month/Year): _____

Option 2

I voluntarily choose to answer questions about my farm and animals, but I do not consent for my employees to take part in this study.

Name of Employer:

_____

Postal Address:

_____

Email of Employer

_____

Phone Number of Employer

_____

Employer Signature: _____

Date (Day/Month/Year): _____

Farm Name: _____ Farm No.: _____

National Institute for Communicable Diseases
Centre for Emerging and Zoonotic Diseases
1 Modderfontein Road, Sandringham, 2131
Tel: +27 (0)11 386 6428

Option 3

I do not provide voluntarily consent for my employees to take part in this study, nor will I answer questions about my farm and animals.

Name of Employer:

_____

__

Postal address:

_____

_____

Employer Signature: _____

Date (Day/Month/Year): _____

Sheep Cohort RVFV Project

Farm ID:_____

Farm Name: _____

INFORMED CONSENT FORM

We, the undersigned, hereby agree that the animal(s), as specified below, may be used by the researcher(s), as specified below, in the procedures as explained below:

1. To be completed by the researcher(s)

- **NAME OF THE RESEARCHER(S):**
  Dr. Claudia Cordel, Dr. Melinda Rostal, Prof. Janusz Paweska, Dr. Pierre Nel, Dr. David Zimmerman, Sr. J.C. Bester and/or Dr. William Karesh

- **NAME OF RESEARCH PROJECT:**
  Understanding Rift Valley Fever in the Republic of South Africa

- **PURPOSE OF RESEARCH PROJECT:**
  To understand how previous exposure to or vaccination for Rift Valley fever virus in livestock or antelope may impact the severity and occurance of Rift Valley fever outbreaks. The results of this research will be used to better predict when the next outbreak may occur and to prevent significant disease in both animals and people.

- **DETAILED PROCEDURE(S) TO BE PERFORMED:**
  Sheep: Animals will be restrained for a blood sample to be collected, given a vaccine or saline injection and marked using two ear-tags and an ear tattoo. The animals will then be released.

  During the first month following vaccination, please keep the vaccinated study sheep separate from the non-vaccinated study sheep while grazing and in the kraal. After the initial vaccination, all study sheep will be sampled again one month later. The sheep may be placed in different flocks for the month and then put back into the same flock. A temporary barrier may be placed in the kraal during the night to keep the animals separate.

  Thereafter all of the study sheep can be flocked together if desired and will be restrained every three months for a blood sample. The animals are to remain on the farm and not be sold or slaughtered until the project is terminated in four years. Any lambs born to the sheep enrolled in the study belong to you, the farm owner, and there are no restrictions on selling, slaughtering or any other use of these lambs.

- **RISK(S) INVOLVED IN SPECIFIED PROCEDURE:**
  There is very little risk to the sheep, they might have some bruising at the site of blood collection or on their ears for a few days.

- **IDENTIFICATION OF ANIMAL TO BE USED:**

All sheep will be identified. Up to three identification methods will be used – microchipping, tatooing and ear tags.

- **UNMISTAKEABLE DISTINGUISHING DESCRIPTION OF ANIMAL TO BE USED:** Sheep will permenantly identified as described above.

- **SICKNESS OR LOSS OF STUDY ANIMAL:**
  During the four-year study there may be animal losses. Should an study sheep become sick or injured please call the project vet, Dr. Claudia Cordel at 051 433 3022 or 084 908 0407. Likewise if a study sheep dies during the study Dr. Cordel must be called to conduct a post-mortem exam in order to receive pro-rated compensation for that animal during that year.

2. To be completed by the animal's owner or person duly authorized to sign on his/her behalf:

   - **NAME OF OWNER: _____**

   - **PHONE NUBER AND EMAIL ADDRESS:_____**

   - **DO YOU CONFIRM THAT THE ANIMALS SELECTED FOR THIS STUDY HAVE NEVER BEEN VACCINATED against Rift Valley Fever?**
     ☐ Yes ☐ No

   - **HAVE YOU RECEIVED DETAILED INFORMATION REGARDING THE PROPOSED STUDY?**
     ☐ Yes ☐ No

   - **HAVE ALL THE RISKS INVOLVED IN THE PROCEDURE BEEN EXPLAINED TO YOU AND DO YOU FULLY UNDERSTAND THESE RISKS?**
     ☐ Yes ☐ No

   - **DO YOU GRANT FULL CONSENT FOR THE PROCEDURE TO BE PERFORMED?**
     ☐ Yes ☐ No

   - **DO YOU AGREE TO REMAIN PART OF THE PROJECT FOR 4 YEARS AND NOT TO SLAUGHTER OR SELL ANY ANIMALS?**
     ☐ Yes ☐ No

   - **DO YOU AGREE TO COMPENSATION AS FOLLOWS R_____PER SHEEP FOR THE FIRST YEAR (half at the time of vaccination and ear tagging and half 7 months after that time) AND R_____FOR EACH YEAR YOUR ANIMALS REMAIN IN THE STUDY FOR UP TO 4 YEARS.**

3. The undersigned parties further agree that no further compensation will be payable to the animal's owner or anybody else other than that agreed to above at the time of signatures below and that all research associated costs will be covered by the researcher(s). The University of Pretoria is not responsible for the payment of compensation as this is an independent research program run by EcoHealth Alliance, ExecuVet LTD. and NICD.

4. Should an animal that is part of the project die during the project the undersigned parties agree that the owner or another representative will take a blood sample, refridgerate it and call the project veterinarian immediately.

5. The undersigned parties further agree that this form would serve to fully indemnify the University of Pretoria and the undersigned researcher(s) against any future claims resulting from the specified procedure by or on behalf of the animal's owner. Any disputes will be settled via an independent third party between the farmer and ExceuVet.

6. The undersigned parties further agree that no material of any kind, including data and research findings, obtained or resulting from the procedure, would be passed on to any third party or used for any purpose other than that specified in this form, except with the written consent of the undersigned owner of the animal.

_____ _____

SIGNATURE RESEARCHER(S) **SIGNATURE OWNER**

_____ _____

SIGNATURE WITNESS **DATE**

- **DO YOU AGREE TO ALLOW NICD TO STORE YOUR ANIMAL'S BIOLOGICAL SAMPLES (WITHOUT PERSONAL INFORMATION) IN A FREEZER BANK TO BE USED FOR OTHER RESEARCH STUDIES ?**
  ☐ Yes ☐ No

_____ _____

SIGNATURE OWNER **DATE**

BANK DETAILS FOR TRANSFER OF COMPENSTATION FUNDS:

ACCOUNT NAME: _____

BANK: _____

BRANCH CODE:_____

ACCOUNT NUMBER: _____

Livestock Cross-sectional RVFV Study

Farm ID:_____

Farm Name: _____

NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES
Division in the National Health Laboratory Service

EcoHealth Alliance

INFORMED CONSENT FORM

We, the undersigned, hereby agree that the animal(s), as specified below, may be used by the researcher(s), as specified below, in the procedures as explained below:

1. To be completed by the researcher(s)

- **NAME OF THE RESEARCHER(S):**
  Dr. Claudia Cordel, Dr. Melinda Rostal, Prof. Janusz Paweska, Dr. Pierre Nel, Dr. David Zimmerman, Dr. William Karesh, Ms. Veerle Msimang and/or Dr. Peter Thompson.

- **NAME OF RESEARCH PROJECT:**
  Understanding Rift Valley Fever and Congo fever in the Republic of South Africa

- **PURPOSE OF RESEARCH PROJECT:**
  To understand how previous exposure to or vaccination for Rift Valley fever virus in livestock or antelope may impact the severity and occurance of Rift Valley fever outbreaks. The results of this research will be used to better predict when the next outbreak may occur and to prevent significant disease in both animals and people. The blood samples will be tested for Rift Valley fever virus and Congo fever (neither of which are government controlled diseases in livestock). The tests used are mainly to detect previous exposure to the viruses and do not indicate any specific infection or require any treatment or action.

- **DETAILED PROCEDURE(S) TO BE PERFORMED:**
  Animals will be restrained for blood collection. Sheep: They will be tattooed and/or an ear tag placed in one or both ears, and a microchip maybe placed at the base of their left ear.

- **RISK(S) INVOLVED IN SPECIFIED PROCEDURE:**
  There is very little risk during blood collection and identification, the animal may have some bruising at the site of blood collection.

- **IDENTIFICATION OF ANIMAL TO BE USED:**
  Sheep will be identified to inform the owner of the exposure history of the sheep to Rift valley fever virus and/or vaccine. For certain sheep, the researchers will approach you, the owner, to resample the sheep requested (you will be asked to sign a new consent form in this case).

- **UNMISTAKEABLE DISTINGUISHING DESCRIPTION OF ANIMAL TO BE USED:**
  Locally bred sheep, goats and cattle will be used in the project and they will be selected randomly on the farm.

2. To be completed by the animal's owner or person duly authorized to sign on his/her behalf:

- **NAME OF OWNER: _____**
- **PHONE NUMBER AND EMAIL ADDRESS:_____**
- **ADDRESS TO MAIL RESULTS: _____**

- **WILL YOU COMPLETE THE FARM SURVEY THAT ASKS ABOUT YOUR FARMING PRACTICES (NO PERSONAL QUESTIONS WILL BE ASKED)?**
- ☐Yes ☐No

- **HAVE YOU RECEIVED DETAILED INFORMATION REGARDING THE PROPOSED STUDY?**
  ☐Yes ☐No

- **HAVE ALL THE RISKS INVOLVED IN THE PROCEDURE BEEN EXPLAINED TO YOU AND DO YOU FULLY UNDERSTAND THESE RISKS?**
  ☐Yes ☐No

- **DO YOU GRANT FULL CONSENT FOR THE PROCEDURE TO BE PERFORMED?**
  ☐Yes ☐No

- **DO YOU AGREE TO NOT TO SLAUGHTER OR SELL THE IDENTIFIED SHEEP UNTIL THE PROJECT INFORMS YOU OF THE TESTING RESULTS (UP TO 6 MONTHS)?** ☐Yes ☐No

3. The undersigned parties further agree that no compensation will be payable to the animal's owner or anybody else and that all research associated costs will be covered by the researcher(s).

4. The undersigned parties further agree that this form would serve to fully indemnify the University of Pretoria and the undersigned researcher(s) against any future claims resulting from the specified procedure by or on behalf of the animal's owner.

5. The undersigned parties further agree that no material of any kind, including data and research findings, obtained or resulting from the procedure, would be passed on to any third party or used for any purpose other than that specified in this form, except with the written consent of the undersigned owner of the animal.

_____ _____
SIGNATURE RESEARCHER(S) **SIGNATURE OWNER**

_____ _____
SIGNATURE WITNESS **DATE**

- **DO YOU AGREE TO ALLOW NICD TO STORE YOUR ANIMAL'S BIOLOGICAL SAMPLES (WITHOUT PERSONAL INFORMATION) IN A FREEZER BANK TO BE USED FOR OTHER RESEARCH STUDIES ?** ☐Yes ☐No

_____ _____
SIGNATURE OWNER **DATE**

- **DO YOU AGREE TO ALLOW NICD TO TEST SAMPLES FROM CATTLE YOU PREVIOUSLY ALLOWED US TO SAMPLE (IN 2015 OR 2016) FOR CONGO FEVER ?** ☐Yes ☐No

_____ _____
SIGNATURE OWNER **DATE**

Farmer Survey: Towards a better understanding of
Rift Valley Fever and Brucellosis Epidemiology in South Africa

PRIVATE DOMESTIC FARMER QUESTIONNAIRE

Appendix 5.

Cross-sectional Study Farm Surveys

You are at the start of Farmer Survey. Swipe the screen as shown below to go backward and forward.

To be filled out by survey administrator:

Admin 1. Farm Identifier:

Admin 2. Farm Name:

Admin 3. Date of interview:

Admin 4. Collect the current GPS coordinates.

Admin 4a. Enter the latitude and longitude manually using the GPS.
If automatic GPS coordinate does not work then:

Latitude (Degrees) _____

Latitude (Minutes) _____

Latitude (Seconds) _____

Longitude (Degrees) _____

Longitude (Minutes) _____

Longitude (Seconds) _____

Admin 5. Filled in by: _____ *Leave this blank if you are not a survey coordinator.*

The remainder of the survey should be filled out by the participant.

Q1 Do you primarily farm domestic or wild animals? ☐

Domestic ☐Wildlife

Q2 Is the land your animals are grazed on: ☐

Communal ☐Private

Q3 What animal species are/were kept on the farm in the past month (CURRENTLY)? *Select all that apply.*
Do not include animals that are not owned by the farm. Variants should be listed under the same species.

- ☐ Cattle
- ☐ Sheep
- ☐ Goat
- ☐ Pig
- ☐ Buffalo
- ☐ Springbok
- ☐ Blesbok
- ☐ Kudu
- ☐ Other

Q3a What animal species are/were kept on the farm in the PAST 12 MONTHS? *Select all that apply.*

Do not include animals that are not owned by the farm. Variants should be listed under the same species.

- ☐ Cattle
- ☐ Sheep
- ☐ Goat
- ☐ Pig
- ☐ Buffalo
- ☐ Springbok
- ☐ Blesbok
- ☐ Kudu
- ☐ Other

Q3b Only display relevant questions pertaining to answers in 3 and 3a

Q3b Cattle *If none, enter zero*
What is the ACTUAL number of cattle NOW?
How many of your cattle are < 2 years of age?
How many of your cattle are between 2-4 years of age?
How many or your cattle are >4 years of age?
What is the AVERAGE number of cattle kept on the farm during one month?

Q3b Sheep *If none, enter zero*
What is the ACTUAL number of sheep now?
How many of your sheep are < 2 years of age?
How many of your sheep are between 2-4 years of age?
How many or your sheep are >4 years of age?
What is the AVERAGE number of sheep kept on the farm during one month?

Q3b Goat *If none, enter zero*
What is the ACTUAL number of goats now?
How many of your goats are < 2 years of age?
How many of your goats are between 2-4 years of age?
How many or your goats are >4 years of age?
What is the AVERAGE number of goats kept on the farm during one month?

Q3b Pigs *If none, enter zero*
What is the ACTUAL number of pigs now?
What is the AVERAGE number of pigs kept on the farm during one month?

Q3b Buffalo *If none, enter zero*
What is the ACTUAL number of buffalo now?
What is the AVERAGE number of buffalo kept on the farm during one month?

Q3b Springbok *If none, enter zero*
What is the ACTUAL number of springbok now?
What is the AVERAGE number of springbok kept on the farm during one month?

Q3b Blesbok *If none, enter zero*
What is the ACTUAL number of blesbok now?
What is the AVERAGE number of blesbok kept on the farm during one month?

Q3b Kudu *If none, enter zero*
What is the ACTUAL number of kudu now?
What is the AVERAGE number of kudu kept on the farm during one month?

Q3b Others *If none, enter zero*
List all other species that are/were kept on the farm in the past month (CURRENTLY). Indicate how many of each
species are/were kept on the farm in the past month. *Please separate answers with commas. Example: 2 horses, 3 camels.*

Q3b List all other species that were kept on the farm in the PAST 12 MONTHS. Indicate the average number of species kept on the farm during one month. *Please separate answers with commas.*
Example: 2 horses, 3 camels

Q4 Are any of the farms' animals housed, grazed or allowed to spend time with other species on the farm?
☐ Yes ☐ No

If yes, Which animals are grazed or allowed to spend time with other species on the farm? *Select all that apply.*
Only display relevant choices selected in 3 and 3a
☐ Cattle
☐ Sheep
☐ Goat
☐ Pig
☐ Buffalo
☐ Springbok
☐ Blesbok
☐ Kudu
☐ Other

If other Please list other animals that are grazed or allowed to spend time with other species. *Please separate answers with commas. Examples: horses, camels*

Q4a Only display relevant questions pertaining to answers in 4
Select all animal species on the farm that the cattle mix with. *Select all that apply.*
☐ Sheep
☐ Goat
☐ Pig
☐ Buffalo
☐ Springbok
☐ Blesbok
☐ Kudu
☐ Other

If other What other species do the cattle mix with? *Please separate answers with commas.*
Examples: horses, camels

Q4a Select all animal species on the farm that the sheep mix with. *Select all that apply.*
☐ Cattle
☐ Goat
☐ Pig
☐ Buffalo
☐ Springbok
☐ Blesbok
☐ Kudu
☐ Other

If other What other species do the sheep mix with? *Please separate answers with commas.*
Examples: horses, camels

Q4a Select all animal species on the farm that the goats mix with. *Select all that apply.*
☐ Cattle
☐ Sheep
☐ Pig
☐ Buffalo
☐ Springbok

☐ Blesbok
☐ Kudu
☐ Other

If other What other species do the goats mix with? *Please separate answers with commas.*
Examples: horses, camels

Q4a Select all animal species on the farm that the pigs mix with. *Select all that apply.*
☐ Cattle
☐ Sheep
☐ Goat
☐ Buffalo
☐ Springbok
☐ Blesbok
☐ Kudu
☐ Other

If other What other species do the pigs mix with? *Please separate answers with commas.*
Examples: horses, camels

Q4a Select all animal species on the farm that the buffalo mix with. *Select all that apply.*
☐ Cattle
☐ Sheep
☐ Goat
☐ Pig
☐ Springbok
☐ Blesbok
☐ Kudu
☐ Other

If other What other species do the buffalo mix with? *Please separate answers with commas.*
Examples: horses, camels

Q4a Select all animal species on the farm that the springbok mix with. *Select all that apply.*
☐ Cattle
☐ Sheep
☐ Goat
☐ Pig
☐ Buffalo
☐ Blesbok
☐ Kudu
☐ Other

If other What other species do the springbok mix with? *Please separate answers with commas.*
Examples: horses, camels

Q4a Select all animal species on the farm that the blesbok mix with. *Select all that apply.*
☐ Cattle
☐ Sheep
☐ Goat
☐ Pig
☐ Buffalo
☐ Springbok
☐ Kudu
☐ Other animal

If other What other species do the blesbok mix with? *Please separate answers with commas.*

Examples: horses, camels

Q4a Select all animal species on the farm that the kudu mix with. *Select all that apply.*

- ☐ Cattle
- ☐ Sheep
- ☐ Goat
- ☐ Pig
- ☐ Buffalo
- ☐ Springbok
- ☐ Blesbok
- ☐ Other

If other What other species do the kudu mix with? *Please separate answers with commas.*

Examples: horses, camels

Please list any additional farm animals interactions not already indicated. *Please separate answers with commas.*

Example: horses mix with camels, ducks mix with chickens

Q5 Are the farm's animals grazed or allowed to spend time with wild roaming antelope/hooved animals?

☐ Yes ☐ No

Q5 If yes Which of the farms' animals are grazed or allowed to spend time with wild roaming antelope/hooved animals? *Select all that apply.* Only display relevant choices pertaining to answers in 3 and 3a

- ☐ Cattle
- ☐ Sheep
- ☐ Goat
- ☐ Pig
- ☐ Buffalo
- ☐ Springbok
- ☐ Blesbok
- ☐ Kudu
- ☐ Other

If other Please list any additional farm animals that mix with wild animals that are not already indicated. *Please separate answers with commas. Example: camels mix with steinbuck.*

Q5a Which wild animals (not farmed) graze or spend time with the animals on the farm? *Select all that apply.*

- ☐ Buffalo
- ☐ Springbok
- ☐ Blesbok
- ☐ Kudu
- ☐ Sable antelope
- ☐ Roan antelope
- ☐ Impala
- ☐ Waterbuck
- ☐ Reedbuck
- ☐ Bontebok
- ☐ Hartebeest
- ☐ Blue wildebeest
- ☐ Black wildebeest
- ☐ Eland
- ☐ Giraffe
- ☐ Zebra

- ☐ Tsessebe
- ☐ Gemsbok
- ☐ Nyala
- ☐ Other

If other Please list any other wild antelope/hooved animals that spend time with the farm animals. *Please separate answers with commas. Example: farmed steinbuck mix with wild springbok, farmed camels mix with wild springbok.*

Q6 Where is each of your animal species kept during the night? *Select all that apply.*
Only display relevant choices pertaining to answers in 3 and 3a or 3-1 and 3-1a

Cattle
- ☐ Open kraal
- ☐ Kraal with roof
- ☐ Communal kraal
- ☐ Veld
- ☐ Other

Q6 Sheep
- ☐ Open kraal
- ☐ Kraal with roof
- ☐ Communal kraal
- ☐ Veld
- ☐ Other

Q6 Goat
- ☐ Open kraal
- ☐ Kraal with roof
- ☐ Communal kraal
- ☐ Veld
- ☐ Other

Q6 Pig
- ☐ Open kraal
- ☐ Kraal with roof
- ☐ Communal kraal
- ☐ Veld
- ☐ Other

Q6 Buffalo
- ☐ Open kraal
- ☐ Kraal with roof
- ☐ Communal kraal
- ☐ Veld
- ☐ Other

Q6 Springbok
- ☐ Open kraal
- ☐ Kraal with roof
- ☐ Communal kraal
- ☐ Veld
- ☐ Other

Q6 Blesbok
- ☐ Open kraal
- ☐ Kraal with roof
- ☐ Communal kraal
- ☐ Veld
- ☐ Other

Q6 Kudu

☐ Open kraal
☐ Kraal with roof
☐ Communal kraal
☐ Veld
☐ Other

Q6 Other

☐ Open kraal
☐ Kraal with roof
☐ Communal kraal
☐ Veld
☐ Other

If other, only options selected in 3 (3-1) and 3a (3a-1) will show up
If other Please list any other location(s) where your cattle are kept during the night.
Please separate answers with commas.

If other Please list any other location(s) where your sheep are kept during the night.
Please separate answers with commas.

If other Please list any other location(s) where your goats are kept during the night.
Please separate answers with commas.

If other Please list any other location(s) where your pigs are kept during the night.
Please separate answers with commas.

If other Please list any other location(s) where your buffalo are kept during the night.
Please separate answers with commas.

If other Please list any other location(s) where your springbok are kept during the night.
Please separate answers with commas.

If other Please list any other location(s) where your blesbok are kept during the night.
Please separate answers with commas.

If other Please list any other location(s) where your kudu are kept during the night.

If other Please list any other location(s) where your other animals are kept during the night.
Please separate answers with commas.

Q7 Do any animals spend time in the house or compounds occupied by people on the farm?
Refers only to animals you own. ☐ Yes ☐ No

If yes Which animal species? *Select all that apply.* Only display relevant choices pertaining to answers in 3 and 3a or 3-1 and 3-1a

☐ Cattle
☐ Sheep
☐ Goat
☐ Pig
☐ Buffalo
☐ Springbok
☐ Blesbok
☐ Kudu
☐ Other

If other Please list any other animals that spend time in the house or compounds occupied by people. *Please separate answers with commas. Examples: dogs, cats.*

Q8 Do the farm animals have contact with animals from other farms or owned by different farmers?

☐ Yes ☐ No

Q8a if yes: How often do they have contact in a typical week? *Answer in times per week.*

Q8b if yes: Under which context do they meet? When… *Select all that apply.*
☐ Grazing
☐ Housing
☐ Feeding
☐ Contact through fence
☐ Other

If other: Please list other contexts in which the farm animals have contact with animals from other farms. *Please separate answers with commas.*

Q9 Approximately how many of each does the farm have within access of the animals? *If none, enter zero.*

Water spring(s) / Lake(s) / Natural dam(s) - permanent source
River, stream – constantly flowing source
Pan(s)- seasonal source
Dam(s) - human made source
Water troughs provided
Other outdoor water source

If other: Please describe the other water source(s). *Please separate answers with commas.*

Q9a Water Sources
If Water springs/ lakes/natural dams: On average what percentage of animals on the farm have access to the water spring(s) / Lake(s) / Natural dam(s) (permanent source) at any given time?

Q9a If River, stream - constantly flowing source: On average what percentage of animals on the farm have access to the river, stream - constantly flowing source at any given time?

Q9a If Pan(s) – seasonal source: On average what percentage of animals on the farm have access to the pan(s)- seasonal source at any given time?

Q9a If Dam(s) - human made source: On average what percentage of animals on the farm have access to the dam(s) - human made source at any given time?

Q9a If water troughs: On average what percentage of animals on the farm have access to the water troughs at any given time?

Q9b How many of each of the following water sources have had water available on the farm during the past year? *For example, 10 pans have had water. If none, enter zero.*

Water spring(s) / Lake(s) / Natural dam(s) - permanent source
River, stream – constantly flowing source
Pan(s)- seasonal source
Dam(s) - human made source
Water troughs provided
Other outdoor water source

If other: Please describe the other water source(s). *Please separate answers with commas.*

Q9c What is the longest period of time during which the following water sources have had water during the past year? *For example, Pans on the farm have been flooded for 5 months.*
Allow decimals

Water has been available from spring(s) / Lake(s) / Natural dam(s) for:_____ months.

Water has been available from river(s), stream(s) (a constantly flowing source) for: _____ months.
Water has been available from pan(s) (a seasonal source) for:_____ months.
Water has been available from dam(s) or a human made source for:_____ months.
Water has been available from water troughs provided for:_____ months.
Water has been available from other outdoor water source(s) for:_____ months.

If other: Please describe the other water source(s). *Please separate answers with commas.*

Q10 Approximately how far do your animals walk each day to get to the grazing area? *Tap below to answer in km or minutes walking. If kept on the veld put 0.*

Q10a Do you rotate grazing areas for the herds? ☐ Yes ☐ No

Q11 Have you purchased/gained (from gift or birth) animals in the past 12 months?
☐ Yes ☐ No

If yes: Which of the following animals have you purchased/gained (from gift or birth) in the past 12 months?
Select all that apply.
☐ Cows
☐ Heifer
☐ Bull
☐ Steer
☐ Calf
☐ Doe goat
☐ Billy goat
☐ Goat kid
☐ Ewe
☐ Ram
☐ Lamb
☐ Sow
☐ Boar
☐ Piglet
☐ Springbok
☐ Blesbok
☐ Kudu
☐ Other

Q11a Asked only for the species indicated as purchased/gained in Q11:
Approximately how many cows were… *If none, enter zero*
Purchased in the past 12 months
Given to you in the past 12 months
Otherwise gained in the past 12 months

In what other ways were cows gained in the past 12 months?

Q11a Approximately how many heifers were… *If none, enter zero*
Purchased in the past 12 months
Given to you in the past 12 months
Otherwise gained in the past 12 months

In what other ways were heifers gained in the past 12 months?

Q11a Approximately how many bulls were… *If none, enter zero*
Purchased in the past 12 months
Given to you in the past 12 months
Otherwise gained in the past 12 months

In what other ways were bulls gained in the past 12 months?

Q11a Approximately how many steers were… *If none, enter zero*
Purchased in the past 12 months
Given to you in the past 12 months
Otherwise gained in the past 12 months

In what other ways were steers gained in the past 12 months?

Q11a Approximately how many calves were… *If none, enter zero*
Purchased in the past 12 months
Given to you in the past 12 months
Born in the past 12 months
Otherwise gained in the past 12 months

In what other ways were calves gained in the past 12 months?

Q11a Approximately how many doe goats were… *If none, enter zero*
Purchased in the past 12 months
Given to you in the past 12 months
Otherwise gained in the past 12 months

In what other ways were doe goats gained in the past 12 months?

Q11a Approximately how many billy goats were… *If none, enter zero*
Purchased in the past 12 months
Given to you in the past 12 months
Otherwise gained in the past 12 months

In what other ways were billy goats gained in the past 12 months?

Q11a Approximately how many goat kids were… *If none, enter zero*
Purchased in the past 12 months
Given to you in the past 12 months
Born in the past 12 months
Otherwise gained in the past 12 months

In what other ways were goat kids gained in the past 12 months?

Q11a Approximately how many ewes were… *If none, enter zero*
Purchased in the past 12 months
Given to you in the past 12 months
Otherwise gained in the past 12 months

In what other ways were ewes gained in the past 12 months?

Q11a Approximately how many rams were… *If none, enter zero*
Purchased in the past 12 months
Given to you in the past 12 months
Otherwise gained in the past 12 months

In what other ways were rams gained in the past 12 months?

Q11a Approximately how many lambs were… *If none, enter zero*

Purchased in the past 12 months
Given to you in the past 12 months
Born in the past 12 months
Otherwise gained in the past 12 months

In what other ways were lambs gained in the past 12 months?

Q11a Approximately how many sows were… *If none, enter zero*

Purchased in the past 12 months
Given to you in the past 12 months
Otherwise gained in the past 12 months

In what other ways were sows gained in the past 12 months?

Q11a Approximately how many boars were… *If none, enter zero*

Purchased in the past 12 months
Given to you in the past 12 months
Otherwise gained in the past 12 months

In what other ways were boars gained in the past 12 months?

Q11a Approximately how many piglets were… *If none, enter zero*

Purchased in the past 12 months
Given to you in the past 12 months
Born in the past 12 months
Otherwise gained in the past 12 months

In what other ways were piglets gained in the past 12 months?

Q11a Approximately how many springbok were… *If none, enter zero*

Purchased in the past 12 months
Given to you in the past 12 months
Born in the past 12 months
Otherwise gained in the past 12 months

In what other ways were springbok gained in the past 12 months?

Q11a Approximately how many blesbok were… *If none, enter zero*

Purchased in the past 12 months
Given to you in the past 12 months
Born in the past 12 months
Otherwise gained in the past 12 months

In what other ways were blesbok gained in the past 12 months?

Q11a Approximately how many kudu were… *If none, enter zero*

Purchased in the past 12 months
Given to you in the past 12 months
Born in the past 12 months
Otherwise gained in the past 12 months

In what other ways were kudu gained in the past 12 months?

List all other species of animals that were gained in the past 12 months. *Please separate with commas.*

Q11a Approximately how many other animals were… *If none, enter zero*

Purchased in the past 12 months

Given to you in the past 12 months

Born in the past 12 months

Otherwise gained in the past 12 months

In what other ways were other animals gained in the past 12 months?

Q11b If they indicated that they had purchased any animals in Q11:
On average, how many times do you purchase animals during one year? *If none, enter zero. Scroll down to view all.*

Q11b On average, how many… *If none, enter zero. Scroll down to view all.*

Cows do you purchase at one time?

Heifers do you purchase at one time?

Bulls do you purchase at one time?

Steers do you purchase at one time?

Calves do you purchase at one time?

Doe goats do you purchase at one time?

Billy goats do you purchase at one time?

Goat kids do you purchase at one time?

Ewes do you purchase at one time?

Rams do you purchase at one time?

Lambs do you purchase at one time?

Sows do you purchase at one time?

Boars do you purchase at one time?

Piglets do you purchase at one time?

Springbok do you purchase at one time?

Blesbok do you purchase at one time?

Kudu do you purchase at one time?

Other animals do you purchase at one time?

Q11c. Have you sold/lost/slaughtered (ceremonial or for food consumption) animals in the PAST 12 MONTHS?
☐ Yes ☐ No

Q11c Which of the following animals have you sold/lost/slaughtered (ceremonial or for food consumption)?
Select all that apply.

☐ Cows
☐ Heifer
☐ Bull
☐ Steer
☐ Calf
☐ Doe goat
☐ Billy goat
☐ Goat kid
☐ Ewe
☐ Ram
☐ Lamb

- ☐ Sow
- ☐ Boar
- ☐ Piglet
- ☐ Springbok
- ☐ Blesbok
- ☐ Kudu
- ☐ Other

Q11d Asked only for the species indicated in Q11:
Approximately how many cows were… *If none, enter zero*

Sold in the past 12 months

Stolen from you in the past 12 months

Predated in the past 12 months
Died in the past 12 months
Were slaughtered in the past 12 months
otherwise lost in the past 12 months

In what other ways were cows lost in the past 12 months?

Q11d Approximately how many heifers were… *If none, enter zero*

Sold in the past 12 months

Stolen from you in the past 12 months

Predated in the past 12 months
Died in the past 12 months
Were slaughtered in the past 12 months
otherwise lost in the past 12 months

In what other ways were heifers lost in the past 12 months?

Q11d Approximately how many bulls were… *If none, enter zero*

Sold in the past 12 months

Stolen from you in the past 12 months

Predated in the past 12 months
Died in the past 12 months
Were slaughtered in the past 12 months
otherwise gained in the past 12 months

In what other ways were bulls lost in the past 12 months?

Q11d Approximately how many steers were… *If none, enter zero*

Sold in the past 12 months

Stolen from you in the past 12 months

Predated in the past 12 months
Died in the past 12 months
were slaughtered in the past 12 months
otherwise lost in the past 12 months

In what other ways were steers lost in the past 12 months?

Q11d Approximately how many calves were… *If none, enter zero*

Sold in the past 12 months

Stolen from you in the past 12 months

Predated in the past 12 months
Died in the past 12 months
were slaughtered in the past 12 months
otherwise lost in the past 12 months

In what other ways were calves lost in the past 12 months?

Q11d Approximately how many doe goats were… *If none, enter zero*

Sold in the past 12 months

Stolen from you in the past 12 months

Predated in the past 12 months
Died in the past 12 months
Were slaughtered in the past 12 months
otherwise lost in the past 12 months

In what other ways were doe goats lost in the past 12 months?

Q11d Approximately how many billy goats were… *If none, enter zero*

Sold in the past 12 months

Stolen from you in the past 12 months

Predated in the past 12 months
Died in the past 12 months
Were slaughtered in the past 12 months
otherwise lost in the past 12 months

In what other ways were billy goats lost in the past 12 months?

Q11d Approximately how many goat kids were… *If none, enter zero*

Sold in the past 12 months

Stolen from you in the past 12 months

Predated in the past 12 months
Died in the past 12 months
were slaughtered in the past 12 months
otherwise lost in the past 12 months

In what other ways were goat kids lost in the past 12 months?

Q11d Approximately how many ewes were… *If none, enter zero*

Sold in the past 12 months

Stolen from you in the past 12 months

Predated in the past 12 months
Died in the past 12 months
Were slaughtered in the past 12 months
Otherwise lost in the past 12 months

In what other ways were ewes lost in the past 12 months?

Q11d Approximately how many rams were… *If none, enter zero*

Sold in the past 12 months

Stolen from you in the past 12 months

Predated in the past 12 months
Died in the past 12 months
Were slaughtered in the past 12 months
Otherwise lost in the past 12 months

In what other ways were rams lost in the past 12 months?

Q11d Approximately how many lambs were… *If none, enter zero*

Sold in the past 12 months

Stolen from you in the past 12 months

Predated in the past 12 months
Died in the past 12 months
were slaughtered in the past 12 months
otherwise lost in the past 12 months

In what other ways were lambs lost in the past 12 months?

Q11d Approximately how many sows were… *If none, enter zero*

Sold in the past 12 months

Stolen from you in the past 12 months

Predated in the past 12 months
Died in the past 12 months
Were slaughtered in the past 12 months
Otherwise lost in the past 12 months

In what other ways were sows lost in the past 12 months?

Q11d Approximately how many boars were… *If none, enter zero*

Sold in the past 12 months

Stolen from you in the past 12 months

Predated in the past 12 months
Died in the past 12 months
Were slaughtered in the past 12 months
Otherwise lost in the past 12 months

In what other ways were boars lost in the past 12 months?

Q11d Approximately how many piglets were… *If none, enter zero*

Sold in the past 12 months

Stolen from you in the past 12 months

Predated in the past 12 months
Died in the past 12 months
Were slaughtered in the past 12 months
Otherwise lost in the past 12 months

In what other ways were piglets lost in the past 12 months?

Q11d Approximately how many springbok were… *If none, enter zero*

Sold in the past 12 months

Stolen from you in the past 12 months

Predated in the past 12 months
Died in the past 12 months
Were slaughtered in the past 12 months
Otherwise lost in the past 12 months

In what other ways were springbok lost in the past 12 months?

Q11d Approximately how many blesbok were… *If none, enter zero*

Sold in the past 12 months

Stolen from you in the past 12 months

Predated in the past 12 months
Died in the past 12 months
Were slaughtered in the past 12 months
Otherwise lost in the past 12 months

In what other ways were blesbok lost in the past 12 months?

Q11d Approximately how many kudu were… *If none, enter zero*

Sold in the past 12 months

Stolen from you in the past 12 months

Predated in the past 12 months
Died in the past 12 months
Were slaughtered in the past 12 months
Otherwise lost in the past 12 months

In what other ways were kudu lost in the past 12 months?

Q11d List all other species of animals that were lost in the past 12 months. *Please separate with commas.*

Approximately how many other animals were… *If none, enter zero. Example: 3 steinbuck, 2 camels.*

Sold in the past 12 months

Stolen from you in the past 12 months

Predated in the past 12 months
Died in the past 12 months
Were slaughtered in the past 12 months
Otherwise lost in the past 12 months

Q11e Asked if they indicated any species had been sold/lost/slaughtered in Q11
On average, how many times do you sell animals during one year? *If you do not sell animals, enter 0.*
Q11f On average, approximately how many…. *Scroll down to view all.*

Cows do you sell at one time?

Heifers do you sell at one time?

Bulls do you sell at one time?

Steers do you sell at one time?

Calves do you sell at one time?

Doe goats do you sell at one time?

Billy goats do you sell at one time?

Goat kids do you sell at one time?

Ewes do you sell at one time?

Rams do you sell at one time?

Lambs do you sell at one time?

Sows do you sell at one time?

Boars do you sell at one time?

Piglets do you sell at one time?

Springbok do you sell at one time?

Blesbok do you sell at one time?

Kudu do you sell at one time?
Approximately how many other animals
do you sell at one time?

Q11g If they indicated that they had bought or sold any animals in Q11c: Where did you purchase your animals from OR sell your animals to in the past 12 months? *Select all that apply. Scroll down to view all.*

Auction	☐ Bought	☐ Sold
Other farms/farmers	☐ Bought	☐ Sold
Abattoir		☐ Sold
Other	☐ Bought	☐ Sold

If indicated: How many auctions did you purchase your animals from?
If you did not purchase any animals at auctions, enter "0"
How many auctions did you sell your animals at?
If you did not sell any animals at auctions, enter "0"
How many other farms did you purchase your animals from?
If you did not purchase any animals at other farms, enter "0"
How many other farms did you sell your animals at?
If you did not sell any animals at other farms, enter "0"
How many abattoirs did you sell your animals at?
If you did not sell any animals at abattoirs, enter "0"

Approximately how far away in Km is the auction where the animal(s) were purchased? *Scroll down to view all.*
Approximately how far away in Km is the second auction where the animal(s) were purchased?
List all other auctions where the animal(s) were purchased and indicate their distance away in Km.

Approximately how far away in Km is the auction where the animal(s) were sold?
Approximately how far away in Km is the second auction where the animal(s) were sold?
List all other auctions where the animal(s) were sold and indicate their distance away in Km.

Approximately how far away in Km is the farm where the animal(s) were purchased? *Scroll down to view all.*
Approximately how far away in Km is the second farm where the animal(s) were purchased?
List all other farms where the animal(s) were purchased and indicate their distance away in Km.

Approximately how far away in Km is the farm where the animal(s) were sold?
Approximately how far away in Km is the second farm where the animal(s) were sold?
List all other farms where the animal(s) were sold and indicate their distance away in Km.

Approximately how far away in Km is the abattoir where the animal(s) were sold? *Scroll down to view all.*
Approximately how far away in Km is the second abattoir where the animal(s) were sold?
List all other abattoirs where the animal(s) were sold and indicate their distance away in Km.

If other: Please specify other places animals were bought or sold.

Q12 What protocols do you implement when introducing new animals into the herd? *Select all that apply.*

- ☐ Quarantine
- ☐ Antibiotics
- ☐ Vaccinations
- ☐ Dipping/tick treatment
- ☐ Other
- ☐ None

If quarantine is selected: How many days are new animals quarantined? *Answer in days*

If other is selected: What other protocols do you implement when introducing new animals into the herd?

Q13 Are the farmed animals or their products intended for international export? ☐ Yes ☐ No

If yes: To where do you export?

Q14 Please estimate the size of the farm in hectares.

Q15 Do you employ people on your farm? ☐ Yes ☐ No

Q15a if yes: Are the workers: ☐ Permanent staff ☐ Seasonal workers

Q15b if permanent selected: How many permanent staff are employed?

Q15b If seasonal selected: How many seasonal staff are employed?

Q16 Do you or any of your employees (and/or families) live on the farm? ☐ Yes ☐ No
If yes: How many
Adults?
Children?

Q17 What is the main industry of the farm?
☐ Meat
☐ Wool (hides)
☐ Dairy
☐ Resale
☐ Dual purpose: meat/dairy
☐ Dual purpose: meat/wool
☐ Tourism/hunting

Q17a If main industry milk or dual meat/dairy: Is the milk produced on the farm pasteurized/boiled?
☐ Yes ☐ No
Is raw (non-boiled) milk consumed by anyone on the farm? ☐ Yes ☐ No

Q18 How would you classify your production system?
☐ Feedlot
☐ Commercial (excluding feedlot)
☐ Semi-commercial
☐ Cash Sales
☐ No cash sales

Q19 Who takes care of animals? *Select all that apply.*
☐ Owner
☐ Farm worker
☐ Children
☐ Other

Q19a If owner takes care of animals: What animal specie(s) do(es) the owner take care of? *Select all that apply.*
☐ Cattle
☐ Sheep
☐ Goat
☐ Pig
☐ Buffalo
☐ Springbok
☐ Blesbok
☐ Kudu
☐ Other

If other: What other species does the owner take care of? *Please separate answers with commas. Example: Camels, horses.*

Q19a If farm workers take care of animals: What animal specie(s) do(es) the farm workers take care of? *Select all that apply.*
☐ Cattle

- ☐ Sheep
- ☐ Goat
- ☐ Pig
- ☐ Buffalo
- ☐ Springbok
- ☐ Blesbok
- ☐ Kudu
- ☐ Other

If other: What other species do the farm workers take care of? *Please separate answers with commas. Example: Camels, horses.*

Q19a If children take care of animals: What animal specie(s) do(es) the children take care of? *Select all that apply.*

- ☐ Cattle
- ☐ Sheep
- ☐ Goat
- ☐ Pig
- ☐ Buffalo
- ☐ Springbok
- ☐ Blesbok
- ☐ Kudu
- ☐ Other

If other: What other species do the children take care of? *Please separate answers with commas. Example: Camels, horses.*

If answered other in Q19 or Q19-1: List all others that take care of animals and indicate the species they take care of. *Example: veterinarians take care of ducks.*

Q20 Are animals slaughtered on the farm? ☐ Yes ☐ No

Q20 If yes to slaughtering: Where are animals slaughtered?

- ☐ A designated outdoor area for the herd
- ☐ Owner/worker's home
- ☐ Local abattoir
- ☐ Other

Q20a if yes to slaughtering: Who assists in the slaughter?

- ☐ Owner
- ☐ Farm workers
- ☐ Abattoir
- ☐ Spiritual leader
- ☐ Local leader
- ☐ Other

If other: Who else assists with slaughtering? *Please separate answers with commas.*

Q21 Have any animals had an abortion in the past 3 months ☐ Yes ☐ No

Q21a If abortion yes: How many animal species had abortions?

Q21b If abortion yes: Which animal species had abortions?

- ☐ Cattle
- ☐ Sheep
- ☐ Goat
- ☐ Pig
- ☐ Buffalo
- ☐ Springbok
- ☐ Blesbok

☐ Kudu
☐ Other

Which other animal species had abortions? *Please separate answers with commas. Example: Camels, horses.*

Q21c If yes: Did anyone assist in the handling of the animals or removal of aborted foetus?
☐ Yes ☐ No

Q21d If yes: How many people were involved in assisting with the abortion?

Q21e If yes: Who was involved in assisting with the abortion? *Select all that apply.*
☐ Veterinarian
☐ Owner
☐ Farm worker
☐ Animal health technician
☐ Other
If other: Who else assisted with the abortion?

Q21f If abortion yes: How was the aborted material disposed of? *Select all that apply.*
☐ Buried
☐ Left (scavenged)
☐ Burnt
☐ Eaten by mother
☐ Other
If other yes: In what other way was the aborted material disposed of?

Q22 Please indicate whether you implement any of the following practices on the farm: *Scroll down to view all.*

Do you maintain fencing around the property?	☐ Yes	☐ No
Do you prevent nose-nose contact between animal species?	☐ Yes	☐ No
Do you keep different animal species in different/divided areas on the farm?	☐ Yes	☐ No
Do you have separate equipment for different animal species?	☐ Yes	☐ No
Do you clean and disinfect equipment used for different herds (same species)?	☐ Yes	☐ No
Do you feed, treat, and work with quarantined/sick animal(s) after you are finished with healthy animals?	☐ Yes	☐ No
Do you keep pregnant animals separate from herd?	☐ Yes	☐ No
Do you clean and disinfect vehicles before and after transporting animals?	☐ Yes	☐ No
Do you vaccinate your animals?	☐ Yes	☐ No
Do you use tick control (dip animals or give injection)	☐ Yes	☐ No
Do you use mosquito/biting fly control?	☐ Yes	☐ No
Do you quarantine new animals?	☐ Yes	☐ No
Do you use other biosafety practices?	☐ Yes	☐ No

Q22a If yes to maintain fencing in Q22: What type of fencing do you maintain around property? *Select all that apply.*
☐ Metal/wire
☐ Wood
☐ Electric
☐ Other

If other: What other type of fencing do you maintain around property? *Please separate answers with commas.*

Q22a Please indicate whether you used tick control on cattle in the past 12 months? ☐ Yes ☐ No

Q22a If yes to tick control in Q22: How many times per year do you use tick control on the animals?

Q22a If yes to tick control in Q22: What seasons is the tick control used on the animals?
☐ Summer (December-February)
☐ Fall (March-May)
☐ Winter (June-August)
☐ Spring (September-November)

Q22b If yes to tick control in Winter, How frequently did you use tick control during the winter? *Indicate number of times*

Q22b If yes to tick control in Spring, How frequently did you use tick control during the spring? *Indicate number of times*

Q22b If yes to tick control in Summer, How frequently did you use tick control during the summer? *Indicate number of times*

Q22b If yes to tick control in Fall, How frequently did you use tick control during the fall? *Indicate number of times*

Q22c What method was used? *Select all that apply*
☐ Dipping tank
☐ Foot bath
☐ Pour-on
☐ Lick in drum with pour-on
☐ Other
☐ Hand spraying
☐ Hand dressing
☐ Spray race
☐ Injection

If other: Please list any other method applied. *Please separate answers with commas.*

Q22a If "mosquito/biting fly" control selected in Q22: How many times per year do you use mosquito or fly control?

Q22a If "mosquito/biting fly" control selected in Q22: What mosquito/biting fly control measures do you use? *Please separate answers with commas.*

Q22a If other biosafety measures indicated: Please describe any other biosafety measures you use. *Please separate answers with commas. Example: all in all out.*

Q23 How far away is the closest farm (from the centre of your farm)? *Answer at least 1 of the following:* Km:
OR minutes drive:
OR minutes walking:

Q24 Has brucellosis been confirmed on your farm at any time in the past? ☐ Yes ☐ No

If yes: Which animals were infected? *Select all that apply.*
☐ Cattle
☐ Sheep
☐ Goat
☐ Pig
☐ Buffalo
☐ Springbok
☐ Blesbok

☐ Kudu
☐ Other

If other: What other animal species were confirmed having brucellosis? *Please separate answers with commas. Example: Camels, horses*

Q24a If yes to Q24 or Q24-1 or Q24-2: When was brucellosis confirmed? *Enter the month and year.*

Enter Date
Month (Enter 1-12)
Year

Q24b Has there been any suspicion of brucellosis on the farm in the past 3 months? ☐ Yes ☐ No

Q24c Has Rift Valley fever been confirmed on your farm at any time in the past? ☐ Yes ☐ No

If yes: Did a veterinarian confirm the diagnosis?
☐ Yes, a state veterinarian ☐ Yes, a private veterinarian ☐ No

If yes: Which animals were infected? *Select all that apply.*
☐ Cattle
☐ Sheep
☐ Goat
☐ Pig
☐ Buffalo
☐ Springbok
☐ Blesbok
☐ Kudu
☐ Other

If other: What other animal species were confirmed having Rift Valley fever? *Please separate answers with commas. Example: Camels, horses.*

For each animal infected in 24c: Approximately how many animals from each species died from Rift Valley fever during the 2010-2011 outbreak that affected your farm? *If none, enter 0.*

Adult cattle _____
Calves _____
Adult sheep _____
Lambs _____
Adult goats _____
Goat kids _____
Pig _____
Buffalo _____
Springbok _____
Blesbok _____
Kudu _____
Other _____

For each animal infected in 24c: Approximately how many animals from each species aborted due to Rift Valley fever during the 2010-2011 outbreak that affected your farm? *If none, enter 0.*

Cows _____
Ewes _____
Lambs _____
Does _____
Pigs _____
Buffalo _____
Springbok _____
Blesbok _____

Kudu _____
Other _____

Q24d If yes to Q24c: When was Rift Valley fever confirmed? *Enter the month and year.*
Enter Date
Month (Enter 1-12)
Year

Q24e Has there been any suspicion of Rift Valley fever on the farm in the past 3 months?
☐ Yes ☐ No

Q25 Have any animals been vaccinated for brucellosis? ☐ Yes ☐ No

If yes: Which animals were vaccinated? *Select all that apply.*
☐ Cattle
☐ Sheep
☐ Goat
☐ Pig
☐ Buffalo
☐ Springbok
☐ Blesbok
☐ Kudu
☐ Other

If yes: What other animals have been vaccinated for brucellosis? *Please separate answers with commas. Example: Camels, horses.*

If yes: When was the most recent vaccine given? *Enter the month and year.*
Enter Date
Month (Enter 1-12)
Year

If yes: What percentage of animals currently on the farm are vaccinated?

If yes: Which vaccine(s) were used?
☐ RB51
☐ S19
☐ RB51 and S19
☐ Other

If other: Please list any other vaccine given. *Please separate answers with commas.*

Q25a Have any animals been vaccinated for Rift Valley fever? ☐ Yes ☐ No

If yes: Which animals were vaccinated? *Select all that apply.*
☐ Cattle
☐ Sheep
☐ Goat
☐ Pig
☐ Buffalo
☐ Springbok
☐ Blesbok
☐ Kudu
☐ Other

If yes: What other animals have been vaccinated for Rift Valley fever? *Please separate answers with commas. Example: Camels, horses.*

If yes: When was the most recent vaccine given? *Enter the month and year.*
Enter Date

Farmer Survey: Towards a better understanding of Rift Valley Fever and Brucellosis Epidemiology in South Africa

Month (Enter 1-12)
Year

If yes: What is your protocol for Rift Valley fever vaccination on your farm?
- ☐ Once for all young
- ☐ Every year
- ☐ Only before/during outbreaks
- ☐ Never

If yes: What percentage of animals currently on the farm are vaccinated?

If yes: Which vaccine(s) were used?
- ☐ Smithburn
- ☐ Clone 13
- ☐ Inactivated
- ☐ Clone 13 and inactivated
- ☐ modified-live
- ☐ Unknown
- ☐ Other

If other: Please list any other vaccine given. *Please separate answers with commas.*

You have successfully completed the survey! Thank you for your participation!
Please hand your device back to the administrator

Attention Survey Administrators: Please add any notes here

COMMUNAL FARMER QUESTIONNAIRE

You are at the start of Farmer Survey. Swipe the screen as shown below to go backward and forward.

To be filled out by survey administrator:

Admin 1. Farm Identifier:

Admin 2. Farm Name:

Admin 3. Date of interview:

Admin 4. Collect the current GPS coordinates.

Admin 4a. Enter the latitude and longitude manually using the GPS.
If automatic GPS coordinate does not work then:

Latitude (Degrees) _____

Latitude (Minutes) _____

Latitude (Seconds) _____

Longitude (Degrees) _____

Longitude (Minutes) _____

Longitude (Seconds) _____

Admin 5. Filled in by: _____ *Leave this blank if you are not a survey coordinator.*

The remainder of the survey should be filled out by the participant.

Q1 Do you primarily farm domestic or wild animals? ☐

Domestic ☐Wildlife

Q2 Is the land your animals are grazed on: ☐

Communal ☐Private

Q3-1 What animal species are/were kept in the communal area in the past month (CURRENTLY)? *Select all that apply.*

Please only indicate animals owned specifically by you.

- ☐ Cattle
- ☐ Sheep
- ☐ Goat
- ☐ Pig
- ☐ Buffalo
- ☐ Springbok
- ☐ Blesbok
- ☐ Kudu
- ☐ Other

Q3-1a What animal species are/were kept in the communal in the PAST 12 MONTHS? *Select all that apply.*

Please only indicate animals owned specifically by you.

- ☐ Cattle

- ☐ Sheep
- ☐ Goat
- ☐ Pig
- ☐ Buffalo
- ☐ Springbok
- ☐ Blesbok
- ☐ Kudu
- ☐ Other

Q3-1b Only display relevant questions pertaining to answers in 3-1 and 3-1a

Q3-1b Cattle *If none, enter zero*
What is the ACTUAL number of your cattle now?
How many of your cattle are < 2 years of age?
How many of your cattle are between 2-4 years of age?
How many or your cattle are >4 years of age?
What is the AVERAGE number of cattle you keep in the communal area during one month?

Q3-1b Sheep *If none, enter zero*
What is the actual number of your sheep now?
How many of your sheep are < 2 years of age?
How many of your sheep are between 2-4 years of age?
How many or your sheep are >4 years of age?
What is the AVERAGE number of sheep you keep in the communal area during one month?

Q3-1b Goat *If none, enter zero*
What is the ACTUAL number of your goats now?
How many of your goats are < 2 years of age?
How many of your goats are between 2-4 years of age?
How many or your goats are >4 years of age?
What is the AVERAGE number of goats you keep in the communal area during one month?

Q3-1b Pigs *If none, enter zero*
What is the ACTUAL number of your pigs now?
What is the AVERAGE number of pigs you keep in the communal area during one month?

Q3-1b Buffalo *If none, enter zero*
What is the ACTUAL number of your buffalo now?
What is the AVERAGE number of buffalo you keep in the communal area during one month?

Q3-1b Springbok *If none, enter zero*
What is the ACTUAL number of your springbok now?
What is the AVERAGE number of springbok you keep in the communal area during one month?

Q3-1b Blesbok *If none, enter zero*
What is the ACTUAL number of your blesbok now?
What is the AVERAGE number of blesbok you keep in the communal area during one month?

Q3-1b Kudu *If none, enter zero*
What is the ACTUAL number of your kudu now?
What is the AVERAGE number of kudu you keep in the communal area during one month?

Q3-1b Others *If none, enter zero*

List all other species that you keep/kept in the communal area in the past month (CURRENTLY). Indicate how many of each species are/were kept on the farm in the past month. *Please separate answers with commas.*
Example: 2 horses, 3 camels.

List all other species that you kept in the communal area in the PAST 12 MONTHS. Indicate the average number of species kept on the farm during one month. *Please separate answers with commas.*
Example: 2 horses, 3 camels.

Q3-1c How many herds use the communal land?
Q3-1d How many owners graze their animals in the communal herd (including yourself)?
Q3-1e Approximately how many animals are in the herd (including your animals)?

Q4-1 Are any of your animals housed, grazed or allowed to spend time with other species on the communal land?
☐ Yes ☐ No

Q4-1 If yes Which of YOUR animals are grazed or allowed to spend time with other species on the communal land?
Select all that apply. Only display relevant choices pertaining to answers in 3-1 and 3-1a
- ☐ Cattle
- ☐ Sheep
- ☐ Goat
- ☐ Pig
- ☐ Buffalo
- ☐ Springbok
- ☐ Blesbok
- ☐ Kudu
- ☐ Other

Q4-1a Only display relevant questions pertaining to answers in 4-1
Select all animal species on the communal land that the cattle mix with. *Select all that apply.*
- ☐ Sheep
- ☐ Goat
- ☐ Pig
- ☐ Buffalo
- ☐ Springbok
- ☐ Blesbok
- ☐ Kudu
- ☐ Other

If other What other species do the cattle mix with? *Please separate answers with commas.*
Examples: horses, camels.

Q4-1a Select all animal species on the communal land that the sheep mix with. *Select all that apply.*
- ☐ Cattle
- ☐ Goat
- ☐ Pig
- ☐ Buffalo
- ☐ Springbok
- ☐ Blesbok
- ☐ Kudu
- ☐ Other

If other What other species do the sheep mix with? *Please separate answers with commas.*
Examples: horses, camels.

Q4-1a Select all animal species on the communal land that the goats mix with. *Select all that apply.*

- ☐ Cattle
- ☐ Sheep
- ☐ Pig
- ☐ Buffalo
- ☐ Springbok
- ☐ Blesbok
- ☐ Kudu
- ☐ Other

If other What other species do the goats mix with? *Please separate answers with commas.*
Examples: horses, camels.

Q4-1a Select all animal species on the communal land that the pigs mix with. *Select all that apply.*

- ☐ Cattle
- ☐ Sheep
- ☐ Goat
- ☐ Buffalo
- ☐ Springbok
- ☐ Blesbok
- ☐ Kudu
- ☐ Other

If other What other species do the pigs mix with? *Please separate answers with commas.*
Examples: horses, camels.

Q4-1a Select all animal species on the communal land that the buffalo mix with. *Select all that apply.*

- ☐ Cattle
- ☐ Sheep
- ☐ Goat
- ☐ Pig
- ☐ Springbok
- ☐ Blesbok
- ☐ Kudu
- ☐ Other

If other What other species do the buffalo mix with? *Please separate answers with commas.*
Examples: horses, camels.

Q4-1a Select all animal species on the communal land that the springbok mix with. *Select all that apply.*

- ☐ Cattle
- ☐ Sheep
- ☐ Goat
- ☐ Pig
- ☐ Buffalo
- ☐ Blesbok
- ☐ Kudu
- ☐ Other

If other What other species do the springbok mix with? *Please separate answers with commas.*
Examples: horses, camels.

Q4-1a Select all animal species on the communal land that the blesbok mix with. *Select all that apply.*

☐ Cattle
☐ Sheep
☐ Goat
☐ Pig
☐ Buffalo
☐ Springbok
☐ Kudu
☐ Other

If other What other species do the blesbok mix with? *Please separate answers with commas.*
Examples: horses, camels.

Q4-1a Select all animal species on the communal land that the kudu mix with. *Select all that apply.*
☐ Cattle
☐ Sheep
☐ Goat
☐ Pig
☐ Buffalo
☐ Springbok
☐ Blesbok
☐ Other

If other What other species do the kudu mix with? *Please separate answers with commas.*
Examples: horses, camels.

Please list any additional animals interactions not already indicated. *Please separate answers with commas.*
Example: horses mix with camels, ducks mix with chickens.

Q5-1 Are the herd's animals grazed or allowed to spend time with wild roaming antelope/hooved animals?
☐ Yes ☐ No

Q5-1 If yes Which of the herds' animals are grazed or allowed to spend time with wild roaming antelope/hooved animals? *Select all that apply.* Only display relevant choices pertaining to answers in 3-1 and 3-1a
☐ Cattle
☐ Sheep
☐ Goat
☐ Pig
☐ Buffalo
☐ Springbok
☐ Blesbok
☐ Kudu
☐ Other

If other Please list any additional herd animals that mix with wild animals that are not already indicated. *Please separate answers with commas. Example: camels mix with steinbuck.*

Q5-1a Which wild animals (not farmed) graze or spend time with the animals on the farm? *Select all that apply.*
☐ Buffalo
☐ Springbok
☐ Blesbok
☐ Kudu
☐ Sable antelope
☐ Roan antelope
☐ Impala
☐ Waterbuck

- ☐ Reedbuck
- ☐ Bontebok
- ☐ Hartebeest
- ☐ Blue wildebeest
- ☐ Black wildebeest
- ☐ Eland
- ☐ Giraffe
- ☐ Zebra
- ☐ Tsessebe
- ☐ Gemsbok
- ☐ Nyala
- ☐ Other

If other Please list any other wild antelope/hooved animals that spend time with the farm animals. *Please separate answers with commas. Example: farmed steinbuck mix with wild springbok, farmed camels mix with wild springbok.*

Q6-1 Where is each of your animal species kept during the night? *Select all that apply.*
Only display relevant choices pertaining to answers in 3 and 3a or 3-1 and 3-1a

Cattle
- ☐ Open kraal
  - ☐ Kraal with roof
  - ☐ Communal kraal
  - ☐ Veld
  - ☐ Other

Q6-1 Sheep
- ☐ Open kraal
- ☐ Kraal with roof
- ☐ Communal kraal
- ☐ Veld
- ☐ Other

Q6-1 Goat
- ☐ Open kraal
- ☐ Kraal with roof
- ☐ Communal kraal
- ☐ Veld
- ☐ Other

Q6-1 Pig
- ☐ Open kraal
- ☐ Kraal with roof
- ☐ Communal kraal
- ☐ Veld
- ☐ Other

Q6-1 Buffalo
- ☐ Open kraal
- ☐ Kraal with roof
- ☐ Communal kraal
- ☐ Veld
- ☐ Other

Q6-1 Springbok
- ☐ Open kraal
- ☐ Kraal with roof
- ☐ Communal kraal
- ☐ Veld
- ☐ Other

Q6-1 Blesbok

☐ Open kraal
☐ Kraal with roof
☐ Communal kraal
☐ Veld
☐ Other

Q6-1 Kudu
☐ Open kraal
☐ Kraal with roof
☐ Communal kraal
☐ Veld
☐ Other

Q6-1 Other
☐ Open kraal
☐ Kraal with roof
☐ Communal kraal
☐ Veld
☐ Other

If other, only options selected in 3 (3-1) and 3a (3a-1) will show up
If other Please list any other location(s) where your cattle are kept during the night.
Please separate answers with commas.

If other Please list any other location(s) where your sheep are kept during the night.
Please separate answers with commas.

If other Please list any other location(s) where your goats are kept during the night.
Please separate answers with commas.

If other Please list any other location(s) where your pigs are kept during the night.
Please separate answers with commas.

If other Please list any other location(s) where your buffalo are kept during the night.
Please separate answers with commas.

If other Please list any other location(s) where your springbok are kept during the night.
Please separate answers with commas.

If other Please list any other location(s) where your blesbok are kept during the night.
Please separate answers with commas.

If other Please list any other location(s) where your kudu are kept during the night.

If other Please list any other location(s) where your other animals are kept during the night.
Please separate answers with commas.

Q7-*1* Do any animals spend time in the house or compounds occupied by people on the farm?
Refers only to animals you own. ☐ Yes ☐ No

If yes Which animal species? *Select all that apply.* Only display relevant choices pertaining to answers in 3 and 3a or 3-1 and 3-1a
☐ Cattle
☐ Sheep
☐ Goat
☐ Pig
☐ Buffalo
☐ Springbok
☐ Blesbok
☐ Kudu
☐ Other

If other Please list any other animals that spend time in the house or compounds occupied by people. *Please separate answers with commas. Examples: dogs, cats.*

Q8-1 Do your animals have contact with animals from other communal herds or private farmers? ☐ Yes ☐ No

Q8-1a If yes: How often do they have contact in a typical week? *Answer in times per week.*

Q8-1b If yes Under which context do they meet? When… *Select all that apply.*
☐ Grazing
☐ Housing
☐ Feeding
☐ Contact through fence
☐ Other

If other: Please list other contexts in which the farm animals have contact with animals from other farms. *Please separate answers with commas.*

Q8-1c Do your animals mix with a private farm or a communal herd? ☐ Communal ☐ Private

Q9-1 Approximately how many of each does the communal area have within access of the animals? *If none, enter zero.*

Water spring(s) / Lake(s) / Natural dam(s) - permanent source
River, stream - constantly flowing source
Pan(s)- seasonal source
Dam(s) - human made source
Water troughs provided
Other outdoor water source

If other: Please describe the other water source(s). *Please separate answers with commas.*

Q9-1a Approximately how far do the communal herd animals walk each day to get water? *Tap below to answer in km or minutes walking.*
Km:
OR minutes walking:

Q9-1b How many of each of the following water sources have had water available on the communal land during the past year? *For example, 10 pans have had water. If none, enter zero.*

Water spring(s) / Lake(s) / Natural dam(s) - permanent source
River, stream – constantly flowing source
Pan(s)- seasonal source
Dam(s) - human made source
Water troughs provided
Other outdoor water source

If other: Please describe the other water source(s). *Please separate answers with commas.*

Q9-1c What is the longest period of time during which the following water sources have had water during the past year? *For example, Pans on the farm have been flooded for 5 months.*
Allow decimals

Water has been available from spring(s) / Lake(s) / Natural dam(s) for:_____ months.
Water has been available from river(s), stream(s) (a constantly flowing source) for: _____ months.
Water has been available from pan(s) (a seasonal source) for:_____ months.
Water has been available from dam(s) or a human made source for:_____ months.
Water has been available from water troughs provided for:_____ months.
Water has been available from other outdoor water source(s) for:_____ months.

If other: Please describe the other water source(s). *Please separate answers with commas.*

Q10-1 Approximately how far do your animals walk each day to get to the grazing area? Tap below to answer in km or minutes walking. *If kept on the veld put 0.*

Km:
OR minutes walking:

Q10-1a Do you move your herd between communal grazing areas? ☐ Yes ☐ No

If yes: After how much time do you return your herd to the grazing area you started off with? *Please indicate in number of days.*

Q11 Have you purchased/gained (from gift or birth) animals in the past 12 months?
☐ Yes ☐ No

If yes: Which of the following animals have you purchased/gained (from gift or birth) in the past 12 months?
Select all that apply.

☐ Cows
- ☐ Heifer
- ☐ Bull
- ☐ Steer
- ☐ Calf
- ☐ Doe goat
- ☐ Billy goat
- ☐ Goat kid
- ☐ Ewe
- ☐ Ram
- ☐ Lamb
- ☐ Sow

☐ Boar
☐ Piglet
☐ Springbok
☐ Blesbok
☐ Kudu
☐ Other

Q11a Asked only for the species indicated as purchased/gained in Q11:
Approximately how many cows were… *If none, enter zero*

Purchased in the past 12 months
Given to you in the past 12 months
Otherwise gained in the past 12 months

In what other ways were cows gained in the past 12 months?

Q11a Approximately how many heifers were… *If none, enter zero*

Purchased in the past 12 months
Given to you in the past 12 months
Otherwise gained in the past 12 months

In what other ways were heifers gained in the past 12 months?

Q11a Approximately how many bulls were… *If none, enter zero*

Purchased in the past 12 months
Given to you in the past 12 months
Otherwise gained in the past 12 months

In what other ways were bulls gained in the past 12 months?

Q11a Approximately how many steers were… *If none, enter zero*
Purchased in the past 12 months
Given to you in the past 12 months
Otherwise gained in the past 12 months

In what other ways were steers gained in the past 12 months?

Q11a Approximately how many calves were… *If none, enter zero*
Purchased in the past 12 months
Given to you in the past 12 months
Born in the past 12 months
Otherwise gained in the past 12 months

In what other ways were calves gained in the past 12 months?

Q11a Approximately how many doe goats were… *If none, enter zero*
Purchased in the past 12 months
Given to you in the past 12 months
Otherwise gained in the past 12 months

In what other ways were doe goats gained in the past 12 months?

Q11a Approximately how many billy goats were… *If none, enter zero*
Purchased in the past 12 months
Given to you in the past 12 months
Otherwise gained in the past 12 months

In what other ways were billy goats gained in the past 12 months?

Q11a Approximately how many goat kids were… *If none, enter zero*
Purchased in the past 12 months
Given to you in the past 12 months
Born in the past 12 months
Otherwise gained in the past 12 months

In what other ways were goat kids gained in the past 12 months?

Q11a Approximately how many ewes were… *If none, enter zero*
Purchased in the past 12 months
Given to you in the past 12 months
Otherwise gained in the past 12 months

In what other ways were ewes gained in the past 12 months?

Q11a Approximately how many rams were… *If none, enter zero*
Purchased in the past 12 months
Given to you in the past 12 months
Otherwise gained in the past 12 months

In what other ways were rams gained in the past 12 months?

Q11a Approximately how many lambs were… *If none, enter zero*

Purchased in the past 12 months
Given to you in the past 12 months
Born in the past 12 months
Otherwise gained in the past 12 months

In what other ways were lambs gained in the past 12 months?

Q11a Approximately how many sows were… *If none, enter zero*
Purchased in the past 12 months
Given to you in the past 12 months
Otherwise gained in the past 12 months

In what other ways were sows gained in the past 12 months?

Q11a Approximately how many boars were… *If none, enter zero*
Purchased in the past 12 months
Given to you in the past 12 months
Otherwise gained in the past 12 months

In what other ways were boars gained in the past 12 months?

Q11a Approximately how many piglets were… *If none, enter zero*
Purchased in the past 12 months
Given to you in the past 12 months
Born in the past 12 months
Otherwise gained in the past 12 months

In what other ways were piglets gained in the past 12 months?

Q11a Approximately how many springbok were… *If none, enter zero*
Purchased in the past 12 months
Given to you in the past 12 months
Born in the past 12 months
Otherwise gained in the past 12 months

In what other ways were springbok gained in the past 12 months?

Q11a Approximately how many blesbok were… *If none, enter zero*
Purchased in the past 12 months
Given to you in the past 12 months
Born in the past 12 months
Otherwise gained in the past 12 months

In what other ways were blesbok gained in the past 12 months?

Q11a Approximately how many kudu were… *If none, enter zero*
Purchased in the past 12 months
Given to you in the past 12 months
Born in the past 12 months
Otherwise gained in the past 12 months

In what other ways were kudu gained in the past 12 months?

List all other species of animals that were gained in the past 12 months. *Please separate with commas.*

Q11a Approximately how many other animals were… *If none, enter zero*

Purchased in the past 12 months

Given to you in the past 12 months

Born in the past 12 months

Otherwise gained in the past 12 months

In what other ways were other animals gained in the past 12 months?

Q11b If they indicated that they had purchased any animals in Q11:
On average, how many times do you purchase animals during one year? *If none, enter zero. Scroll down to view all.*

Q11b On average, how many… *If none, enter zero. Scroll down to view all.*

Cows do you purchase at one time?

Heifers do you purchase at one time?

Bulls do you purchase at one time?

Steers do you purchase at one time?

Calves do you purchase at one time?

Doe goats do you purchase at one time?

Billy goats do you purchase at one time?

Goat kids do you purchase at one time?

Ewes do you purchase at one time?

Rams do you purchase at one time?

Lambs do you purchase at one time?

Sows do you purchase at one time?

Boars do you purchase at one time?

Piglets do you purchase at one time?

Springbok do you purchase at one time?

Blesbok do you purchase at one time?

Kudu do you purchase at one time?

Other animals do you purchase at one time?

Q11c. Have you sold/lost/slaughtered (ceremonial or for food consumption) animals in the PAST 12 MONTHS?

☐ Yes ☐ No

Q11c Which of the following animals have you sold/lost/slaughtered (ceremonial or for food consumption)?
Select all that apply.

☐ Cows
- ☐ Heifer
- ☐ Bull
- ☐ Steer

☐ Calf
- ☐ Doe goat
- ☐ Billy goat
- ☐ Goat kid

☐ Ewe
- ☐ Ram
- ☐ Lamb

☐ Sow

- ☐ Boar
- ☐ Piglet
- ☐ Springbok
- ☐ Blesbok
- ☐ Kudu
- ☐ Other

Q11d Asked only for the species indicated in Q11:
Approximately how many cows were… *If none, enter zero*

Sold in the past 12 months

Stolen from you in the past 12 months

Predated in the past 12 months
Died in the past 12 months
Were slaughtered in the past 12 months
otherwise lost in the past 12 months

In what other ways were cows lost in the past 12 months?

Q11d Approximately how many heifers were… *If none, enter zero*

Sold in the past 12 months

Stolen from you in the past 12 months

Predated in the past 12 months
Died in the past 12 months
Were slaughtered in the past 12 months
otherwise lost in the past 12 months

In what other ways were heifers lost in the past 12 months?

Q11d Approximately how many bulls were… *If none, enter zero*

Sold in the past 12 months

Stolen from you in the past 12 months

Predated in the past 12 months
Died in the past 12 months
Were slaughtered in the past 12 months
otherwise gained in the past 12 months

In what other ways were bulls lost in the past 12 months?

Q11d Approximately how many steers were… *If none, enter zero*

Sold in the past 12 months

Stolen from you in the past 12 months

Predated in the past 12 months
Died in the past 12 months
were slaughtered in the past 12 months
otherwise lost in the past 12 months

In what other ways were steers lost in the past 12 months?

Q11d Approximately how many calves were… *If none, enter zero*

Sold in the past 12 months

Stolen from you in the past 12 months

Predated in the past 12 months
Died in the past 12 months
were slaughtered in the past 12 months
otherwise lost in the past 12 months

In what other ways were calves lost in the past 12 months?

Q11d Approximately how many doe goats were… *If none, enter zero*

Sold in the past 12 months

Stolen from you in the past 12 months

Predated in the past 12 months
Died in the past 12 months
Were slaughtered in the past 12 months
otherwise lost in the past 12 months

In what other ways were doe goats lost in the past 12 months?

Q11d Approximately how many billy goats were… *If none, enter zero*

Sold in the past 12 months

Stolen from you in the past 12 months

Predated in the past 12 months
Died in the past 12 months
Were slaughtered in the past 12 months
otherwise lost in the past 12 months

In what other ways were billy goats lost in the past 12 months?

Q11d Approximately how many goat kids were… *If none, enter zero*

Sold in the past 12 months

Stolen from you in the past 12 months

Predated in the past 12 months
Died in the past 12 months
were slaughtered in the past 12 months
otherwise lost in the past 12 months

In what other ways were goat kids lost in the past 12 months?

Q11d Approximately how many ewes were… *If none, enter zero*

Sold in the past 12 months

Stolen from you in the past 12 months

Predated in the past 12 months
Died in the past 12 months
Were slaughtered in the past 12 months
Otherwise lost in the past 12 months

In what other ways were ewes lost in the past 12 months?

Q11d Approximately how many rams were… *If none, enter zero*

Sold in the past 12 months

Stolen from you in the past 12 months

Predated in the past 12 months
Died in the past 12 months
Were slaughtered in the past 12 months
Otherwise lost in the past 12 months

In what other ways were rams lost in the past 12 months?

Q11d Approximately how many lambs were… *If none, enter zero*

Sold in the past 12 months

Stolen from you in the past 12 months

Predated in the past 12 months

Died in the past 12 months
were slaughtered in the past 12 months
otherwise lost in the past 12 months

In what other ways were lambs lost in the past 12 months?

Q11d Approximately how many sows were… *If none, enter zero*

Sold in the past 12 months

Stolen from you in the past 12 months

Predated in the past 12 months
Died in the past 12 months
Were slaughtered in the past 12 months
Otherwise lost in the past 12 months

In what other ways were sows lost in the past 12 months?

Q11d Approximately how many boars were… *If none, enter zero*

Sold in the past 12 months

Stolen from you in the past 12 months

Predated in the past 12 months
Died in the past 12 months
Were slaughtered in the past 12 months
Otherwise lost in the past 12 months

In what other ways were boars lost in the past 12 months?

Q11d Approximately how many piglets were… *If none, enter zero*

Sold in the past 12 months

Stolen from you in the past 12 months

Predated in the past 12 months
Died in the past 12 months
Were slaughtered in the past 12 months
Otherwise lost in the past 12 months

In what other ways were piglets lost in the past 12 months?

Q11d Approximately how many springbok were… *If none, enter zero*

Sold in the past 12 months

Stolen from you in the past 12 months

Predated in the past 12 months
Died in the past 12 months
Were slaughtered in the past 12 months
Otherwise lost in the past 12 months

In what other ways were springbok lost in the past 12 months?

Q11d Approximately how many blesbok were… *If none, enter zero*

Sold in the past 12 months

Stolen from you in the past 12 months

Predated in the past 12 months
Died in the past 12 months
Were slaughtered in the past 12 months
Otherwise lost in the past 12 months

In what other ways were blesbok lost in the past 12 months?

Q11d Approximately how many kudu were… *If none, enter zero*

Sold in the past 12 months

Stolen from you in the past 12 months

Predated in the past 12 months
Died in the past 12 months
Were slaughtered in the past 12 months
Otherwise lost in the past 12 months

In what other ways were kudu lost in the past 12 months?

Q11d List all other species of animals that were lost in the past 12 months. *Please separate with commas.*

Approximately how many other animals were… *If none, enter zero. Example: 3 steinbuck, 2 camels.*

Sold in the past 12 months

Stolen from you in the past 12 months

Predated in the past 12 months
Died in the past 12 months
Were slaughtered in the past 12 months
Otherwise lost in the past 12 months

Q11e Asked if they indicated any species had been sold/lost/slaughtered in Q11
On average, how many times do you sell animals during one year? *If you do not sell animals, enter 0.*

Q11f On average, approximately how many…. *Scroll down to view all.*

Cows do you sell at one time?

Heifers do you sell at one time?

Bulls do you sell at one time?

Steers do you sell at one time?

Calves do you sell at one time?

Doe goats do you sell at one time?

Billy goats do you sell at one time?

Goat kids do you sell at one time?

Ewes do you sell at one time?

Rams do you sell at one time?

Lambs do you sell at one time?

Sows do you sell at one time?

Boars do you sell at one time?

Piglets do you sell at one time?

Springbok do you sell at one time?

Blesbok do you sell at one time?

Kudu do you sell at one time?

Approximately how many other animals do you sell at one time?

Q11g If they indicated that they had bought or sold any animals in Q11c: Where did you purchase your animals from OR sell your animals to in the past 12 months? *Select all that apply. Scroll down to view all.*

Auction	☐ Bought	☐ Sold
Other farms/farmers	☐ Bought	☐ Sold
Abattoir		☐ Sold
Other	☐ Bought	☐ Sold

If indicated: How many auctions did you purchase your animals from?
If you did not purchase any animals at auctions, enter "0"
How many auctions did you sell your animals at?
If you did not sell any animals at auctions, enter "0"
How many other farms did you purchase your animals from?
If you did not purchase any animals at other farms, enter "0"
How many other farms did you sell your animals at?
If you did not sell any animals at other farms, enter "0"
How many abattoirs did you sell your animals at?
If you did not sell any animals at abattoirs, enter "0"

Approximately how far away in Km is the auction where the animal(s) were purchased? *Scroll down to view all.*
Approximately how far away in Km is the second auction where the animal(s) were purchased?
List all other auctions where the animal(s) were purchased and indicate their distance away in Km.

Approximately how far away in Km is the auction where the animal(s) were sold?
Approximately how far away in Km is the second auction where the animal(s) were sold?
List all other auctions where the animal(s) were sold and indicate their distance away in Km.

Approximately how far away in Km is the farm where the animal(s) were purchased? *Scroll down to view all.*
Approximately how far away in Km is the second farm where the animal(s) were purchased?
List all other farms where the animal(s) were purchased and indicate their distance away in Km.

Approximately how far away in Km is the farm where the animal(s) were sold?
Approximately how far away in Km is the second farm where the animal(s) were sold?
List all other farms where the animal(s) were sold and indicate their distance away in Km.

Approximately how far away in Km is the abattoir where the animal(s) were sold? *Scroll down to view all.*
Approximately how far away in Km is the second abattoir where the animal(s) were sold?
List all other abattoirs where the animal(s) were sold and indicate their distance away in Km.

If other: Please specify other places animals were bought or sold.

Q12-1 What protocols do you implement when introducing new animals into the herd? *Select all that apply.*

- ☐ Quarantine
- ☐ Antibiotics
- ☐ Vaccinations
- ☐ Dipping/tick treatment
- ☐ Other
- ☐ None

If quarantine is selected: How many days are new animals quarantined? *Answer in days*

If other is selected: What other protocols do you implement when introducing new animals into the herd?

Q13-1 Are the farmed animals or their products intended for international export? ☐ Yes ☐ No

If yes: To where do you export?

Q14-1 Please estimate the size of the communal area in hectares

Q15-1 Is employment provided through your communal herd? ☐ Yes ☐ No

Q15-1a if yes: Are the workers: ☐ Permanent staff ☐ Seasonal workers

Q15-1b if permanent selected: How many permanent staff are employed?

Q15-1b If seasonal selected: How many seasonal staff are employed?

Q16-1 Do you or any of your employees (and/or families) live on the farm? ☐ Yes ☐ No
If yes: How many
Adults?
Children?

Q17-1 Why do you keep your animals?
☐ Meat
☐ Wool (hides)
☐ Milk
☐ Resale/Cash sale
☐ Tourism/hunting
☐ Bartering
☐ Ceremonial Use
☐ Symbol of wealth
☐ Draft

Q17-1a If meat was selected: What is the meat used for: ☐ Home Consumption ☐ Selling *Select all that apply.*

Q17-1a
If milk was selected: What is the milk used for: ☐ Home Consumption ☐ Selling *Select all that apply.*
If home consumption: Is the milk pasteurized/boiled prior to consumption? ☐ Yes ☐ No
If selling: Is the milk pasteurized/boiled prior to selling? ☐ Yes ☐ No

Q18 How would you classify your production system?
☐ Feedlot
☐ Commercial (excluding feedlot)
☐ Semi-commercial
☐ Cash Sales
☐ No cash sales

Q19-1 Who takes care of animals? *Select all that apply.*
☐ Owner
☐ Employed farm worker
☐ Children
☐ No one
☐ Other

Q19-1a If owner takes care of animals: What animal specie(s) do(es) the owner take care of? *Select all that apply.*
☐ Cattle
☐ Sheep
☐ Goat
☐ Pig
☐ Buffalo
☐ Springbok
☐ Blesbok
☐ Kudu
☐ Other

If other: What other species does the owner take care of? *Please separate answers with commas. Example: Camels, horses.*

Q19-1a If farm workers take care of animals: What animal specie(s) do(es) the employed farm workers take care of? *Select all that apply.*

- ☐ Cattle
- ☐ Sheep
- ☐ Goat
- ☐ Pig
- ☐ Buffalo
- ☐ Springbok
- ☐ Blesbok
- ☐ Kudu
- ☐ Other

If other: What other species do the farm workers take care of? *Please separate answers with commas. Example: Camels, horses.*

Q19-1a If children take care of animals: What animal specie(s) do(es) the children take care of? *Select all that apply.*

- ☐ Cattle
- ☐ Sheep
- ☐ Goat
- ☐ Pig
- ☐ Buffalo
- ☐ Springbok
- ☐ Blesbok
- ☐ Kudu
- ☐ Other

If other: What other species do the children take care of? *Please separate answers with commas. Example: Camels, horses.*

If answered other in Q19 or Q19-1: List all others that take care of animals and indicate the species they take care of. *Example: veterinarians take care of ducks.*

Q20-1 Are animals slaughtered out of the herd? ☐ Yes ☐ No

Q20-1 If yes to slaughtering: Where are animals slaughtered?

- ☐ A designated outdoor area for the herd
  - ☐ Owner/worker's home
- ☐ Local abattoir
- ☐ Other

Q20-1 If yes: Where else do you slaughter your animals? *Please separate answers with commas.*

Q20-1a if yes to slaughtering: Who assists in the slaughter?

- ☐ Owner
- ☐ Farm workers
- ☐ Abattoir
- ☐ Spiritual leader
- ☐ Local leader
- ☐ Other

If other: Who else assists with slaughtering? *Please separate answers with commas.*

Q21-1 Have any animals had an abortion in the past 3 months ☐ Yes ☐ No

Q21-1a If abortion yes: How many animal species had abortions?

Q21-1b If abortion yes: Which animal species had abortions?

- ☐ Cattle
- ☐ Sheep
- ☐ Goat

☐ Pig
☐ Buffalo
☐ Springbok
☐ Blesbok
☐ Kudu
☐ Other

Which other animal species had abortions? *Please separate answers with commas. Example: Camels, horses.*

Q21-1c If yes: Did anyone assist in the handling of the animals or removal of aborted foetus? ☐ Yes ☐ No

Q21-1d If yes: How many people were involved in assisting with the abortion?

Q21-1e If yes: Who was involved in assisting with the abortion? *Select all that apply.*
☐ Veterinarian
☐ Owner
☐ Farm worker
☐ Animal health technician
☐ Other
If other: Who else assisted with the abortion?

Q21-1f If abortion yes: How was the aborted material disposed of? *Select all that apply.*
☐ Buried
☐ Left (scavenged)
☐ Burnt
☐ Eaten by mother
☐ Other
If abortion yes: In what other way was the aborted material disposed of?

Q22-1 Please indicate whether any of the following practices are implemented on or in the communal area: *Scroll down to view all.*

Is fencing maintained around the property?	☐ Yes	☐ No
Is nose-nose contact between animal species prevented?	☐ Yes	☐ No
Are different animals species kept in different/divided areas on the farm?	☐ Yes	☐ No
Is there separate equipment for different animals species?	☐ Yes	☐ No
Are quarantined/sick animal(s) fed, treated, and worked with after the healthy animals?	☐ Yes	☐ No
Are pregnant animals kept separate from herd?	☐ Yes	☐ No
Are vehicles cleaned and disinfected before and after transporting animals?	☐ Yes	☐ No
Do you vaccinate your animals?	☐ Yes	☐ No
Is tick control used (are the animals dipped or an injection given)	☐ Yes	☐ No
Is mosquito/biting fly control used?	☐ Yes	☐ No
Do you quarantine new animals?	☐ Yes	☐ No

Are other bio safety practices used? ☐ Yes ☐ No

Q22-1a If yes to maintain fencing in Q22: What type of fencing do you maintain around property?
☐ Metal/Wire
☐ Wood
☐ Electric
☐ Other

If fencing yes: What other type of fencing do you maintain around property? *Please separate answers with commas.*

Q22-1 Please indicate whether you used tick control on cattle in the past 12 months? ☐ Yes ☐ No

Q22-1a If yes tick control in Q22-1: How many times per year are the animals treated for ticks?

Q22-1a If yes to tick control in Q22-1: What season is the tick control used on the animals?*Select all that apply.*
☐ Summer (December-February)
☐ Fall (March-May)
☐ Winter (June-August)
☐ Spring (September-November)

Q22-1b If yes to tick control in Winter, How frequently did you use tick control during the winter? *Indicate number of times*

Q22-1b If yes to tick control in Spring, How frequently did you use tick control during the spring? *Indicate number of times*

Q22-1b If yes to tick control in Summer, How frequently did you use tick control during the summer? *Indicate number of times*

Q22-1b If yes to tick control in Fall, How frequently did you use tick control during the fall? *Indicate number of times*

Q22-1c What method was used? *Select all that apply*
☐ Dipping tank
☐ Foot bath
☐ Pour-on
☐ Lick in drum with pour-on
☐ Hand spraying
☐ Hand dressing
☐ Spray race
☐ Injection
☐ Other

If other: Please list any other method applied. *Please separate answers with commas.*

Q22-1a If "mosquito/biting fly" control selected in Q22-1: How many times per year do you use mosquito or fly control?

Q22-1a If "mosquito/biting fly" control selected in Q22-1: What mosquito/biting fly control measures do you use? *Please separate answers with commas.*

Q22-1a If "Other biosafety practices…" selected in Q22-1: Please describe any other biosafety measures you use. *Please separate answers with commas. Example: all in all out.*

Q23-1 Is the nearest farm private or communal? ☐ Private ☐ Communal

Q23-1a How far away is the closest private farm or communal grazing area of a different community (from the centre of your communal area)? *Answer at least 1 of the following*:
Km:
OR minutes drive:

OR minutes walking:

Q24-1 Has brucellosis been confirmed in any communal animals at any time in the past?
☐ Yes ☐ No
If yes: Which animals were infected?
☐ Cattle
☐ Sheep
☐ Goat
☐ Pig
☐ Buffalo
☐ Springbok
☐ Blesbok
☐ Kudu
☐ Other

If other: What other animal species were confirmed having brucellosis? *Please separate answers with commas. Example: Camels, horses.*

Q24-1a If yes to Q24 or Q24-1 or Q24-2: When was brucellosis confirmed? *Enter the month and year.*
Enter Date
Month (Enter 1-12)
Year

Q24-1b Has there been any suspicion of brucellosis in any communal animals in the past 3 months? ☐ Yes ☐ No

Q24-1c Has Rift Valley fever been confirmed in any communal animals at any time in the past? ☐ Yes ☐ No
If yes: Did a veterinarian confirm the diagnosis?
☐ Yes, a state veterinarian ☐ Yes, a private veterinarian ☐ No

If yes: Which animals were infected? *Select all that apply.*
☐ Cattle
☐ Sheep
☐ Goat
☐ Pig
☐ Buffalo
☐ Springbok
☐ Blesbok
☐ Kudu
☐ Other

If other: What other animal species were confirmed having Rift Valley fever? *Please separate answers with commas. Example: Camels, horses.*

For each animal infected in 24-1c: Approximately how many animals from each species died from Rift Valley fever during the 2010-2011 outbreak that affected your farm? *If none, enter 0.*

Adult cattle _____
Calves _____
Adult sheep _____
Lambs _____
Adult goats _____
Goat kids _____
Pig _____
Buffalo _____
Springbok _____
Blesbok _____
Kudu _____
Other _____

For each animal infected in 24-1c: Approximately how many animals from each species aborted due to Rift Valley fever during the 2010-2011 outbreak that affected your farm? *If none, enter 0.*

Cows _____
Ewes _____
Lambs _____
Does _____
Pigs _____
Buffalo _____
Springbok _____
Blesbok _____
Kudu _____
Other _____

Q24-1d If yes to Q24c-1: When was Rift Valley fever confirmed? *Enter the month and year.*

Enter Date
Month (Enter 1-12)
Year

Q24-1e Has there been any suspicion of Rift Valley fever in any communal animals in the past 3 months? ☐ Yes ☐ No

Q25-1 Have any of your animals been vaccinated for brucellosis? ☐ Yes ☐ No

If yes: Which animals were vaccinated? *Select all that apply.*

☐ Cattle
☐ Sheep
☐ Goat
☐ Pig
☐ Buffalo
☐ Springbok
☐ Blesbok
☐ Other

If yes: List any other of your animals that have been vaccinated for brucellosis? *Please separate answers with commas. Example: Camels, horses.*

If yes: When was the most recent vaccine given? *Enter the month and year.*

Enter Date
Month (Enter 1-12)
Year

If yes: What percentage of your animals are vaccinated?

If yes: Which vaccine(s) were used?

☐ RB51
☐ S19
☐ RB51 and S19
☐ Other

If other: Please list any other vaccine given. *Please separate answers with commas.*

Q25a-1 Have any of your animals been vaccinated for Rift Valley fever? ☐ Yes ☐ No

If yes: Which animals were vaccinated? *Select all that apply.*

☐ Cattle
☐ Sheep
☐ Goat
☐ Pig

- ☐ Buffalo
- ☐ Springbok
- ☐ Blesbok
- ☐ Kudu
- ☐ Other

If yes: List any other of your animals that have been vaccinated for Rift Valley fever? *Please separate answers with commas. Example: Camels, horses.*

If yes: What is your protocol for Rift Valley fever vaccination for your animals?

- ☐ Once for all young
- ☐ Every year
- ☐ Only before/during outbreaks
- ☐ Never

If yes: When was the most recent vaccine given? *Enter the month and year.*

Enter Date
Month (Enter 1-12)
Year

If yes: What percentage of animals currently on the farm are vaccinated?

If yes: Which vaccine(s) were used?

- ☐ Smithburn
- ☐ Clone 13
- ☐ Inactivated
- ☐ Clone 13 and inactivated
- ☐ modified-live
- ☐ Unknown
- ☐ Other

If other: Please list any other vaccine given. *Please separate answers with commas.*

You have successfully completed the survey! Thank you for your participation!
Please hand your device back to the administrator

Attention Survey Administrators: Please add any notes here

Appendix 6:
Longitudinal Sheep Study Questionnaire

COHORT QUESTIONNAIRE

Farm animals Information

To be filled out by survey administrator:

Admin 1. Farm Identifier:

Admin 2. Farm Name:

Admin 3. Date of interview:

Admin 4. Collect the current GPS coordinates.

Admin 4a. Enter the latitude and longitude manually using the GPS.
If automatic GPS coordinate does not work then:

Latitude (Degrees) _____

Latitude (Minutes) _____

Latitude (Seconds) _____

Longitude (Degrees) _____

Longitude (Minutes) _____

Longitude (Seconds) _____

Admin 5. Filled in by: _____

Admin 6. Primary Event Number (since the beginning of the study in May 2016):

- ☐ PRE
- ☐ 16-01
- ☐ 16-02
- ☐ 16-03
- ☐ 16-04
- ☐ 16-05
- ☐ 16-06
- ☐ 16-07
- ☐ 16-08
- ☐ 16-09
- ☐ 16-10
- ☐ 16-11
- ☐ 16-12
- ☐ 16-13
- ☐ 16-14

The remainder of the survey should be filled out by the participant.

Q3 What animal species are/were kept on the farm in the past three months? *Select all that apply.*

Do not include animals that are not owned by the farm.

- ☐ Cattle
- ☐ Sheep
- ☐ Goat
- ☐ Pig
- ☐ Buffalo
- ☐ Springbok
- ☐ Blesbok
- ☐ Kudu
- ☐ Other animal

Q3b Only display relevant questions pertaining to answers in 3 and 3a

Q3b Cattle
What is the ACTUAL number of cattle NOW?
How many of your cattle are < 2 years of age?
How many of your cattle are between 2-4 years of age?
How many or your cattle are >4 years of age?

Q3b Sheep
What is the ACTUAL number of sheep now?
How many of your sheep are < 2 years of age?
How many of your sheep are between 2-4 years of age?
How many or your sheep are >4 years of age?

Q3b Goats
What is the ACTUAL number of goats now?
How many of your goats are < 2 years of age?
How many of your goats are between 2-4 years of age?
How many or your goats are >4 years of age?

Q3b Pigs
What is the ACTUAL number of pigs now?

Q3b Buffalo
What is the ACTUAL number of buffalo now?

Q3b Springbok
What is the ACTUAL number of springbok now?

Q3b Blesbok
What is the ACTUAL number of blesbok now?

Q3b Kudu
What is the ACTUAL number of kudu now?

Q3b Other animals
List all other species that are/were kept on the farm in the past month (CURRENTLY). Indicate how many of each
species are/were kept on the farm in the past month. *Please separate answers with commas. Example: 2 horses, 3 camels*

Q8 Have the farm animals had contact with animals from other farms in the past 3 months?
☐ Yes ☐ No

Q8a if yes: How often do they have contact in a typical week? *Answer in times per week.*

Q8b if yes: Under which context do they meet? When…
Select all that apply.

- ☐ Grazing
- ☐ Housing
- ☐ Feeding
- ☐ Contact through fence
- ☐ Other

If other: Please list other contexts in which the farm animals have contact with animals from other farms.
Please separate answers with commas.

Q9 During the past 3 months have the sheep on the farm had access to a pan(s)- seasonal source of water?
☐ Yes ☐ No

Q9a If yes: During the past 3 months what percentage of sheep on the farm have access to the pan(s)- seasonal water source when it was available?

Q10a During the past 3 months have you rotated the grazing areas for the flocks?
☐ Yes ☐ No

Q11 Have you purchased/gained (from gift or birth) animals in the past 3 months?
☐ Yes ☐ No

If yes: Which of the following animals have you purchased/gained (from gift or birth) in the past 3 months?
Select all that apply.

- ☐ Cow
- ☐ Heifer
- ☐ Bull
- ☐ Steer
- ☐ Calf
- ☐ Doe goat
- ☐ Billy goat
- ☐ Goat kid
- ☐ Ewe
- ☐ Ram
- ☐ Lamb
- ☐ Sow
- ☐ Boar
- ☐ Piglet
- ☐ Springbok
- ☐ Blesbok
- ☐ Kudu
- ☐ Other

Q11a Asked only for the species indicated as purchased/gained in Q11:
Approximately how many cows were… *If none, enter zero*

Purchased in the past 3 months
Given to you in the past 3 months
Otherwise gained in the past 3 months

In what other ways were cows gained in the past 3 months?

Q11a Approximately how many heifers were… *If none, enter zero*

Purchased in the past 3 months
Given to you in the past 3 months
Otherwise gained in the past 3 months

In what other ways were heifers gained in the past 3 months?

Q11a Approximately how many bulls were… *If none, enter zero*
Purchased in the past 3 months
Given to you in the past 3 months
Otherwise gained in the past 3 months

In what other ways were bulls gained in the past 3 months?

Q11a Approximately how many steers were… *If none, enter zero*
Purchased in the past 3 months
Given to you in the past 3months
Otherwise gained in the past 3 months

In what other ways were steers gained in the past 3 months?

Q11a Approximately how many calves were… *If none, enter zero*
Purchased in the past 3 months
Given to you in the past 3 months
Born in the past 3 months
Otherwise gained in the past 3 months

In what other ways were calves gained in the past 3 months?

Q11a Approximately how many doe goats were… *If none, enter zero*
Purchased in the past 3 months
Given to you in the past 3 months
Otherwise gained in the past 3 months

In what other ways were doe goats gained in the past 3 months?

Q11a Approximately how many billy goats were… *If none, enter zero*
Purchased in the past 3 months
Given to you in the past 3 months
Otherwise gained in the past 3 months

In what other ways were billy goats gained in the past 3 months?

Q11a Approximately how many goat kids were… *If none, enter zero*
Purchased in the past 3 months
Given to you in the past 3 months
Born in the past 3 months
Otherwise gained in the past 3 months

In what other ways were goat kids gained in the past 3 months?

Q11a Approximately how many ewes were… *If none, enter zero*
Purchased in the past 3 months
Given to you in the past 3 months
Otherwise gained in the past 3 months

In what other ways were ewes gained in the past 3 months?

Q11a Approximately how many rams were… *If none, enter zero*
Purchased in the past 3 months
Given to you in the past 3 months
Otherwise gained in the past 3 months

In what other ways were rams gained in the past 3 months?

Q11a Approximately how many lambs were… *If none, enter zero*
Purchased in the past 3 months
Given to you in the past 3 months
Born in the past 3 months
Otherwise gained in the past 3 months

In what other ways were lambs gained in the past 3 months?

Q11a Approximately how many sows were… *If none, enter zero*
Purchased in the past 3 months
Given to you in the past 3 months
Otherwise gained in the past 3 months

In what other ways were sows gained in the past 3 months?

Q11a Approximately how many boars were… *If none, enter zero*
Purchased in the past 3 months
Given to you in the past 3 months
Otherwise gained in the past 3 months

In what other ways were boars gained in the past 3 months?

Q11a Approximately how many piglets were… *If none, enter zero*
Purchased in the past 3 months
Given to you in the past 3 months
Born in the past 3 months
Otherwise gained in the past 3 months

In what other ways were piglets gained in the past 3 months?

Q11a Approximately how many springbok were… *If none, enter zero*
Purchased in the past 3 months
Given to you in the past 3 months
Born in the past 3 months
Otherwise gained in the past 3 months

In what other ways were springbok gained in the past 3 months?

Q11a Approximately how many blesbok were… *If none, enter zero*
Purchased in the past 3 months
Given to you in the past 3 months
Born in the past 3 months
Otherwise gained in the past 3 months

In what other ways were blesbok gained in the past 3 months?

Q11a Approximately how many kudu were… *If none, enter zero*

Purchased in the past 3 months
Given to you in the past 3 months
Born in the past 3 months
Otherwise gained in the past 3 months

In what other ways were kudu gained in the past 3 months?

List all other species of animals that were gained in the past 3 months. *Please separate with commas.*

Q11a Approximately how many other animals were… *If none, enter zero*

Purchased in the past 3 months
Given to you in the past 3 months
Born in the past 3 months
Otherwise gained in the past 3 months

In what other ways were other animals gained in the past 3 months?

Q11b If they indicated that they had purchased any animals in Q11:
On AVERAGE, how many times did you purchase animals during the past 3 months? *If none, enter zero*

Q11c Have you sold/lost/slaughtered (ceremonial or for food consumption) animals in the past 3 months?

☐ Yes ☐ No

Which of the following animals have you sold/lost/slaughtered (ceremonial or for food consumption)?
Select all that apply.

☐ Cow
☐ Heifer
☐ Bull
☐ Steer
☐ Calf
☐ Doe goat
☐ Billy goat
☐ Goat kid
☐ Ewe
☐ Ram
☐ Lamb
☐ Sow
☐ Boar
☐ Piglet
☐ Springbok
☐ Blesbok
☐ Kudu
☐ Other

Q11d Asked only for the species indicated in Q11:
Approximately how many cows were… *If none, enter zero*

Sold in the past 3 months
Stolen from you in the past 3 months
Predated in the past 3 months
Died in the past 3 months

Were slaughtered in the past 3 months
otherwise lost in the past 3 months

In what other ways were cows lost in the past 3 months?

Q11d Approximately how many heifers were… *If none, enter zero*

Sold in the past 3 months

Stolen from you in the past 3 months

Predated in the past 3 months
Died in the past 3 months
Were slaughtered in the past 3 months
otherwise lost in the past 3 months

In what other ways were heifers lost in the past 3 months?

Q11d Approximately how many bulls were… *If none, enter zero*

Sold in the past 3 months

Stolen from you in the past 3 months

Predated in the past 3 months
Died in the past 3 months
Were slaughtered in the past 3 months
otherwise lost in the past 3 months

In what other ways were bulls lost in the past 3 months?

Q11d Approximately how many steers were… *If none, enter zero*

Sold in the past 3 months

Stolen from you in the past 3 months

Predated in the past 3 months
Died in the past 3 months
were slaughtered in the past 3 months
otherwise lost in the past 3 months

In what other ways were steers lost in the past 3 months?

Q11d Approximately how many calves were… *If none, enter zero*

Sold in the past 3 months

Stolen from you in the past 3 months

Predated in the past 3 months
Died in the past 3 months
Were slaughtered in the past 3 months
otherwise lost in the past 3 months

In what other ways were calves lost in the past 3 months?

Q11d Approximately how many doe goats were… *If none, enter zero*

Sold in the past 3 months

Stolen from you in the past 3 months

Predated in the past 3 months
Died in the past 3 months
Were slaughtered in the past 3 months
otherwise lost in the past 3 months

In what other ways were doe goats lost in the past 3 months?

Q11d Approximately how many billy goats were… *If none, enter zero*

Sold in the past 3 months

Stolen from you in the past 3 months

Predated in the past 3 months
Died in the past 3 months
Were slaughtered in the past 3 months
otherwise lost in the past 3 months

In what other ways were billy goats lost in the past 3 months?

Q11d Approximately how many goat kids were… *If none, enter zero*

Sold in the past 3 months

Stolen from you in the past 3 months

Predated in the past 3 months
Died in the past 3 months
Were slaughtered in the past 3 months
otherwise lost in the past 3 months

In what other ways were goat kids lost in the past 3 months?

Q11d Approximately how many ewes were… *If none, enter zero*

Sold in the past 3 months

Stolen from you in the past 3 months

Predated in the past 3 months
Died in the past 3 months
Were slaughtered in the past 3 months
Otherwise lost in the past 3 months

In what other ways were ewes lost in the past 3 months?

Q11d Approximately how many rams were… *If none, enter zero*

Sold in the past 3 months

Stolen from you in the past 3 months

Predated in the past 3 months
Died in the past 3 months
Were slaughtered in the past 3 months
Otherwise lost in the past 3 months

In what other ways were rams lost in the past 3 months?

Q11d Approximately how many lambs were… *If none, enter zero*

Sold in the past 3 months

Stolen from you in the past 3 months

Predated in the past 3 months
Died in the past 3 months
Were slaughtered in the past 3 months
otherwise lost in the past 3 months

In what other ways were lambs lost in the past 3 months?

Q11d Approximately how many sows were… *If none, enter zero*

Sold in the past 3 months

Stolen from you in the past 3 months

Predated in the past 3 months
Died in the past 3 months
Were slaughtered in the past 3 months

Otherwise lost in the past 3 months

In what other ways were sows lost in the past 12 months?

Q11d Approximately how many boars were… *If none, enter zero*

Sold in the past 3 months

Stolen from you in the past 3 months

Predated in the past 3 months
Died in the past 3 months
Were slaughtered in the past 3 months
Otherwise lost in the past 3 months

In what other ways were boars lost in the past 3 months?

Q11d Approximately how many piglets were… *If none, enter zero*

Sold in the past 3 months

Stolen from you in the past 3 months

Predated in the past 3 months
Died in the past 3 months
Were slaughtered in the past 3 months
otherwise lost in the past 3 months

In what other ways were piglets lost in the past 3 months?

Q11d Approximately how many springbok were… *If none, enter zero*

Sold in the past 3 months

Stolen from you in the past 3 months

Predated in the past 3 months
Died in the past 3 months
Were slaughtered in the past 3 months
Otherwise lost in the past 3 months

In what other ways were springbok lost in the past 3 months?

Q11d Approximately how many blesbok were… *If none, enter zero*

Sold in the past 3 months

Stolen from you in the past 3 months

Predated in the past 3 months
Died in the past 3 months
Were slaughtered in the past 3 months
Otherwise lost in the past 3 months

In what other ways were blesbok lost in the past 3 months?

Q11d Approximately how many kudu were… *If none, enter zero*

Sold in the past 3 months

Stolen from you in the past 3 months

Predated in the past 3 months
Died in the past 3 months
Were slaughtered in the past 3 months
Otherwise lost in the past 3 months

In what other ways were kudu lost in the past 3 months?

Q11d List all other species of animals that were lost in the past 3 months. *Please separate with commas.*

Approximately how many other animals were… *If none, enter zero*

Sold in the past 3 months

Stolen from you in the past 3 months

Predated in the past 3 months
Died in the past 3 months
Were slaughtered in the past 3 months
Otherwise lost in the past 3 months

In what other ways were other animals lost in the past 3 months?

Q11e Asked if they indicated any species had been sold/lost/slaughtered in Q11
On average, how many times did you sell animals during the past 3 months? (*If you do not sell animals, enter zero)*

Q11g If they indicated that they had bought or sold any animals in Q11: Where did you purchase your animals from OR sell your animals to in the past 3 months? (Select all that apply)

	Bought	Sold
Auction	☐	☐
Other farms/farmers	☐	☐
Abattoir	☐	☐
Other	☐	☐

If yes: How many auctions did you purchase your animals from?
If you did not purchase any animals at auctions, enter "0"
How many auctions did you sell your animals at?
If you did not sell any animals at auctions, enter "0"
How many other farms did you purchase your animals from?
If you did not purchase any animals at other farms, enter "0"
How many other farms did you sell your animals at?
If you did not sell any animals at other farms, enter "0"
How many abattoirs did you sell your animals at?
If you did not sell any animals at abattoirs, enter "0"

Approximately how far away in Km is the auction where the animal(s) were purchased?
Approximately how far away in Km is the second auction where the animal(s) were purchased?
List all other auctions where the animal(s) were purchased and indicate their distance away in Km.

Approximately how far away in Km is the auction where the animal(s) were sold?
Approximately how far away in Km is the second auction where the animal(s) were sold?
List all other auctions where the animal(s) were sold and indicate their distance away in Km.

Approximately how far away in Km is the farm where the animal(s) were purchased?
Approximately how far away in Km is the second farm where the animal(s) were purchased?
List all other farms where the animal(s) were purchased and indicate their distance away in Km.

Approximately how far away in Km is the farm where the animal(s) were sold?
Approximately how far away in Km is the second farm where the animal(s) were sold?
List all other farms where the animal(s) were sold and indicate their distance away in Km.

Approximately how far away in Km is the abattoir where the animal(s) were sold?
Approximately how far away in Km is the second abattoir where the animal(s) were sold?
List all other abattoirs where the animal(s) were sold and indicate their distance away in Km.

If other: Please specify other places animals were bought or sold.

Q21 Have any animals had an abortion in the past 3 months ☐ Yes ☐ No

Q21a If abortion yes: How many animal species had abortions?

Q21b If abortion yes: Which animal species had abortions?
- ☐ Cattle
- ☐ Sheep
- ☐ Goat
- ☐ Pig
- ☐ Buffalo
- ☐ Springbok
- ☐ Blesbok
- ☐ Kudu
- ☐ Other animal

Which other animal species had abortions? *Please separate answers with commas. Example: Camels, horses*

Q21c If abortion yes: Did anyone assist in the handling of the animals or removal of aborted foetus? ☐ Yes ☐ No

Q21d If abortion yes: How many people were involved in assisting with the abortion?

Q21e If abortion yes: Who was involved in assisting with the abortion? *Select all that apply.*
- ☐ Veterinarian
- ☐ Owner
- ☐ Farm worker
- ☐ Animal Health
- ☐ Technician
- ☐ Other

If other: Who else assisted with the abortion?

Q21f If abortion yes: How was the aborted material disposed of? *Select all that apply.*
- ☐ Buried
- ☐ Left (scavenged)
- ☐ Burnt
- ☐ Eaten by mother
- ☐ Other

If abortion yes: In what other way was the aborted material disposed of?

Q24 Has brucellosis been confirmed on your farm the past 3 months? ☐ Yes ☐ No

If yes: Which animals were infected? *Select all that apply.*
- ☐ Cattle
- ☐ Sheep
- ☐ Goat
- ☐ Pig
- ☐ Buffalo
- ☐ Springbok
- ☐ Blesbok
- ☐ Other animal

If other : What other animal species were confirmed as having brucellosis? *Please separate answers with commas. Example: Camels, horses*

Q24a If yes to Q24 or Q24-1 or Q24-2: When was brucellosis confirmed?
Month (Enter 1-12)

Year

Q24b Has there been any suspicion of brucellosis on the farm in the past 3 months? ☐ Yes ☐ No

Q24c Has Rift Valley fever been confirmed on your farm the past 3 months? ☐ Yes ☐ No

If yes: Which animals were infected? *Select all that apply.*
- ☐ Cattle
- ☐ Sheep
- ☐ Goat
- ☐ Pig
- ☐ Buffalo
- ☐ Springbok
- ☐ Blesbok
- ☐ Other animal

If other: What other animal species were confirmed having Rift Valley fever? *Please separate answers with commas. Example: Camels, horses*

Q24d If yes to Q24c or Q24c-1 or Q24c-2: When was Rift Valley fever confirmed?
Month (Enter 1-12)
Year

Q24e Has there been any suspicion of Rift Valley fever on the farm in the past 3 months?
☐ Yes ☐ No

Q25a Have any animals been vaccinated for Rift Valley fever the past 3 months? ☐ Yes ☐ No

If yes: Which animals were vaccinated? *Select all that apply.*
- ☐ Cattle
- ☐ Sheep
- ☐ Goat
- ☐ Pig
- ☐ Buffalo
- ☐ Springbok
- ☐ Blesbok
- ☐ Other animal

If yes: What other animals have been vaccinated for Rift Valley fever in the past 3 months ?
Please separate answers with commas. Example: Camels, horses

If yes (based on species selected below):
How many cattle were vaccinated for Rift Valley fever in the past 3 months?
How many sheep were vaccinated for Rift Valley fever in the past 3 months?
How many goats were vaccinated for Rift Valley fever in the past 3 months?
How many pigs were vaccinated for Rift Valley fever in the past 3 months?
How many buffalo were vaccinated for Rift Valley fever in the past 3 months?
How many springbok were vaccinated for Rift Valley fever in the past 3 months?
How many blesbok were vaccinated for Rift Valley fever in the past 3 months?
How many other animals were vaccinated for Rift Valley fever in the 3 three months?

If yes: Which vaccine(s) were used?
- ☐ Smithburn
- ☐ Clone 13
- ☐ Inactivated
- ☐ Clone 13 and inactivated
- ☐ modified-live
- ☐ Unknown

☐ Other

If other: Please list any other vaccine given. *Please separate answers with commas.*

Thank You
You have successfully completed the survey! Thank you for your participation!
Please hand your device back to the administrator

Attention Survey Administrators: Please add any notes here

Appendix 7.

Calculation of Basic Reproduction Number Using a Constant Birth Rate

R_0 calculation for simple RVFV transmission model with one host and one vector species with transovarial transmission

Here, we illustrate the derivation of R0 for a simplified system with one host and one vector but including transovarial transmission. This key difference between this and the model used for simulations is that we replace the density-dependent dynamics with an egg laying, b, chosen to maintain a constant vector population size.

Following the notation used in the main text, we present the equations for the susceptible, infected and recovered host population:

$$\frac{dSS}{dt} = b_S \cdot N_H - \theta_{SA} \cdot SS \cdot IA - \mu_S \cdot SS \quad (1)$$

$$\frac{dIS}{dt} = \theta_{SA} \cdot SS \cdot IA - \mu_S \cdot IS - \sigma \cdot IS \quad (2)$$

$$\frac{dRS}{dt} = \sigma \cdot IS - \mu_S \cdot RS \quad (3)$$

the susceptible, exposed and infected adult vectors:

$$\frac{dSA}{dt} = -\theta_{AS} \cdot SA \cdot IS - \mu_A \cdot SA + dev \cdot SAE \quad (4)$$

$$\frac{dEA}{dt} = \theta_{AS} \cdot SA \cdot IS - \mu_A \cdot EA - \epsilon \cdot EA \quad (5)$$

$$\frac{dIA}{dt} = \epsilon \cdot IAE - \mu_A \cdot IA + dev \cdot IAE \quad (6)$$

and the infected and susceptible egg population:

$$\frac{dIAE}{dt} = b \cdot q \cdot IA - dev \cdot IAE - \mu_{AE} \cdot IAE \quad (7)$$

$$\frac{dSAE}{dt} = b \cdot ((1-q) \cdot IA + SA) - dev \cdot SAE - \mu_{AE} \cdot SAE \quad (8)$$

At the disease-free equilibrium, maintaining the vector population size requires

$$\mu_A \cdot SA = dev \cdot SAE \quad \text{and} \quad b \cdot SA = (dev + \mu_{AE})SAE$$

which provides an expression

$$b = \frac{(dev + \mu_{AE})SAE}{SA} = \frac{(dev + \mu_{AE})}{dev}\mu_A = \frac{\mu_A}{p_3}$$

where p_3, given by,

$$p_3 = \frac{dev}{dev + \mu_{AE}}$$

is the proportion of eggs surviving to become adults.

Equations 2, 5, 6 and 7 for SI, EA, IA and IAE respectively represent the infectious subsystem and are used to write down the Jacobian, J, at the disease-free equilibrium where

$$J = \begin{bmatrix} -(\mu_S + \sigma) & 0 & \theta_{SA}N_H & 0 \\ \theta_{AS}N_V p_2 & -(\mu_A + \epsilon) & 0 & 0 \\ 0 & \epsilon & -\mu_A & dev \\ 0 & 0 & b \cdot q & -(dev + \mu_{AE}) \end{bmatrix}$$

Following Diekmann et al. (2010) this is decomposed into the matrix of transmissions (T) and transitions (Σ), where

$$T = \begin{bmatrix} 0 & 0 & \theta_{SA}N_H & 0 \\ \theta_{AS}N_V p_2 & 0 & 0 & 0 \\ 0 & \epsilon & 0 & 0 \\ 0 & 0 & b \cdot q & 0 \end{bmatrix}$$

and

$$\Sigma = \begin{bmatrix} -(\mu_S + \sigma) & 0 & 0 & 0 \\ 0 & -(\mu_A + \epsilon) & 0 & 0 \\ 0 & \epsilon & -\mu_A & dev \\ 0 & 0 & 0 & -(dev + \mu_{AE}) \end{bmatrix}$$

We then require the inverse of the transitions matrix, Σ^{-1} given by:

$$\Sigma^{-1} = -\begin{bmatrix} \frac{1}{(\mu_S+\sigma)} & 0 & 0 & 0 \\ 0 & \frac{1}{(\mu_A+\epsilon)} & 0 & 0 \\ 0 & \frac{1 \quad \epsilon}{\mu_A(\mu_A+\epsilon)} & \frac{1}{\mu_A} & \frac{1 \quad dev}{\mu_A(dev+\mu_{AE})} \\ 0 & 0 & 0 & \frac{1}{dev+\mu_{AE}} \end{bmatrix}$$

where an element $-\Sigma_{ij}^{-1}$ gives the expected time spent by an individual in state i, given that they start in state j.

The next generation matrix, K_L, is given by K_L=-T Σ^{-1}:

$$K_L = -T\Sigma^{-1} = \begin{bmatrix} 0 & \frac{\theta_{SA}N_H \ p_1}{\mu_A} & \frac{\theta_{SA}N_H}{\mu_A} & \frac{\theta_{SA}N_H \ p_1}{\mu_A} \\ \frac{\theta_{AS}N_V \ p_2}{(\mu_S+\sigma)} & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & \frac{bqp_1}{\mu_A} & \frac{bq}{\mu_A} & \frac{bqp_3}{\mu_A} \end{bmatrix}$$

where, p_1, given by

$$p_1 = \frac{\epsilon}{\epsilon + \mu_A}$$

is the proportion of exposed vectors surviving to the infectious state, and p_2, given by

$$p_2 = \frac{dev}{dev + \mu_A}$$

is the proportion of vectors that are adults rather than eggs, and, p_3, given by

$$p_3 = \frac{dev}{dev + \mu_{AE}}$$

is the proportion of eggs surviving to become adults.

$$K_L = -T\Sigma^{-1} = \begin{bmatrix} 0 & \frac{\theta_{SA}N_H\ p_1}{\mu_A} & \frac{\theta_{SA}N_H}{\mu_A} & \frac{\theta_{SA}N_H\ p_3}{\mu_A} \\ \frac{\theta_{AS}N_V\ p_2}{\mu_S+\sigma} & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & \frac{bqp_1}{\mu_A} & \frac{bq}{\mu_A} & \frac{bqp_3}{\mu_A} \end{bmatrix}$$

Reducing K_L by removing the row and columns not associated with a disease entry point we obtain

$$\mathrm{K} = \begin{bmatrix} 0 & \frac{\theta_{SA}N_H\ p_1}{\mu_A} & \frac{\theta_{SA}N_H\ p_3}{\mu_A} \\ \frac{\theta_{AS}N_V\ p_2}{\mu_S+\sigma} & 0 & 0 \\ 0 & \frac{bqp_1}{\mu_A} & \frac{bqp_3}{\mu_A} \end{bmatrix} = \begin{bmatrix} 0 & R_{HV}p_1 & R_{HV}p_3 \\ R_{VH}p_2 & 0 & 0 \\ 0 & \frac{bqp_1}{\mu_A} & \frac{bq}{\mu_A} \end{bmatrix}$$

$$= \begin{bmatrix} 0 & R_{HV}p_1 & R_{HV}p_3 \\ R_{VH}p_2 & 0 & 0 \\ 0 & \frac{qp_1}{p_3} & \mathrm{q} \end{bmatrix}$$

Using b= $\frac{\mu_A}{p_3}$ from above and setting

$$R_{HV} = \frac{\theta_{SA}N_H}{\mu_A} \quad \text{and} \quad R_{VH} = \frac{\theta_{AS}N_V}{\mu_S+\sigma}$$

where R_{HV} is the number of host infections caused a single infected vector in a fully susceptible host population and R_{VH} is the number of vector infections caused a single infected host in a fully susceptible vector population.

The dominant eigenvalue of which gives R_0. Thus, R_0 is the largest solution of

$$\mathrm{Det}\begin{bmatrix} -\lambda & R_{HV}p_1 & R_{HV}p_3 \\ R_{VH}p_2 & -\lambda & 0 \\ 0 & qp_1 & \mathrm{q}-\lambda \\ & p_3 & \end{bmatrix} = 0$$

Giving

$$R_0 = \frac{q}{2} + \sqrt{\frac{q}{2} + R_{HV}p_1R_{VH}p_2}$$

Thus, when there is no transovarial transmission, we obtain

$$R_0 = \sqrt{R_{HV}p_1R_{VH}p_2}$$

which reproduces the form from the classic Ross-Macdonald models, and if there is no transmission between host and vector, we obtain

$$R_0 = q$$

Appendix 8:
wxMaxima code for calculating the Basic Reproduction Number

RVFV model

1 Preamble

```
kill(all)$ /· clear workspace ·/
load(linearalgebra)$ /· gives us jacobian() ·/
load(numericalio)$ /· load CSV file from R ·/
ratprint: false$ /· turn off rational conversion messages ·/;
fpprintprec: 4$ /· show fewer sig.figs in output ·/
```

1.1 Parameters and useful definitions

Read in parameters from CSV file created by R and assign to a hashmap.
Loop through entries (skipping blank first) and assign pfoo[key] = val;

```
mp: read_matrix("~/RVFV/final_params.csv")$
kill(ptmp)$ /· clear ptmp first to ensure getting a hashmap, not a list ·/
for i: 2 thru length(args(mp)) do (
    [key, val]: args(mp)[i],
    ptmp[key]: val
)$
```

These are the main parameters, as extracted from the model written in R

```
→      params: [
    vax = ptmp["vax"], /· Vaccination rate ·/
    σ = ptmp["sigma_sl"], /· Daily recovery rate ·/
    g = ptmp["g"], /· Daily maturation rate of lambs to sheep ·/
    μ_S = ptmp["muS"], /· Daily sheep mortality rate ·/
    sold_S = ptmp["soldS"], /· Daily % of sheep sold ·/
    ρ_S = ptmp["rhoS"], /· RVF specific mortality fraction in sheep ·/
    b_L = ptmp["bL"], /· Daily no. of sheep born ·/
    μ_L = ptmp["muL"], /· Daily lamb mortality rate ·/
    ρ_L = ptmp["rhoL"], /·RVFV specific mortality fraction for lambs ·/
    Abort = ptmp["Abort"], /· Abortion fraction among RVF infected pregnant ewes ·/
    ω_MA = ptmp["omega_MA"], /· Daily waning of maternal antibodies ·/
    NLmax = ptmp["NLmax"], /· Herd size maintained by the carrying capacity in lambs ·/
    Eg_AE = ptmp["EgAE"], /· Daily rate at which adult Aedes (circumluteolus) lays eggs ·/
    EgN_AE = ptmp["EgNAE"], /· No. of female eggs an adult Asian aedes (lineatopennis) lays
    μ_A = ptmp["muA"], /· Daily Aedes mortality rate ·/
    μ_AE = ptmp["muAE"], /· Daily Aedes egg mortality rate ·/
    NAEmax = ptmp["NAEmax"], /· Carrying capacity acting on the number of Aedes eggs that ca
    ψ_A = ptmp["psiA"], /· Fraction of Aedes mcintoshi eggs that hatch ·/
    φ_A = ptmp["phiA"], /· Fraction of Aedes mcintoshi larve/pupae that survive ·/
    bh_A = ptmp["bhA"], /· Aedes hatching fraction if all conditions are met ·/
    dev_ALP = ptmp["dev_ALP"], /· Mean development time of Aedes on days when there was at l
    wait_A = ptmp["waitA"], /· 1 / Time taken before a newly emerged mosquito takes its firs
    μ_ALP = ptmp["mu_ALP"], /· Daily mean mortality rate in Aedes larvae/Pupae on days when
    bite_A = ptmp["biteA"], /· Aedes bite rate on the flock of sheep, roughly 1% of Aedes bi
    θ_Awl = ptmp["thetaA_wl"], /· Daily transmission term that includes transmission to Aede
    ε = ptmp["epsilon"], /· 1 / Extrinsic incubation rate in mosquitoes ·/
    Eg_CE = ptmp["EgCE"], /· Daily Culex rate of laying eggs ·/
    EgN_CE = ptmp["EgNCE"], /· Average no. of female eggs a Culex female lays at a time ·/
    δ = ptmp["delta"], /· Fraction of infected Culex that lay eggs ·/
    ψ_C = ptmp["psiC"], /· Fraction of Culex eggs that hatch in the lab ·/
    φ_C = ptmp["phiC"], /· Fraction of Culex that survive larval/pupal stages ·/
    bh_C = ptmp["bhC"], /· 1 / Culex hatching time ·/
    dev_CLP = ptmp["dev_CLP"], /· Daily Mean development time for Culex pupae/larvae on days
    wait_C = ptmp["waitC"], /· 1 / Time it takes before a newly emerged mosquito takes its f
    μ_C = ptmp["muC"], /· Daily Culex mortality rate ·/
    μ_CLP = ptmp["mu_CLP"], /· Daily Mean Culex larvae/Pupae mortality rate on days when the
    μ_CE = ptmp["muCE"], /· Daily Culex egg mortality rate ·/
    NCEmax = ptmp["NCEmax"], /· Carrying capacity acting on the number of Culex eggs that ca
    θ_Cwl = ptmp["thetaCu_wl"], /· Transmission term that includes transmission to Culex fro
    bite_C = ptmp["biteC"] /· Daily Culex bite rate on the sheep ·/
        ];
```

(params) $[vax=0, \sigma=0.25, g=0.005, \mu_S=1.4\ 10^{-4}, sold_S=0.002, \rho_S=0.063, b_L=0.004, \mu_L=4.0\ 10^{-4}, \rho_L=0.751, Abort=0.9, \omega_{MA}=0.008, NLmax=800, Eg_AE=0.333, EgN_AE=34.58, \mu_A=0.07, \mu_{AE}=0.001, NAEmax=190000, \psi_A=0.9, \varphi_A=0.36, bh_A=0.23, dev_ALP=0.064, wait_A=0.333, \mu_{ALP}=0.036, bite_A=0.15, \theta_{Awl}=3.127\ 10^{-6}, \varepsilon=0.083, Eg_CE=0.333, EgN_CE=118, \delta=0.8, \psi_C=0.82, \varphi_C=0.291, bh_C=0.667, dev_CLP=0.06, wait_C=0.333, \mu_C=0.063, \mu_{CLP}=0.025, \mu_{CE}=0.146, NCEmax=650000, \theta_{Cwl}=3.16\ 10^{-6}, bite_C=0.035]$

These "interesting" parameters are kept separate, to assist later examination:

```
int_params: [
    q = ptmp["q"], /· Transovarial transmission fraction ·/
    T_asl = ptmp["Tasl"], /· Transmission rate to Aedes from sheep/lambs ·/
    T_sla = ptmp["Tsla"], /· Transmission rate to sheep/lamb from Aedes ·/
    T_csl = ptmp["Tcsl"], /· Transmission rate to Culex from sheep/lambs ·/
    T_slc = ptmp["Tslc"] /· Transmission rate to sheep/lambs from Culex ·/
];
```

(int_params) $[q=0.85, T_{asl}=0.41, T_{sla}=0.16, T_{csl}=0.71, T_{slc}=0.31]$

The transmission parameters are derived from the main parameters:

```
trans_params: at([
    β_SLA = bite_A·T_sla / (NS + NL),
    β_ASL = bite_A·T_asl / (NS + NL),
    β_SLC = bite_C·T_slc / (NS + NL),
    β_CSL = bite_C·T_csl / (NS + NL)],
params)$
```

1.2 Host/Vector populations sizes

Since R0 depends on population size, create some different cases to examine
including the situation where only one vector species is present:

```
HV_popns: [NS = 562, NL = 262, NA = 25e3, NC = 500e3]$
HV_A_only: [NS = 562, NL = 262, NA = 25e3, NC = 0]$
HV_C_only: [NS = 562, NL = 262, NA = 0, NC = 500e3]$
```

Some additional cases to examine

```
mean_popns: [NS = 562, NL = 262, NA = 9246,   NAE = 48019, NC = 50418]$
high_popns: [NS = 562, NL = 262, NA = 19822,  NAE = 48019, NC = 110634]$
max_popns:  [NS = 562, NL = 262, NA = 178977, NAE = 48019, NC = 346981]$
```

2 Disease Free Equilibria (DFE)

The aim here is to find the population sizes at DFE,
this can be done more easily by removing all disease components from the equations first.
In the DFE vectors and hosts do not affect each other, and the equations can be decoupled.

Finding the birth rates that fix the populations sizes allows removal of density dependent birth effects
which will simplify the Linearised Infectious Subsystem.
Example: SS ⟶ p_SS*NS where p_SS = SS/(SS+VS)
p_SS will then be used later in the LIS.

Individuals of species X enter at rate birth_X (which we will solve for later).

2.1 Sheep & Lambs

```
dSS: + g·SL − sold_S·SS − μ_S·SS$
dVS: + g·VL − sold_S·VS − μ_S·VS$
/· replacing b_L·(1 − NS/NLmax)·SS with birth_L ·/
dSL: + birth_L − g·SL − vax·SL − μ_L·SL$
dVL: − g·VL + vax·SL − μ_S·VL$
```

Find the DFE:

```
dx: [dSS, dVS, dSL, dVL]$
x:  [SS, VS, SL, VL]$
sol_S: factor(solve(dx, x)[1]);
```

(sol_S)
$$\left[SS=\frac{birth_L\ g}{(\mu_L+vax+g)(\mu_S+sold_S)},VS=\frac{birth_L\ g\ vax}{(\mu_L+vax+g)(\mu_S+g)(\mu_S+sold_S)},\right.$$
$$\left.SL=\frac{birth_L}{\mu_L+vax+g},VL=\frac{birth_L\ vax}{(\mu_L+vax+g)(\mu_S+g)}\right]$$

Find the value of birth_L that gives NS+NL:

```
NS_birth_L: rhs(factor(solve(at(SS+VS+SL+VL = NS+NL, sol_S), birth_L)[1]));
```

(NS_birth_L)
$$\frac{(NS+NL)(\mu_L+vax+g)(\mu_S+g)(\mu_S+sold_S)}{(\mu_S+sold_S+g)(\mu_S+vax+g)}$$

Find numerical values for the DFE, to see how it looks.
Note: this may not exactly match the NS and NL given by the full R model, but should be reasonably close.

```
at(at(at(sol_S, birth_L = NS_birth_L), params), HV_popns);
```

(%o25) $[SS=577.0, VS=0, SL=247.0, VL=0]$

2.2 Aedes

```
/· replacing EgAE·EgNAE·(SA + (1 − q)·IA)·(1 − NAE/NAEmax) with birth_A ·/
dSAE:  + birth_A − bh_A·SAE − μ_AE·SAE$
dSALP: + bh_A·SAE − dev_ALP·SALP − μ_ALP·SALP$
dSAY:  + dev_ALP·SALP − wait_A·SAY − μ_A·SAY$
dSA:   + wait_A·SAY − μ_A·SA$
```

Find the DFE:

```
dx: [dSAE, dSALP, dSAY, dSA]$
x:  [SAE, SALP, SAY, SA]$
sol_A: factor(solve(dx, x)[1]);
```

(sol_A) $$[SAE=\frac{birth_A}{\mu_{AE}+bh_A}, SALP=\frac{bh_A\, birth_A}{(\mu_{AE}+bh_A)(\mu_{ALP}+dev_ALP)}, SAY=\frac{bh_A\, birth_A\, dev_ALP}{(\mu_A+wait_A)(\mu_{AE}+bh_A)(\mu_{ALP}+dev_ALP)}, SA=\frac{bh_A\, birth_A\, dev_ALP\, wait_A}{\mu_A(\mu_A+wait_A)(\mu_{AE}+bh_A)(\mu_{ALP}+dev_ALP)}]$$

Find the value of birth_A that fixes NA.
Note that birth_A = adjusted_birth_A * SA_star, so need to factor that in.

```
sol_birth_A: solve(at(SAE+SALP+SAY+SA = NA, sol_A), birth_A);
```

(sol_birth_A) $$[birth_A=\frac{(NA\,\mu_A\,\mu_{AE}+NA\,bh_A\,\mu_A)\,\mu_{ALP}+NA\,dev_ALP\,\mu_A\,\mu_{AE}+NA\,bh_A\,dev_ALP\,\mu_A}{\mu_A\,\mu_{ALP}+(dev_ALP+bh_A)\,\mu_A+bh_A\,dev_ALP}]$$

```
SA_star: at(SA, sol_A)$
adjusted_birth_A: at(birth_A/SA_star, sol_birth_A);
```

(adjusted_birth_A)
$$\frac{\mu_A\,(\mu_A+wait_A)\,(\mu_{AE}+bh_A)\,(\mu_{ALP}+dev_ALP)}{bh_A\,dev_ALP\,wait_A}$$

Numerical values

```
at(at(at(sol_A, birth_A = adjusted_birth_A), params), HV_popns);
```

(%o36) $[SAE=0.5755\,,SALP=1.324\,,SAY=0.2102\,,SA=1]$

2.3 Culex

```
/* replacing EgCE*EgNCE*SC*(1 - SCE/NCEmax) + δ*EgCE*EgNCE*IC with birth_C */
dSCE: + birth_C - bh_C*SCE - μ_CE*SCE$
dSCLP: + bh_C*SCE - dev_CLP*SCLP - μ_CLP*SCLP$
dSCY: + dev_CLP*SCLP - wait_C*SCY - μ_C*SCY$
dSC: + wait_C*SCY - μ_C*SC$
```

Find the DFE

```
dx: [dSCE, dSCLP, dSCY, dSC]$
x:  [SCE, SCLP, SCY, SC]$
sol_C: factor(solve(dx, x))[1];
```

(sol_C) $[SCE=\frac{birth_C}{\mu_{CE}+bh_C}\,,SCLP=\frac{bh_C\,birth_C}{(\mu_{CE}+bh_C)\,(\mu_{CLP}+dev_CLP)}\,,SCY=\frac{bh_C\,birth_C\,dev_CLP}{(\mu_C+wait_C)\,(\mu_{CE}+bh_C)\,(\mu_{CLP}+dev_CLP)}\,,SC=\frac{bh_C\,birth_C\,dev_CLP\,wait_C}{\mu_C\,(\mu_C+wait_C)\,(\mu_{CE}+bh_C)\,(\mu_{CLP}+dev_CLP)}]$

Find the value of birth_C that fixes NC

```
NC_birth_C: rhs(factor(solve(at(SCE+SCLP+SCY+SC = NC, sol_C), birth_C)[1]));
```

(NC_birth_C)
$$\frac{NC\,\mu_C\,(\mu_{CE}+bh_C)\,(\mu_{CLP}+dev_CLP)}{\mu_C\,\mu_{CLP}+dev_CLP\,\mu_C+bh_C\,\mu_C+bh_C\,dev_CLP}$$

Numerical values

```
at(at(at(sol_C, birth_C = NC_birth_C), params), mean_popns);
at(at(at(sol_C, birth_C = NC_birth_C), params), max_popns);
at(at(at(sol_C, birth_C = NC_birth_C), params), HV_popns);
```

(%o45) $[SCE = 3.089\ 10^3, SCLP = 2.424\ 10^4, SCY = 3.673\ 10^3, SC = 1.941\ 10^4]$

(%o46) $[SCE = 2.126\ 10^4, SCLP = 1.668\ 10^5, SCY = 2.528\ 10^4, SC = 1.336\ 10^5]$

(%o47) $[SCE = 3.064\ 10^4, SCLP = 2.404\ 10^5, SCY = 3.643\ 10^4, SC = 1.925\ 10^5]$

2.4 Population size proportions at DFE

Calculate the proportion of the population for each variable at DFE e.g. pSS is SS / (SS+VS)

```
/* Sheep & Lambs */
p_SS: factor(at(SS/(SS+VS), sol_S))$
p_SL: factor(at(SL/(SL+VL), sol_S))$

/* Aedes */
p_SAE: factor(at(SAE/(SAE+SALP+SAY+SA), sol_A))$
p_SALP: factor(at(SALP/(SAE+SALP+SAY+SA), sol_A))$
p_SAY: factor(at(SAY/(SAE+SALP+SAY+SA), sol_A))$
p_SA: factor(at(SA/(SAE+SALP+SAY+SA), sol_A))$

/* Culex */
p_SCE: factor(at(SCE/(SCE+SCLP+SCY+SC), sol_C))$
p_SCLP: factor(at(SCLP/(SCE+SCLP+SCY+SC), sol_C))$
p_SCY: factor(at(SCY/(SCE+SCLP+SCY+SC), sol_C))$
p_SC: factor(at(SC/(SCE+SCLP+SCY+SC), sol_C))$
```

It is useful to see what p_SS and p_SA look like

```
'p_SS = p_SS;
```

(%o58) $$p_{SS} = \frac{\mu_S + g}{\mu_S + vax + g}$$

```
'p_SA = p_SA;
```

(%o59) $$p_{SA} = \frac{bh_A\ dev_ALP\ wait_A}{(\mu_A + wait_A)(\mu_A\ \mu_{ALP} + dev_ALP\ \mu_A + bh_A\ \mu_A + bh_A\ dev_ALP)}$$

Show numerical values of population sizes

```
numerical_popns: at([p_SS, p_SL, p_SAE, p_SALP, p_SAY, p_SA, p_SCE, p_SCLP, p_SCY, p_SC],
params)$ table_form(addrow(
        matrix(['p_SS, 'p_SL, 'p_SAE, 'p_SALP, 'p_SAY, 'p_SA, 'p_SCE, 'p_SCLP, 'p_SCY,
       'p_SC]),  matrix(numerical_popns)))$
```

(%t61)

p_{SS}	p_{SL}	p_{SAE}	p_{SALP}	p_{SAY}	p_{SA}	p_{SCE}	p_{SCLP}	p_{SCY}	p_{SC}
1.0	1.0	0.1851	0.4257	0.06761	0.3216	0.06127	0.4808	0.07285	0.3851

3 Calculating R0

3.1 Linearised infectious subsystem

Linearise the main equations, using SS ⟶ p_SS*NS etc.
The birth term for Aedes eggs is replaced with a simpler version.

```
/· Sheep & Lambs ·/
dIS: - σ·IS - μ_S·IS - ρ_S·IS + g·IL - sold_S·IS + β_SLC·(p_SS·NS)·IC + β_SLA·(p_SS·NS)·IA$
dIL: - σ·IL - μ_L·IL - ρ_L·IL - g·IL + β_SLC·(p_SL·NL)·IC + β_SLA·(p_SL·NL)·IA$

/· Aedes ·/
dEA:   + β_ASL·(p_SA·NA)·(IL + IS) - ε·EA - μ_A·EA$
dIALP: + bh_A·IAE - dev_ALP·IALP - μ_ALP·IALP$
dIAY:  + dev_ALP·IALP - wait_A·IAY - μ_A·IAY$
dIA:   + wait_A·IAY - μ_A·IA + ε·EA$
/· Note: adjusted_birth_A ·/
dIAE:  + q·adjusted_birth_A·IA - bh_A·IAE - μ_AE·IAE$

/· Culex ·/
dEC: + β_CSL·(p_SC·NC)·(IL + IS) - ε·EC - μ_C·EC$
dIC: + ε·EC - μ_C·IC$
```

Set up vectors x and dx:
Note: trans_params won't be needed again, so substitute them in here

```
dx: at([dIS, dIL, dEA, dIAE, dIALP, dIAY, dIA, dEC, dIC], trans_params)$
x:  [IS, IL, EA, IAE, IALP, IAY, IA, EC, IC]$
```

Create a function to simplify substituting in parameters:

```
setpars(x) := at(x, append(params, int_params))$
```

Now check that all parameters are accounted for (i.e. they don't appear in the parameterised output):

```
→  setpars(dx);
```

(%o74)
$$\left[\frac{0.01085\ IC\ NS}{NS+NL}+\frac{0.024\ IA\ NS}{NS+NL}-0.3151\ IS+0.005\ IL,\frac{0.01085\ IC\ NL}{NS+NL}+\frac{0.024\ IA\ NL}{NS+NL}-1.006\ IL,\frac{0.01978\ (IS+IL)\ NA}{NS+NL}-0.153\ EA,0.113\ IA-0.231\ IAE,0.23\ IAE-0.1\ IALP,0.064\ IALP-0.403\ IAY,0.333\ IAY-0.07\ IA+0.083\ EA,\frac{0.009569\ (IS+IL)\ NC}{NS+NL}-0.146\ EC,0.083\ EC-0.063\ IC\right]$$

3.2 Jacobian J

First calculate the Jacobian J.
The algebraic output is complex, so substitute in parameters to check output:

```
→  J: jacobian(dx, x)$
   setpars(%);
```

(%o76)
$$\begin{pmatrix} -0.3151 & 0.005 & 0 & 0 & 0 & 0 & \frac{0.024\ NS}{NS+NL} & 0 & \frac{0.01085\ NS}{NS+NL} \\ 0 & -1.006 & 0 & 0 & 0 & 0 & \frac{0.024\ NL}{NS+NL} & 0 & \frac{0.01085\ NL}{NS+NL} \\ \frac{0.01978\ NA}{NS+NL} & \frac{0.01978\ NA}{NS+NL} & -0.153 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & -0.231 & 0 & 0 & 0.113 & 0 & 0 \\ 0 & 0 & 0 & 0.23 & -0.1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0.064 & -0.403 & 0 & 0 & 0 \\ 0 & 0 & 0.083 & 0 & 0 & 0.333 & -0.07 & 0 & 0 \\ \frac{0.009569\ NC}{NS+NL} & \frac{0.009569\ NC}{NS+NL} & 0 & 0 & 0 & 0 & 0 & -0.146 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0.083 & -0.063 \end{pmatrix}$$

3.3 Transmissions T and transitions Σ

Create an 9x9 zero matrix and copy the elements from J corresponding to transmissions.

Note: transmissions are:
- vectors from hosts: IA, IC <- IS, IL
- hosts from vectors: IS, IL <- EA, EC
- vertical trans: IAE <- IA

```
T: zeromatrix(9,9)$ /* create empty matrix */
transmissions: [[1,7], [1,9], [2,7], [2,9], [3,1], [3,2], [4,7], [8,1], [8,2]]$
for t in transmissions do ([i,j]: t, T[i,j]: J[i,j])$
setpars(T);
```

(%o80)

$$\begin{pmatrix} 0 & 0 & 0 & 0 & 0 & 0 & \frac{0.024\ NS}{NS+NL} & 0 & \frac{0.01085\ NS}{NS+NL} \\ 0 & 0 & 0 & 0 & 0 & 0 & \frac{0.024\ NL}{NS+NL} & 0 & \frac{0.01085\ NL}{NS+NL} \\ \frac{0.01978\ NA}{NS+NL} & \frac{0.01978\ NA}{NS+NL} & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0.113 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ \frac{0.009569\ NC}{NS+NL} & \frac{0.009569\ NC}{NS+NL} & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix}$$

```
T[4,7];
```

(%o81)

$$\frac{q\,\mu_A\,(\mu_A+wait_A)\,(\mu_{AE}+bh_A)\,(\mu_{ALP}+dev_ALP)}{bh_A\,dev_ALP\,wait_A}$$

Transition matrix Σ

```
Σ: J - T$
setpars(%);
```

(%o83)

$$\begin{pmatrix} -0.3151 & 0.005 & 0 & 0 & 0 & 0 & 0.0 & 0 & 0.0 \\ 0 & -1.006 & 0 & 0 & 0 & 0 & 0.0 & 0 & 0.0 \\ 0.0 & 0.0 & -0.153 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & -0.231 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0.23 & -0.1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0.064 & -0.403 & 0 & 0 & 0 \\ 0 & 0 & 0.083 & 0 & 0 & 0.333 & -0.07 & 0 & 0 \\ 0.0 & 0.0 & 0 & 0 & 0 & 0 & 0 & -0.146 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0.083 & -0.063 \end{pmatrix}$$

3.4 NGM with large domain K_L

```
KL: -T.invert(Σ)$
setpars(%);
```

(%o85)

$$\begin{pmatrix} 0 & 0 & \frac{0.186\ NS}{NS+NL} & \frac{0.1805\ NS}{NS+NL} & \frac{0.1813\ NS}{NS+NL} & \frac{0.2833\ NS}{NS+NL} & \frac{0.3429\ NS}{NS+NL} & \frac{0.09791\ NS}{NS+NL} & \frac{0.1722\ NS}{NS+NL} \\ 0 & 0 & \frac{0.186\ NL}{NS+NL} & \frac{0.1805\ NL}{NS+NL} & \frac{0.1813\ NL}{NS+NL} & \frac{0.2833\ NL}{NS+NL} & \frac{0.3429\ NL}{NS+NL} & \frac{0.09791\ NL}{NS+NL} & \frac{0.1722\ NL}{NS+NL} \\ \frac{0.06276\ NA}{NS+NL} & \frac{0.01996\ NA}{NS+NL} & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0.8757 & 0.85 & 0.8537 & 1.334 & 1.614 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ \frac{0.03036\ NC}{NS+NL} & \frac{0.009659\ NC}{NS+NL} & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix}$$

3.5 The NGM with small domain K

Create a matrix that will keep only states-at-entry (i.e. 1,2,3,4,8)
Note: this could also be done via "K: submatrix(5,6,7,9, KL, 5,6,7,9)"

```
E95: zeromatrix(9,5)$
states_at_entry: [1,2,3,4,8]$
for i: 1 thru 5 do E95[states_at_entry[i], i]: 1$
```

```
K: transpose(E95).KL.E95$
setpars(%);
```

(%o90)

$$\begin{pmatrix} 0 & 0 & \frac{0.186\,NS}{NS+NL} & \frac{0.1805\,NS}{NS+NL} & \frac{0.09791\,NS}{NS+NL} \\ 0 & 0 & \frac{0.186\,NL}{NS+NL} & \frac{0.1805\,NL}{NS+NL} & \frac{0.09791\,NL}{NS+NL} \\ \frac{0.06276\,NA}{NS+NL} & \frac{0.01996\,NA}{NS+NL} & 0 & 0 & 0 \\ 0 & 0 & 0.8757 & 0.85 & 0 \\ \frac{0.03036\,NC}{NS+NL} & \frac{0.009659\,NC}{NS+NL} & 0 & 0 & 0 \end{pmatrix}$$

A table view of K, the column variable creates the row value

```
table_form(addrow(matrix([IS, IL, EA, IAE, EC]), at(setpars(K), HV_popns)))$
```

(%t91)

IS	*IL*	*EA*	*IAE*	*EC*
0	0	0.1269	0.1231	0.06678
0	0	0.05914	0.0574	0.03113
1.904	0.6057	0	0	0
0	0	0.8757	0.85	0
18.42	5.861	0	0	0

3.6 Eigenvalues

Try to calculate the eigenvalues of K:

```
eigs: eigenvalues(setpars(K))$
```

eigenvalues: solve is unable to find the roots of the characteristic polynomial.

Note that K is a 5x5 matrix, so the characteristic polynomial has degree 5, which Maxima cannot solve algebraicially.
To get the eigenvalues, we instead get the characteristic polynomial from K directly, and solve that numerically.

```
(%i96) chpol: charpoly(K, λ)$
```

Calculate R0
- realroots() solves numerically for the real roots of the characteristic polynomial
- map(rhs, roots1) extracts the Right Hand Side of each part of the list
- lmax gives the maximimum of a list (the largest eigenvalue)
- float converts that to a decimal number

```
(%i95) pchpol: setpars(chpol)$

(%i94) realroots(at(pchpol, HV_popns))$
       R0: float(lmax(map(rhs, %)));
(R0)   1.445
```

The case for Aedes or Culex only:

```
(%i93) float(lmax(map(rhs, realroots(at(pchpol, HV_A_only)))));
       float(lmax(map(rhs, realroots(at(pchpol, HV_C_only)))));
(%o97) 1.102
(%o98) 1.189
```

R0 at other population sizes:

```
(%i92) R0_mean: float(lmax(map(rhs, realroots(at(pchpol, mean_popns)))));
       R0_high: float(lmax(map(rhs, realroots(at(pchpol, high_popns)))));
       R0_max: float(lmax(map(rhs, realroots(at(pchpol, max_popns)))));

(R0_mean) 0.9739
(R0_high) 1.114
(R0_max) 2.082
```

This clearly shows that we are in the right ballpark,
as moving from low to high mosquito populations bring R0 over 1.

4 Plots

Since Maxima cannot solve for R0 algebraically, it must be done numerically,
therefore all parameters in chpol must be fixed, except for the few we're interested in exploring.
The remaining parameters will be varied and fixed prior to solving.

```
chpol2: factor(at(chpol, params))$
```

For printing purposes, increase font sizes to ensure labels are visible

```
preamble: "set xlabel font ',24'; set ylabel font ',24'; set xtics font ',16'; set ytics font ',16'; set key font ',24'"
```

4.1 Vertical transmission q

Numerically calculate R0 for varying vertical transmission q.
Show both Aedes and Culex (at max), or just Aedes, highlighting the default value.

No. of points in figure, giving [0, 0.01, ..., 0.99, 1.0]

```
npts: 100$
```

```
zx: makelist(float(z), z, 0, 1, 1/npts)$
y1: makelist(0, length(zx))$
y2: makelist(0, length(zx))$
y3: makelist(0, length(zx))$

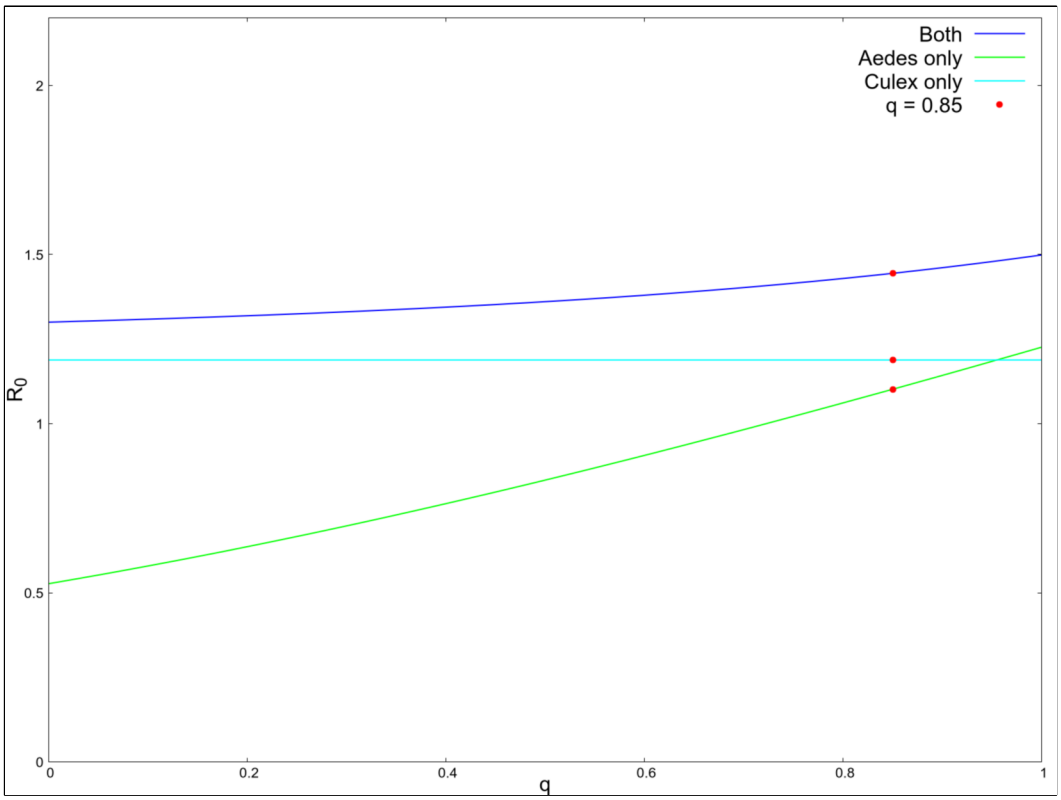
for i: 1 thru length(zx) do (
    int1: [q = zx[i], T_asl = 0.41, T_sla = 0.16, T_csl = 0.71, T_slc = 0.31],
    /·int1: [q = zx[i], T_asl = 0.0, T_sla = 0.0, T_csl = 0.0, T_slc = 0.0],·/
    y1[i]: float(lmax(map(rhs, realroots(at(at(chpol2, HV_popns), int1)))),
    y2[i]: float(lmax(map(rhs, realroots(at(at(chpol2, HV_A_only), int1)))),
    y3[i]: float(lmax(map(rhs, realroots(at(at(chpol2, HV_C_only), int1))))
)$
```

```
/· closest point to given value ·/
pt: floor(at(q, int_params)·npts)+1$
print('q, "=", zx[pt], ": both =", y1[pt], ", Aedes only =", y2[pt], ",
Culex only = ", y3[pt])$
```

$q = 0.85$: *both* = 1.445 , *Aedes only* = 1.102 , *Culex only* = 1.189

```
wxplot2d(
    [
        [discrete, zx, y1],
        [discrete, zx, y2],
        [discrete, zx, y3],
        [discrete, [zx[pt], zx[pt], zx[pt]], [y1[pt], y2[pt], y3[pt]]]
    ],
    ['y, 0, 2.2],
    [style, [lines, 2, 1], [lines, 2, 3], [lines, 2, 6], [points, 3, 2, 1]],
    [xlabel, "{q}"],
    [ylabel, "R_0"],
    [legend, "Both", "Aedes only", "Culex only", sconcat("q = ", zx[pt])],
    [gnuplot_preamble, preamble]
)$
```

(%t112)



4.2 Transmission to Aedes from Sheep & Lambs

Numerically calculate R0 for varying transmission parameter T_asl.

```
zx: makelist(float(z), z, 0, 1, 1/npts)$
y1: makelist(0, length(zx))$
y2: makelist(0, length(zx))$
y3: makelist(0, length(zx))$

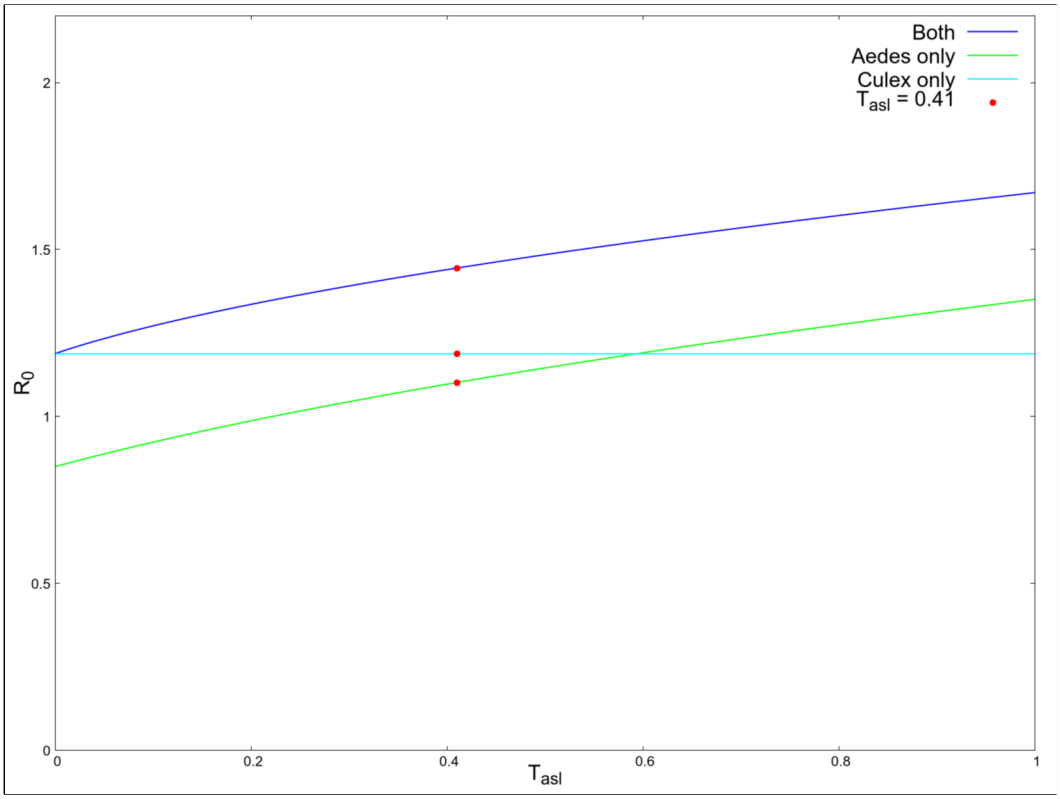
for i: 1 thru length(zx) do (
    int1: [q = 0.85, T_asl = zx[i], T_sla = 0.16, T_csl = 0.71, T_slc = 0.31],
    y1[i]: float(lmax(map(rhs, realroots(at(at(chpol2, HV_popns), int1)))),
    y2[i]: float(lmax(map(rhs, realroots(at(at(chpol2, HV_A_only), int1)))),
    y3[i]: float(lmax(map(rhs, realroots(at(at(chpol2, HV_C_only), int1)))))
)$
```

```
/· closest point to given value ·/
pt: floor(at(T_asl, int_params)·npts)+1$
print('T_asl, " =", zx[pt], ": both =", y1[pt], ", Aedes only =", y2[pt], ",
Culex only = ",
```

T_{asl} = 0.41 : *both* = 1.445 , *Aedes only* = 1.102
, *Culex only* = 1.189

```
wxplot2d(
    [
        [discrete, zx, y1],
        [discrete, zx, y2],
        [discrete, zx, y3],
        [discrete, [zx[pt], zx[pt], zx[pt]], [y1[pt], y2[pt], y3[pt]]]
    ],
    ['y, 0, 2.2],
    [style, [lines, 2, 1], [lines, 2, 3], [lines, 2, 6], [points, 3, 2, 1]],
    [xlabel, "T_{asl}"],
    [ylabel, "R_0"],
    [legend, "Both", "Aedes only", "Culex only", sconcat("T_{asl} = ", zx[pt])],
    [gnuplot_preamble, preamble]
)$
```

(%t120)



4.3 Sheep & Lambs from Aedes

Numerically calculate R0 for varying transmission parameter T_sla.

```
zx: makelist(float(z), z, 0, 1, 1/npts)$
y1: makelist(0, length(zx))$
y2: makelist(0, length(zx))$
y3: makelist(0, length(zx))$

for i: 1 thru length(zx) do (
    int1: [q = 0.85, T_asl = 0.41, T_sla = zx[i], T_csl = 0.71, T_slc = 0.31],
    y1[i]: float(lmax(map(rhs, realroots(at(at(chpol2, HV_popns), int1))))),
    y2[i]: float(lmax(map(rhs, realroots(at(at(chpol2, HV_A_only), int1))))),
    y3[i]: float(lmax(map(rhs, realroots(at(at(chpol2, HV_C_only), int1)))))
)$
```

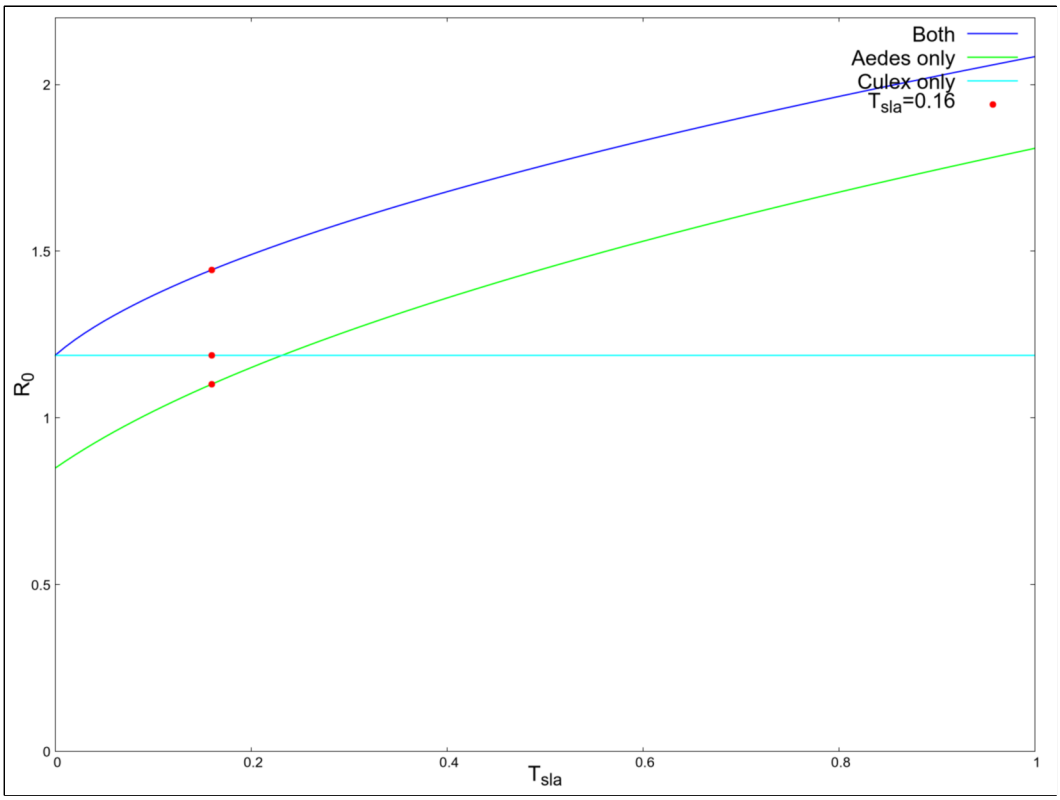
```
/· closest point to given value ·/
pt: floor(at(T_sla, int_params)·npts)+1$
print('T_sla, " =", zx[pt], ": both =", y1[pt], ", Aedes only =", y2[pt], ",
Culex only = ",
```

T_{sla} = 0.16 : *both* = 1.445 , *Aedes only* = 1.102

, *Culex only* = 1.189

```
wxplot2d(
    [
        [discrete, zx, y1],
        [discrete, zx, y2],
        [discrete, zx, y3],
        [discrete, [zx[pt], zx[pt], zx[pt]], [y1[pt], y2[pt], y3[pt]]]
    ],
    ['y, 0, 2.2],
    [style, [lines, 2, 1], [lines, 2, 3], [lines, 2, 6], [points, 3, 2, 1]],
    [xlabel, "T_{sla}"],
    [ylabel, "R_0"],
    [legend, "Both", "Aedes only", "Culex only", sconcat("T_{sla}=", zx[pt])],
    [gnuplot_preamble, preamble]
)$
```

(%t128)



4.4 Culex from Sheep & Lambs

Numerically calculate R0 for varying transmission parameter T_csl.

```
zx: makelist(float(z), z, 0, 1, 1/npts)$
y1: makelist(0, length(zx))$
y2: makelist(0, length(zx))$
y3: makelist(0, length(zx))$

for i: 1 thru length(zx) do (
    int1: [q = 0.85, T_asl = 0.41, T_sla = 0.16, T_csl = zx[i], T_slc = 0.31],
    y1[i]: float(lmax(map(rhs, realroots(at(at(chpol2, HV_popns), int1)))),
    y2[i]: float(lmax(map(rhs, realroots(at(at(chpol2, HV_A_only), int1)))),
    y3[i]: float(lmax(map(rhs, realroots(at(at(chpol2, HV_C_only), int1))))
)$
```

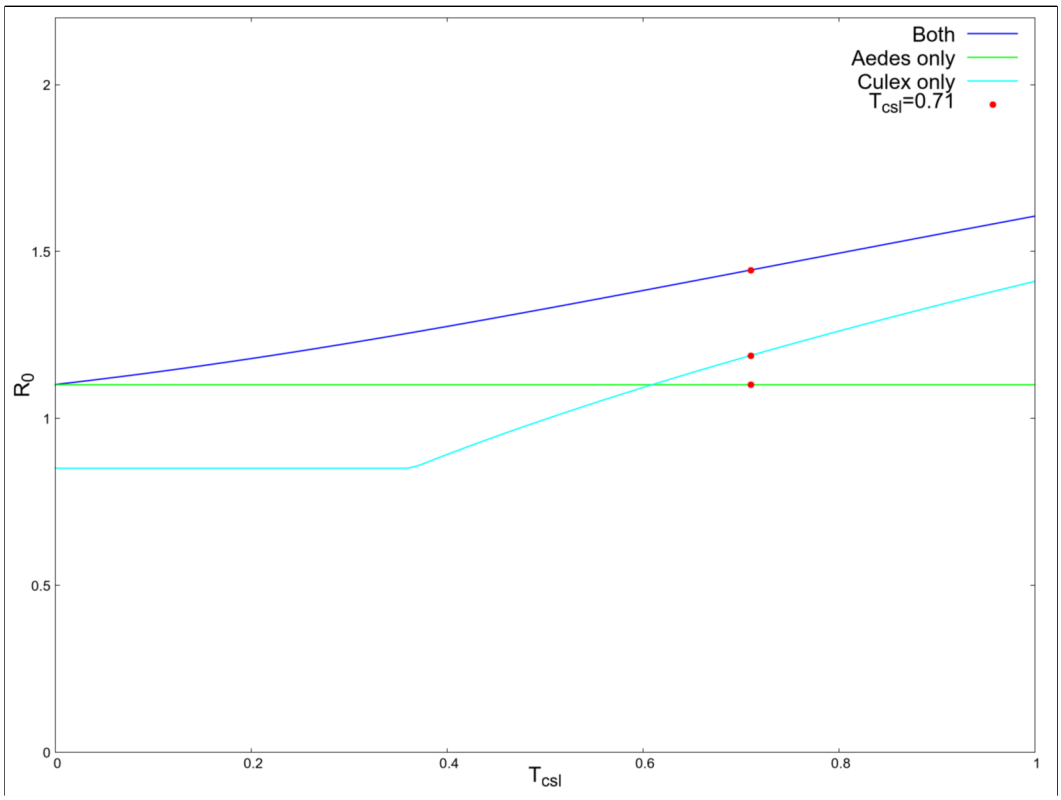
```
/· closest point to given value ·/
pt: floor(at(T_csl, int_params)·npts)+1$
print('T_csl, " =", zx[pt], ": both =", y1[pt], ", Aedes only =", y2[pt],
", Culex only = ",
```

T_{csl} = 0.71 : *both* = 1.445 , *Aedes only* = 1.102

, *Culex only* = 1.189

```
(%i136) wxplot2d(
            [
                [discrete, zx, y1],
                [discrete, zx, y2],
                [discrete, zx, y3],
                [discrete, [zx[pt], zx[pt], zx[pt]], [y1[pt], y2[pt], y3[pt]]]
            ],
    ['y, 0, 2.2],
    [style, [lines, 2, 1], [lines, 2, 3], [lines, 2, 6], [points, 3, 2, 1]],
    [legend, "Both", "Aedes only", "Culex only", sconcat("T_{csl}=", float(zx[pt]))],
    [xlabel, "T_{csl}"], [ylabel, "R_0"],
    [gnuplot_preamble, preamble]
        )$
```

(%t136)



4.5 Sheep & Lambs from Culex

Numerically calculate R0 for varying transmission parameter T_slc.
Show both Aedes and Culex (at max), or just Culex, highlighting the default value.

```
zx: makelist(float(z), z, 0, 1, 1/npts)$
y1: makelist(0, length(zx))$
y2: makelist(0, length(zx))$
y3: makelist(0, length(zx))$

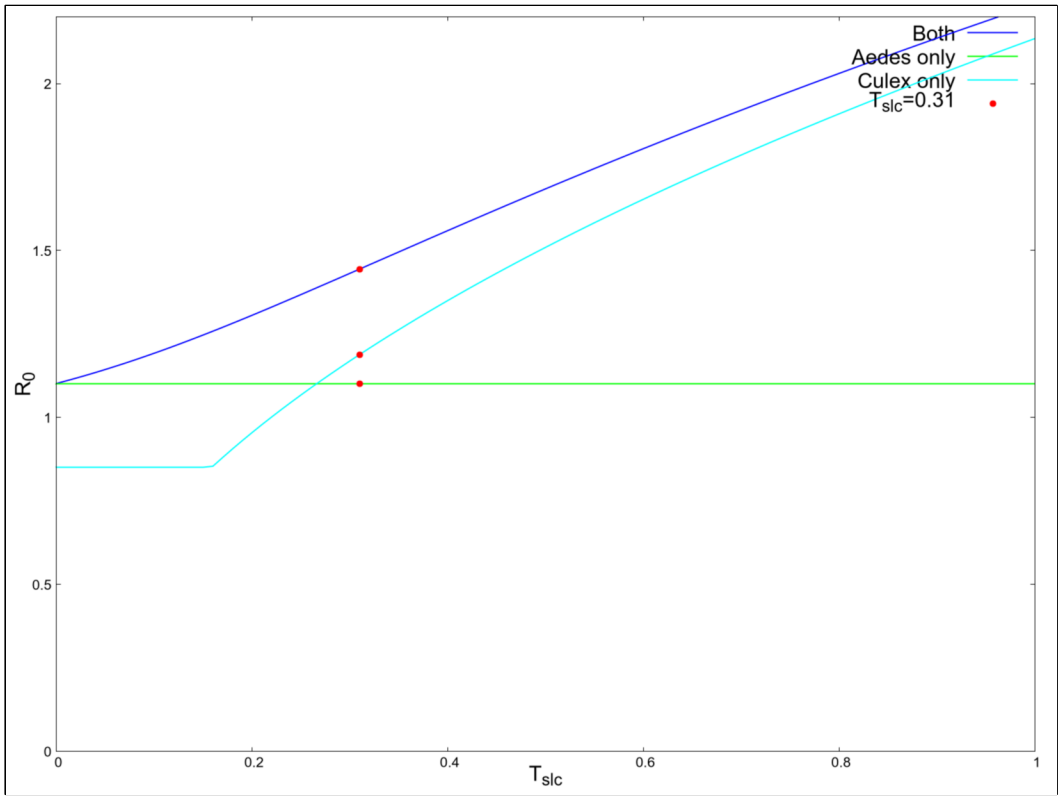
for i: 1 thru length(zx) do (
    int1: [q = 0.85, T_asl = 0.41, T_sla = 0.16, T_csl = 0.71, T_slc = zx[i]],
    y1[i]: float(lmax(map(rhs, realroots(at(at(chpol2, HV_popns), int1)))),
    y2[i]: float(lmax(map(rhs, realroots(at(at(chpol2, HV_A_only), int1)))),
    y3[i]: float(lmax(map(rhs, realroots(at(at(chpol2, HV_C_only), int1)))))
)$
```

```
/· closest point to given value ·/
pt: floor(at(T_slc, int_params)·npts)+1$
print('T_slc, " =", zx[pt], ": both =", y1[pt], ", Aedes only =", y2[pt], ", Culex only = ",
```

T_{slc} = 0.31 : *both* = 1.445 , *Aedes only* = 1.102 , *Culex only* = 1.189

```
wxplot2d(
    [
        [discrete, zx, y1],
        [discrete, zx, y2],
        [discrete, zx, y3],
        [discrete, [zx[pt], zx[pt], zx[pt]], [y1[pt], y2[pt], y3[pt]]]
    ],
    ['y, 0, 2.2],
    [style, [lines, 2, 1], [lines, 2, 3], [lines, 2, 6], [points, 3, 2, 1]],
    [xlabel, "T_{slc}"],
    [ylabel, "R_0"],
    [legend, "Both", "Aedes only", "Culex only", sconcat("T_{slc}=", zx[pt])],
    [gnuplot_preamble, preamble]
)$
```

(%t144)



5 Showing that R0 = q when there is no transmission

Only vertical transmission, so transmissions are
- vertical trans: IAE <- IA

```
J0: jacobian(at(dx, [T_asl = 0, T_sla = 0, T_csl = 0, T_slc = 0]), x)$

T0: zeromatrix(9,9)$
T0[4,7]: J0[4,7]$

Σ0: J0 - T0$
```

NGM with large domain K_L

```
KL0: factor(-T0.invert(Σ0));
```

(KL0)

$$\begin{pmatrix} 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & \frac{q\,\varepsilon\,(\mu_A+wait_A)\,(\mu_{AE}+bh_A)\,(\mu_{ALP}+dev_ALP)}{bh_A\,dev_ALP\,wait_A\,(\mu_A+\varepsilon)} & q & \frac{q\,(\mu_{AE}+bh_A)}{bh_A} & \frac{q\,(\mu_{AE}+bh_A)\,(\mu_{ALP}+dev_ALP)}{bh_A\,dev_ALP} & \frac{q\,(\mu_A+wait_A}{} \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix}$$

The NGM with small domain K
(just a single entry)

```
K0: KL0[4,4];
```

(K0) q

```
;
```

Appendix 9:

Consent Form for Follow-up Questions for Bayesian Analysis

Follow-up RVF Farm Questions (2018)

Informed Consent Form for Follow Up Farm Survey Participants

Project: Understanding Rift Valley fever in the Republic of South Africa

Purpose

Rift Valley fever virus has been the cause of disease outbreaks within South Africa, where the most recent outbreak occurred in 2010-2011. Rift Valley fever virus infection has resulted in significant losses of livestock through mortalities and abortions. In 2015 and 2017 we conducted surveys to check for antibodies in livestock, wildlife and people in the region and asked questions regarding the management of animals on farms. We are focusing on identifying factors associated with occurrence and severity of Rift Valley fever in animals as it relates to deaths and abortions. The results of this research will be used to reduce the risk of Rift Valley fever virus infection in both animals and people.

This project was approved by Witwatersrand Research Ethics Committee – M140306, telephone number 011 717 1234 and the Provincial Department of Health to understand Rift Valley fever better and to minimize outbreaks in South Africa.

Procedures

We would like to ask 10 questions to clarify your responses from the farm questionnaire you have previously completed. The information that you give now will be added to that previous questionnaire data and will be protected in the same confidential manner. Your participation in this follow-up is voluntary. The benefit of participating in the study is that we will improve our understanding of factors that impact the severity of Rift Valley fever in a herd with factors on the farm, the only risk associated with this follow-up is slight inconvenience.

Please ask me any questions regarding this follow-up research at this time.

Respondent agreement:

The farm survey follow-up research study has been explained to me. I voluntarily participate. I had an opportunity for my questions to be answered. I know that I may stop my participation in the research at any time. I understand that if I have questions about this research and my participation, I may contact the project's Field Coordinator, Dr. Claudia Cordel, at 084-908-0407 or email execuvet26@gmail.com. I agree to answer questions about my farm pertaining to the Rift Valley fever outbreak in 2010-2011.

Participant's Signature: _____ Date _ _ / _ _ /201_ Name *(write complete and clearly)* _____

Farm Name _____ Farm ID [ ]

Initials Interviewer:

Follow-up RVF Farm Questions (2018)

For verbal consent:

_____ Date _ _ / _ _ /201_
Participant's Name

I confirm that I have read the entire consent form to the participant and the participant verbally, volunteered to participate in the follow-up:

_____
Name of Project Interviewer
(write complete and clearly)

Appendix 10:

Follow-up Questions Asked of the Farmers for Bayesian Analysis

Follow Up Farm Survey Questionnaire

The first question is:

1. Were you farming with livestock (cattle, sheep or goats) on this property during the 2010-2011 RVF outbreak? _____
2. Did you have about the same numbers of livestock just before the 2010-2011Rift Valley fever outbreak as you had in the past two years when you answered our previous questionnaire(s)? _____

If answer to 2 was "No":

2a. How many cattle did you have going into the 2010-2011 Rift Valley fever outbreak?_____

2b. How many sheep did you have going into the 2010-2011 Rift Valley fever outbreak?_____

2c. How many goats did you have going into the 2010-2011 Rift Valley fever outbreak?_____

3. Previously we asked you if Rift Valley fever had been confirmed on your farm before, and you answered _____.

If answer to 2 was "No":

3a. Even if unconfirmed, did you have cases of abortion or deaths on the farm from Rift Valley fever virus during the 2010-2011 outbreak?

_____

If answer to 2 was "Yes":

3b. To be more specific, did you have cases of abortion or deaths on the farm from Rift Valley fever on the farm during the 2010-2011 outbreak, even if unconfirmed? _____

4. *If 3a or 3b was "Yes":* I am going to ask you about the number of animals affected by the 2010-2011 Rift Valley fever outbreak on your farm. First, I am going to ask about the number of adult animals that had an abortion during the outbreak, then I am going to ask about the number adult and young animals that died during the outbreak. Please do not include any abortions as a deaths, but count abortions and deaths separately.

4a. How many abortions occurred in your herd during the 2010-2011 Rift Valley fever outbreak?

What was the number of Cows that aborted? _____
What was the number of Ewes that aborted? _____
What was the number of Does that aborted? _____

4b. How many of each of the following species and age groups died during the 2010-2011 Rift Valley fever outbreak?

What was the number of Adult Cattle that died_____
What was the number of Calves that died _____
What was the number of Adult Sheep that died _____
What was the number of Lambs that died_____
What was the number of Adult Goats that died _____
What was the number of Goat Kids that died_____

Note, if they give you the same number for deaths and abortions please note it out and ask them confirm. If they only can't remember the number of





deaths vs abortions, please
indicate the numbers for each species affect by either in the notes.

5. Did you vaccinate against Rift Valley fever virus before you first heard that the 2010-2011 outbreak was happening?

If 5. was Yes ask 5a. if no, skip to 6:

5a. Please estimate the percent of your herd that was vaccinated against Rift Valley fever virus at the time of the outbreak. _____%

6. Did you vaccinate against Rift Valley fever virus during the 2010-2011 outbreak?

If 6. was Yes answer 6a, if no, skip to thank you:

6a. Do you think the vaccine worked?